A
cute coronary syndromes (ACS) are severe clinical manifestations of coronary artery lumen occlusion by a thrombus formed on the contact of a ruptured or eroded atherosclerotic plaque.\(^1\)\(^2\) Tissue factor (TF) is highly expressed in atherosclerotic plaques,\(^3\) and TF activity of plaques retrieved from patients with unstable angina (UA) is significantly higher than that found in the plaques of patients with stable angina (SA),\(^4\) which suggests that it may significantly determine thrombus formation after plaque disruption. TF activity is highly dependent on the presence of phosphatidylserine (PS),\(^5\) and it has been shown that this anionic phospholipid is redistributed on the cell surface during apoptotic death,\(^6\) conferring to the cell a potent procoagulant activity.\(^7\)\(^8\) Interestingly, shed membrane apoptotic microparticles rich in PS are produced in considerable amounts within human atherosclerotic plaques and carry almost all TF activity of the plaque lipid core,\(^9\) indicating that they may largely determine plaque thrombogenicity. In the present study, we hypothesized that high levels of cell-derived microparticles with procoagulant potential could also be detectable in the circulating blood of patients with recent clinical signs of plaque disruption and thrombosis and may therefore contribute to the initiation and perpetuation of the thrombotic process.

**Methods**

To isolate the circulating microparticles and determine their procoagulant potential, we prospectively included 39 patients with angina and angiographic documentation of coronary artery disease (CAD) and 12 controls. Among patients with CAD, 12 (9 men; mean age 62±3 years) had SA with no signs of myocardial ischemia at rest, and 27 had ACS: 13 (8 men; mean age 62±4 years) had UA (Braunwald class III) with documented signs of recent myocardial ischemia at rest, and 14 (10 men; mean age 61±4 years) had acute myocardial infarction (MI). Cardiovascular risk factors were not significantly different between the 3 groups of patients with CAD, except for hypercholesterolemia, which was more prevalent in patients with SA (\(P<0.02\)).

All coronary patients were receiving aspirin. Patients with ACS received additional standard antithrombotic therapy before blood sampling. Anti-ischemic medications were equally distributed between groups. Programmed primary coronary angioplasty (85% with stent placement) was performed after blood sampling in 9 patients.
with SA (75%), 12 patients with UA (92%), and 14 patients with MI (100%).

Controls (9 men and 3 women, mean age 58±4 years) were patients with angiographic documentation of absence of CAD (4 patients with chest pain, 4 patients with valvular disease, and 4 patients with dilated cardiomyopathy).

To characterize the cell origins of the microparticles, we included 16 additional consecutive patients with angina and angiographic documentation of CAD (5 with SA, mean age 63±5 years, and 11 with ACS, mean age 62±4 years) and 6 noncoronary patients (5 with valvular disease and 1 with dilated cardiomyopathy, mean age 65±8 years).

Isolation of the Circulating Microparticles and Determination of Their Procoagulant Potential
Blood samples were collected before any mechanical intervention at admission (day 0), except in 6 patients with MI, in whom blood sampling was performed at day 8 after the coronary event. Microparticles were captured by immobilized annexin V, and the anionic phospholipid content was determined by a prothrombinase assay as previously described in detail.9,10 We verified that the method used for the capture of microparticles did not allow the capture of PS-containing lipoproteins.

Determination of the Cell Origins of Circulating Microparticles
Microparticles were captured by specific antibodies (anti-CD3, anti-CD11a, anti-CD31, anti-CD146, and anti-GP Ib).9,10 The morphology of circulating microparticles was recently published.11,12

Statistical Analysis
Results are expressed as mean±SEM. Comparisons between groups were made by a 1-way ANOVA. Simple regression analysis was performed to analyze the relation between values of microparticles captured with anti-CD31, anti-CD146, or anti-GP Ib antibodies. A value of P<0.05 was considered statistically significant.

Results
The level of circulating microparticles in patients admitted with a diagnosis of SA was not significantly different from that in patients admitted for noncoronary heart disease (10.1±1.6 versus 9.9±1.6 nmol/L PS equivalent, respectively). However, the level of procoagulant microparticles was significantly elevated in the circulating blood of patients with UA (18.6±1.9 nmol/L PS) or MI (25.6±4.8 nmol/L PS) compared with patients who presented with signs of SA (P<0.01) or with noncoronary patients (P<0.01). In the patients with ACS, no difference was found in the level of circulating microparticles between those with UA and those with MI (Figure).

