Myeloperoxidase Serum Levels Predict Risk in Patients With Acute Coronary Syndromes

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Background—Polymorphonuclear neutrophils (PMNs) have gained attention as critical mediators of acute coronary syndromes (ACS). Myeloperoxidase (MPO), a hemoprotein abundantly expressed by PMNs and secreted during activation, possesses potent proinflammatory properties and may contribute directly to tissue injury. However, whether MPO also provides prognostic information in patients with ACS remains unknown.

Methods and Results—MPO serum levels were assessed in 1090 patients with ACS. We recorded death and myocardial infarctions during 6 months of follow-up. MPO levels did not correlate with troponin T, soluble CD40 ligand, or C-reactive protein levels or with ST-segment changes. However, patients with elevated MPO levels (>350 μg/L; 31.3%) experienced a markedly increased cardiac risk (adjusted hazard ratio [HR] 2.25 [1.32 to 3.82]; P=0.003). In particular, MPO serum levels identified patients at risk who had troponin T levels below 0.01 μg/L (adjusted HR 7.48 [95% CI 1.98 to 28.29]; P=0.001). In a multivariate model that included other biochemical markers, troponin T (HR 1.99; P=0.023), C-reactive protein (1.25; P=0.044), vascular endothelial growth factor (HR 1.87; P=0.041), soluble CD40 ligand (HR 2.78; P<0.001), and MPO (HR 2.11; P=0.008) were all independent predictors of the patient’s 6-month outcome.

Conclusions—In patients with ACS, MPO serum levels powerfully predict an increased risk for subsequent cardiovascular events and extend the prognostic information gained from traditional biochemical markers. Given its proinflammatory properties, MPO may serve as both a marker and mediator of vascular inflammation and further points toward the significance of PMN activation in the pathophysiology of ACS. (Circulation. 2003;108:1440-1445.)

Key Words: angina ■ myocardial infarction ■ leukocytes ■ prognosis ■ inflammation

Patients with acute coronary syndromes (ACS) are characterized by increased platelet activation and aggregation within the coronary circulation.1 Thrombus formation at a ruptured or eroded plaque and distal embolization of platelet aggregates eventually lead to myocyte necrosis.2 In particular, the occurrence of minor myocardial injury as observed in ACS is reliably assessed by measuring the release of troponins, which have emerged as powerful tools for risk assessment and therapeutic management of patients with ACS.3,4

There is growing evidence that myocardial cell injury not only is related to platelet activation but also is preceded by recruitment and activation of polymorphonuclear neutrophils (PMNs).5,6 PMNs, despite their apparent insignificance in coronary atherogenesis, have been shown to increasingly undergo degranulation within the coronary circulation in ACS.6 One of the principal mediators secreted on PMN activation is myeloperoxidase (MPO), a hemoprotein traditionally viewed as a microbicidal enzyme.7 However, there is accumulating evidence that MPO also displays potent proatherogenic properties. For example, MPO can oxidize LDL cholesterol, thereby propagating uptake by macrophages and perpetuating foam cell formation.8 Furthermore, MPO has been shown to activate metalloproteinases and promote destabilization and rupture of the atherosclerotic plaque surface.9 Also, MPO catalytically consumes endothelium-derived nitric oxide, thereby reducing nitric oxide bioavailability and impairing its vasodilatory and anti-inflammatory functions.10,11

PMNs have been demonstrated to release MPO into the coronary circulation, yielding elevated MPO plasma levels in patients with unstable angina and acute myocardial infarc-
A case-control study revealed that MPO levels in PMN and whole blood were independently associated with the prevalence of stable coronary artery disease.\textsuperscript{12} Appreciating that PMN activation is an early event in ACS, we hypothesized that MPO levels may identify patients at increased risk for cardiovascular events independent of existing myocardial necrosis. We therefore investigated the prognostic information of MPO serum levels in patients with ACS using the database of patients with ACS enrolled in the eTcE3 Anti-Platelet Therapy in Unstable Refractory angina (CAPTURE) trial.\textsuperscript{14}

### Methods

#### Patients

The CAPTURE trial enrolled 1265 patients with ACS (61\% males, aged 61±10 years). All CAPTURE patients had recurrent chest pain at rest associated with ECG changes during treatment with intravenous heparin and nitroglycerin. All patients underwent coronary angiography before randomization that indicated significant coronary artery disease with a culprit lesion >70\% suitable for angioplasty. Heparin was administered from before randomization until at least 1 hour after coronary angioplasty. For all patients, coronary interventions were scheduled between 18 and 24 hours after beginning study treatment. The patients were randomly assigned to abciximab or placebo. Primary end points of the study were mortality and nonfatal myocardial infarction during the 30 days of the follow-up period.\textsuperscript{14} Serum samples were collected 8.7±4.9 hours after the last episode of chest pain.

