High Levels of Systemic Myeloperoxidase Are Associated With Coronary Plaque Erosion in Patients With Acute Coronary Syndromes
A Clinicopathological Study

Giuseppe Ferrante, MD, PhD; Masataka Nakano, MD; Francesco Prati, MD; Giampaolo Niccoli, MD, PhD; Maria T. Mallus, MD, PhD; Vito Ramazzotti, MD; Rocco A. Montone, MD; Frank D. Kolodgie, PhD; Renu Virmani, MD; Filippo Crea, MD, PhD

Background—Systemic levels of myeloperoxidase predict prognosis in patients with acute coronary syndromes and are considered a marker of plaque vulnerability. It is not known whether myeloperoxidase is associated with different coronary morphologies (ie, rupture or erosion of the culprit lesion) in patients with acute coronary syndrome.

Methods and Results—Twenty-five consecutive patients (aged 67±11 years; 15 men [60%]; 13 [52%] with non–ST-segment elevation acute coronary syndrome and 12 [48%] with acute ST-segment elevation myocardial infarction) were enrolled. Optical coherence tomography classified the culprit lesion as ruptured in 18 (72%) or eroded in 7 patients (28%) and detected intraluminal thrombus in 89% of ruptured plaques and 100% of eroded plaques. Baseline systemic levels of serum myeloperoxidase were significantly higher in patients with an eroded plaque than in those with a ruptured plaque (median, 2500 ng/mL; 25th to 75th percentile, 1415 to 2920 versus median, 707 ng/mL; 25th to 75th percentile, 312 to 943; \(P=0.001\)), whereas C-reactive protein levels did not differ significantly (median, 11.3 mg/L; 25th to 75th percentile, 1.3 to 28.5 versus median, 3.9 mg/L; 25th to 75th percentile, 1.3 to 17.8; \(P=0.76\), respectively). In addition, the density of myeloperoxidase-positive cells within thrombi overlying plaques in postmortem coronary specimens retrieved from sudden coronary death victims was significantly higher in lesions with erosion (n = 11) than ruptures (n = 11) (median, 1584; 25th to 75th percentile, 1088 to 2135 cells/mm² versus median, 579; 25th to 75th percentile, 442 to 760 cells/mm²; \(P=0.0012\)).

Conclusions—Systemic myeloperoxidase levels are significantly elevated in patients with acute coronary syndrome presenting with eroded culprit plaque compared with patients presenting with ruptured culprit plaque. Consistently, in postmortem coronary specimens, luminal thrombi superimposed on eroded plaques contain a higher density of myeloperoxidase-positive cells than thrombi superimposed on ruptured plaques. This study supports the concept that elevations in selective inflammatory biomarkers reflect specific acute complications of coronary atherosclerosis. (Circulation. 2010;122:2505-2513.)

Key Words: acute coronary syndromes • myeloperoxidase • optical coherence tomography • plaque

P laque rupture with subsequent thrombus formation is considered the main mechanism responsible for acute coronary syndromes (ACS) and is reported in approximately two thirds of patients dying of acute myocardial infarction (MI) in postmortem histopathological studies,1,2 whereas plaque erosion is detected in approximately one third.3,4 Furthermore, plaque erosion has been found in approximately one quarter of patients with ST-segment elevation MI (STEMI) in vivo by optical coherence tomography (OCT).5 The precursor of ruptured culprit lesions in patients with ACS is thin cap fibroatheroma, characterized by a relatively larger necrotic core and overlying thin cap with infiltrating macrophages and focal disruption.6 Conversely, eroded plaques have a different histological profile as they are enriched in selective proteoglycans, mainly hyaluronan at the plaque/thrombus interface,7 which typically lacks an endothelial layer.

