Myeloperoxidase: A Useful Biomarker for Cardiovascular Disease Risk Stratification?

Roger K. Schindhelm,* Leonard P. van der Zwan,1 Tom Teerlink,2 and Peter G. Scheffer2

BACKGROUND: Inflammation and oxidative stress are associated with atherosclerosis. Myeloperoxidase (MPO) is linked to both inflammation and oxidative stress by its location in leukocytes and its role in catalyzing the formation of oxidizing agents. Recent evidence suggests that MPO activity precipitates atherogenesis. Measurement of MPO in plasma may therefore contribute to cardiovascular disease (CVD) risk stratification.

CONTENT: Cross-sectional studies, case-control studies, and prospective-cohort studies investigating the relation between MPO and CVD have been evaluated. Differences in study populations, sample materials, sample handling, and assays were ascertained. Potential causal mechanisms linking MPO to accelerated atherosclerosis are discussed here. A majority of studies indicate that measurement of MPO in plasma was associated with improved CVD risk stratification above and beyond risk stratification results obtained with markers used in routine clinical practice. However, comparison of these epidemiological studies with regard to MPO and outcome is hampered because the reported MPO concentration depends on the assay method, sampling material, and preanalytical and analytical procedures. The link between MPO and CVD can, at least partly, be explained by MPO-dependent oxidation of LDL and HDL, subsequently leading to cholesterol accumulation in the arterial wall. Furthermore, MPO may reduce the bioavailability of nitric oxide, resulting in endothelial dysfunction. Finally, MPO destabilizes atherosclerotic plaques.

SUMMARY: Increasing evidence suggests that MPO is causally linked to atherosclerosis and its measurement may improve CVD risk estimation. Before MPO can be used in routine clinical practice, however, standardization of sampling and laboratory procedures is needed.

Cardiovascular disease (CVD) is the leading cause of death in Western societies. CVD mortality and morbidity are promoted by major CVD risk factors, such as hyperlipidemia, hypertension, and smoking. The sequence of events leading to CVD includes endothelial dysfunction, atherosclerotic plaque formation, and rupture (1). Inflammation has been implicated in all these stages in the evolution of atherosclerotic plaques (1). Moreover, oxidative stress is currently considered a key event in CVD development (2).

Myeloperoxidase (MPO) is an enzyme linked to both inflammation and oxidative stress. It is abundantly expressed in the azurophilic granules of most leukocyte subspecies, including neutrophils and monocytes (3). MPO is released by leukocytes in a state of inflammation and catalyzes the formation of several reactive species, including hypochlorous acid, and thus has a role in host defense against microorganisms (3).

Paradoxically, MPO has been implicated in initiation and propagation of atherosclerosis. In the past few years, evidence emerging from epidemiological studies has shown that higher concentrations of MPO are associated with an increased CVD risk, independent of classical CVD risk factors (4–15). However, comparison of these epidemiological studies with regard to MPO and CVD outcome is hampered by the fact that various assay methods and sampling procedures have been applied. The differences that are attributable to the use of various methods may have important implications for the interpretation of the results of these studies.

This review presents epidemiological evidence for MPO as a relevant biomarker with respect to CVD outcome, reviews the pathophysiological mechanisms

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3 Nonstandard abbreviations: CVD, cardiovascular disease; MPO, myeloperoxidase; CAD, coronary artery disease; OR, odds ratio; HR, hazard ratio; CRP, C-reactive protein; AMI, acute myocardial infarction; ACS, acute coronary syndrome; NOS, nitric oxide synthase; ApoA-1, apolipoprotein A-1.
linking MPO to CVD, and addresses preanalytical and analytical issues in measurement of MPO.

**Myeloperoxidase and Cardiovascular Disease: Epidemiological Studies**

A number of epidemiological studies have been reported that address the association of MPO with CVD and markers of atherosclerosis in a wide range of patient populations, including patients with established atherosclerosis and patients with an acute presentation with chest pain (Table 1).

