Myeloperoxidase, Subclinical Atherosclerosis, and Cardiovascular Disease Events

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OBJECTIVES We evaluated whether myeloperoxidase (MPO) predicts future cardiovascular disease (CVD) events in asymptomatic adults and whether subclinical atherosclerosis may affect this relation.

BACKGROUND Myeloperoxidase is a leukocyte-derived enzyme-generating reactive oxidant species that has been shown to predict risk of CVD in selected populations.

METHODS We studied 1,302 asymptomatic adults (mean age 59 years, 47% women) without known CVD who were followed for 3.8 years. We measured MPO by the use of immunoassay. Coronary artery calcium (CAC), a measure of subclinical atherosclerosis, was measured by computed tomography with the Agatston score categorized as none/minimal (0 to 9), mild (10 to 99), and moderate/significant (≥100). Cox regression, adjusted for age, sex, and other risk factors, examined the relation of CAC and/or MPO with incident CVD events.

RESULTS Persons with MPO levels at or above compared with below the median (257 pM) were more likely (p < 0.05 to p < 0.001) to be women, have a higher body mass index, greater low-density lipoprotein cholesterol, greater systolic and diastolic blood pressure, and lower high-density lipoprotein cholesterol. Mean MPO levels increased according to CAC categories (p trend = 0.02). Incident CVD events were more likely in those at or above versus below the median MPO level (4.6% vs. 2.3%, p = 0.02), even after adjustment for age, sex, CAC, and risk factors (hazard ratio [HR]: 1.9, 95% confidence interval: 1.0 to 3.6, p = 0.04). Combining CAC and MPO categories, CVD incidence ranged from 0.6% in those with a CAC score of 0 to 9 to 7.1% (adjusted HR: 9.2, p < 0.001) in those with CAC scores of ≥100 and MPO below the median and 14.0% (adjusted HR: 19.5, p < 0.0001) in those with CAC scores of ≥100 and MPO at or above the median.

CONCLUSIONS Our study suggests persons with both increased levels of both MPO and CAC are at an increased risk of CVD events. Imaging of subclinical atherosclerosis combined with assessment of biomarkers of plaque vulnerability may help improve CVD risk stratification. (J Am Coll Cardiol Img 2009;2:1093–9) © 2009 by the American College of Cardiology Foundation
Mycoperoxidase (MPO) is a leukocyte-derived enzyme-generating reactive oxidant species that may be atherogenic (1). Potential mechanisms that may relate to MPO promoting vascular disease include the following: stimulating conversion of low-density lipoprotein (LDL) into an atherogenic form, selectively modifying apolipoprotein A-1 and generating dysfunctional high-density lipoprotein (HDL), promoting endothelial dysfunction, promoting vulnerable plaque, and promoting myocardial dysfunction and abnormal ventricular remodeling after myocardial infarction (MI).

In 1,090 patients with acute coronary syndrome, those with increased levels of MPO (>350 μg/l; 31.3%) had an increased cardiac risk (adjusted hazard ratio [HR]: 2.25 [95% confidence interval (CI): 1.32 to 3.82], p = 0.003) (2). In 512 patients with acute MI, levels of MPO above the median predicted mortality (odds ratio: 1.8, 95% CI: 1.0 to 3.0, p = 0.034) (3). Also, levels of MPO were associated with the future risk of coronary artery disease in apparently healthy individuals in the European Prospective Investigation into Cancer-Norfolk Prospective Population Study, a nested case-control study (1,138 cases and 2,237 control patients) with 8-year follow-up, with an adjusted odds ratio for the highest quartile of MPO 1.36, 95% CI: 1.07 to 1.73 (4). Coronary artery calcium (CAC) is a well-established measure of subclinical atherosclerosis, and the authors of numerous cohort studies (5) show its strong relation to future cardiovascular events and mortality in asymptomatic individuals.