Clinical Perspective on p 2513

Inflammation is known to play a key role in ACS, in which systemic levels of acute-phase reactant proteins, specifically C-reactive protein (CRP), predict prognosis,8,9 correlate with the number of ruptured plaques detected in vivo by intravascular ultrasound,10 and show an inverse correlation with the
thickness of the fibrous cap of the culprit lesion as assessed by OCT. In an autopsy study of patients who died suddenly, CRP levels were elevated compared with controls but did not present differences in relation to culprit lesion morphology (ie, erosion or rupture). Alternatively, several studies have documented the presence of widespread coronary neutrophil activation in patients with unstable angina. Furthermore, serum levels of myeloperoxidase, a hemoprotein stored in azurophilic granules and released on neutrophil activation, predict the outcome of patients with ACS and are considered a marker of plaque vulnerability. It is unknown, however, whether myeloperoxidase is associated with different underlying coronary morphologies with luminal thrombi. We therefore assessed whether systemic levels of myeloperoxidase in ACS patients reflect the presence of erosion or rupture of the culprit plaque assessed in vivo by OCT, which is by far superior in the identification of plaque rupture compared with other intravascular imaging modalities. We also investigated whether intraluminal thrombi overlying eroded or ruptured plaques in postmortem coronary specimens from patients who died suddenly differ in their content of myeloperoxidase-positive cells.

Methods

Patients

Between January and December 2007, consecutive patients with a diagnosis of non-ST-segment elevation ACS (NSTE-ACS) (non-ST-segment elevation MI [NSTEMI] or unstable angina class II to IIIB according to the Braunwald classification) undergoing coronary angiography within 48 hours from admission or with a diagnosis of STEMI, either undergoing primary percutaneous coronary intervention within 24 hours of symptom onset or with a MI within the last 30 days before admission, were considered for inclusion in this study. Patient enrollment was performed at San Giovanni Hospital. NSTE-ACS was classified as NSTEMI (MI with persistent ST-segment elevation) or unstable angina according to the Braunwald classification. The diagnosis of STEMI was based on typical symptoms and new ST-segment elevation at the J point in ≥2 contiguous leads (>0.2 mV in V1 through V3 and >0.1 mV in other leads). Exclusion criteria were renal insufficiency (creatinine clearance <30 mL/min estimated with the Cockcroft-Gault formula), cardiogenic shock, and failure to advance the OCT image wire across the culprit lesion. All patients gave their written informed consent for participation in the study, the ethics committee approved the protocol study, and the study was performed according to the Declaration of Helsinki.

OCT Procedure

Coronary angiography was performed via the transfemoral approach in all cases with the use of a 6F or 7F sheath. The culprit lesion was identified by means of angiography, ECG ST-segment alterations, and regional wall motion abnormalities on echocardiographic assessment. All patients received aspirin (250 mg IV loading dose, followed by aspirin 75 to 100 mg daily) and clopidogrel (oral loading dose of 300 to 600 mg followed by 75 mg daily) before coronary angiography. Unfractionated heparin (initial weight-adjusted intravenous bolus ≤4000 IU, with repeat boluses to achieve an activated clotting time of 250 to 300 seconds) was administered. The use of intracoronary or intravascular platelet IIb/IIIa inhibitors was left to the operators’ discretion. OCT was performed with a nonocclusive technique in all cases, with the use of Image wire and Image system (Lightlab, Boston, Mass) with either a 2-mm/s (M2) or 3-mm/s (M2-X) pullback speed machine. A 2.5F Transit (Cordis, Miami Lakes, Fla) microcatheter was placed distal to the culprit lesion over the standard guidewire used for the angioplasty procedure, and the OCT image wire was then exchanged. The culprit lesion was imaged during continuous manual or automated injection of intracoronary iso-osmolar contrast media (Visipaque). Therefore, lesions with occlusive massive thrombosis that could not be adequately flushed with contrast media were excluded from the study.