**ESTABLISHED ATHEROSCLEROSIS**

The first epidemiological report assessing the association between MPO and CVD was a case-control study published by Zhang and coworkers in 2001 (15). The authors studied the relation of MPO, expressed either as mass per milligram of neutrophil protein or as MPO mass per milliliter of blood, with coronary artery disease (CAD) risk in a case-control design with 158 patients with established CAD and 175 controls. CAD patients had significantly higher concentrations of MPO compared to the controls, and it was demonstrated by multivariate logistic regression analysis adjusted for traditional CVD risk factors that MPO was associated with an 11.9-fold (95% CI, 5.5–25.5, upper vs lower quartile) increased risk for CAD for leukocyte MPO, and a 20.4-fold (95% CI, 8.9–47.7, upper vs lower quartile) increased risk for CAD for blood MPO.

In a cross-sectional study by Duzguncinar et al., MPO was increased in patients with CAD and correlated to the extent and severity of atherosclerosis of the coronary vessels (16). Meuwese and coworkers studied the association of MPO with CAD in 1138 patients with CAD and 2237 controls (11). These investigators demonstrated that MPO (upper vs lower quartile) was related to CAD after adjustment for traditional risk factors [odds ratio (OR), 1.36 (95% CI, 1.07–1.73)]. In a case-control study in 680 patients, 382 patients with stable CAD and 194 controls with normal coronary angiograms, MPO was higher in patients with CAD compared to controls (14). In addition, MPO concentrations were found to correlate with the presence of CAD [OR, 2.08 (95% CI, 1.54–2.81)]. Mocatta and coworkers studied the 5-year all-cause mortality rate in 512 patients with established myocardial infarction (12). Patients with MPO values above the median had an almost 2-fold increased risk [hazard ratio (HR) 1.81 (95% CI, 1.07–3.05)] of all-cause mortality compared to those with MPO values below the median. The predictive value of MPO for mortality and myocardial infarction was studied by Baldus et al. in a group of 1090 patients with acute coronary syndromes during a 6-month follow-up period (4). Although MPO did not correlate with established markers of CVD risk and inflammation, including troponin-T, soluble CD40 ligand, and C-reactive protein (CRP), patients with increased MPO (upper vs lower tertile) had a 2.25-fold (95% CI, 1.32–3.82) increased risk of adverse events, defined as reinfarction or death. In a multivariate model, MPO was the strongest independent predictor of CVD outcome. In 38 patients with ST-segment myocardial infarction presenting with cardiogenic shock and treated with percutaneous coronary interventions, baseline MPO was an independent predictor of in-hospital mortality [OR, 3.9 (95% CI: 1.8–7.5)], after adjustment for clinical, laboratory, and angiographic variables (8). Brevetti et al. studied the predictive value of MPO vs CRP for fatal and nonfatal CVD events in 156 patients with peripheral artery disease (6). Despite the relatively low number of events (n = 17) during 6 months of follow-up, the authors demonstrated that MPO was a strong predictor for adverse events [HR 6.80 (95% CI, 1.20–38.69)], whereas CRP was not [HR 0.88 (95% CI, 0.60–1.29)]. Exner et al. studied the progression of stenosis of the internal carotid artery in 1019 asymptomatic CAD patients with a follow-up of 7.5 months (9). Patients with progressive stenosis had significantly higher baseline MPO concentrations compared to patients with stable disease. Interestingly, the relation between MPO and progression of stenosis was modified by HDL cholesterol level. An MPO concentration above the median was associated with a 2.6-fold increased risk (95% CI, 1.4–4.8) of disease progression, but only in patients with HDL cholesterol concentrations below the median. Altogether, the results of these studies indicate that MPO may be a valuable novel marker for CVD events. However, one should keep in mind that publication bias due to unpublished negative findings cannot be ruled out. Stefanescu and coworkers found no independent association between MPO and all-cause mortality in 382 patients with stable CAD during 3.5 years of follow-up (17). In contrast to the above-mentioned studies, all of which had reported significant associations between MPO and CVD, 1 case-control study in HIV patients showed no significant independent association of MPO with CVD events (18). Another study, in patients undergoing elective coronary angiography, showed no significant differences in MPO concentrations for those with proven stable CAD compared to those without proven CAD (19). A possible explanation for this negative finding might be the lower risk in stable vs unstable CAD. Indeed, cardiovascular events are thought to be more likely in unstable CVD (20). These observations may thus indicate that MPO is a particularly useful biomarker in high-risk populations.

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Table 1. Overview of prospective and case-control studies assessing the association of myeloperoxidase with cardiovascular disease.

