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Association of myeloperoxidase with total and cardiovascular mortality in individuals undergoing coronary angiography—The LURIC study

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Abstract

Background

The phagocytic enzyme myeloperoxidase (MPO) acts as a front-line defender against microorganisms. However, increased MPO levels have been found to be associated with complex and calcified atherosclerotic lesions and incident cardiovascular disease. Therefore, this study aimed to investigate a predictive role of MPO, a biomarker of inflammation and oxidative stress, for total and cardiovascular mortality in patients referred to coronary angiography.

Methods and results

MPO plasma concentrations along with eight MPO polymorphisms were determined in 3036 participants of the Ludwigshafen Risk and Cardiovascular Health study (median follow-up 7.75 years). MPO concentrations were positively associated with age, diabetes, smoking, markers of systemic inflammation (interleukin-6, fibrinogen, C-reactive protein, serum amyloid A) and vascular damage (vascular cellular adhesion molecule-1 and intercellular adhesion molecule-1) but negatively associated with HDL-cholesterol and apolipoprotein A-I. After adjustment for cardiovascular risk factors MPO concentrations in the highest versus the lowest quartile were associated with a 1.34-fold risk (95% CI: 1.09–1.67) for total mortality. In the adjusted model the hazard ratio for cardiovascular mortality in the highest MPO quartile was 1.42 (95% CI: 1.07–1.88). Five MPO polymorphisms were positively associated with MPO concentrations but not with mortality. Using Mendelian randomization, we did not obtain evidence for a causal association of MPO with either total or cardiovascular mortality.

Conclusions

MPO concentrations but not genetic variants at the *MPO* locus are independently associated with risk for total and cardiovascular mortality in coronary artery disease patients.

Keywords: Myeloperoxidase, Inflammation, High-density lipoprotein, Cardiovascular mortality, Risk factor

1. Introduction

Myeloperoxidase (MPO), a member of the heme peroxidase family, is abundantly expressed in neutrophils and monocytes at sites of inflammation [1]. Under physiological conditions MPO reacts with halides, thiocyanate and nitrite and the corresponding MPO-derived oxidation products play an important role in phagocyte's antimicrobial armoury by killing invading pathogens thereby contributing to

host defence [1,2]. However, persistent activation of MPO results in elevated levels of reactive chlorine species and MPO-derived oxidants have been linked with neurodegenerative disorders, carcinogenesis, lung disease and respiratory damage, rheumatoid arthritis, kidney damage and atherosclerosis [1,3–6].

An association between MPO levels and the risk of coronary artery disease (CAD) has first been reported in 2001 [7]. Since then, numerous studies have addressed the role of MPO as a circulating inflammatory marker in chronic heart failure [8,9], acute coronary syndrome (ACS) [10–12], and CAD [13]. Besides various prospective and cross-sectional studies examining the relationship between MPO and the presence of atherosclerosis or the risk of future CAD, evidence came up that a certain MPO polymorphism in the promoter region (− 463G/A) might be associated with CAD [14]. Furthermore, this polymorphism was assumed to determine MPO levels and to be associated with plasma levels of common risk factors for atherosclerosis and even the progression of atherosclerosis but also to predict a likely risk for cancer [15].

Several studies have investigated the predictive value of MPO for short-term recurrence of cardiovascular events, while only limited studies have concentrated on MPO as a marker for long-term cardiovascular risk. Therefore, the present study aimed at investigating (i) whether MPO concentration is an independent predictor of total and cardiovascular mortality in patients undergoing coronary angiography, (ii) the relationship between genetic polymorphisms of MPO and plasma MPO levels, and (iii) the association of these MPO polymorphisms with total and/or cardiovascular mortality.

2. Materials and methods

2.1. Study design and participants

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study includes 3316 consecutive white Caucasian patients hospitalized for coronary angiography between June 1997 and May 2001 [16]. The Ethics Review Committee at the “Landesärztekammer Rheinland-Pfalz” (Mainz, Germany) approved the study. Informed written consent was obtained from each participant. Clinical indications for angiography were chest pain or non-invasive tests consistent with myocardial ischaemia. With the exception of ACSs, the patients had to present in a stable clinical condition without major concomitant non-cardiovascular disease.