OCT Image Analysis

OCT image analysis was performed offline by 2 expert investigators (G.F., F.P.) who were blinded to the clinical presentation; discordance was resolved by consensus. The culprit lesion morphology was described according to previously reported criteria. The culprit lesion was classified as ruptured or eroded, and the presence of thrombus was reported. Plaque rupture was defined as the presence of fibrous cap discontinuity leading to a communication between the inner (necrotic) core of the plaque and the lumen, with or without the presence of a flap. Plaque erosion was defined as the presence of intracoronary thrombus adjacent to the luminal surface of the plaque in the absence of detectable signs of overlying fibrous cap rupture, according to the expert review document on methodology, terminology, and clinical applications of OCT and in agreement with the postmortem histopathological classification of coronary pathologies.

A thrombus was defined as an irregular mass protruding into the lumen or adjacent to the luminal surface. Because massive and red thrombi may reduce the ability to assess underlying structures because of the limited depth penetration of OCT laser light (∼1.5 mm) or the high backscattering with signal-free shadowing appearance, respectively, all OCT cross sections acquired within the coronary segment with thrombus were reviewed, one by one, for its entire longitudinal extension, for sites where vessel wall and plaque morphology could be assessed, as proposed in the expert document on OCT.

Biochemical Analyses

Venous blood samples were drawn from the forearm before coronary angiography with the use of standard venipuncture. Collected samples were immediately placed on ice and stored at −80°C. Biomarkers analyses were performed at Catholic University of Rome. Serum high-sensitivity CRP was measured with the use of a latex-enhanced immunone nephelometric assay by BN II analyzer (Siemens Healthcare Diagnostics, Deerfield, Ill), as described previously, and expressed as milligrams per liter. Serum myeloperoxidase was measured by enzyme-linked immunosorbent assay according to procedures recommended by the manufacturer (Calbiochem). This assay provides a detection limit of 1.5 ng/ml.

Autopsy Specimens

Case enrollment involved examination of sudden coronary deaths received in consultation from the Maryland medical examiner’s office. The lesions were classified according to the scheme reported previously. Histological sections (n=27 lesions) from the CVPath Institute registry with rupture or erosion were prescreened, and 22 plaques (ruptures [n=11] or erosions [n=11]) were selected on the basis of the availability of sections suitable for immunohistochemical staining and morphometric analysis.

Immunohistochemical Analysis

Paraffin sections were dewaxed and incubated with primary antibodies targeted against myeloperoxidase (dilution 1:2500; Dako, Carpinteria, Calif) or the neutrophil marker cathepsin G (dilution 1:800; Dako). Before staining occurred, antigen retrieval was performed in EDTA buffer with steam heat. The labeling of primary antibodies was achieved with the use of a biotinylated link antibody (Envision, Dako), and positive staining was visualized by a 3,3′-diaminobenzidine substrate chromogen system. The sections were then rinsed in phosphate-buffered saline and counterstained with Gill hematoxylin. For quantitative analysis, ≥2 representative images (at ×400 magnification) were acquired from areas within the thrombus, luminal fibrous cap, if present, or, in the case of erosion, the plaque with 200 μm nearest the
thrombus interface. The mean values of total and positive cells were then calculated and expressed as cell density (cells per square millimeter).

**Dual Immunostaining**

Paraffin sections were dewaxed and stained for the macrophage marker CD68 (dilution 1:800; Dako) after heat retrieval. The primary antibody was then labeled with a 4+ universal goat-link biotinylated secondary antibody (Biocare Medical, Concord, Calif) with a universal 4+ streptavidin alkaline phosphatase conjugate. Positive staining was then visualized with a Ferangi blue chromogen (Biocare). Staining was then performed for myeloperoxidase (dilution 1:8000; 1 hour) after denaturing and incubating in an avidin-biotin block (Dako). The labeling of secondary antibody was performed as described above; however, the chromogen was substituted for Vulcan Fast Red (Biocare).