#### Biochemical Analysis

Serum samples were centrally stored at −80°C. Determination of cardiac markers was performed blinded to patients’ histories and the allocated treatment at the research laboratory of the University of Frankfurt. MPO serum levels were measured by ELISA according to procedures recommended by the manufacturer (Calbiochem). This assay provides a detection limit of 1.5 \( \mu \text{g/L} \). Using internal controls, total imprecision over the 8-week period was 8.4\%. No trend of the test results toward higher or lower levels was observed during the 8-week study period. Vascular endothelial growth factor and soluble CD40 ligand (sCD40L) were measured by ELISA (both R&D Systems). The diagnostic threshold value was 300 \( \mu \text{g/L} \) for vascular endothelial growth factor\textsuperscript{15} and 5.0 \( \mu \text{g/L} \) for sCD40L.\textsuperscript{16} Cardiac troponin T (TnT) was determined with a 1-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics). The cutoff level for TnT was set at 0.01 \( \mu \text{g/L} \).\textsuperscript{17} High-sensitivity C-reactive protein was measured with the Behring BN II Nephelometer (Dade Behring Inc). A diagnostic threshold value of 10 \( \mu \text{g/mL} \) was used.\textsuperscript{18,19}

#### Statistical Methods

After blind assessment of the biochemical markers, test results were merged with the database. To distinguish between patients with different degrees of cardiac risk, an exploratory data analysis was chosen. The Cox proportional-hazards regression model was used to estimate the relative risk for cardiovascular events, and patients were categorized according to tertiles of MPO concentration.\textsuperscript{20} Post hoc analysis of tertiles was performed with the Cox proportional-hazards regression model with MPO tertiles as a categorical variable; the first tertile served as the reference group. Receiver operating characteristics curve analysis over the dynamic range of the MPO assay was used to identify the threshold level for MPO that provided the highest predictive value to stratify patients with ACS according to risk. The effect of baseline characteristics (with P<0.01 necessary to enter a variable into the model) and other biochemical markers on any observed associations between MPO levels and cardiovascular events was analyzed with stepwise Cox proportional-hazards models. All results for continuous variables are expressed as mean±SD. Comparisons between groups were analyzed by \( t \) test (2-tailed).

### Results

Baseline samples were available for 1090 patients enrolled in the CAPTURE trial (86.2\%). The baseline characteristics for this substudy population were not different from the total study population with respect to age, gender, cardiovascular risk profile, and concomitant treatment before and after randomization. The reduction of cardiac events in the abciximab group of the substudy population was comparable to the entire CAPTURE study population before PTCA (2.2\% placebo versus 0.9\% abciximab; \( P=0.07 \)), after PTCA (7.9\% versus 3.5\%; \( P=0.001 \)), and at 30 days (9.0\% versus 4.2\%; \( P=0.001 \)).