CAD has been defined angiographically using the maximum luminal narrowing estimated by visual analysis. We defined clinically relevant CAD using as a criterion the occurrence of at least one stenosis of ≥ 20% as pre-specified in the study protocol [16] in at least one of 15 coronary arterial segments according to Austen et al. [17]. Individuals with stenoses of less than 20% were considered not having CAD. Of the 3036 individuals 645 (21.3%) had no angiographic CAD. Out of 2391 CAD patients 479 (20%) had their diagnosis

made seven days or less before the day of blood sampling. Alternatively we defined CAD as at least one stenosis of ≥ 50% narrowing (see: Supplementary Tables IIIA and IIIB). The severity of CAD was assessed using the Friesinger score [18].

Diabetes mellitus was diagnosed when (i) plasma glucose was > 1.25 g/L in the fasting state or > 2.00 g/L 2 h after the oral glucose load, respectively, or (ii) when HbA1c was ≥ 6.5% or (iii) when individuals were receiving oral anti-diabetics or insulin. Hypertension was diagnosed (i) if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mm Hg or (ii) if individuals were on anti-hypertensive medication: angiotensin-converting enzyme (ACE)-inhibitors, angiotensin-II type-1 (AT1) receptor antagonists, beta-blockers, calcium channel blockers, and diuretics. The estimated glomerular filtration rate (eGFR) was calculated using the chronic kidney disease epidemiologic collaboration (CKD-EPI) formula [19]. Left ventricular function had been graded semi-quantitatively by contrast ventriculography into normal or slightly, moderately, or severely impaired [16].

The primary and secondary outcome measures were total and cardiovascular mortality, respectively. Information on vital status was obtained from local person registries. For deceased patients, information on the cause of death was extracted from death certificates obtained from local health authorities. No patients were lost to follow-up. Of the 3036 subjects included in this examination, 708 deaths (23.3%) had occurred during a median observation time of 7.75 years. Cardiovascular death included the following categories: sudden death, fatal myocardial infarction (MI), death due to congestive heart failure, death immediately after intervention to treat CAD, fatal stroke, and other causes of death due to CAD. Cause of death of 23 participants was unknown. These individuals were included in the calculation of total mortality but not in the analysis of cardiovascular mortality.

2.2. Laboratory measurements

Fasting blood samples were drawn by venipuncture before coronary angiography according to a standardized protocol. Centrifugation (2600 *g*, 4 °C, 15 min) was performed within 15–20 min of blood collection. Whole blood, plasma, and DNA were stored at − 80 °C. The standard laboratory methods used were described elsewhere [16]. Lipoproteins were separated by a combined ultracentrifugation–precipitation method. C-reactive protein (CRP, N high sensitivity CRP) and serum amyloid A (SAA, N Latex SAA) were measured by immunonephelometry (Behring Nephelometer II, Dade Behring, Marburg, Germany). Fibrinogen was determined using the Clauss method (STA fibrinogen, Roche, Mannheim, Germany). Plasma MPO levels were determined using a commercially available sandwich ELISA assay with two polyclonal anti-MPO antibodies (Immundiagnostik AG, Bensheim, Germany). Intra-assay and inter-assay variations were 3.9% and 8.3%, respectively; the limit of detection was 1.6 ng/mL. ELISA assays (R&D Systems Inc., Minneapolis, MN) were used to measure plasma levels of interleukin-6 (IL-6), vascular cellular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured by an electrochemoluminescence

enzyme immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany). During the recruitment period troponin T (TnT) was determined by ECLIA (Roche Diagnostics, Mannheim, Germany) using a cut-off at 0.1 μg/L; post hoc, we used a fourth generation ECLIA from Roche Diagnostics with high sensitivity.

2.3. Analysis of MPO polymorphisms

Genomic DNA was isolated from whole blood by standard methods [16]. MPO genotypes were determined by 5′ exonuclease assays (TaqMan). Applied Biosystems 'Assay-by-Design' custom service (Applied Biosystems, Vienna, Austria) was used for the design and manufacture of primer and probe sets. Sequences of primers and probes are presented in Table I (Supplementary). Endpoint fluorescence data were exported into Excel format and analysed as scatter plot. Samples were analysed in batches, each containing 95 samples and a negative control (water instead of DNA).