**Statistical Analysis**

The distribution of continuous variables was assessed by visual inspection of their frequency histograms and with the use of the Shapiro-Wilk test. Continuous variables are expressed as mean±SD or median and 25th to 75th percentiles, according to a gaussian or nonnormal distribution, respectively. Because of the small sample size, Mann-Whitney U test was used for the comparison of continuous variables. Categorical variables were expressed as percentage and compared by χ² or Fisher exact tests as appropriate. Correlations between continuous variables were assessed with the use of Pearson correlation test or Spearman correlation rank test as appropriate. Myeloperoxidase and CRP levels are reported as median and 25th to 75th percentiles. A 2-tailed P<0.05 was the level of statistical significance. STATA 10.1 (StataCorp LP, College Station, Tex) statistical software was used.

**Results**

**Patients**

Twenty-five patients were included (mean±SD age, 67±11 years; 15 men [60%]). Thirteen patients (52%) had NSTE-ACS, of whom 4 (16%) had unstable angina and 9 (36%) had NSTEMI. Twelve patients (48%) had STEMI, of whom 5 (20%) had an acute STEMI and underwent primary percutaneous coronary intervention and 7 (28%) had a subacute or recent STEMI. Baseline clinical characteristics are listed in Table 1. In the overall study population, median CRP serum level was 4.2 mg/L (25th to 75th percentile, 1.3 to 17.8), the prevalence of thrombus was 16 of 18 (89%) in patients with eroded culprit lesion versus 33.5 mm (25th to 75th percentile, 33.6 to 41.6) in those with ruptured culprit lesion. A 2-tailed P<0.05 was the level of statistical significance. STATA 10.1 (StataCorp LP, College Station, Tex) statistical software was used.

**OCT Assessment of Culprit Lesion**

A total of 25 vessels were imaged with OCT, and 25 culprit lesions were identified. No patient presented with massive thrombosis that could affect the assessment of underlying plaque rupture. The culprit lesion showed rupture in 18 cases (72%) and erosion in 7 (28%). Examples of plaque rupture or plaque erosion are reported in Figure 1. Median total length of coronary segments assessed by OCT in the culprit vessel was 35.0 mm (25th to 75th percentile, 33.6 to 41.6) in patients with eroded culprit lesion versus 33.5 mm (25th to 75th percentile, 26.5 to 36.7) in those with ruptured culprit lesion (P=0.15). The prevalence of thrombus was 16 of 18 (89%) in ruptured plaques and 100% in eroded plaques. Baseline clinical and angiographic characteristics did not differ significantly between patients with eroded culprit plaque and those with ruptured culprit plaque; however, patients with erosion tended to be more frequently women (P=0.07) and smokers (P=0.06) (Table 1).

**Table 1. Patient Characteristics Overall and According to Culprit Lesion Morphology**

<table>
<thead>
<tr>
<th>Lesion type</th>
<th>All Patients</th>
<th>Plaque Rupture</th>
<th>Plaque Erosion</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=25)</td>
<td>(n=18)</td>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>Age, mean±SD, y</td>
<td>67±11</td>
<td>67±11</td>
<td>67±14</td>
<td>0.74</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>15 (60)</td>
<td>13 (72.2)</td>
<td>2 (28.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>22 (88)</td>
<td>15 (83.3)</td>
<td>7 (100)</td>
<td>0.37</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>7 (28)</td>
<td>6 (33.3)</td>
<td>1 (14.3)</td>
<td>0.63</td>
</tr>
<tr>
<td>Diagnosis, n (%)</td>
<td>3 (12)</td>
<td>4 (22.2)</td>
<td>2 (28.6)</td>
<td>0.73</td>
</tr>
<tr>
<td>LAD</td>
<td>11 (44)</td>
<td>9 (50)</td>
<td>2 (28.6)</td>
<td>0.35</td>
</tr>
<tr>
<td>LCX</td>
<td>2 (8)</td>
<td>1 (5.6)</td>
<td>1 (14.3)</td>
<td>0.20</td>
</tr>
<tr>
<td>RCA</td>
<td>12 (48)</td>
<td>8 (44.4)</td>
<td>4 (57.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Multivessel disease, n (%)</td>
<td>13 (52)</td>
<td>10 (55.6)</td>
<td>3 (42.9)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

LAD indicates left anterior descending artery; LCX, left circumflex artery; and RCA, right coronary artery.

