Persistent Systemic Inflammation in Unstable Angina Is Largely Unrelated to the Atherothrombotic Burden

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OBJECTIVES The aim of this study was to assess the relationship between systemic inflammation, atherosclerosis, and thrombosis in two distinct clinical models of atherothrombosis.

BACKGROUND Persistent unstable angina (UA) is commonly associated with coronary thrombosis and persistent systemic inflammation.

METHODS We assessed circulating markers of activation of the thrombotic and fibrinolytic cascades and systemic soluble and cellular markers of inflammation on admission in 40 patients with persisting UA (Braunwald class IIIB; group 1) and 30 patients with Leriche-Fontaine stage IIB-III peripheral artery disease awaiting revascularization (group 2).

RESULTS The extent of atherosclerosis (p < 0.01) and activation of the coagulation system were greater in group 2, which had higher thrombin-antithrombin III complexes and D-dimer levels (2.7 and 24.4 μg/l, respectively), than in group 1 (2.0 μg/l and 12.9 μg/l, p = 0.02 and p = 0.0001, respectively). In contrast, C-reactive protein and interleukin-6 levels were higher in group 1 (7.6 pg/ml and 7.8 pg/ml, respectively) than in group 2 (4.5 pg/ml and 3.0 pg/ml, p < 0.01 and p = 0.03, respectively). Moreover, neutrophil activation was only found in group 1 (neutrophil myeloperoxidase content –4.0 arbitrary units vs. +3.4 arbitrary units in group 2, p < 0.0001). These differences persisted during the initial three days of hospitalization.

CONCLUSIONS Such a large, consistent discrepancy between atherothrombotic burden and systemic inflammation suggests that atherothrombosis, by itself, is an unlikely cause of persisting, recurring UA. An understanding of the primary inflammatory mechanisms of persistent and recurrent coronary instability could open the way to novel therapeutic strategies. (J Am Coll Cardiol 2005;45:238–43) © 2005 by the American College of Cardiology Foundation

The spectrum of clinical presentation of acute coronary syndromes is varied. At one extreme end of the spectrum, some patients present with acute myocardial infarction not preceded by anginal symptoms and, after this event, may remain totally asymptomatic for years or decades. At the other end, some patients present with infarction preceded (1), and the average risk of major cardiac events at four to six months exceeds 10% under the current optimal medical (2) or interventional strategies (3). Eventually, only after six months, the increased risk transiently conferred by instability reverts back to that of stable or “quiescent” phases of ischemic heart disease. Coronary thrombi composed of layers of different ages are common at autopsy in unstable patients (4), consistent with a “staccato” pattern of clinical exacerbations of instability and with a nearly 50% reduction of infarction rates achieved by antiplatelet and antithrombotic treatments (1). However, thrombosis, by itself, may not be the only plausible explanation of the long persistence and common recurrence of coronary instability. A possible role of an acute coronary inflammatory process is suggested by: 1) elevated levels of circulating inflammatory markers observed in patients with refractory UA and with acute infarction preceded by UA (5–9); 2) persistence of elevated C-reactive protein (CRP) serum levels after acute instability associated with a seven-fold greater risk of recurrent UA or of infarction (10,11); and 3) the adverse prognostic value of increasing levels of interleukin (IL)-1 receptor antagonist and IL-6 during the first two days after admission in patients with persisting UA (12).

Thus, the intriguing association between coronary thrombosis and inflammation observed in patients with UA raises the question of whether systemically detectable inflammation is largely a reflection of ongoing atherothrom-
Abbreviations and Acronyms
CRP = C-reactive protein
IL = interleukin
PAD = peripheral artery disease
UA = unstable angina

Atherothrombosis or whether it may reflect independent, primary causal mechanisms of inflammation directly responsible for persisting, recurrent coronary instability.

To investigate the relative role of atherothrombosis and that of primary inflammatory triggers in UA, we compared the levels of circulating markers of systemically detectable thrombotic and inflammatory activation in two distinct clinical syndromes, both characterized by atherothrombosis.

We assessed systemic soluble and cellular markers of inflammation together with markers of activation of the thrombotic and fibrinolytic cascades and seropositivity to putative infectious agents in 40 patients with Braunwald class IIIB UA and negative troponin T, as well as in 30 patients with Leriche-Fontaine stage IIB-III peripheral artery disease (PAD) awaiting revascularization procedures, as potential distinct clinical models of atherothrombotic diseases.