<table>
<thead>
<tr>
<th>First author, year (ref. #)</th>
<th>Sample type</th>
<th>Study population</th>
<th>n, Cases (controls)</th>
<th>Men, %</th>
<th>Age, years</th>
<th>Follow-up, mo</th>
<th>Outcome</th>
<th>Risk, 95% CI</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple 2007 (23)</td>
<td>Heparin plasma</td>
<td>Patients with ACS</td>
<td>457</td>
<td>57% (No event) 48% (Event)</td>
<td>57 (16) (No event) 62 (18) (Event)</td>
<td>4</td>
<td>All-cause mortality</td>
<td>0.9 (0.4–2.1)</td>
<td>Above vs below 99th percentile</td>
</tr>
<tr>
<td>Baldus 2003 (4)</td>
<td>Serum</td>
<td>Patients with ACS</td>
<td>1090</td>
<td>71 (Low MPO) 69 (High MPO)</td>
<td>61.4 (10.5) (Low MPO) 62.5 (10.4) (High MPO)</td>
<td>6</td>
<td>MI* or mortality</td>
<td>2.25 (1.32–3.82)</td>
<td>High vs low MPO</td>
</tr>
<tr>
<td>Brennan 2003 (5)</td>
<td>Plasma</td>
<td>Patients with chest pain</td>
<td>142 (462)</td>
<td>55 (No MI) 70 (MI)</td>
<td>61.4 (13.8) (No MI) 66.5 (12.8) (MI)</td>
<td>1 and 6</td>
<td>MI</td>
<td>4.7 (2.9–7.7)</td>
<td>4th vs 1st Quartile MPO</td>
</tr>
<tr>
<td>Brevetti 2008 (6)</td>
<td>Serum</td>
<td>Patients with PAD</td>
<td>156</td>
<td>77</td>
<td>67.1 (8.2)</td>
<td>12–24</td>
<td>CVD events</td>
<td>6.80 (1.20–38.7)</td>
<td>High vs low MPO</td>
</tr>
<tr>
<td>Cavusoglu 2007 (7)</td>
<td>Plasma</td>
<td>Patients with ACS</td>
<td>193</td>
<td>100</td>
<td>65.0 (9.3) (Low MPO) 64.7 (10.8) (High MPO)</td>
<td>24</td>
<td>MI</td>
<td>1.60 (1.09–2.36)</td>
<td>Per 1 SD of log(MPO)</td>
</tr>
<tr>
<td>Dominguez 2008 (8)</td>
<td>Serum</td>
<td>Patients STEMI and CS</td>
<td>38</td>
<td>78 (Survivors) 65 (Non-survivors)</td>
<td>66 (10) (Survivors) 75 (12) (Non-survivors)</td>
<td>NA</td>
<td>In-hospital mortality</td>
<td>3.9 (1.8–7.5)</td>
<td>MPO, continuous</td>
</tr>
<tr>
<td>El-Bejjani 2008 (18)</td>
<td>Plasma</td>
<td>HIV-infected adults</td>
<td>62 (62)</td>
<td>94</td>
<td>46.0 (40–52) 45.3 (39–51)</td>
<td>12</td>
<td>CVD events</td>
<td>NA</td>
<td>Cases vs controls</td>
</tr>
<tr>
<td>Exner 2006 (9)</td>
<td>Serum</td>
<td>Asymptomatic CAD patients</td>
<td>1019</td>
<td>62</td>
<td>69 (61–76)</td>
<td>7.5</td>
<td>Progression of ICA stenosis</td>
<td>2.57 (1.39–4.75)</td>
<td>Above vs below MPO median</td>
</tr>
<tr>
<td>Khan 2007 (10)</td>
<td>EDTA plasma</td>
<td>Patients with STEMI</td>
<td>384 (257)</td>
<td>21 (1st quartile) 15 (4th quartile)</td>
<td>61.8 (12.3) (1st quartile) 67.6 (11.9) (4th quartile)</td>
<td>&gt;1</td>
<td>Death and nonfatal MI</td>
<td>6.91 (1.79–26.73)</td>
<td>Above vs below median log(MPO)</td>
</tr>
<tr>
<td>Meares 2007 (11)</td>
<td>Serum</td>
<td>Healthy individuals</td>
<td>1138 (2237)</td>
<td>63 (Controls) 64 (Cases)</td>
<td>65.3 (7.7) (Controls) 65.5 (7.8) (Cases)</td>
<td>96</td>
<td>CAD</td>
<td>1.36 (1.07–1.73)</td>
<td>Log(MPO), continuous</td>
</tr>
<tr>
<td>Mocatta 2007 (12)</td>
<td>EDTA plasma</td>
<td>Patients with MI</td>
<td>512</td>
<td>80</td>
<td>61.7 (11.0)</td>
<td>60</td>
<td>All-cause mortality</td>
<td>1.81 (1.07–3.05)</td>
<td>Above vs below MPO median</td>
</tr>
<tr>
<td>Morrow 2008 (13)</td>
<td>Plasma</td>
<td>Patients with ACS</td>
<td>1524</td>
<td>68 (Below median) 66 (Above median)</td>
<td>61 (52–69) 61 (53–70)</td>
<td>1</td>
<td>Nonfatal MI or hospitalization</td>
<td>2.10 (1.36–3.23)</td>
<td>Log(MPO), continuous</td>
</tr>
<tr>
<td>Ndrepepa 2008 (14)</td>
<td>EDTA plasma</td>
<td>Patients with stable CAD</td>
<td>680 (194)</td>
<td>42 (Controls) 73 (Cases)</td>
<td>58.7 (7) (Controls) 74.5 (Cases)</td>
<td>NA</td>
<td>ACS</td>
<td>2.08 (1.54–2.81)</td>
<td>MPO, continuous</td>
</tr>
<tr>
<td>Stefanescu 2008 (17)</td>
<td>EDTA plasma</td>
<td>Patients with stable CAD</td>
<td>382</td>
<td>46 (1st tertile) 72 (2nd tertile) 68.6 (3rd tertile)</td>
<td>64.1 (1st tertile) 66.1 (2nd tertile) 67.4 (3rd tertile)</td>
<td>42</td>
<td>All-cause mortality</td>
<td>1.06 (0.71–1.59)</td>
<td>High vs low and intermediate MPO</td>
</tr>
<tr>
<td>Zhang 2001 (15)</td>
<td>Leukocytes/ blood</td>
<td>Patients with and without established CAD</td>
<td>158 (175)</td>
<td>58 (Controls) 80 (Cases)</td>
<td>55 (10) (Controls) 64 (13) (Cases)</td>
<td>NA</td>
<td>NA</td>
<td>11.9 (5.5–25.5)</td>
<td>20.4 (8.9–47.2)</td>
</tr>
</tbody>
</table>