**Relation Between Biomarkers and Culprit Lesion Morphology**

Serum myeloperoxidase levels were strikingly higher in patients with erosion than in those with rupture (median, 2500 ng/mL; 25th to 75th percentile, 1415 to 2920 versus median, 707 ng/mL; 25th to 75th percentile, 312 to 943; P=0.001) (Figure 2), whereas CRP levels were not significantly different between the 2 groups (median, 11.3 mg/L; 25th to 75th percentile, 642 to 1415). No correlation was found between CRP and myeloperoxidase levels (percentile, 667 to 1538); however, the chromogen was substituted for Vulcan Fast Red (Biocare). Staining was then performed for myeloperoxidase (dilution 1:8000; 1 hour) after denaturing and incubating in an avidin-biotin block (Dako). The labeling of secondary antibody was performed as described above; however, the chromogen was substituted for Vulcan Fast Red (Biocare).
percentile, 1.3 to 28.5 versus median, 3.9 mg/L; 25th to 75th percentile, 1.3 to 17.8;
P/P/H₁₁₀₀₅₀.7₆) (Figure 3).

Immunohistochemical Analysis of Autopsy Cases

The density of myeloperoxidase-positive cells within the thrombus was significantly greater in erosions than ruptures (erosions: median, 1584; 25th to 75th percentile, 1088 to 2135 cells/mm² versus ruptures: median, 579; 25th to 75th percentile, 442 to 760 cells/mm²; P=0.0012) (Figure 4), whereas values within the fibrous cap or plaque/thrombus interface (absence of a necrotic core) were similar (erosions: median, 301; 25th to 75th percentile, 176 to 617 cells/mm² versus ruptures: median, 291; 25th to 75th percentile, 225 to 1122 cells/mm²; P=0.97) (Figure 5). Although the density of cathepsin G–positive neutrophils within thrombi was nearly 2-fold greater for erosions, median values did not achieve statistical significance in comparison to rupture (erosions: median, 822; 25th to 75th percentile, 298 to 1039 cells/mm² versus ruptures: median, 462; 25th to 75th percentile, 325 to 626 cells/mm²; P=0.18). Notably, the density of cathepsin G–positive neutrophils within the fibrous cap or plaque/thrombus interface was remarkably low (<200 cells/mm²) and was similar between morphologies (erosions: median, 178; 25th to 75th percentile, 65 to 333 cells/mm² versus ruptures: median, 117; 25th to 75th percentile, 54 to 311 cells/mm²; P=0.67). Accordingly, the median ratios of cathepsin-positive neutrophils to the total population of cells expressing myeloperoxidase within thrombi were markedly lower, especially for plaque erosion (erosion: 0.46; rupture: 0.71), thus indicating the significant contribution of myeloperoxidase expression by macrophages, which was confirmed by dual immunostaining for both myeloperoxidase and CD68 (Figure 6).

Discussion

This study reports a significant elevation in the levels of circulating myeloperoxidase in ACS patients presenting with culprit plaque erosion (assessed by OCT) compared with those with culprit lesions attributed to ruptures. Moreover, examination of culprit plaques from sudden coronary deaths showed that luminal thrombi associated with erosion contain a higher density of myeloperoxidase-positive cells than thrombi superimposed to ruptures. The concordance of information provided by clinical imaging and postmortem analy-
ses, together with an absence of a significant difference in the systemic levels of CRP between ACS patients with eroded or ruptured plaques, lends strong support to the concept that elevations in specific inflammatory biomarkers may reflect different morphologies of coronary atherosclerosis and highlights the heterogeneity of coronary mechanisms of ACS. Of note, in our clinical study the prevalence of plaque erosion was similar to that reported in previous studies.3–5 We also confirmed a higher prevalence of erosion in female patients and smokers compared with patients with plaque rupture, which achieved borderline significance despite the small number of patients.3,4,25