2.4. Statistical analysis

Normal distribution was assessed using QQ-Plot and Shapiro–Wilk test. Parameters not normally distributed were transformed logarithmically for statistical analyses. We established quartiles of continuous variables according to the values in individuals without angiographic CAD. Associations of categorical and continuous variables were analysed by logistic regression and univariate analysis of variance (ANOVA), respectively, with co-variables. We studied the effects of gender, age, CAD, cardiovascular risk factors, and inflammatory markers on MPO using ANOVA models in which we included those factors not under examination as co-variables. To assess the predictive value of models with and without MPO for cardiovascular and total mortality we used reclassification analysis including receiver operating characteristic (ROC) curves, net reclassification improvement, and integrated discrimination index. The association of MPO polymorphisms and MPO plasma concentrations was analysed using ANOVA models assuming a co-dominant (homozygosity for the major MPO allele, heterozygosity and homozygosity for the minor allele) or a dominant (homozygosity for the major MPO allele and presence of at least one minor allele) effect of the minor allele. We undertook genetic instrumental variable analysis using a combined variable of two single nucleotide polymorphisms (SNPs) (c.− 822C > A, rs2243827; g.5237G > A, rs11575868; category 0 [no minor allele], category 1 [heterozygosity or homozygosity for rs2243827 or rs11575868], category 2 [heterozygosity or homozygosity for rs2243827 and rs11575868]). Cox proportional hazard models were used to examine the association of the instrumental variable with total and/or cardiovascular mortality. To examine the relationship of MPO levels on total and cardiovascular mortality, hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox proportional hazard models according to quartiles of MPO. Multivariable adjustment was carried out for age, sex, intake of lipid lowering drugs, CAD status, and risk factors as indicated. All statistical tests are 2-sided; $P < 0.05$ is considered significant. We used the SPSS 17.0 statistical package

(SPSS Inc., Chicago, IL), STATA 11 (StataCorp LP 4905 Lakeway Drive, College Station, Texas 77845-4512, USA), and the R version 2.15.2 (R Foundation for Statistical Computing).

3. Results

3.1. Study participants

Compared with non-CAD patients, patients with angiographically confirmed CAD were significantly older and more likely to be male (Table 1). Current or past smoking, type 2 diabetes, hypertension, cardiovascular medication, impaired left ventricular function, and cerebrovascular and peripheral artery disease were more prevalent in the CAD group. Fifty-three percent of the CAD patients had a history of MI. The CAD patients had significantly higher systolic blood pressure, fasting glucose, triglycerides, and significantly lower HDL-cholesterol (HDL-C) and eGFR compared to patients without CAD. Low-density lipoprotein-cholesterol (LDL-C) was slightly lower in CAD patients than in patients without CAD, even after adjustment for the use of lipid-lowering drugs. Body mass index, diastolic blood pressure, and MPO concentration were similar in both groups (Table 1).

3.2. Association of MPO with cardiovascular risk factors and CAD status

We examined the effect of gender, age, angiographic CAD, and risk factors on MPO levels in a general linear model where we included those factors not under examination as co-variables (Table 2). MPO concentrations were independent of gender but significantly increased with age. The concentration of MPO was not associated with the severity of atherosclerosis (Friesinger coronary score, Table 2) [18]. Significantly higher MPO concentrations were found in patients with diabetes mellitus ($P = 0.010$) but not in patients with the metabolic syndrome without diabetes mellitus. Current smokers had significantly higher MPO levels compared to non-smokers ($P = 0.015$). MPO levels decreased with increasing quartiles of HDL-C and apolipoprotein A-I, with 13.3% ($P = 0.010$) and 12.0% ($P = 0.048$) lower mean MPO concentrations, respectively, in the fourth compared to the first quartile. Hypertension, body mass index, LDL-C, apolipoprotein B and triglycerides were not significantly associated with MPO levels. However, patients on lipid lowering drug therapy (primarily statins) had on average 8.1% ($P = 0.018$) lower MPO concentrations compared to untreated subjects. Multiple linear regression with forward selection of the independent variables listed in Table 2 selected five significant predictors of MPO concentration in the order of HDL-C > age > use of lipid-lowering drugs > smoking status > diabetes mellitus.

We were interested to see if MPO concentrations were increased in ACS. We stratified our study population into persons without CAD, stable CAD, unstable CAD, those presenting with acute non-ST elevation myocardial infarction (NSTEMI) or STEMI (less than 7 days after onset of symptoms), STEMI between 7 and 15 days after onset of symptoms or STEMI more than 15 days ago. As expected TnT

(Supplementary Fig. I) was markedly elevated in acute NSTEMI or STEMI, while MPO levels were only slightly increased (Supplementary Fig. II).

3.3. Association of MPO with inflammatory markers

We then examined whether MPO concentrations were associated with markers of acute/chronic inflammation. Levels of the inflammatory marker IL-6, as well as levels of major acute-phase reactants (CRP, SAA, fibrinogen) increased in parallel to MPO (Fig. 1). This relationship was independent of conventional coronary risk factors. Furthermore, MPO levels were significantly associated with circulating levels of VCAM-1 and ICAM-1, accepted markers of vascular damage.