*MI, myocardial infarction; PAD, peripheral artery disease; STEMI, ST-segment elevation MI; CS, cardiogenic shock; ICA, internal carotid artery; NA, not applicable.
ACUTE PRESENTATION WITH CHEST PAIN
In a cross-sectional study by Esporcatte et al., an MPO concentration higher than 100 pmol/L had a diagnostic sensitivity of 92% and specificity of 40% as a marker for identifying patients with an acute myocardial infarction (AMI) presenting with acute chest pain and non–ST elevation electrocardiogram findings. In this study, AMI was defined by troponin I concentration of >1.0 µg/L (21). In contrast, a study by Apple et al. found no additional diagnostic value of MPO (99th percentile) compared to troponin I in patients with clinically diagnosed acute coronary syndrome (ACS) (22). Although MPO and troponin I showed similar diagnostic sensitivities, the diagnostic specificity of MPO was considerably lower than troponin I. Furthermore, a previous study by Apple et al. (23) showed that in patients presenting with symptoms suggestive of ACS, increased troponin I was significantly associated with all-cause mortality, whereas increased MPO (99th percentile) was not. Morrow and coworkers studied the predictive value of MPO, soluble CD40 ligand, troponin I, and CRP in 1524 patients with ACS in a tirofiban intervention trial for survival within 180 days. Patients with increased MPO concentrations (above median) were at higher risk for nonfatal MI or rehospitalization for ACS at 30 days. Furthermore, MPO was associated with recurrent ischemic events, after adjustment for CRP, troponin I, soluble CD40 ligand, and other major CVD risk factors (13). The clinical value of MPO to predict AMI and adverse events after 30 days and 6 months of follow-up in patients with acute chest pain was assessed by Brennan and coworkers (5). MPO was higher in patients with established AMI than in those without established AMI at presentation. Patients initially negative for troponin T but subsequently positive for troponin T had higher MPO concentrations than patients with no increase in troponin T. Patients with high MPO (upper vs lower quartile) were more likely to have a major cardiac event at 30 days [OR 4.7 (95% CI, 2.8–7.7)] and 6 months [OR 4.7 (95% CI, 2.9–7.7)] of follow-up. Khan et al. studied the incidence of nonfatal myocardial infarction and death in 384 patients with ST-segment myocardial infarction and observed that those with MPO values above the median had an almost 7-fold (HR 6.91 [95% CI, 1.79–26.73]) increased risk of adverse outcomes (10). In a study by Cavusoglu et al., MPO was found to be independently associated with MI [OR 1.60 (95% CI, 1.09–2.36)] in 193 men with ACS during 2 years of follow-up (7). Another cross-sectional study showed that MPO had a higher diagnostic sensitivity and specificity in identifying patients with AMI than the total white blood cell count and 3-chlorotyrosine (24).

