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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-

tion.[6,12] A case-control study revealed that MPO levels in PMN and whole blood were independently associated with the prevalence of stable coronary artery disease.[13] 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 c7E3 Anti-Platelet Therapy in Unstable Refractory angina (CAPTURE) trial.[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.[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 μg/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 μg/L for vascular endothelial growth factor[15] and 5.0 μg/L for sCD40L.[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 μg/L.[17] High-sensitivity C-reactive protein was measured with the Behring BN II Nephelometer (Dade Behring Inc). A diagnostic threshold value of 10 mg/L was used.[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.[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 $P=0.10$ 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).
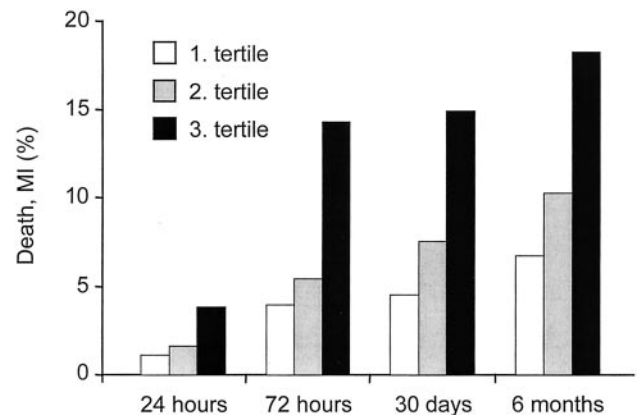

Figure 1. Association between MPO serum levels and cardiac event rate according to MPO tertile in placebo group (n=547). Range of MPO was as follows: ≤222 μg/L (1st tertile), 223 to 350 μg/L (2nd tertile), and >350 μg/L (3rd tertile). Differences in event rates between tertiles were significant at 72 hours ($P<0.001$), 30 days ($P<0.001$), and 6 months ($P<0.001$) of follow-up. MI indicates myocardial infarction.

Comparison of categorical variables was generated by the Pearson χ^2 test. Probability values <0.05 were considered statistically significant. All analyses were performed with SPSS 11.0 (SPSS Inc).

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%; $P=0.001$).[14]

MPO Serum Levels and Cardiac Risk

MPO was detectable in baseline serum samples of all study patients, with a median of 287 μg/L (range 1.5 to 1112 μg/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 μg/L (n=178); MPO-2, 222 to 350 μg/L (n=187); and MPO-3, above 350 μg/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, $P=0.012$).
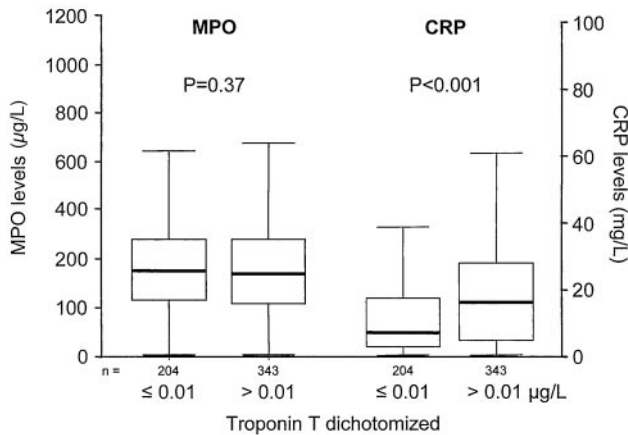
Figure 2. MPO and C-reactive protein (CRP) serum levels, respectively, according to baseline TnT status (n=547).

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 μg/L, whereas C-reactive protein serum levels were significantly higher in patients with TnT levels >0.01 μg/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 μg/L MPO. Of 547 placebo patients, 171 (31.3%) had MPO serum levels ≥350 μg/L, and 376 patients had levels <350 μg/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 5.1% for patients with low MPO serum levels (P=0.001; Figure 3A). During the subsequent 6 months of follow-up, event rate curves for patients with high and 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).

