Thrombogenic potential of human coronary atherosclerotic plaques

Diego Ardissino, Piera Angelica Merlino, Kenneth A. Bauer, Ezio Bramucci, Maurizio Ferrario, Raffaella Coppola, Raffaella Fetiveau, Stefano Lucreziotti, Robert D. Rosenberg, and Pier Mannuccio Mannucci

Higher levels of tissue factor (the initiator of blood coagulation) have been found in coronary atherosclerotic plaques of patients with unstable coronary artery disease, but it is not established whether they are associated with a different thrombotic response to in vivo plaque rupture. In 40 patients undergoing directional coronary atherectomy, prothrombin fragment 1 + 2, a marker of thrombin generation, was measured in intracoronary blood samples obtained proximally and distally to the coronary atherosclerotic plaque before and after the procedure. Before the procedure, plasma prothrombin fragment 1 + 2 levels were significantly increased across the lesion in patients with unstable, but not in those with stable, coronary disease (unstable, median increase, 0.37 nM; range, −0.35–1.16 nM) (stable, median increase, −0.065 nM; range, −0.58–1.06 nM) (P = .0021). After plaque removal, an increase in prothrombin fragment 1 + 2 across the lesion was observed only in patients with unstable coronary disease (unstable, median increase, 0.25 nM; range, −1.04–4.9 nM) (stable, 0.01 nM; range, −0.48–3.59 nM) (P = .036). There was a correlation between the tissue factor content of the plaque and the increase in thrombin generation across the lesion (ρ = 0.33; P = .038). The higher tissue factor content found in plaques obtained from patients with unstable coronary disease was associated with a local increase in thrombin generation, thus suggesting a link with the in vivo thrombogenicity of the plaque. (Blood. 2001;98:2726-2729)

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Intracoronary blood sampling
Thrombin generation was quantitated by measuring the plasma levels of prothrombin fragment 1 + 2, a 31-kd polypeptide released from the amino terminal end of prothrombin during its conversion to thrombin, which is considered to be an indirect quantitative index of the thrombin generated by the prothrombinase complex. Although previous studies have usually measured prothrombin fragment levels in peripheral venous blood, our goal was to measure it in coronary artery blood collected by means of a specially designed sampling catheter that allows a blood flow of 80 mL/min. A potential advantage of this technique over peripheral venous sampling is that it avoids dilution of the fragment in the systemic circulation; a potential disadvantage is that blood withdrawal through a catheter may itself artifactualy increase the levels of the fragment. To evaluate potential artifactual increases, the fragment was measured in venous blood withdrawn directly from a clean antecubital venipuncture made by means of a 19-gauge needle at the same time blood was withdrawn through the sampling catheter in 24 patients who had received 325 mg aspirin and 70 U/kg heparin. There was virtually no difference in plasma prothrombin fragment 1 + 2 levels between the blood samples obtained directly from the vein and those obtained from the sampling catheter (0.84 nM/L ± 0.08 vs 0.86 nM/L ± 0.05, respectively). To assess the variability over time of prothrombin fragment 1 + 2 levels in the samples drawn directly from the coronary artery by means of the sampling catheter, 2 sets of blood samples were drawn from the same 24 patients with an interval of 25 minutes (the average time of the directional coronary atherectomy procedure). An increase was defined as abnormal when fragment levels were above the 95th percentile of the distribution of the changes between the 2 samples (0.11 nM).

Blood processing and measurement of thrombin generation
Samples were collected through the sampling catheter directly into refrigerated plastic tubes containing an anticoagulant mixture consisting of a thrombin inhibitor, EDTA, and aprotinin. The anticoagulant blood ratio used was 1:9 (vol/vol). Blood samples were immediately centrifuged at 2500g for 25 minutes at 4°C; the plasma was frozen on dry ice and stored at −80°C until use. All samples were analyzed by laboratory personnel who had no knowledge of the clinical data. Plasma levels of prothrombin fragment 1 + 2 were measured by means of a previously described double-antibody radioimmunoassay, which has a coefficient of variation of 8%.8

Coronary angiography and directional coronary atherectomy
Selective coronary arteriography was performed in multiple views using the Judkins technique. Greater than 70% narrowing in the diameter of the coronary arteries was considered significant coronary artery stenosis. Patients were classified as having 1-, 2-, or 3-vessel disease according to the number of vessels with significant coronary stenoses. Angiographic morphology of the lesions was prospectively assessed and was considered complex if the lesions had irregular borders, overhanging edges, ulcerations, or thromboses.