METHODS

Study population. GROUP 1: UNSTABLE ANGINA. We enrolled 40 consecutive patients (34 men, age 60 ± 12 years) admitted to our coronary care unit with Braunwald class IIIB UA, without evidence of acute myocardial necrosis detected by troponin T release, and submitted to coronary angiography. We excluded patients with positive troponin T (≥0.1 mg/l), known or suspected thrombotic disorders, malignancy, infection, inflammatory disease, or recent surgery or trauma (<4 weeks). All patients were treated with aspirin, intravenous nitrates, calcium antagonists, and/or beta-blockers. Heparin was not used as a first-step treatment. In addition, we also excluded patients with Doppler evidence of PAD or a systolic ankle/brachial pressure ratio ≤0.95.

GROUP 2: PERIPHERAL ARTERY DISEASE. We enrolled 30 consecutive patients (21 men, age 63 ± 16 years) with Leriche-Fontaine stage IIIB to III PAD with claudication appearing after <100 m of walking, admitted for revascularization by a Rotablator percutaneous procedure or surgery. General exclusion criteria adopted for UA patients were also applied to PAD. In addition, we also excluded patients with evidence of trophic limb lesions and those with a previous myocardial infarction and echocardiographic evidence of regional wall motion alteration at rest or after a dipyridamole stress test.

Informed consent. The Ethics Committee of the Catholic University of Rome approved the protocol, and all patients gave their written, informed consent.

Blood sampling and laboratory methods. The number of coronary and peripheral artery flow-limiting stenoses with >70% diameter reduction and total occlusion and of stenoses between 50% and 70% was assessed visually by two independent observers, and discordant findings were resolved by a third observer.

Peripheral blood samples were taken on hospital admission and after 6, 12, 24, 48, and 72 h in both groups of patients.

We measured high-sensitivity CRP and IL-6 as markers of an acute-phase response, neutrophil myeloperoxidase content as an indicator of neutrophil activation, thrombin-antithrombin III complexes and prothrombin fragment 1+2 as markers of thrombin generation, and D-dimers as markers of formed intravascular fibrin undergoing lysis. Seropositivity for Chlamydia pneumoniae, cytomegalovirus, and Helicobacter pylori was also assessed. Troponin T, a specific marker of myocardial necrosis, was measured to rule out the possible role of myocardial cell damage in inducing the inflammatory response.

Samples for thrombin-antithrombin III complexes, prothrombin fragment 1+2, D-dimers, and IL-6 were immediately transferred into pre-cooled tubes containing citrate, theophylline, adenosine, and dipyridamole (CTAD tubes; Dade-Behring, Marburg, Germany) and centrifuged at 2,000 rpm for 20 min. Plasma aliquots were stored at −80°C. Commercially available ELISA assays (Enzygnost thrombin-antithrombin III complex, micro-prothrombin fragment 1+2, and D-dimer ELISA kit, Dade-Behring; Quantikine human IL-6, R&D Systems, Minneapolis, Minnesota) were used.

Serum samples were obtained for CRP and troponin T analysis and for assessment of seropositivity for C. pneumoniae, cytomegalovirus, and H. pylori. C-reactive protein was assayed in an ultra-sensitive CRP assay by nephelometry (BN-II, Dade-Behring) with a sensitivity of 0.2 mg/l. Troponin T was measured by a commercial enzyme immunoassay (Boehringer Mannheim, Mannheim, Germany). Seropositivity for C. pneumoniae was assessed by the micro-immunofluorescence method (Chlamydia MIF kit, MRL Diagnostics, Cypress, California); seropositivity for C. pneumoniae was defined as the presence of specific antibodies at a serum dilution of 1:16. Seropositivity for cytomegalovirus and H. pylori was assessed by a commercial ELISA for specific immunoglobulin G (Dade-Behring); seropositivity for cytomegalovirus and H. pylori was defined as the presence of a serum antibody titer >6 U/l and 10 U/l, respectively.

Samples for myeloperoxidase intracellular content were immediately transferred into tubes containing 1:9 EDTA solution and quickly analyzed using a Bayer H3 hematology analyzer (Bayer-Diagnostic Co., Tarrytown, New York), as previously described (8,13). The computer software calculates an index of intracellular myeloperoxidase activity (intracellular myeloperoxidase index), which quantifies the mean myeloperoxidase activity of the whole neutrophil population in arbitrary units. In normal subjects,
Table 1. Clinical and Angiographic Findings

<table>
<thead>
<tr>
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<th>Unstable Angina (n = 40)</th>
<th>Peripheral Artery Disease (n = 30)</th>
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</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>60 ± 12</td>
<td>63 ± 16</td>
</tr>
<tr>
<td>Male gender</td>
<td>34 (85%)</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>28 (41%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>16 (40%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>20 (50%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>13 (32%)</td>
<td>16 (55%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>22 (55%)</td>
<td>11 (36%)</td>
</tr>
<tr>
<td>Arterial stenoses &gt;70% and occlusions</td>
<td>2.5 ± 0.9</td>
<td>5.5 ± 1.6*</td>
</tr>
<tr>
<td>Arterial stenoses 50% to 70%</td>
<td>1.1 ± 0.5</td>
<td>4.7 ± 2.3*</td>
</tr>
</tbody>
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*p < 0.01 vs. unstable angina. Data are presented as the mean value ± SD or number (%) of patients.