However, the association of MPO with subclinical atherosclerosis is not established. In addition, there are limited data on the combined utility of imaging of subclinical atherosclerosis and assessment of biomarkers, such as MPO, for identifying patients at increased risk of cardiovascular events. We evaluated whether MPO predicts future cardiovascular disease (CVD) events in asymptomatic adults and specifically whether this may depend on the extent of atherosclerotic burden present.

METHODS

We studied 1,302 adults (mean age 59 years, 47% women) without known cardiovascular disease or symptoms, within the EISNER (Early Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research) research study. The current study was approved by the Cedars-Sinai Medical Center Institutional Review Board (institutional research board numbers 3351, 3354, and 3974). Follow-up for incident CVD events (MI, revascularization, stroke, or CVD death) was available for an average of 3.8 years. Cardiovascular events were reviewed and adjudicated by study physicians. Standardized assessment of cardiovascular risk factors was available among participants. Serum and ethylene diamine tetraacetic acid plasma samples were stored at −70°C for up to 4 years until all baseline samples were accumulated.

We measured MPO using an immunoassay (BioSite, an Inverness Medical Company, Waltham, Massachusetts). The ethylene diamine tetraacetic acid plasma sample, once treated with reagents on the test device, reacts with fluorescent antibody conjugates, providing fluorescent signals from which the marker concentrations are calculated on the basis of reference plasma samples. A moderately high correlation of 0.79 with a near 1:1 correspondence (slope = 1.07) was observed between measurements of the BioSite assay compared with that of the Prognostix CardioMPO assay (protocol CLN0002, BioSite, Inc., personal communication) over a wide range of measurements extending to 5,000 pM. A coefficient of variability of 12% has been reported at a mean level of 306 pM (lot W33318, Biosite, Inc.) (William Arnold, PhD, personal communication, April 2009).

Subjects were scanned by the use of either electron beam computed tomography (General Electric/Imatron, South San Francisco, California) or by a multidetector scanner (Siemens, Munich, Germany). The imaging protocol involved an experienced licensed radiologic technician acquiring a single scan on each patient consisting of approximately 30 to 40 3-mm slices encompassing the heart from the carina to the apex, performed at 50% electrocardiographic triggering in an attempt to minimize motion artifact. Instructions on breath-holding also were given to minimize misregistration. Foci of CAC were identified and scored by an experienced technician, who used semiautomatic commercial software (ScImage, Inc., Los Altos, California), and the scoring was verified by an imaging cardiologist.

The software initially calculated lesion-specific scores as the product of the area of each calcified focus and peak computed tomography number (scored as 1 if 130 to 199 Hounsfield Units [HU], 2 if 200 to 299 HU, 3 if 300 to 399 HU, and ≥4 if ≥400 HU). These were summed across all lesions
identified within left main, left anterior descending, left circumflex, and right coronary arteries to provide arterial specific calcium scores and across arteries to provide a total Agatston calcium score used for analysis (6). The quantity of CAC was categorized as none/minimal (0 to 9), mild (10 to 99), moderate (100 to 399), or significant (≥400).

A fasting lipid profile (total and HDL cholesterol [HDL-C], triglycerides, with calculated LDL cholesterol [LDL-C] based on the Friedwald equation) plus glucose was performed on each study participant by a point-of-care analyzer (Cholestech, an Inverness Medical Company). Two readings of blood pressure (with mean systolic and diastolic readings used for analysis) and weight and height for calculation of body mass index also were obtained. A brief medical history to assess previous history of cardiac disease, diabetes, and medication usage also was taken. Diabetes was defined as a self-reported history of being told by a physician that diabetes was present, taking diabetes medications, or having a fasting glucose of 7.0 mmol/l (126 mg/dl) or greater or a casual glucose of 11.1 mmol/l (200 mg/dl) or greater. A 10-year risk of coronary heart disease was estimated by the Framingham risk score algorithms of the National Cholesterol Education Program (7). Those with diabetes were defined as coronary heart disease risk equivalents and assigned a risk score of 20% (or greater if determined as such by the Framingham risk score calculation).