All in all, these studies indicate that measurement of MPO in patients presenting with acute chest pain provides clinically relevant information. It should be noted, however, that between-study differences in cut-off values for both MPO and the cardiac troponins might have affected the diagnostic and prognostic value of MPO compared to troponin.

MPO as a Causal Factor in the Pathogenesis of Atherosclerosis
MPO is important in host defense against pathogens. On the other hand, as discussed above, a considerable number of epidemiological and clinical studies have demonstrated an association between increased concentrations of MPO and CVD, independent of classical risk factors. The source of MPO in plasma is activated leukocytes. Release of MPO and subsequent formation of reactive species (MPO-derived reactive species) (3) may be triggered by several mechanisms. First, it is known that inflammation induces recruitment and activation of white blood cells. Second, minimally modified LDL particles in the intima may trigger the influx of monocytes that mature into resident macrophages, some of which express MPO. Third, neutrophils in the blood stream are attracted and bound to sites of damaged endothelium. MPO released by these adherent leukocytes is initially bound to the vascular endothelium and subsequently transcytosed to the subendothelial matrix (25). Therefore, both local release by resident macrophages and transcytosis of intraluminally produced MPO are sources of MPO in the vascular wall.

The microenvironment of the subendothelial space is especially conducive to MPO activity. Mitochondrial respiration, NAD(P)H oxides, xanthine oxidase, and uncoupled nitric oxide synthase (NOS) are major sources of the highly reactive superoxide radical, which is actively converted into hydrogen peroxide by superoxide dismutase. Although less reactive than the superoxide radical, hydrogen peroxide is the cosubstrate for all MPO-catalyzed reactions. MPO amplifies the oxidative potential of hydrogen peroxide by producing a variety of reactive oxidants, including chlorinating and nitrating species. In the following sections some targets of MPO-derived reactive substances are briefly described, but it should be noted that many more cellular macromolecules and processes are adversely affected by vascular MPO activity. Key features of the adverse role of MPO in the vascular wall are depicted in Fig. 1.

LDL OXIDATION BY MPO
The oxidative modification of LDL is an early event in atherosclerosis, and oxidized LDL contributes to atherogenesis by promoting cholesterol deposition and transformation of macrophages into foam cells (2). Retention of LDL in the subendothelial space makes
LDL a major target for oxidation by prooxidants produced by arterial wall cells. Sources of oxidants include NAD(P)H oxidases, xanthine oxidase, lipoxygenases, mitochondrial respiration, uncoupled NOS, and MPO. MPO is a highly cationic protein and can bind to endothelial cells, leukocytes, and LDL. The association of MPO with LDL may enhance oxidation of this lipoprotein (26). MPO generates a number of reactive species, including hypochlorous acid, chloramines, tyrosyl radicals, and nitrogen dioxide, that oxidize the protein, lipid, and antioxidant constituents of LDL (27). Many of the primary oxidation products are unstable and serve as reactive intermediates that promote further oxidative modifications of LDL and may also lead to cross-linking and aggregation. However, a limited number of stable oxidation products have been identified that may serve as biomarkers of MPO-catalyzed oxidation. The modified tyrosine residues 3-nitrotyrosine and 3-chlorotyrosine are among the best characterized of these stable oxidation products (28). Although MPO-generated reactive nitrogen species are involved in the conversion of tyrosine into 3-nitrotyrosine, other mechanisms may contribute to the formation of 3-nitrotyrosine as well. Notably, peroxynitrite, the reaction product of nitric oxide and superoxide, is also involved in the generation of 3-nitrotyrosine. In contrast to 3-nitrotyrosine, 3-chlorotyrosine is uniquely produced by MPO, and may therefore serve as a unique molecular fingerprint for MPO-catalyzed oxidation. Hazen and Heinecke reported that 3-chlorotyrosine was 6-fold higher in human advanced atherosclerotic lesions compared with normal aortic tissue. Moreover, the concentration of 3-chlorotyrosine in LDL isolated from atherosclerotic intima was 30-fold higher than in circulating LDL (29). Mocatta et al. (12) measured chlorotyrosine in total plasma protein, however, and found no differences between groups that varied by 5-fold in mean MPO con-
The vascular intima, resulting in conversion of LDL into proatherogenic properties of LDL. MPO-catalyzed carbamylation might enhance the antiinflammatory and antioxidative properties and reverse-cholesterol transport, HDL also possesses.