CAD = coronary artery disease.

this index is about 0. Positive values appear when the neutrophils are rich in myeloperoxidase, and negative values indicate depletion of myeloperoxidase, due to neutrophil activation and degranulation.

Statistical analysis. As the data were not normally distributed, non-parametric tests were used. Results are expressed as the median value, 25% to 75% interquartiles, and range. The Mann–Whitney U test was used to evaluate differences between groups, and discontinuous variables were tested by the chi-square test. Correlation between variables was assessed by the Spearman correlation test. Continuous variables containing clinical data are expressed as the mean value ± SD and were evaluated by the Student t test. A p value of <0.05 was considered statistically significant. All tests were two-tailed.

RESULTS

Clinical variables and angiographic findings. The two groups were similar in terms of age, gender, smoking status, hypertension, diabetes, and family history of ischemic heart disease (Table 1). The number of flow-limiting arterial stenoses was significantly higher in PAD than in UA (stenosis >70% and occlusion: 5.5 ± 1.6 vs. 2.5 ± 0.9, respectively; stenoses between 50% and 70%: 4.7 ± 2.3 vs. 1.1 ± 0.5, respectively; p < 0.01) (Table 1, Fig. 1). In PAD, the presence of flow-limiting coronary stenoses was excluded by the echocardiographic dipyridamole stress test, and in UA, by the presence of peripheral artery stenosis by a systolic ankle/brachial pressure ratio ≥0.95.

Activation of thrombotic and fibrinolytic cascade. Activation of the thrombotic and fibrinolytic cascades was significantly greater in patients with PAD than in those with UA. On admission, thrombin-antithrombin III complexes and D-dimers levels were significantly higher in patients with PAD than in those with UA, being 2.7 µg/l (range 0.9 to 47.0 µg/l) versus 2.0 µg/l (range 1.2 to 7.4 µg/l) (p = 0.02) (Fig. 2A) and 24.4 µg/l (range 9.1 to 182.5 µg/l) versus 12.9 µg/l (range 1.9 to 60.0 µg/l) (p = 0.0001) (Fig. 2B), respectively. Moreover, the levels of thrombin-antithrombin III complexes and D-dimers remained consistently higher in patients with PAD than in those with UA throughout the first 72 h of hospitalization (p = 0.01; data not shown). Prothrombin fragment 1+2 median levels were not significantly different between UA (1.0 nmol/l, range 0.2 to 8.3 nmol/l) and PAD (1.0 nmol/l, range 0.3 to 7.5 nmol/l) (p = NS).

Inflammatory markers. In contrast, the inflammatory markers CRP and IL-6 were significantly higher in patients with PAD than in those with PAD, being 7.6 mg/l (range 1.4 to 114 mg/l) versus 4.5 mg/l (range 0.7 to 22.2 mg/l) (p < 0.01) for CRP (Fig. 2C) and 7.8 pg/ml (range 3 to 90 pg/ml) versus 3.0 pg/ml (range 3 to 15 pg/ml) (p = 0.03) for IL-6 (Fig. 2D), respectively. Furthermore, neutrophil activation was only observed in UA (intracellular myeloperoxidase index –4.0 arbitrary units [−14.5 to +7.7] vs. +3.4 arbitrary units[−5 to +8.5] in PAD, p < 0.0001) (Fig. 2E). These differences were maintained throughout the first 72 h of hospitalization (p = 0.01). The neutrophil count did not differ between UA and PAD patients. A correlation between CRP and IL-6 levels was statistically significant in UA and PAD patients (r = 0.45, p = 0.01 and r = 0.34, p < 0.05, respectively). The correlation between the intra-
cellular myeloperoxidase index and CRP was statistically significant in patients with UA \((r = 0.62, p = 0.03)\) but not in patients with PAD \((r = 0.13, p = \text{NS})\). No correlation was found between CRP and thrombin-antithrombin III complexes, D-dimers, or prothrombin fragment 1 levels in UA or PAD patients.

The prevalence of seropositivity for infectious agents was similar in UA and PAD: 62% versus 40% for \textit{C. pneumoniae} \((p = \text{NS})\), 89% versus 87% for cytomegalovirus \((p = \text{NS})\), and 83% versus 78% for \textit{H. pylori} \((p = \text{NS})\). There was no correlation between CRP levels and antibody titers against \textit{C. pneumoniae}, cytomegalovirus, and \textit{H. pylori} in UA or PAD groups.