#### MPO Serum Levels and Cardiac Risk

MPO was detectable in baseline serum samples of all study patients, with a median of 287 \( \mu \text{g/L} \) (range 1.5 to 1112 \( \mu \text{g/L} \)). Because other markers, such as TnT and sCD40L, have been shown to interact with the treatment effect of the glycoprotein IIb/IIIa receptor antagonist abciximab, the exploratory analysis was restricted to the placebo group (n=547). Patients were stratified into tertiles according to their measured MPO serum levels: MPO-1, <222 \( \mu \text{g/L} \) (n=178); MPO-2, 222 to 350 \( \mu \text{g/L} \) (n=187); and MPO-3, above 350 \( \mu \text{g/L} \) (n=182), respectively. For the initial 24-hour period, the combined end points of mortality and nonfatal myocardial infarction revealed a trend between MPO tertiles (\( P=0.17 \); Figure 1). For the later follow-up time points (72 hours, 30 days, and 6 months), event rates showed significant differences among MPO tertiles (Figure 1). Post hoc analysis of tertiles with the Cox proportional-hazards regression model revealed that only the third MPO tertile significantly differed from the first MPO tertile, which served as a reference (72 hours, \( P=0.004 \); 30 days, \( P=0.008 \); and 6 months, \( P=0.012 \)).
When MPO serum levels were linked to traditional risk markers, neither TnT (r=0.04), vascular endothelial growth factor (r=0.03), C-reactive protein serum levels (r=0.02), nor sCD40L, a marker of platelet activation previously found to be predictive of adverse outcome in the CAPTURE population \(^{(16)}\) (r=0.06), correlated. Moreover, MPO serum levels did not differ between patients with TnT serum levels above and below 0.01 \(\mu\text{g}/\text{L}\), whereas C-reactive protein serum levels were significantly higher in patients with TnT levels >0.01 \(\mu\text{g}/\text{L}\) (Figure 2).

**Risk Stratification According to Serum MPO Status**

On the basis of the above results, we categorized the study population using a threshold level of 350 \(\mu\text{g}/\text{L}\) MPO. Of 547 placebo patients, 171 (31.3\%) had MPO serum levels \(\geq 350\) \(\mu\text{g}/\text{L}\), and 376 patients had levels <350 \(\mu\text{g}/\text{L}\). As illustrated in Table 1, there were few significant differences in baseline characteristics between the 2 groups. Patients with elevated MPO serum levels were more frequently diabetics, and more of them had a history of coronary events. For patients with high MPO serum levels, the combined end point of death and nonfatal myocardial infarction was significantly different compared with patients with low MPO serum levels (Table 2). After 72 hours, 14.0\% of patients with high MPO serum levels suffered death and nonfatal myocardial infarction compared with patients with low MPO serum levels (25.8\% versus 18.1\%; \(P=0.003\)). During the subsequent 6 months of follow-up, the combined end point of death and nonfatal myocardial infarction did not continue to diverge (Figure 3B). Accordingly, the significant difference between patients with high and low MPO serum levels was entirely related to an increased event rate during the initial 72 hours after onset of symptoms. The crude event rates were 14.6\% versus 6.4\% \(P=0.003\) at 30 days and 18.1\% versus 8.8\% \(P=0.002\) at 6 months. This difference was mainly driven by an increased rate of nonfatal myocardial infarctions. The single end point mortality at 6-month follow-up did not differ between groups (2.1\% versus 1.8\%; \(P=1.00\)). Consistently, urgent revascularization procedures, including percutaneous coronary intervention and CABG, were significantly more frequent in patients with high MPO serum levels (13.7\% versus 8.1\%; \(P=0.014\)). Nonurgent revascularization procedures during 6 months of follow-up were similar between patients with high and low MPO serum levels (25.8\% versus 26.4\%; \(P=0.95\)).

**Multivariate Risk Stratification**

In a multivariate analysis that included baseline characteristics and biochemical markers (TnT, vascular endothelial growth factor, C-reactive protein, sCD40L, HDL, and HDL), MPO serum levels were significantly higher in patients with TnT serum levels \(\geq 0.01\) \(\mu\text{g}/\text{L}\) vs 5.1\% for patients with low MPO serum levels (Table 2). In Table 2, the combined end point of death and nonfatal myocardial infarction was significantly different compared with patients with low MPO serum levels, respectively, did not continue to diverge (Figure 3B). Accordingly, the significant difference between patients with high and low MPO serum levels was entirely related to an increased event rate during the initial 72 hours after onset of symptoms. The crude event rates were 14.6\% versus 6.4\% \(P=0.003\) at 30 days and 18.1\% versus 8.8\% \(P=0.002\) at 6 months. This difference was mainly driven by an increased rate of nonfatal myocardial infarctions. The single end point mortality at 6-month follow-up did not differ between groups (2.1\% versus 1.8\%; \(P=1.00\)). Consistently, urgent revascularization procedures, including percutaneous coronary intervention and CABG, were significantly more frequent in patients with high MPO serum levels (13.7\% versus 8.1\%; \(P=0.014\)). Nonurgent revascularization procedures during 6 months of follow-up were similar between patients with high and low MPO serum levels (25.8\% versus 26.4\%; \(P=0.95\)).