Multivariate Risk Stratification

In a multivariate analysis that included baseline characteristics and biochemical markers (TnT, vascular endothelial

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

	MPO Low (n=376)	MPO High (n=171)	P
Male	71.4	69.2	0.34
Age, y	61.4±10.5	62.5±10.4	0.32
Troponin T ≥0.01 μg/L	62.1	61.2	0.84
C-reactive protein ≥10 mg/L	45.9	42.7	0.52
sCD40L >5.0 μg/L	35.5	43.2	0.11
White blood cell count, ×10⁹/L	8.9±3.1	9.2±2.7	0.71
ST-segment depression	48.5	48.5	1.00
T-wave inversion	54.8	44.8	0.038
History of angina >7 days	55.3	57.4	0.64
History of infarction <30 days	10.5	16.6	0.022
History of infarction >30 days	20.3	20.4	0.97
PTCA	13.6	20.7	0.024
CABG	2.2	4.9	0.16
Risk factors			
Diabetes	8.2	12.5	0.024
Hypertension	35.4	36.9	0.59
Current smokers	42.5	40.0	0.63
Medication at baseline			
Aspirin	97.9	98.1	1.00
Intravenous heparin	99.0	98.9	0.98
Intravenous nitrates	99.4	99.3	1.00
β-Blockers	63.5	62.9	0.91

Values are percentages unless otherwise indicated.

TABLE 2.   Multivariate Cox Proportional-Hazards Regression Model for Death and Nonfatal Myocardial Infarction During 6 Months of Follow-Up Derived From the Placebo Group of the CAPTURE Trial

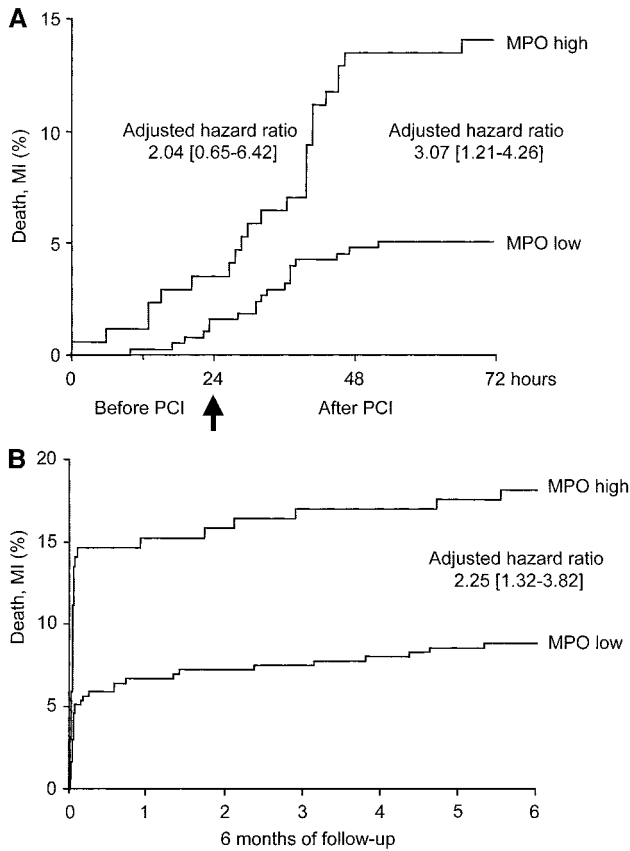| Variable	Adjusted Hazard Ratio	95% CI	P
Gender	0.98	0.67 to 1.52	0.87
Age >65 y	1.19	0.69 to 1.52	0.57
Diabetes mellitus	1.16	0.59 to 2.64	0.65
Hypercholesterolemia	1.05	0.65 to 1.32	0.78
Current smoker	0.98	0.59 to 1.34	0.65
Hypertension	1.05	0.89 to 1.29	0.99
History of coronary revascularization	0.92	0.64 to 1.25	0.82
ST-segment depression	1.02	0.65 to 1.51	1.00
MPO >350 μg/L	2.25	1.32 to 3.82	0.003

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.

