Association Between Myeloperoxidase Levels and Risk of Coronary Artery Disease

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Numerous epidemiologic studies have evaluated several inflammatory markers, including C-reactive protein, various cytokines, adhesion molecules, and white blood cell (WBC) count for their clinical usefulness in predicting risk of cardiovascular disease. Recent investigations have suggested that myeloperoxidase (MPO), an abundant enzyme secreted from activated neutrophils, monocytes, and certain tissue macrophages (such as in atherosclerotic plaques), may be involved in the development of coronary artery disease (CAD).

Myeloperoxidase synthesis occurs during myeloid differentiation in bone marrow and is completed within granulocytes prior to their entry into the circulation. The enzyme is stored within primary granules of neutrophils and monocytes and is not released until leukocyte activation and degranulation. Myeloperoxidase forms free radicals and diffusible oxidants with antimicrobial activity. However, MPO also promotes oxidative damage of host tissues at sites of inflammation, including atherosclerotic lesions. Immunohistochemical studies have demonstrated the presence of MPO in human atherosclerotic lesions, and mass spectrometry studies have shown that oxidation products generated by MPO are enriched in low-density lipoprotein (LDL) recovered from human atheroma and in low-density lipoprotein (LDL) recovered from diseased arterial tissue. Myeloperoxidase has been implicated as an enzymatic catalyst of LDL oxidation in vivo, converting the lipoprotein into a high-up take form for macrophages leading to cholesterol deposition and foam cell formation. MPO also is capable of using nitric oxide as a physiologic substrate, thereby serving as a catalytic sink for the nitric oxide, perhaps contributing to endothelial dysfunction.

Based on the links between MPO, oxidation of LDL, and the functional deficiency of nitric oxide in atherosclerotic vessels, we hypothesized that levels of MPO in leukocytes might serve to identify individuals at increased risk for CAD. In this study we evaluated whether levels of MPO are associated with the presence of angiographically documented CAD.

Methods

Study Population

Based on logistic regression power calculations (assuming equal-size groups),

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we estimated that 326 patients were needed to provide 80% power to detect a statistically significant (α = .05) odds ratio (OR) of at least 2.0 for presence of CAD in the highest MPO quartile compared with the lowest quartile. Patients (n = 333) were identified from 2 practices within the Cardiology Department of the Cleveland Clinic Foundation. From July to September 2000, 85 consecutive patients were enrolled from the Preventive Cardiology Clinic and simultaneously, 125 consecutive patients were enrolled from the catheterization laboratory. Based on CAD prevalence in these patients, 116 additional control subjects were needed. All patients who did not have significant CAD as determined by coronary artery catheterization during the preceding 6 months were identified from the catheterization database, and then 140 were randomly selected (based on area code/telephone number) and invited to participate for MPO measurement.

Coronary artery disease was defined by a history of documented myocardial infarction, prior coronary revascularization intervention (coronary artery bypass graft surgery or percutaneous coronary intervention), or as the presence of ≥50% stenosis in 1 or more coronary arteries identified during cardiac catheterization. Exclusion criteria for the CAD group were an acute coronary event within 3 months preceding enrollment, end-stage renal disease, or prior bone marrow transplantation. The control group consisted of patients who had undergone diagnostic coronary angiography that revealed no evidence of significant CAD, or as the presence of ≥50% stenosis in 1 or more coronary arteries identified during cardiac catheterization. Exclusion criteria for control subjects were 1 or more coronary vessels with stenosis ≥50%, valvular heart disease, left ventricular dysfunction, end-stage renal disease, bone marrow transplantation, or evidence of infection or active inflammatory diseases as revealed by history and physical examination. All patients were older than 45 years and afebrile.

Clinical history was assessed for diabetes mellitus, smoking (past and present), hypertension, and whether any first-degree relatives had CAD (men by age 50 years and women by age 60 years). Diabetes was ascertained with a physician diagnosis of diabetes or fasting plasma glucose level higher than 126 mg/dL (7.0 mmol/L). Hypertension was defined as a physician diagnosis of chronic hypertension or patient’s blood pressure measured at higher than 140/90 mm Hg on examination. Current smoking was defined as smoking more than 5 cigarettes within the past 3 months. Study protocol and consent forms were approved by the Cleveland Clinic Foundation Institutional Review Board and written informed consent was obtained from all patients.