Serum myeloperoxidase levels are derived mainly from neutrophils and therefore are considered a marker of neutrophil activation.26,27 Elevated levels of serum myeloperoxidase are associated with poor outcome in patients with ACS,16 predict the risk of MI in patients with chest pain presenting to the emergency department, and have been considered a marker of plaque vulnerability.17 Myeloperoxidase is a basic, highly cationic protein, localized to the azurophilic granules in the myeloid series of hematopoietic cells. It is synthetized at the promyelocyte stage of development and can be detected in peripheral neutrophils,26 although previous studies have also reported the presence of circulating monocytes with high or low myeloperoxidase expression.28,29 Myeloperoxidase30,31 and its oxidation products32–34 have been detected in human atherosclerotic lesions. In addition, neutrophils may infiltrate ruptured or eroded culprit lesions in patients with ACS.35

Whether elevations in systemic levels of myeloperoxidase have a causal role in determining plaque erosion with subsequent thrombus formation or occur secondary to the event cannot be ascertained from our clinical study. In our postmortem examination, the density of myeloperoxidase-positive cells in the fibrous cap of ruptures or plaque near the thrombus interface of erosion was not significantly different, in agreement with previous studies showing the presence of myeloperoxidase at sites of both eroded and ruptured plaques.31 Instead, myeloperoxidase-positive cells were sig-

---

**Figure 1.** Examples of ruptured and eroded culprit plaques with thrombus assessed in vivo by OCT. A, Cross-sectional image showing a thin cap fibroatheroma with evidence of rupture of the fibrous cap at the mid portion and presence of flap (arrow), with communication between the inner core of the plaque (arrowhead) and the lumen. T indicates thrombus. *Guidewire causing typical artifact. B, Longitudinal view of the scanned coronary segment from same patient as in A; white vertical straight line marks the level corresponding to cross-sectional image in A. C, Cross-sectional image showing an eroded plaque. Multiple intraluminal thrombotic masses are adjacent to the surface of the plaque, with no evidence of fibrous cap or of rupture. *Guidewire causing typical artifact. D, Longitudinal view of the scanned coronary segment from same patient as in C; white vertical straight line marks the level corresponding to cross-sectional image in C.

**Figure 2.** Serum levels of myeloperoxidase (MPO) in patients with ruptured culprit plaque compared with those with eroded culprit plaque, as assessed by OCT. Box plot represents median, interquartile range, minimum, and maximum values.

**Figure 3.** Serum levels of CRP in patients with ruptured culprit plaque compared with those with eroded culprit plaque, as assessed by OCT. Box plot represents median, interquartile range, minimum, and maximum values.
nificantly increased in thrombi overlying eroded plaques as opposed to ruptures, suggesting their role as important players of thrombus formation associated with erosion. Myeloperoxidase can exert prothrombotic activity via the generation of oxidant reactive species as it catalyzes lipid peroxidation in vivo, therefore leading to tissue factor activation and tissue factor pathway inhibitor inactivation. Myeloperoxidase reacts with hydrogen peroxide to form an enzyme substrate complex with strong oxidant activity and can bind firmly to negatively charged glycosaminoglycans and proteoglycans in the extracellular matrix.

In a previous postmortem study of patients dying suddenly of acute MI, Sugiyama et al identified a novel subset of macrophages containing myeloperoxidase, infiltrating the subendothelium at sites of coronary plaque erosion or rupture, with few neutrophils in the same coronary lesions. They further demonstrated the importance of granulocyte/monocyte colony-stimulating factor in regulating the retention of myeloperoxidase during the differentiation of monocytes into macrophages and the fact that CD40 ligand, lysophosphatidylcholine, or cholesterol crystals, which are usually present in human atheromas, stimulate myeloperoxidase-containing macrophages to produce hypochlorous acid (HOCl), a specific myeloperoxidase-derived reactive oxygen species, which serves as a metal-independent oxidizing agent in vivo.