Follow-up for CVD events consisted of administering patient questionnaires, patient interviews, and/or using hospital records to obtain outcome data on consented patients. The incidence of MIs, strokes, and deaths were verified by 2 physicians from independent review of admission reports, discharge summaries, and consultation/lab reports. Revascularizations were verified by hospital records. All deaths were verified by National Death Index and/or independent review of death reports by 2 physicians. For analytic purposes, total CVD events included MI or cardiac death, late revascularizations (>90 days), and stroke.

Continuous variables were assessed between 2 groups by the use of the Student t test, whereas categorical variables were assessed by use of the Pearson chi-square test or Fisher exact test for cell counts of ≤5. Cuzick’s nonparametric test for trend for continuous variables (e.g., mean MPO by CAC category) and the log-rank test of trend for comparison of CVD event rates across MPO and/or CAC categories were applied. The Student t test or chi-square test of proportions was used initially to examine the relationships of risk factors between those with MPO levels at or above versus below the median. Also, we used multiple logistic regression to examine for risk factors independently associated with increased MPO levels. Cox regression, adjusted for age, sex, and other risk factors (LDL-C, HDL-C, systolic blood pressure, diabetes, and current smoking), was used to examine the relationship of MPO and CAC to CVD events. Analyses were performed with MPO examined above versus below the median and also continuously by natural log transformation. Finally, from the Cox model-predicted relative hazards, we examined using receiver-operator characteristic analyses, whether MPO and/or CAC provided incremental value to prediction of CVD events over models with standard risk factors alone. Curves were compared with the method of DeLong et al. (8).

RESULTS

Table 1 displays the clinical characteristics of age, sex, risk factors, by MPO level at or above the median (257 pM) versus below the median level. Patients with greater MPO levels were significantly more likely to be women, have greater body mass index, greater LDL-C, lower HDL-C, and to have greater systolic and diastolic blood pressure. There was a trend (p = 0.15) for greater CAC levels in those with higher versus lower MPO levels. Moreover, Figure 1 shows mean MPO levels to be greater according to increasing CAC categories

<table>
<thead>
<tr>
<th>Variable</th>
<th>MPO Below Median (&lt;257 pmol/l) n = 649</th>
<th>MPO At or Above Median (≥257 pmol/l) n = 653</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>58.3 ± 8.2</td>
<td>58.8 ± 8.7</td>
<td>0.36</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>43.8</td>
<td>49.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.6 ± 4.7</td>
<td>28.6 ± 5.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>7.7</td>
<td>8.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>6.9</td>
<td>6.6</td>
<td>0.80</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>132.4 ± 37.8</td>
<td>138.7 ± 40.5</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>55.4 ± 16.7</td>
<td>52.5 ± 16.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>131.7 ± 17.5</td>
<td>134.8 ± 17.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>81.6 ± 10.7</td>
<td>82.9 ± 11.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Framingham risk (%)</td>
<td>8.3 ± 6.9</td>
<td>9.0 ± 7.3</td>
<td>0.08</td>
</tr>
<tr>
<td>CAC scores (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–9</td>
<td>58.7*</td>
<td>53.5</td>
<td></td>
</tr>
<tr>
<td>10–99</td>
<td>19.6</td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>100–399</td>
<td>14.2</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>≥400</td>
<td>7.6</td>
<td>9.3</td>
<td></td>
</tr>
</tbody>
</table>

*p = 0.15 comparing distribution of MPO levels across CAC categories.

BP = blood pressure, CAC = coronary artery calcium; HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, MPO = myeloperoxidase.
(p trend = 0.02), although after adjustment for other risk factors this relationship was attenuated. In a separate multiple logistic regression (results not shown), factors independently associated with the odds of having greater MPO levels were body mass index (p = 0.001) and LDL-C (p = 0.001), with HDL-C being inversely associated (p = 0.001) and male sex being associated with a lesser likelihood (odds ratio: 0.54, p < 0.001) of having greater MPO levels.