Measurements

Samples were coded to ensure anonymity and all analyses were performed in a blinded fashion. After an overnight fast, blood was drawn into tubes containing EDTA and used to quantify WBC, LDL-C, high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and fasting triglycerides (TG). Neutrophils were isolated by buoyant density centrifugation. Neutrophile preparations were at least 98% homogeneous by visual inspection. Leukocyte preparations were supplemented to 0.2% cetyltrimethylammonium bromide for cellular lysis, incubated at room temperature for 10 minutes, snap frozen in liquid nitrogen, and stored at −80°C until analysis.

Functional MPO was quantified by peroxidase activity assay of neutrophil lysates. Briefly, detergent-lysed cells (10⁴ per mL; triplicate samples) were added to 20-mM phosphate buffer (pH 7.0) containing 14.4-mM guaiacol, 0.34-mM H₂O₂, and 200-µM diethylene triamine penta-acetic acid and the formation of guaiacol oxidation product was monitored at 430 nm at 25°C. A millimolar absorbance coefficient of 26.6 M⁻¹ cm⁻¹ for the diguaiacol oxidation product was used to calculate peroxidase activity. One unit of MPO activity is defined as the amount that consumes 1 µmol of H₂O₂ per minute at 25°C. Myeloperoxidase activity reported is normalized either per milligram of neutrophil protein (leukocyte-MPO) or per milliliter of blood (blood-MPO). The total content of MPO in blood is dependent on MPO levels per leukocyte and the total number of leukocytes. Since neutrophils possess more than 95% of the MPO content in blood, blood-MPO (MPO units per milliliter of blood) was estimated by multiplying the units of MPO activity per neutrophil by the absolute neutrophil count (per microliter of blood, ×10⁰⁰). Protein concentration was determined as previously described.

Levels of leukocyte-MPO in an individual were reproducible, demonstrating less than ±7% variations in subjects over time (n = 6 men evaluated every 1-3 months for >2-year period). The coefficient of variance for determination of leukocyte-MPO, as determined by analysis of samples multiple times consecutively, was 4.2%. Leukocyte-MPO determination for 10 samples analyzed on 3 separate days yielded a coefficient of variance of 4.6%. The coefficient of variance for determination of blood-MPO, as determined by analysis of samples multiple times consecutively, was 4.2%. Blood-MPO determination for 10 samples analyzed on 3 separate days yielded a coefficient of variance of 4.8%.

Myeloperoxidase mass per neutrophil was determined using an enzyme-linked immunosorbent assay (ELISA). Capture plates were made by incubating 96-well plates overnight with polyclonal antibody (Dako, Glostrup, Denmark) raised against the heavy chain of human MPO (10 µg/mL in 10 mM of phosphate-buffered saline, pH 7.2). Plates were washed and sandwich ELISAs were performed on leukocyte lysates using alkaline phosphatase–labeled antibody to human MPO. Myeloperoxidase mass was calculated based on standard curves generated with known amounts of human MPO purified from leukocytes as described. Purity of isolated MPO was established by demonstrating an RZ of 0.87 (A₄₅₀/A₂₈₀), sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis, and in-gel tetramethylenen-
MYELOPEROXIDASE AND CORONARY ARTERY DISEASE