**TABLE 1. Baseline Characteristics According to MPO Status for the Placebo Group of the CAPTURE Trial (n=547)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>MPO Low (n=376)</th>
<th>MPO High (n=171)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>71.4</td>
<td>69.2</td>
<td>0.34</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.4±10.5</td>
<td>62.5±10.4</td>
<td>0.32</td>
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<tr>
<td>Troponin T (\geq 0.01) (\mu\text{g}/\text{L})</td>
<td>62.1</td>
<td>61.2</td>
<td>0.84</td>
</tr>
<tr>
<td>C-reactive protein (\geq 10) (\text{mg}/\text{L})</td>
<td>45.9</td>
<td>42.7</td>
<td>0.52</td>
</tr>
<tr>
<td>sCD40L &gt;5.0 (\mu\text{g}/\text{L})</td>
<td>35.5</td>
<td>43.2</td>
<td>0.11</td>
</tr>
<tr>
<td>White blood cell count, (\times10^9/\text{L})</td>
<td>8.9±3.1</td>
<td>9.2±2.7</td>
<td>0.71</td>
</tr>
<tr>
<td>ST-segment depression</td>
<td>48.5</td>
<td>48.5</td>
<td>1.00</td>
</tr>
<tr>
<td>T-wave inversion</td>
<td>54.8</td>
<td>44.8</td>
<td>0.038</td>
</tr>
<tr>
<td>History of angina &gt;7 days</td>
<td>55.3</td>
<td>57.4</td>
<td>0.64</td>
</tr>
<tr>
<td>History of infarction &lt;30 days</td>
<td>10.5</td>
<td>16.6</td>
<td>0.022</td>
</tr>
<tr>
<td>History of infarction &gt;30 days</td>
<td>20.3</td>
<td>20.4</td>
<td>0.97</td>
</tr>
<tr>
<td>PTCA</td>
<td>13.6</td>
<td>20.7</td>
<td>0.024</td>
</tr>
<tr>
<td>CABG</td>
<td>2.2</td>
<td>4.9</td>
<td>0.16</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>8.2</td>
<td>12.5</td>
<td>0.024</td>
</tr>
<tr>
<td>Hypertension</td>
<td>35.4</td>
<td>36.9</td>
<td>0.59</td>
</tr>
<tr>
<td>Current smokers</td>
<td>42.5</td>
<td>40.0</td>
<td>0.63</td>
</tr>
<tr>
<td>Medication at baseline</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>97.9</td>
<td>98.1</td>
<td>1.00</td>
</tr>
<tr>
<td>Intravenous heparin</td>
<td>99.0</td>
<td>98.9</td>
<td>0.98</td>
</tr>
<tr>
<td>Intravenous nitrates</td>
<td>99.4</td>
<td>99.3</td>
<td>1.00</td>
</tr>
<tr>
<td>(\beta)-Blockers</td>
<td>63.5</td>
<td>62.9</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Values are percentages unless otherwise indicated.
growth factor, C-reactive protein, sCD40L, and white blood cell count). MPO remained an independent and powerful predictor of increased cardiac risk both at 30 days of follow-up (adjusted hazard ratio 1.8 [95% CI 1.1 to 3.3]; P=0.013) and at 6 months of follow-up (adjusted hazard ratio 2.1 [95% CI 1.7 to 5.2]; P=0.006; Table 3). Division of the patients into 6 groups based on their MPO and TnT levels revealed that MPO identified a subgroup of patients with low TnT serum levels who had significantly increased cardiac risk: patients with TnT serum levels ≤0.01 μg/L but MPO serum levels above 350 μg/L were at significantly higher risk than patients who had low levels for both TnT and MPO (15.9% versus 2.0%; P=0.001; Figure 4A). Furthermore, the predictive value of MPO was independent of systemic inflammation as evidenced by C-reactive protein. High MPO serum levels indicated increased cardiac risk both in patients with medium C-reactive protein serum levels (20.0% versus 5.9%; P<0.001) and in those with low C-reactive protein serum levels (17.8% versus 0%; P<0.001; Fig. 4b). Also, MPO predicted adverse outcome independent of sCD40L; in patients with low TnT levels (<0.01 μg/L) and low sCD40L levels (<5 μg/L), high MPO levels remained predictive for increased cardiac risk (9.1% versus 2.3%; P=0.028; Figure 5).