Figure 2 shows CVD event risk to increase by quartile of MPO level (trend p = 0.05). The overall incidence of CVD events was 3.5%. Persons at or above versus below the median MPO level had a 2-fold greater CVD event rate at 4.6% versus 2.3% (p = 0.02 or 1.2%/year vs. 0.6%/year, p = 0.02). In Cox proportional hazards regression (results not shown), adjusted for age, sex, LDL-C and HDL-C, systolic blood pressure, diabetes, and smoking, increased MPO (at or above the median vs. below) levels remained independently predictive of CVD events (HR: 2.0, 95% CI: 1.05 to 3.74, p = 0.034). This relationship was slightly weaker when MPO was examined continuously (natural log-transformed): HR: 1.7; CI: 0.98 to 3.1 per natural log unit, p = 0.06.

Table 2 shows the incidence of CVD events and age, sex, and risk factor-adjusted HRs for CVD events according to CAC and MPO categories together. Although CAC was the main factor associated with increased CVD event rates, MPO levels at or above versus below the median level remained an independent predictor of CVD events (HR: 1.9, p = 0.04).

In a similar model with CAC and MPO categories combined (Table 3), in those with both moderate and significant CAC (scores ≥100) and MPO levels at or above the median, the risk of CVD events was increased. The incidence of CVD ranged from 0.6% in those with CAC 0 to 9 (regardless of MPO) to 7.1% (HR: 9.2, p < 0.001) in those with CAC ≥100 and lower MPO and 14.0% (HR: 19.5, p < 0.0001) in those with CAC ≥100 and higher MPO. Event risk is also displayed in Figure 3.

Finally, when we examined the overall utility of MPO and/or CAC to improve the prediction of CVD events, compared with age, sex, and risk factors alone where the area under the curve (AUC) was 0.755, significant improvement in prediction of CVD events was obtained from models additionally
containing combined MPO-CAC categories (AUC = 0.838, p = 0.0037) and CAC categories alone (AUC = 0.833, p = 0.005) but not MPO alone (AUC = 0.762, p = 0.59). Moreover, there was no improvement from adding MPO to a model with age, sex, risk factors, and CAC (AUC = 0.838 vs. 0.833, p = 0.53).

**DISCUSSION**

We show in this report levels of MPO to be modestly associated with the degree of subclinical atherosclerosis burden noted by increased levels of CAC. Further, we confirm other reports of a modest increase in cardiovascular event risk associated with greater MPO levels among those with acute coronary syndrome (2), myocardial infarction (3), and those asymptomatic without known coronary disease (4), which remained significant even after adjusting for other risk factors. Of note, among persons with at least a moderate degree of subclinical atherosclerosis (CAC scores ≥100) who also had increased levels of MPO, CVD event risk was increased.

Numerous prospective studies (2,3,9–12) among persons with stable or unstable coronary artery disease have shown an association between elevated MPO levels and future CVD event risk. The EPIC-Norfolk study is the only other epidemiologic study of initially healthy individuals to examine the relationship of MPO with future event risk and found a 1.5-fold increased risk of coronary heart disease events among persons in the highest quartile of MPO levels, even after adjusting for standard risk factors (4). We note the risk of CVD events to be increased once MPO levels are above the median (approximately 257 pm) in our population, a finding that persists after adjustment for other risk factors.

This is the first report examining the relation of MPO with CAC as a measure of subclinical atherosclerosis. We find a significant (p = 0.02) trend of increasing mean MPO levels according to increased levels of CAC, although this relationship was attenuated after adjustment for other risk factors. Others have previously reported MPO levels to independently predict other measures of subclinical atherosclerosis such as endothelial dysfunction (13) and carotid stenosis in persons with low HDL-C (14) but not carotid atherosclerosis in persons with familial hypercholesterolemia (15).