Before directional coronary atherectomy, patients were given 160 to 325 mg aspirin and heparin U/kg 70 adjusted as needed to maintain an activated clotting time of more than 250 seconds. Directional coronary atherectomy was performed using standard procedures. Briefly, the atherectomy device was directed over a previously inserted 300-cm-long guide wire and positioned at the level of the stenosis, and the support balloon was then inflated up to 1 atm. The cutter was then retracted, and the inflation pressure of the balloon was increased to a maximum of 3 atm. The driving motor was started, and the rotating cutter slowly was advanced to cut and collect the protruding plaque in the collection chamber located at the tip of the catheter. After each pass, the balloon was deflated and either removed or repositioned.

Plaque processing and measurement of tissue factor procoagulant activity
After thawing, the lipid-bound proteins in the plaques were solubilized using 1% Triton X-100 in phosphate-buffered saline for 2 hours at room temperature and then were centrifuged at 50 000g for 1 hour at 20°C. Tissue factor activity was analyzed in the supernatant using a previously published chromogenic factor Xa generation assay.9

Statistical analysis
The descriptive statistics include mean values and standard deviations, or median values and ranges, as appropriate. Between-group differences were tested by using the Student unpaired t test or the Mann Whitney U test. Prevalences were compared by means of the χ² test. Correlation between tissue factor activity and prothrombin fragment 1 + 2 was calculated as Spearman rank correlation (ρ). All tests were 2-tailed, and P < .05 was regarded as statistically significant.

Results
Clinical and angiographic characteristics of the study population are reported in Table 1. There was a higher prevalence of lesions with complex morphology in the patients with unstable coronary artery disease than in those with stable disease (P < .0001).
Before directional coronary atherectomy, there was no difference in the plasma prothrombin fragment 1 + 2 levels measured proximally to the lesion between the patients with stable or unstable coronary artery disease, whereas higher fragment levels were found distally to the lesion in unstable patients (P = .0045). No significant increase in fragment 1 + 2 levels was observed across the lesion in stable patients, whereas a significant increase was observed in unstable patients (Table 2) (P = .002). The median change in prothrombin fragment levels across the lesion was significantly greater in patients with unstable coronary artery disease (P = .0021); there was a median increase of 0.37 nM range, −0.35-1.16 nM) in unstable patients and a median decrease of −0.065 nM range, −0.58-1.06) in stable patients. An abnormal increase in the fragment was observed in 71% of the unstable patients but in only 28% of the stable patients (χ² = 7.24; P = .007).
After plaque removal, there was no difference in the fragment levels measured proximally to the lesion between the patients with stable and unstable coronary artery disease, whereas significantly higher levels were found distally to the lesion in patients with unstable disease (P = .027). No significant increase in fragment 1 + 2 was observed

Table 1. Clinical, angiographic, and procedural characteristics of patients with stable or unstable coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>All (N = 40)</th>
<th>Stable (n = 22)</th>
<th>Unstable (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y) (%)</td>
<td>53 ± 7.6</td>
<td>54.4 ± 7.3</td>
<td>52.5 ± 8.2</td>
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<td>Males (%)</td>
<td>37 (92)</td>
<td>20 (91)</td>
<td>17 (94)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>17 (43)</td>
<td>10 (45)</td>
<td>7 (39)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>20 (50)</td>
<td>12 (55)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>17 (43)</td>
<td>7 (32)</td>
<td>10 (56)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>2 (5)</td>
<td>1 (5)</td>
<td>1 (3)</td>
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<tr>
<td>Family history (%)</td>
<td>21 (53)</td>
<td>12 (55)</td>
<td>10 (56)</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>15 (38)</td>
<td>10 (45)</td>
<td>5 (28)</td>
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<tr>
<td><strong>Angiographic and procedural characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. diseased vessels (%)</td>
<td>1 (%)</td>
<td>22 (55)</td>
<td>12 (55)</td>
</tr>
<tr>
<td>2 (%)</td>
<td>11 (29)</td>
<td>7 (32)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>3 (%)</td>
<td>7 (17)</td>
<td>3 (14)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Lesion morphology*</td>
<td>21 (53)</td>
<td>18 (82)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>Smooth (%)</td>
<td>19 (47)</td>
<td>4 (18)</td>
<td>15 (83)</td>
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<td>Complex (%)</td>
<td>5.3 (2.9)</td>
<td>5.6 (2.9)</td>
<td>5.0 (2.7)</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as means ± SD or medians and ranges as appropriate. Means were compared using the Student unpaired t test; medians were compared using the Mann-Whitney U test. Frequencies were compared using the χ² test.