Table 1. Patient Characteristics*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n = 175)</th>
<th>Patients With CAD (n = 158)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>55 (10)</td>
<td>64 (13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Women, %</td>
<td>42</td>
<td>20</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>5</td>
<td>23</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>31</td>
<td>58</td>
<td>.73</td>
</tr>
<tr>
<td>Family history of CAD, %</td>
<td>53</td>
<td>54</td>
<td>.73</td>
</tr>
<tr>
<td>History of smoking, %</td>
<td>49</td>
<td>78</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>10</td>
<td>9</td>
<td>.81</td>
</tr>
<tr>
<td>Any lipid-lowering medications, %</td>
<td>27</td>
<td>70</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Statins, %</td>
<td>25</td>
<td>65</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Aspirin, %</td>
<td>71</td>
<td>84</td>
<td>.006</td>
</tr>
<tr>
<td>ACE inhibitors, %</td>
<td>18</td>
<td>44</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>β-Blockers, %</td>
<td>27</td>
<td>59</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Calcium channel blockers, %</td>
<td>15</td>
<td>24</td>
<td>.03</td>
</tr>
<tr>
<td>Total cholesterol, median (IQR), mg/dL†</td>
<td>203 (166-234)</td>
<td>203 (174-234)</td>
<td>.93</td>
</tr>
<tr>
<td>LDL cholesterol, median (IQR), mg/dL†</td>
<td>132 (89-144)</td>
<td>122 (90-146)</td>
<td>.76</td>
</tr>
<tr>
<td>HDL cholesterol, median (IQR), mg/dL†</td>
<td>49 (40-56)</td>
<td>43 (36-49)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fasting triglycerides, median (IQR), mg/dL‡</td>
<td>121 (91-198)</td>
<td>159 (117-240)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>WBC count, mean (SD), x10³/µL</td>
<td>7.4 (3.0)</td>
<td>8.4 (3.2)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Absolute neutrophil count, mean (SD), x10³/µL</td>
<td>3.8 (1.9)</td>
<td>5.2 (2.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Framingham Global Risk Score, mean (SD)</td>
<td>5.5 (3.8)</td>
<td>8.0 (3.0)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*CAD indicates coronary artery disease; ACE, angiotensin-converting enzyme; IQR, interquartile range; LDL, low-density lipoprotein; HDL, high-density lipoprotein; and WBC, white blood cell.
†To convert cholesterol values from mg/dL to mmol/L, multiply by 0.0259.
‡To convert triglyceride values from mg/dL to mmol/L, multiply by 0.0113.

RESULTS

Patient Demographics

The clinical and biochemical characteristics of study participants are shown in Table 1. Patients with CAD were older, more likely to be men, and more likely to have a history of diabetes, hypertension, and smoking. Patients with CAD also had increased fasting TG levels and were more likely to use lipid-lowering medications (predominantly statins), aspirin, and other cardiovascular medications. The Framingham Global Risk Score, absolute neutrophil count, and WBC count were significantly increased in patients with CAD.

MPO Levels and Prevalence of CAD

Myeloperoxidase activity per milligram of neutrophil protein (leukocyte-MPO) was significantly greater for patients with CAD than for controls (median values, 18.1 U/mg vs 13.4 U/mg, respectively; P < .001 for trend and for difference) (Figure). Individuals in the highest quartile of leukocyte-MPO levels had increased risk of CAD compared with those in the lowest quartile (OR, 8.8; 95% CI, 4.4-17.5; P < .001 for trend) (Table 2). In an analysis that quantified MPO mass per neutrophil using an ELISA in a random subset of subjects (n = 111), the results were highly correlated (r = 0.95) with the activity measurements (data not shown). Rates of CAD were also associated with increasing blood-MPO quartiles (P < .001 for trend) (Figure, Table 2). As shown in other studies, the Framingham Global Risk Score and...
WBC counts were associated with rates of CAD (Table 2).

**Leukocyte-MPO and CAD Risk Factors**

Leukocyte-MPO levels were independent of age, sex, diabetes, hypertension, smoking (ever or current), WBC count, LDL-C, TG, and Framingham Global Risk Score. Weak negative correlations between leukocyte-MPO and both TC \((r = -0.15; P = .005)\) and HDL-C \((r = -0.14; P = .01)\) were observed. A positive correlation was observed between leukocyte-MPO and absolute neutrophil count \((r = 0.20; P < .001)\) and family history of CAD (median leukocyte-MPO: 15.9 [with family history] vs 14.1 [without family history]; \(P = .05\)). Similar correlations were noted for blood-MPO.

**Multivariable Adjustments for Single and Multiple Risk Factors**

Odds ratios for leukocyte-MPO and blood-MPO quartiles were adjusted for individual traditional CAD risk factors. Since rates for CAD in the second and third quartiles of leukocyte-MPO appeared comparable (Table 2), they were combined for all further analyses and are referred to as the midrange levels in univariate and multivariable models. Odds ratios for both the middle (second plus third) and highest (fourth), relative to the lowest (first), quartiles of both leukocyte-MPO and blood-MPO remained associated with CAD status following adjustments for individual traditional CAD risk factors, WBC count, and Framingham Global Risk Score (data not shown), with ORs ranging from 8.4 (95% CI, 4.2-16.9; \(P < .001\)) after adjustment for HDL-C to 13.5 (95% CI, 6.3-29.1; \(P < .001\)) after adjustment for smoking. Diabetes, hypertension, smoking, and to a lesser degree age, HDL-C, WBC count, and Framingham Global Risk Score, also remained significant predictors for CAD status following single-factor adjustments. Similar results were observed for blood-MPO following single-factor adjustments for individual traditional CAD risk factors (data not shown).