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

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

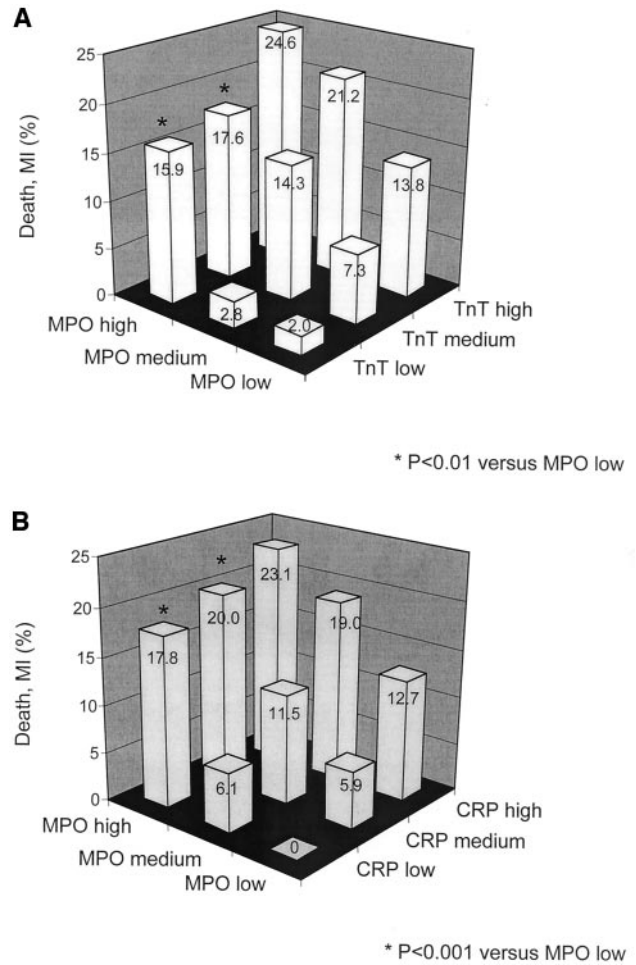| Variable	Adjusted Hazard Ratio	95% CI	P
Troponin T >0.01 μg/L	1.99	1.16 to 3.64	0.023
C-reactive protein tertiles	1.25	1.02 to 1.68	0.044
Vascular endothelial growth factor >300 μg/L	1.87	1.03 to 3.51	0.041
sCD40L >5 μg/L	2.78	1.57 to 4.91	<0.001
MPO >350 μg/L	2.11	1.21 to 3.67	0.008

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.

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
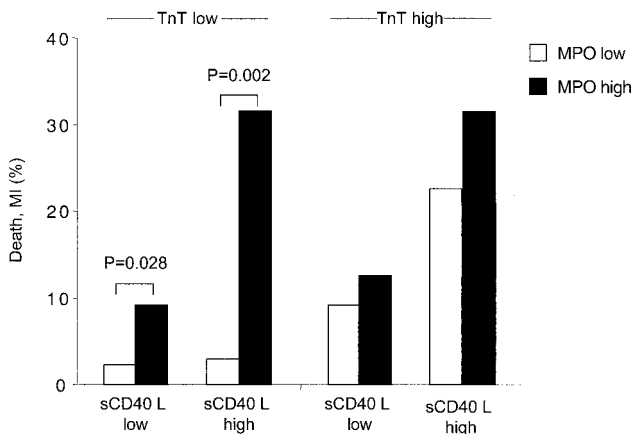
Figure 5. MPO identifies patients with adverse events (death, nonfatal myocardial infarction) independent of TnT (diagnostic threshold level for TnT was 0.01 μg/L) and sCD40L (diagnostic threshold level for sCD40L was 5 μg/L) status (n=547). MI indicates myocardial infarction.

ratio 0.22 [95% CI 0.09 to 0.54]; $P<0.001$) and 30 days of follow-up (adjusted hazard ratio 0.24 [95% CI 0.10 to 0.58]; $P=0.001$). This significant difference was maintained at 6 months of follow-up (0.39 [95% CI 0.20 to 0.76]; $P=0.006$). However, in a multivariate model that included TnT, sCD40L, and MPO, sCD40L ($P<0.001$) remained the only independent predictor of the effect of abciximab.