**DISCUSSION**

Our study shows that atherothrombosis is unlikely by itself to account for the elevation of systemic markers of inflammation, which characterizes patients with persisting UA. Indeed, levels of thrombin-antithrombin III complexes (a marker of thrombin generation) and D-dimers (a marker of formed intravascular fibrin) were nearly twice as high in patients with severe PAD (Leriche-Fontaine IIB-III) awaiting revascularization than in patients with severe UA (Braunwald class IIIB), consistent with a significantly greater number of flow-limiting stenosis, total occlusions, or stenoses >70% and of stenoses 50% to 70%. By contrast, levels of CRP and IL-6, two major markers of inflammation, were significantly higher in patients with UA than in patients with PAD. Moreover, neutrophil activation, as assessed by its intracellular content of myeloperoxidase, was only observed in UA but not in PAD patients and was linearly correlated with CRP levels. Thus, although severe PAD requiring revascularization procedures is associated with a much greater systemic activation of the coagulation cascade, as compared with UA, it is characterized by a much smaller elevation of CRP and IL-6 levels and by no detectable neutrophil activation in the peripheral blood. Such differences remained significant in all successive blood samples taken up to 72 h after admission.

Elevation of CRP and IL-6 and neutrophil activation have been consistently reported in patients with UA but not in patients with chronic, stable effort angina and a greater severity of coronary atherosclerosis \((6–8)\) or in patients with active variant angina and a greater ischemic burden \((8,14)\). At a variance from CRP and IL-6 serum levels, which in patients with Braunwald class IIIIB UA may remain elevated for several months after discharge \((12,15)\), neutrophil activation appears limited to the acute symptomatic phase of instability of the disease, as it is not found at discharge \((8)\), and appears to take place selectively in the coronary vascular bed of unstable patients \((13)\). These observations are consistent with the prognostic value of myeloperoxidase levels in patients with acute coronary syndromes \((16)\). Conversely, in patients with PAD, neutrophil activation was only
reported after induced claudication (17), but not at rest (17,18).

The greater systemic levels of inflammatory markers in UA, compared with PAD, cannot be explained by differences in the prevalence of traditional risk factors (Table 1) or in seropositivity for infectious agents possibly involved in atherosclerotic syndromes, as the prevalence of seropositivity for *C. pneumoniae*, cytomegalovirus, and *H. pylori* was not significantly different between patients with UA and those with PAD, in agreement with previous studies (19,20).

Our data show significant differences not only in the magnitude but also in the pattern of systemic markers of inflammation and activation of the coagulation system in two prototypical clinical models of atherothrombosis, UA, and peripheral vascular disease. In particular, UA is characterized by a marked systemic up-regulation of inflammatory activation, which considerably exceeds the amount attributable to the atherothrombotic burden in PAD. The persistence and recurrence of coronary instability is more suggestive of persistent, recurrent thrombogenic stimuli than of a process of repair of a single initial injury resulting from a fissured or eroded atherosclerotic plaque.

**Study limitations.** We assessed the atherosclerotic burden only on the basis of the number of stenoses >70% and 50% to 70%, which was more than double in patients with peripheral vascular disease (Table 1). However, in postmortem studies, the number of coronary flow-limiting stenoses strongly correlated with the percentage of coronary intimal surface covered by raised fibrous plaque (21). This correlation is likely to hold true also for PAD. A greater atherosclerotic burden and a larger endothelial involvement are consistent with the markedly higher levels of D-dimers and thrombin-antithrombin III complexes in patients with PAD also observed in previous studies (22–24). Yet, such markedly larger atherosclerotic burden in peripheral vascular disease was not associated with neutrophil degranulation, which was present in UA and with lower high sensitivity CRP and IL-6 serum levels, as compared with UA.

**Atherothrombosis, inflammation, and plaque instability.** Collectively, our findings suggest the hypothesis that the recurring phases of coronary instability may not necessarily develop when the atherosclerotic burden has reached a critical threshold, but may as a result of transient superimposed "primary" coronary inflammatory stimuli. Such triggers of instability eventually wane with a return of the patient to a stable phase and baseline ischemic risk, related to atherosclerotic and risk factor burden. This hypothesis would be consistent with findings of multiple complex (25,26), fissured (27,28), and inflamed (29) coronary artery plaques found in patients with persistent, recurrent instability, suggestive of simultaneous multifocal plaque instability and with widespread coronary inflammation, also involving angiographically normal arteries (13). Such widespread coronary inflammation could be related to perturbation of the immune response (30,31), antigenic mimicry, and auto- toimmune mechanisms (32,33). Understanding these inflammatory mechanisms could open the way to the development of novel therapeutic strategies designed to block inflammatory triggers of persistent, recurrent coronary thrombus formation, without interfering heavily with hemostatic mechanisms—so far the only available strategy to prevent local coronary thrombosis—at the price of an increased risk of bleeding.

**REFERENCES**