*P < .0001.
across the excised lesion in stable patients, whereas a significant increase was observed in unstable patients (Table 2) \( (P = .043) \). The change in fragment levels across the lesion was significantly greater in the patients with unstable coronary disease \( (P = .036) \), with a median increase of 0.25 nM \( (\text{range}, -1.04-4.91) \) compared with 0.01 \( (\text{range}, -0.48-3.59) \) in stable patients. The median changes in prothrombin fragment 1 + 2 across the lesion before and after directional coronary atherectomy were not significantly different in either stable or unstable patients. An abnormal increase in the fragment was observed in 65% of the unstable patients and in 27% of the stable patients \( (\chi^2 = 5.4; P = .01) \). Percentages of patients with abnormal increases before and after directional coronary atherectomy were not significantly different between the stable and the unstable patients. However, although the greater changes in the fragment were observed after directional coronary atherectomy in stable and unstable patients, these were more pronounced in the unstable patients.

Median weights were as follows: extracted plaque, 5.7 mg \( \text{(range}, 0.15-15.1 \text{ mg)} \); coronary plaque of patients with stable disease, 4.5 mg \( \text{(range}, 0.15-9.7 \text{ mg)} \); coronary plaque of patients with unstable disease, 7.2 mg \( \text{(range}, 0.8-15.1 \text{ mg)} \). Tissue factor activity per milligram plaque weight ranged from 0 to 5.3 mU/mg. It was greater in the plaques extracted from patients with unstable disease than from patients with stable disease \( (0.258 \text{ mU/mg} \text{ [range}, 0.08-5.30 \text{ mU/mg}] \text{ vs} 0.156 \text{ mU/mg} \text{ [range}, 0.1-9.4 \text{ mU/mg}] \); \( P = .011 \). There was a statistically significant positive correlation between the tissue factor activity of the removed plaque and the increase in thrombin generation across the lesion after the procedure \( (\rho = 0.33; \ P = .038) \).

### Discussion

Tissue factor has been detected within the necrotic cores of endo-atherectomy specimens from patients with carotid atherosclerosis and in atherectomy specimens from patients with coronary artery disease. Furthermore, the tissue factor found in atherosclerotic plaques has been shown to be functionally active in vitro because it is capable of generating activated factor X. In an ex vivo model, Toschi et al. and Badimon et al. used a perfusion chamber to evaluate the platelet and fibrinogen deposition on aortic atherosclerotic plaques exposed to flowing blood, and they found a positive correlation between the amount of tissue factor detected by means of in situ binding assays and both platelet and fibrinogen deposition. Tissue factor staining of the lipid-rich core was more intense than that of all of the other plaque components, and there was also a higher level of plaque and fibrinogen deposition. Finally, a recent study has shown that a thrombus could be histologically detected only in coronary atherectomy specimens characterized by immunohistochemically detectable tissue factor. All these findings strongly support the view that the presence and amount of functional tissue factor in coronary atherosclerotic plaques may be critical for thrombus formation in acute coronary events. However, it is still unknown whether higher levels of tissue factor in coronary plaques in vivo is associated with increased thrombus formation after plaque rupture.

This study confirms that tissue factor activity is greater in the coronary atherosclerotic plaques extracted from patients with unstable angina or myocardial infarction than in those extracted from patients with stable angina. The new finding is that the local (intracoronary) measurement of thrombin generation makes it possible to demonstrate that greater tissue factor activity in atherosclerotic plaque is associated with a greater increase in thrombin generation across the lesion, before and after plaque disruption induced by directional coronary atherectomy. Of the patients with unstable coronary artery disease, 71% had abnormal increases in thrombin generation before the procedure and 65% had them after the procedure; of those with stable disease, 27% had abnormal increases in thrombin generation across the lesion before the procedure and 25% had them after the procedure. Perhaps the greater increase in thrombin generation observed before the procedure in patients with unstable coronary artery disease occurred because most of them already had a ruptured plaque that exposed tissue factor. Patients with stable angina have smooth, fibrous, nonulcerated plaques with little exposure of tissue factor. We surmise that in patients with unstable plaque, the thrombogenic material is already exposed and so the lesions induced by atherectomy do not further increase such a thrombogenic stimulus. On the other hand, in patients who have stable plaque at baseline, the lesions induced by atherectomy theoretically expose a new surface to prothrombin activation, but the local thrombotic stimulus is too weak and inadequate to reach the level of prothrombin activation seen in unstable patients. The highest values of thrombin generation across the lesion were observed in patients with unstable coronary artery disease after atherectomy. We hypothesize that this strong thrombogenic stimulus may lead to the thrombotic complications occasionally observed during percutaneous coronary interventions despite intensive antithrombotic therapy.

On the whole, these findings further support the notion that plaque rupture and exposure of the atheromatous core is not the only precipitating event in determining coronary thrombosis. The nature of the plaque components exposed to flowing blood, together with the local rheologic and systemic blood factors in unstable patients, are also critical in determining the extent of the prothrombic response. In this respect, tissue factor appears to be a critical plaque component.

### References


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