In multivariable analyses with simultaneous adjustment for each of the single risk factors that were significantly associated with CAD in the preceding step, leukocyte-MPO remained the strongest predictor of CAD risk for both the middle vs the low quartile (adjusted OR, 8.5; 95% CI, 3.7-

**Table 2. Odds Ratio of CAD Prevalence According to MPO Levels, WBC Count, and Framingham Global Risk Score**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile</th>
<th>Quarte</th>
<th>Quarte</th>
<th>Quarte</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte-MPO</td>
<td>U/mg PMN</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>U/MN protein</td>
<td>≤11.8</td>
<td>11.9-15.3</td>
<td>15.4-19.8</td>
<td>≥19.9</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>CAD rate, %</td>
<td>24/91</td>
<td>35/76 (46)</td>
<td>36/83 (43)</td>
<td>63/83 (76)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Unadjusted OR</td>
<td>1.0</td>
<td>2.4 (1.2-4.6)</td>
<td>2.1 (1.1-4.0)</td>
<td>8.8 (4.4-17.5)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Model 1, OR</td>
<td>1.0</td>
<td>8.5 (3.7-19.7)</td>
<td>20.3 (7.9-52.1)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Model 2, OR</td>
<td>1.0</td>
<td>4.2 (2.1-8.1)</td>
<td>11.9 (5.5-25.5)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Blood-MPO</td>
<td>U/mg PMN × ANC</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>U/MN protein</td>
<td>≤2.9</td>
<td>3.0-4.1</td>
<td>4.2-5.7</td>
<td>≥5.8</td>
<td></td>
</tr>
<tr>
<td>CAD rate, %</td>
<td>16/91</td>
<td>35/83 (42)</td>
<td>41/79 (52)</td>
<td>66/80 (83)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Unadjusted OR</td>
<td>1.0</td>
<td>3.4 (1.7-6.8)</td>
<td>5.1 (2.5-10.2)</td>
<td>22.1 (10.0-48.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Model 1, OR</td>
<td>1.0</td>
<td>3.6 (1.8-7.5)</td>
<td>15.6 (6.2-36.7)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Model 2, OR</td>
<td>1.0</td>
<td>5.3 (2.7-10.5)</td>
<td>20.4 (8.9-47.2)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>WBC count</td>
<td>%</td>
<td>≤5.78</td>
<td>7.33-9.02</td>
<td>≥9.03</td>
<td></td>
</tr>
<tr>
<td>CAD rate, %</td>
<td>24/85</td>
<td>36/82 (56)</td>
<td>38/83 (46)</td>
<td>50/83 (60)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Unadjusted OR</td>
<td>1.0</td>
<td>3.2 (1.7-6.2)</td>
<td>2.1 (1.1-4.1)</td>
<td>3.9 (2.0-7.3)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Adjusted OR</td>
<td>1.0</td>
<td>3.0 (1.6-5.7)</td>
<td>4.3 (2.1-8.9)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Framingham Global Risk Score</td>
<td>≤4</td>
<td>5</td>
<td>8-9</td>
<td>≥10</td>
<td></td>
</tr>
<tr>
<td>CAD rate, %</td>
<td>25/86</td>
<td>41/114 (36)</td>
<td>41/83 (65)</td>
<td>51/70 (73)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Unadjusted OR</td>
<td>1.0</td>
<td>1.4 (0.8-2.5)</td>
<td>4.5 (2.3-9.1)</td>
<td>6.5 (3.2-13.2)</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR</td>
<td>1.0</td>
<td>1.8 (1.0-3.3)</td>
<td>7.2 (3.5-17.5)</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