**Effect of Abciximab Related to MPO Serum Levels**

Cox proportional-hazards regression model indicated that the effect of treatment with abciximab tended to be higher in patients with high MPO serum levels (P=0.027). Patients with elevated MPO serum levels who received abciximab were at significantly lower risk at 72 hours (adjusted hazard

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**TABLE 3. Multivariate Cox Proportional-Hazards Regression Model of Multiple Biomarkers for Prediction of Death and Nonfatal Myocardial Infarction During 6 Months of Follow-Up**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted Hazard Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin T &gt;0.01 μg/L</td>
<td>1.99</td>
<td>1.16 to 3.64</td>
<td>0.023</td>
</tr>
<tr>
<td>C-reactive protein tertiles</td>
<td>1.25</td>
<td>1.02 to 1.68</td>
<td>0.044</td>
</tr>
<tr>
<td>Vascular endothelial growth factor &gt;300 μg/L</td>
<td>1.87</td>
<td>1.03 to 3.51</td>
<td>0.041</td>
</tr>
<tr>
<td>sCD40L &gt;5 μg/L</td>
<td>2.78</td>
<td>1.57 to 4.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MPO &gt;350 μg/L</td>
<td>2.11</td>
<td>1.21 to 3.67</td>
<td>0.008</td>
</tr>
</tbody>
</table>

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**Figure 3.** Kaplan-Meier event rate curves showing cumulative incidence of death or nonfatal myocardial infarction at 72-hour (A) and 6-month (B) follow-up according to baseline MPO serum level (diagnostic threshold 350 μg/L; n=547); arrow indicates percutaneous coronary intervention (PCI). Hazard ratios were adjusted for differences in baseline characteristics. MI indicates myocardial infarction.

**Figure 4.** Predictive value of MPO for incidence of death and nonfatal myocardial infarction according to TnT serum levels (A) and C-reactive protein (CRP) serum levels (B). Diagnostic threshold levels were 222 and 350 μg/L for MPO, 0.01 and 0.1 μg/L for TnT, and 5 and 15 mg/L for C-reactive protein (n=547). MI indicates myocardial infarction.
MPO, sCD40L, and TnT all emerged as independent predictors of adverse outcome. The combination of MPO and sCD40L was especially revealing in patients with low TnT levels. With the cutoff for TnT being lowered to 0.01 μg/L, neither TnT nor MPO but only sCD40L independently identified those patients who derived benefit from abciximab, which suggests that recruitment and degranulation of PMNs is a primary event and is followed by release of other systemic mediators and acute-phase proteins such as C-reactive protein.

Another important finding of the present study is that MPO, sCD40L, and TnT all emerged as independent predictors of adverse outcome. The combination of MPO and sCD40L was especially revealing in patients with low TnT levels. With the cutoff for TnT being lowered to 0.01 μg/L, neither TnT nor MPO but only sCD40L independently identified those patients who derived benefit from abciximab, which may underscore the specificity of a treatment regimen directed against a receptor expressed on activated platelets. This may further imply that neutrophil activation represents an adjunct pathophysiological event in ACS that is distinctly different from platelet activation. Eventually, platelet and neutrophil activation may contribute to the same "down-
stream” event, that is, myocardial injury as reflected by the release of troponins.

In conclusion, the present study revealed that MPO is a powerful predictor of adverse outcome in patients with ACS. Particularly in individuals with low TnT levels, MPO identified patients at increased risk for future cardiovascular events. This suggests that MPO unmasks states of acute inflammation in the coronary circulation indicative of increased neutrophil activation, which ultimately precedes myocardial injury. Thus, MPO levels not only stratify risk in patients with ACS but also shed light on the underlying pathophysiology, which is activation and degranulation of PMN being a critical component of acute coronary inflammation. The data obtained from the present study extend the understanding of ACS in that they reveal that neutrophil activation is a thus far underrecognized event during coronary inflammation. Given the emerging body of evidence for proinflammatory properties of MPO, the enzyme by itself may not only be a marker of neutrophil activation but also may be a direct contributor to the inflammatory milieu during ACS. Although future prospective studies are warranted to confirm these results, the present findings support the rationale to further evaluate MPO for risk stratification in patients with ACS and encourage the development of pharmacological strategies to modulate the catalytic activity of this enzyme.

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References
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