In a subsequent in vitro study, the same group reported a pathogenetic role for myeloperoxidase in determining plaque
erosion, in which they showed that HOCl may cause direct human endothelial cell death and detachment by both apoptotic and oncotic cell death pathways and that HOCl, at lower sublethal concentrations, increases the expression of tissue factor by endothelial cells, leading to a prothrombotic state. Therefore, the accumulation of myeloperoxidase in the subendothelial space, due to the presence of such myeloperoxidase-containing macrophages and the deposit of myeloperoxidase via endothelial transcytosis, is likely to be involved in plaque erosion. In agreement with Sugiyama et al, we found the presence of 2 subpopulations of myeloperoxidase-positive cells, neutrophils and macrophages, in both eroded and ruptured plaques, more so in the thrombi overlying them. Of note, myeloperoxidase may also mediate coronary endothelial apoptosis via other mechanisms. Wang et al have reported that myeloperoxidase catalyses the formation of cyanate from the cosubstrates thiocyanate and H$_2$O$_2$ and that cyanate leads to carbamylation of proteins and lipoproteins by modifying lysine residues to form homocitrulline. This process occurs in human atherosclerotic plaques; in particular, carbamylation of high-density lipoproteins prevents their binding to SR-B1 scavenger receptor, which has an inhibitor role in endothelial cell apoptosis, thus eventually resulting in enhanced endothelial cell apoptosis. Of note, smokers have higher cyanate blood levels. Interestingly, in our study smokers also presented with higher levels of myeloperoxidase. These findings could also provide a possible explanation for the association between cigarette smoking and plaque erosion reported in previous studies as well as in this study.

These data, however, do not explain why thrombi overlying eroded plaques contain a larger amount of myeloperoxidase-positive cells than those overlying ruptured plaques. Interestingly, Kolodgie et al reported an intense immunostaining pattern for hyaluronan and its receptor, CD44, along the plaque/thrombus interface in plaque erosion, with little hyaluronan accumulation in stable plaques or at sites of plaque rupture and localization of CD44 mainly to inflammatory cells in ruptured plaques. This suggests that in plaque erosion, the loss of the endothelial layer could be triggered by a selective accumulation of hyaluronan followed by platelet adhesion via CD44 and subsequent thrombus formation, in part mediated by a direct action of hyaluronan on fibrin polymerization. A recent study in endotoxemic mice with experimental lipopolysaccharide-induced sepsis has demonstrated the existence of a specific mechanism of neutrophil recruitment and sequestration in the liver sinusoids mediated by neutrophil CD44 adhesion to hyaluronan. Moreover, monocytes express CD44, which has been shown to mediate macrophage recruitment and adhesion to endothelial cells of lung capillaries after inhalation of lipopolysaccharide in mice. We therefore hypothesize that in patients with ACS, a selective accumulation of hyaluronan in eroded plaques may promote the adhesion and accumulation of circulating neutrophils and monocyte-expressing myeloperoxidase, which in turn may enhance endothelial cell death in erosion and promote thrombus formation.