Two patients experienced recurrent ischemic syndromes during hospitalization: 1 patient in the UA group developed recurrent documented myocardial ischemia, and 1 in the MI group had reocclusion of his stented culprit coronary artery. Interestingly, these 2 patients had very high baseline levels of circulating procoagulant microparticles (35.6 and 62.3 nmol/L PS, respectively).

Circulating microparticles captured with anti-CD146 or anti-CD31 antibody were elevated in patients with ACS (compared with both stable coronary and noncoronary patients), whereas those captured with anti-GP Ib, anti-CD3, or anti-CD11a were not (Table). The values obtained with anti-CD31 antibody were not correlated with those obtained with anti-GP Ib antibody but were highly correlated with those obtained with anti-CD146 antibody (P<0.001). Given that there were almost no CD3-bearing microparticles, these findings indicate that microparticles captured with anti-CD31 or anti-CD146 were most likely of endothelial origin.

Discussion
In the present study, the levels of circulating procoagulant microparticles in patients who presented with SA were not different from those found in noncoronary patients. However, we found significantly elevated levels of circulating procoagulant microparticles in the patients who presented with ACS in comparison with both SA patients and noncoronary patients. These results suggest that an increase in circulating microparticles more specifically reflects the complications of atherosclerosis (ie, thrombus formation occurring on the contact of a disrupted atheromatous plaque). Because only patients with ACS received heparin, it could be argued that differences in levels of microparticles reflect differences in medications. This is unlikely, however, because heparin has been reported either to have no effect or to decrease microparticle generation from thrombin-activated platelets.13

Soejima et al14 recently reported that plasma TF antigen levels are significantly elevated in patients with UA compared with those measured in patients with SA and are associated with a poor prognosis. However, these authors did not measure TF activity, nor did they determine the origin of the TF antigen. Our findings extend those of Soejima et al and suggest that the procoagulant potential of the circulating blood is, at least in part, related to the presence of elevated levels of circulating procoagulant microparticles. Moreover, the prevalence of microparticles bearing CD146 and CD31 suggests a potentially important role for acute endothelial injury in this process. This may reflect the endothelial erosion at the site of plaque disruption, the endothelial injury on exposure of plaque microvessels to inflammatory cells, and/or the endothelial injury associated with myocardial ischemia. It should be noted that our data do not exclude the possibility that a certain amount of circulating microparticles may also originate from other cell types, including smooth muscle cells and cardiomyocytes.

The detection of elevated levels of circulating procoagulant microparticles 8 days after the acute ischemic syndrome is in line with the observation of persistent intracoronary thrombi 24 hours to 30 days after the ischemic episode.15 In the
present study, the 2 patients who experienced documented recurrent myocardial ischemia or coronary reoclusion with reinfarction had very high basal levels of circulating microparticles. This observation suggests that the level of circulating microparticles could be useful as an indicator of the persistence or recurrence of thrombus and therefore as a prognostic marker of the recurrence of ischemic events. This hypothesis needs to be tested in a large multicenter study.

In addition to their direct effect in promotion and amplification of the coagulation cascade, the circulating microparticles may also act in a variety of inflammatory processes\textsuperscript{16,17} and may be responsible for dissemination of the procoagulant and proinflammatory potentials to sites remote from the microenvironment of their formation.\textsuperscript{9}

In conclusion, high levels of procoagulant microparticles are present in the circulating blood of patients with ACS and may participate in the generation and perpetuation of intracoronary thrombi. The high levels of circulating microparticles of endothelial origin suggest an important role for endothelial injury in thrombus formation.

Acknowledgment

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References


### Antigenic Characterization of the Circulating Microparticles in Patients With SA, ACS or Noncoronary Heart Disease (NC)

<table>
<thead>
<tr>
<th>Capture Antibody</th>
<th>SA (n=5)</th>
<th>ACS (n=11)</th>
<th>NC (n=6)</th>
<th>P, 1-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD146</td>
<td>3.3±0.9</td>
<td>8.2±1.4</td>
<td>2.6±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD31</td>
<td>1.6±0.4</td>
<td>8.8±3.0</td>
<td>1.2±0.3</td>
<td>0.06</td>
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<tr>
<td>GP Ib</td>
<td>2.6±0.5</td>
<td>6.9±1.8</td>
<td>6.5±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>CD3</td>
<td>0</td>
<td>0.2±0.2</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>CD11a</td>
<td>0.6±0.3</td>
<td>1.7±0.9</td>
<td>0.2±0.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as nmol/L PS equivalent.
Elevated Levels of Shed Membrane Microparticles With Procoagulant Potential in the Peripheral Circulating Blood of Patients With Acute Coronary Syndromes

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