*Unadjusted odds ratios (ORs) and confidence intervals (CIs) were calculated using logistic regression models comparing the risk of each of the upper 3 quartiles for the given parameter to the lowest quartile. For adjusted analyses, quartiles 2 and 3 were combined and are referred to as midrange levels. Unadjusted trends for increasing CAD rates with increasing parameter (MPO level or WBC) were evaluated using the Cochrane-Armitage trend test. The Hosmer-Lemeshow goodness-of-fit test against the null hypothesis showed a good fit for each model, producing a test statistic of 8.4 \((P = .00)\) for leukocyte-MPO and 6.7 \((P = .00)\) for blood-MPO with Model 1, and 8.5 \((P = .00)\) for leukocyte-MPO and 13.1 \((P = .01)\) for blood-MPO with model 2. Generalized R² values for model 1 were 52% (leukocyte-MPO) and 50% (blood-MPO); for Model 2, 34% (leukocyte-MPO) and 39% (blood-MPO). CAD indicates coronary artery disease; MPO, myeloperoxidase; WBC, white blood cell; PMN, polymorphonuclear leukocyte; and ANC, absolute neutrophil count. 1P for trend across quartiles.

Model 1 consisted of covariates significant after single-factor adjustments (age, sex, diabetes, hypertension, smoking history, HDL-C, WBC count) and MPO quartiles and tested for independence of each relative to the others in predicting CAD status.

Model 2 consisted of Framingham Global Risk assessment, WBC count, and MPO quartiles.

Adjusted ORs for WBC count and Framingham were calculated with simultaneous adjustment for levels of leukocyte-MPO, WBC count, and Framingham scores.

‡Quartile 2: \(P = .31; \) quartiles 3 and 4: \(P < .001.

¶Adjusted range vs low: \(P = .06; \) high vs low: \(P < .001.

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19.7) and the high vs the low quartile (adjusted OR, 20.3; 95% CI, 7.9–52.1). With further adjustment for Framingham Global Risk Score and WBC count, the ORs for leukocyte-MPO were 4.2 (middle vs low quartile) and 11.9 (high vs low quartile). Blood-MPO also remained a strong predictor of CAD risk following multivariable adjustments (Table 2). The adjusted ORs for Framingham Global Risk Score and WBC count were also significant.

COMMENT
The results of this study suggest that MPO may serve as an independent marker of CAD. Levels of functional MPO per leukocyte and per milliliter of blood were associated with the risk of CAD in a study population that was characterized angiographically for disease status, even following multivariable adjustments for traditional risk factors and WBC count.

Several studies support potential links between MPO and the development of CAD. Myeloperoxidase has been implicated as a participant in atherosclerosis through mechanisms related to its role in inflammation,9,22 LDL oxidation,11,13,23,24 and nitric oxide consumption leading to endothelial dysfunction.16 Myeloperoxidase generates an array of diffusible oxidants9 and is capable of initiating lipid peroxidation25,26 and promoting protein nitration27,28 and crosslinking,29 processes known to occur during the evolution of atherosclerosis.9,10,30–32 Myeloperoxidase also binds to LDL in plasma33 and promotes site-specific oxidation of the lipoprotein.35 Both immunohistochemical and mass spectrometry studies demonstrate that MPO is present in, and promotes oxidative modification of, targets within human atheroma at all stages of lesion development.9,10,22 Furthermore, LDL recovered from human atherosclerotic lesions is enriched in multiple oxidation products formed specifically by MPO, such as chlorotyrosine14 and Schiff base adducts of p-hydroxyphenylacetaldehyde (a tyrosine oxidation product17) with both apolipoprotein B100 lysine residues12 and aminophospholipids.13

There are several clues to the potential functional consequences of MPO-catalyzed oxidation in the artery wall. Iso- lated human monocytes use MPO to oxidatively convert LDL into an athero- genic particle capable of promoting cholesterol accumulation and foam cell formation.14 Uptake occurs via the scavenger receptor CD36,17 a receptor that appears to play a major role in foam cell formation in vivo.36 Myeloperoxidase may thus be involved in the atherosclerotic process directly by promoting lesion development.