**Study Limitations**

A main limitation of our study is the sensitivity of OCT, which falls below the dimension of individual endothelial cells. Therefore, the diagnosis of plaque erosion in a clinical setting was not based on the direct visualization of loss of the endothelial layer covering the underlying plaque; rather, it was made when no fibrous cap rupture could be identified beneath the thrombus by OCT. Furthermore, thrombi may reduce the ability to assess underlying structures. However, in our study, patients with massive occlusive thrombosis were excluded because OCT was not feasible. In addition, to optimize the identification of plaque rupture, we have implemented the recommendations proposed in the expert review document on methodology, terminology, and clinical applications of OCT. Of note, the frequency of culprit lesions attributed to erosion in our in vivo study was 28%, and female sex and cigarette smoking tended to be associated with plaque erosion, in agreement with previous postmortem and OCT studies in the setting of acute MI. Finally, although we cannot exclude the possibility of misclassification of some ruptured plaques as eroded, the remarkable concordance between our in vivo and separate postmortem findings strengthens the overall validity of our results. Other limitations are the small sample size and the heterogeneous composition of the patient population; the fact that histopathological analyses were performed on thrombi and plaques from postmortem specimens, which are prone to selection bias; and the fact that the measurement of inflammatory biomarkers was performed in a different population of in vivo patients, which therefore did not allow assessment of the relation between systemic levels and local expression of the same inflammatory biomarkers. Finally, the extent of coronary atherosclerosis differed among patients in the clinical study because approximately half of the patients enrolled had >1 diseased vessel, which may have introduced a confounding factor in the relation between myeloperoxidase levels and culprit lesion morphology assessed by OCT. The number of diseased vessels, however, was similar in patients with plaque rupture and in those with plaque erosion, and the levels of myeloperoxidase did not differ in relation to the number of diseased coronary vessels.

**Conclusions**

This study shows that patients with ACS presenting with culprit plaque erosion have higher systemic levels of myeloperoxidase than those with culprit plaque rupture, as assessed in vivo with OCT. Luminal thrombi overlying eroded plaques in sudden coronary death victims have a higher density of myeloperoxidase-positive cells than thrombi overlying ruptured plaques in postmortem specimens. This study supports the concept that elevations in specific inflammatory biomarkers reflect different acute complications of coronary atherosclerosis and underscores the heterogeneity of coronary mechanisms of ACS.

**Sources of Funding**

This study was supported in part by CVPath Institute, Gaithersburg, Md. Dr Ferrante was awarded a research grant from the European
Association of Percutaneous Cardiovascular Interventions (year 2007).

Disclosures

None.

References


37. Penn MS, Patel CV, Cui MZ, DiCorletto PE, Chisolm GM. LDL increases inactivating factor on vascular smooth muscle cell surfaces: hydrogen


**CLINICAL PERSPECTIVE**

This study reports a significant elevation in the levels of circulating myeloperoxidase in patients with an acute coronary syndrome presenting with culprit plaque erosion (assessed by optical coherence tomography) compared with those with culprit plaque rupture. It also reports that examination of culprit plaques from sudden coronary deaths shows that luminal thrombi associated with erosion contain a higher density of myeloperoxidase-positive cells than thrombi superimposed to ruptures. Myeloperoxidase can exert prothrombotic activity via the generation of oxidant reactive species as it catalyzes lipid peroxidation in vivo, thus leading to tissue factor activation and tissue factor pathway inhibitor inactivation. Furthermore, myeloperoxidase reacts with hydrogen peroxide to form an enzyme substrate complex with strong oxidant activity and can bind firmly to negatively charged glycosaminoglycans and proteoglycans in the extracellular matrix. The concordance of information provided in our study by coronary imaging and postmortem analyses highlights the notion that mechanisms of coronary instability are heterogeneous. The only therapeutic target in acute coronary syndromes is currently represented by coronary thrombosis. It is unlikely, however, that more potent antithrombotic drugs can further improve the outcome of acute coronary syndromes because of the associated increased hemorrhagic risk. Our study suggests that coronary thrombosis can be triggered by different stimuli and mechanisms and that a smart treatment of acute coronary syndromes can possibly be guided by intravascular imaging.
High Levels of Systemic Myeloperoxidase Are Associated With Coronary Plaque Erosion in Patients With Acute Coronary Syndromes: A Clinicopathological Study
Giuseppe Ferrante, Masataka Nakano, Francesco Prati, Giampaolo Niccoli, Maria T. Mallus, Vito Ramazzotti, Rocco A. Montone, Frank D. Kolodgie, Renu Virmani and Filippo Crea

Circulation. published online November 29, 2010;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2010/11/29/CIRCULATIONAHA.110.955302

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/