Our study showed that the extent of subclinical atherosclerosis may help identify where biomarkers such as MPO may be most useful for identifying persons at greater risk. Although this relationship has not been examined with other subclinical atherosclerosis measures, other studies have examined the prognostic importance of MPO combined with other biomarkers. The EPIC-Norfolk study showed persons with MPO levels above the median who also had C-reactive protein levels ≥2 mg/l were at a significant 2.4-fold greater risk of coronary heart disease events, greater than what increased levels of MPO conferred alone (1.5-fold greater risk) (4).

In addition, Khan et al. (12) showed the combination of MPO and N-terminal pro-B-type natriuretic peptide levels to improve prognostication in high-risk patients. We also demonstrate from receiver-operating characteristics analyses that although combined MPO-CAC groups significantly improved prediction of CVD events over age, sex, and risk factors alone, this finding was explained exclusively by CAC. The level of MPO alone did not add incremental value to prediction over age, sex, and risk factors with or without CAC. Our data

<table>
<thead>
<tr>
<th>CAC Score</th>
<th>MPO Level, pM</th>
<th>Incidence of CVD Events, n (%)</th>
<th>Adjusted HR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–9</td>
<td>&lt;257</td>
<td>4/730 (0.55)</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>10–99</td>
<td>≥257</td>
<td>6/154 (3.9)</td>
<td>5.2 (1.4–18.8)</td>
<td>0.012</td>
</tr>
<tr>
<td>≥100</td>
<td>&lt;257</td>
<td>10/141 (7.1)</td>
<td>9.2 (2.7–31.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥100</td>
<td>≥257</td>
<td>21/150 (14.0)</td>
<td>19.5 (6.3–60.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Notes adjusted for age, sex, LDL-C, HDL-C, diabetes, smoking, and systolic blood pressure. Note: Reliability of the estimates may be affected by the small number of events in the reference group. Abbreviations as in Table 2.

![Figure 3. Event Risk of CVD (%) by Combined MPO and CAC Group](image-url)

The cumulative proportion of subjects with CVD events is shown according to combined MPO and CAC group; log-rank test for trend p < 0.001. Abbreviations as in Figures 1 and 2.
showing CAC to add to prediction over risk factors is consistent with other previous reports (16–18). **Study limitations.** Our primary end point was a composite of CVD events, including revascularizations; we did not have an adequate number of individual hard end points (e.g., MIs or strokes) to examine these end points separately. In addition, our results apply principally to asymptomatic individuals who comprised our population; findings may differ for other higher-risk populations (e.g., acute coronary syndromes) where MPO has been shown to be an even stronger risk factor. Also, although our Cox model could be overfitted with 7 covariates, we are able to demonstrate stability in our key estimates based on a simpler model adjusting for Framingham risk score only, which results in essentially unchanged estimates for combined CAC and MPO categories. Finally, our study population was composed primarily of Caucasian patients, so our findings cannot be generalized to more diverse populations.

Our study demonstrates the potential utility of a biomarker such as MPO in combination with imaging measures to help identify higher-risk persons. Further studies are needed to examine which biomarkers perform best with which specific imaging modalities and in which patient subgroups (e.g., asymptomatic, intermediate risk, stable coronary disease, younger patients, or acute coronary syndrome). Biomarkers used for this purpose should be low cost, free of radiation, and easily measured at the time of imaging to provide sustained utility in the clinical setting.

**CONCLUSIONS**

Our report suggests that persons who have both increased levels of MPO and CAC have an increased risk of CVD events. Although greater CAC scores imply there is generally more extensive disease and thus a greater likelihood of vulnerable plaques, adding a marker such as MPO helps refine this relationship by further identifying those with active versus stable disease. Therefore, MPO may serve as a marker of plaque vulnerability, which is more common in those with greater CAC scores. Imaging of subclinical atherosclerosis combined with biomarker assessment of MPO and/or other factors that may relate to plaque vulnerability may help to better identify groups of patients at high risk of CVD events.

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**REFERENCES**


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