**IMPAIRMENT OF HDL FUNCTION BY MPO**

In addition to playing a central role in cholesterol efflux and reverse-cholesterol transport, HDL also possesses antiinflammatory and antioxidative properties. Although the exact mechanisms are not fully understood, some of the apolipoproteins and enzymes associated with HDL particles have antioxidative capacities. In this respect the protective role of HDL in MPO-mediated LDL oxidation is important. Mechanisms by which HDL can prevent or delay oxidation in the vessel wall include binding of transition metal ions and removal of oxidized (phospho)lipids and short-chain aldehydes from cells and LDL. After uptake by HDL, these oxidation products are either hydrolyzed by HDL-associated enzymes, such as platelet-activating factor acetylhydrolase and paraoxonase, or remain associated with HDL and are eventually eliminated from the circulation after hepatic uptake of HDL. The antiinflammatory activity of HDL is probably closely linked to its antioxidative activity, because many oxidized lipids possess potent proinflammatory properties and can trigger arterial inflammation. HDL particles decrease expression of adhesion molecules on endothelial cells and inhibit adhesion of monocytes to these cells and entry of inflammatory cells into the intima.

In metabolic diseases associated with accelerated atherosclerosis, HDL particles may become functionally defective. Dysfunctional HDL particles lack atheroprotective properties and promote proinflammatory effects. Over the past few years it has become clear that MPO is involved in rendering HDL dysfunctional. Apolipoprotein A-I (apoA-I) is a preferred target for MPO-catalyzed oxidation, as evidenced by an approximately 100-fold enrichment of both 3-nitrotyrosine and 3-chlorotyrosine in apoA-I recovered from circulatory HDL compared to other proteins in the circulation.

**MPO REDUCES THE BIOAVAILABILITY OF NITRIC OXIDE**

NO produced by endothelial NOS is a powerful vasodilator and as such plays a critical role in the regulation of vascular tone. Additionally, NO suppresses binding of circulating cells to the endothelium and inhibits proliferation of smooth muscle cells in the vascular wall. NO is a critical element in vascular homeostasis, and consequently insufficient production and/or increased scavenging of NO may impair vascular function and accelerate atherosclerosis. There are strong indications that MPO, by several mechanisms, may reduce the bioavailability of NO. First, NO serves as a substrate for peroxidases and MPO may thus serve as a catalytic sink for NO. Second, scavenging of NO by MPO-derived reactive substances may further reduce the bioavailability of NO. Third, hypochlorous acid can react with nitrogen atoms of the NOS substrate arginine to produce chlorinated arginine species that are inhibitors of all isoforms of NOS and have been shown to impair endothelium-dependent relaxation of rat aortic rings. Finally, it has been demonstrated that hypochlorous acid is a potent inducer of uncoupling of endothelial NOS, thereby turning NOS into a superoxide-producing enzyme. Although the relative impact of these mechanisms is currently unknown, it is clear that MPO, by catalytic as well as noncatalytic processes, depletes NO in the vascular wall. In agreement with this notion was the observation, in hu-
man study participants, of a strong inverse association between MPO serum concentrations and brachial artery flow-mediated dilation, which remained significant after adjustment for classic CVD risk factors and CRP (41). In a study by Baldus et al., release of vascular MPO from the subendothelial space by intravenous administration of heparin resulted in an improvement of endothelium-dependent vascular function, reflected by increases of brachial flow-mediated dilation and acetylcholine-induced forearm blood flow (42).