Myeloperoxidase also may play a role in the pathogenesis of acute coronary syndromes through plaque destabilization.22 Circulating leukocytes release MPO during acute coronary syndromes.35 Macrophages containing MPO and MPO-dependent oxidation products are selectively enriched in atheromas that have undergone plaque rupture and ulceration.22 Moreover, hypochlorous acid (HOCl), a primary oxidant generated by MPO,38 may promote extracellular matrix degradation in vivo.22 Myeloperoxidase-generated HOCl both activates latent matrix metalloproteinases and inactivates their physiological inhibitors (eg, tissue inhibitor of metalloproteinase 1).39–41 Myeloperoxidase thus may influence plaque stability and the propensity for provoking thrombosis.22

Myeloperoxidase also may contribute to CAD through promoting endothelial dysfunction.15 Nitric oxide modulates MPO catalytic activity32 and serves as a physiological substrate for MPO.30 Myeloperoxidase attenuates nitric oxide–dependent smooth muscle relaxation43 and preliminary studies with preconstricted vascular rings show that MPO attenuates nitric oxide–mediated vasorelaxant responses.44 Thus, MPO may serve as a catalytic sink for nitric oxide, limiting its bioavailability and function.9,16

Although multiple lines of evidence suggest potential mechanisms for MPO in the development of cardiovascular disease, there are limited data in humans or animals. A cross-sectional study of 92 MPO-deficient individuals reported that MPO deficiency (a genetic disorder that occurs in 1:2000 to 1:5000 individuals) is associated with a decreased prevalence of cardiovascular events.45 A functional polymorphism in the promoter region of the gene for MPO resulting in decreased enzyme expression recently was reported to be associated with decreased risk of CAD.46 Recent studies with MPO knockout mice demonstrated increased atherosclerotic lesion development.47 However, further investigation demonstrated species-specific differences between mouse and human, including the absence of MPO and its oxidation products within lesions among wild-type mice.47

This study, to our knowledge, is the first direct attempt to correlate levels of MPO in leukocytes and blood with angiographically documented CAD status. While elevated MPO levels were associated with CAD, there was considerable variation among individuals in the levels of MPO present within leukocytes. Differences in leukocyte-MPO content, more than leukocyte counts, appear to play a role in the association between MPO and CAD status. Heterogeneity in MPO levels within circulating monocytes48,49 and atheromatous macrophages22 have also been reported. While monocytes and certain subpopulations of macrophages are the likely source of MPO in atheroma,10,22,28,30 we used neutrophils in our study as a surrogate because they are easier to isolate, contain more than 95% of the circulating MPO, and because cell-specific (ie, monocyte vs neutrophil) differences in MPO regulation have not been reported. Indeed, little is known about the factors that regulate MPO expression in vivo, either at the level of bone marrow or within the artery wall. While a significant correlation was noted between leukocyte-MPO and the absolute neutrophil count, the mechanism for this interaction is unknown. In our study, leukocyte- and blood-MPO levels were stable within an individual over time, with no significant differences in the specific activity (ie, units per milligram of MPO protein) of MPO within leuko-
cytes isolated from CAD patients and controls (P= .92). Also, quantification of MPO through either functional assays (eg, peroxidase activity) or by mass (eg, ELISA) yielded consistent findings.

The association between MPO levels and CAD was apparent despite increased use of lipid-lowering drugs, aspirin, and other cardiovascular agents (β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors) in the CAD group. These medications did not appear to alter MPO levels as there were no significant differences in leukocyte- or blood-MPO levels in controls taking vs not taking each of these agents (P> .27 for all comparisons). Moreover, leukocyte- and blood-MPO remained significant predictors of CAD status for patients taking vs not taking each medication class. For example, the adjusted OR for leukocyte-MPO (model 2) considering only patients not taking lipid-lowering medications (middle vs low quartile: OR, 5.0; P= .06; high vs low quartile: OR, 15.4; P< .001) were similar to the overall ORs (Table 2). Similar results were seen for blood-MPO. In addition, we examined the possible effects of aspirin intake on MPO levels by monitoring MPO indices in 12 healthy men at baseline and following 2 weeks of aspirin use (325 mg/d). We found no differences in leukocyte-MPO or blood-MPO levels at baseline vs 2 weeks.

In summary, MPO levels are associated with the presence of angiographically proven coronary atherosclerosis. If these findings are confirmed in other prospective studies, MPO levels by monitoring MPO indices in healthy populations and prove to be predictive of future cardiac events in longitudinal studies and of MPO as a potential therapeutic target for CAD appear warranted.

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