Discussion

The results of the present study establish MPO serum levels as a powerful independent prognostic determinant of clinical outcome in patients with ACS. Most notably, in patients with TnT serum levels below 0.01 μg/L, elevated MPO serum levels identify a subgroup of patients who have significantly increased cardiac risk (adjusted hazard ratio 7.48 [95% CI 1.98 to 28.29]; $P=0.001$). In conjunction with TnT, MPO identified 95% of all adverse events in CAPTURE.

Risk stratification in patients with ACS remains a major objective for the selection of optimal medical and interventional treatment regimens. Troponins are the most established prognostic markers in that they reliably predict adverse cardiac events and identify those patients who derive major benefit from intensified antiplatelet therapy with glycoprotein IIb/IIIa receptor antagonists.[4] Because troponins reflect myocardial injury and necrosis, efforts remain to identify patients at risk during an earlier stage of the disease.

Pathophysiologically, platelet activation and thrombus formation are considered a central contributor to the development of ischemia and myocardial injury, as demonstrated by the profound benefit of antiaggregatory regimens in ACS. sCD40L, a protein released by platelets on activation, has been demonstrated to predict adverse outcome in ACS and proved to identify those patients who derive benefit from glycoprotein IIb/IIIa receptor antagonists.[16] In contrast to platelet activation, recruitment and degranulation of PMNs only recently gained attention in ACS and has been characterized as a localized event within the coronary circulation.[6,21–23] One of the principal enzymes secreted by activated neutrophils is MPO, a hemoprotein that accounts for 4% of

total cellular protein. PMNs are the foremost cellular source of intravascular MPO and contribute to 95% of total circulating MPO content.[13]

MPO serum levels in patients enrolled in the CAPTURE trial were up to 20-fold higher than those reported previously for healthy subjects.[24] Given the site-specific degranulation of PMNs in the coronary circulation[6] and the fact that blood was obtained from a peripheral vein, the systemic MPO levels obtained in the present study presumably reflect even higher local MPO concentrations in the coronary circulation. As reported earlier, MPO binds to glycosaminoglycans on endothelial cell surfaces and is released by administration of heparin derivatives.[25] Because all CAPTURE patients received heparin, circulating MPO levels in the present study may also represent MPO that was bound to the coronary endothelium. Here, MPO may contribute to a local proinflammatory milieu, eg, by catabolism of endothelium-derived nitric oxide.[11,26]

Interestingly, MPO levels were equally distributed among patients with low and high TnT serum levels (Figure 2), which indicates that elevated MPO serum levels are not temporally related to myocardial injury. More importantly, MPO identified patients at risk for cardiovascular events who had low baseline TnT serum levels. These data suggest that MPO release actually precedes myocardial injury and that MPO elevation identifies patients with unstable atherosclerotic plaque formation even before complete microvascular obstruction. In addition, ECG evidence of myocardial ischemia did not correlate with MPO levels (Table 2), which further reinforces the notion that MPO release is a prerequisite rather than a consequence of myocardial injury.

Surprisingly, MPO levels did not correlate with C-reactive protein, a systemic marker of inflammation and the most well-characterized measure for identifying patients with stable coronary artery disease who are at risk for future cardiovascular events.[27] Mean C-reactive protein levels in the CAPTURE study were 18.3±26.9 mg/L. Because C-reactive protein correlated with TnT levels (Figure 2), elevated C-reactive protein serum levels most likely reflect both a robust vascular inflammatory response and myocardial injury.[5] However, in patients with low CRP levels, MPO identified those with increased risk for cardiac events (Figure 4B), 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.

Acknowledgments

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