MPO AND PLAQUE VULNERABILITY
Plaque destabilization and rupture are thought to be essential processes in inducing acute cardiovascular events. For example Rossi et al. found a higher incidence of unstable plaques in patients with AMI (20). MPO may play a role in plaque destabilization by activating metalloproteinases, thereby weakening the fibrous cap. This notion is supported by several studies that showed a positive association of MPO and CVD mortality in patients with acute cardiovascular events, whereas 2 studies that included patients with stable CAD failed to show a predictive value of MPO for CVD (17, 19). Additional evidence for a role of MPO in plaque destabilization is obtained from pathophysiologic studies. Plaque injury activates neutrophils, which may lead to MPO release (43). Malle et al. observed colocalization of MPO and hypochlorite-modified proteins in human atherosclerotic lesions (44). Sugiyama et al. reported that, in contrast to macrophages in fatty streaks that contain little or no MPO, macrophages in eroded or ruptured plaques are rich in MPO (45). Consistent with this observation, these investigators found a higher concentration of proteins modified by hypochlorous acid in eroded and ruptured plaques compared to stable plaques. Taken together, existing data provide strong evidence that MPO may be involved in turning late-stage atherosclerosis into acute cardiovascular events.

Laboratory Analyses of MPO: Methodology and Pitfalls
Differences in study populations or analytical procedures may influence the estimated predictive value of MPO in CVD risk stratification. Most results from the above-mentioned studies are based on measurement of MPO mass. Although a high correlation between MPO mass and MPO activity \( r = 0.95 \) has been reported (15), it cannot be completely ruled out that measurement of MPO activity instead of concentration would have influenced the results. As a matter of fact, the preferred assay procedure (mass or activity) is currently not known. It should be noted, however, that ex vivo measurement of MPO activity does not necessarily reflect the in vivo activity of the enzyme. Enzymatic activity of MPO strongly depends on the local concentration of the cosubstrate hydrogen peroxide, which is relatively high in the microenvironment of the subendothelial space, especially in individuals with risk factors associated with increased oxidative stress, such as smoking, hypertension, and type 2 diabetes. Laboratory measurement of MPO activity is usually performed in the presence of saturating amounts of substrate and hydrogen peroxide and therefore represents maximal enzyme activity that can most likely be regarded as a proxy for enzyme mass.

The type of analytical specimen also differs between studies; whole blood, leukocytes, and plasma have all been used. Additionally, measurement of MPO in plasma has been performed after an intravenous bolus injection of heparin, which mobilizes MPO from vascular compartments and therefore yields higher values compared to baseline plasma concentrations, especially in patients with CAD (42). Heparin may induce MPO release in white blood cells (46), and neutrophils are activated during coagulation of blood (47). The effect of collection tubes on MPO concentrations has recently been studied in patients presenting with symptoms of ACS (48). MPO concentrations were higher in serum and samples collected in heparin tubes than in samples collected in EDTA or citrate tubes. Moreover, samples collected into EDTA tubes stored on ice or at room temperature were the most stable. Plasma MPO concentrations remained relatively stable in heparin samples stored on ice, but storage at room temperature for 2 hours led to a 4-fold increase in MPO concentrations. From these results, Shih and coworkers concluded that EDTA should be the preferred anticoagulant for samples collected for MPO determination.

An additional issue that hampers comparison of reported MPO concentrations is the fact that some authors report MPO concentrations on a molar or weight basis, whereas others normalize to total protein by reporting MPO/protein ratios.

Clearly, reported measures of MPO are not directly comparable and are dependent on the type of specimen used. In fact, quite a few reports do not include information on the anticoagulant used to obtain plasma. Thus standardization of method protocols and blood collection tube type is urgently required.

Concluding Remarks
Epidemiological studies in a wide range of patient populations clearly indicate that MPO, in addition to traditional markers, is an important CVD risk marker, especially in patients with unstable CAD. The causative role of MPO in initiating CVD and acute cardiovascular events is supported by in vitro experiments and pathophysiologic observations, indicating that MPO is involved in all
stages of atherogenesis from endothelial dysfunction to plaque rupture. Before MPO can be used routinely in clinical practice for CVD risk stratification, however, a better understanding of and recommendations for pre-analytical and analytical procedures are important.

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