3.4. Association of MPO polymorphisms with MPO plasma concentration

Next, we investigated the association of SNPs in the MPO gene [20] with plasma MPO concentrations. The genotype distributions did not differ significantly from Hardy–Weinberg equilibrium. The minor allele frequencies of the MPO SNPs ranged from 0.03 to 0.21 (Table 3A). Assuming a dominant model, statistically significant associations with MPO levels were found for five SNPs. Three polymorphisms (c.− 822C > A, c.− 765T > C, c.− 653G > A [previously termed − 463G/A]) are located in the 5′ region and may alter MPO expression. For these SNPs the presence of at least one minor allele was associated with an increase of MPO concentrations by 13.3, 10.0, and 10.0%, respectively. Homozygosity of the minor allele in these SNPs did not further increase MPO concentration (co-dominant model, Table 3B). The c.2149T > C SNP (Ile717Val) was associated with an increase of MPO levels by 18.9% (Table 3A).

We constructed an instrumental variable based on genotypes from two SNPs that have shown to be related with MPO plasma concentration (c.− 822C > A (rs2243827) and g.5237G > A (rs11575868)). MPO concentrations were significantly different between genotypes with higher concentration in the category 1 (Supplementary Table II) compared to the categories 0 (no minor allele) and 2 (two minor alleles). Thus, we fitted the effect by a regression model that pools categories 0 and 2. The mean difference between two genotype categories (0/2 versus 1) was 26.2 ng/mL. The variance of the MPO concentration explained by the instrumental variable was 0.43% (R-square).

3.5. MPO and mortality from all causes

Among the 3036 individuals studied, 708 deaths (23.3%) occurred during a median follow-up of 7.75 years. Compared with individuals in the lowest MPO quartile, the unadjusted HRs for death in the second to the fourth MPO quartile were 1.28 (95% CI: 1.02–1.60, $P = 0.033$), 1.27 (95% CI: 1.01–1.60, $P = 0.038$), and 1.76 (95% CI: 1.42–2.17, $P < 0.001$), respectively (Table 4 [Model 1], Fig. 2).

Inclusion of age and gender as co-variables only slightly altered these estimates (Table 4 [Model 2]). Although HRs decreased after additional adjustment for the CAD status at presentation (no CAD, stable CAD, or unstable CAD) and established cardiovascular risk factors, MPO retained its prognostic importance in the fourth quartile (Table 4 [Model 3]).

Among the 2391 subjects with angiographic CAD, 627 participants (26.2%) died during follow-up. In this subgroup, HRs for death were similar to those obtained in the entire study population (Table 4 [Models 1–3], Fig. 2). Similar results were obtained in a subgroup of patients with CAD defined ≥ 50% of stenosis (Supplementary Table IIIA). In patients without CAD (n = 645, 81 deaths), MPO levels were not significantly associated with total mortality (Table 4 [Models 2–3]). Considering the subgroup of individuals without CAD or with stable CAD (n = 2083, 486 deaths), HRs were slightly higher compared to the entire study population (Supplementary Table IIIA).

3.6. MPO and cardiovascular mortality

As death certificates were not available from 23 patients, the analysis for cardiovascular mortality included 3013 individuals in total. Among these, 442 (14.6%) died from cardiovascular causes, 55 (1.8%) of infection, 92 (3.1%) of cancer, and 96 (3.2%) died of other causes. HRs for death from cardiovascular causes according to MPO levels were slightly higher compared to those obtained for mortality from all causes in the entire study population and in the CAD subgroup, respectively (Table 5 [Models 1–3], Fig. 2). In the highest MPO quartile unadjusted (Model 1) and fully adjusted HRs (Model 3) for the entire study population were 1.90 (95% CI: 1.45–2.48, $P < 0.001$) and 1.42 (95% CI: 1.07–1.88, $P = 0.010$), respectively. For CAD patients HRs in the fourth MPO quartile were 1.86 (1.40–2.45, $P < 0.001$, unadjusted model) or 1.48 (1.10–1.98, $P = 0.009$, fully adjusted model) (Table 5). In patients without CAD (n = 645, 43 cardiovascular deaths), MPO levels were not significantly associated with cardiovascular mortality (Table 5 [Models 1–3]). Comparable results were obtained in a subgroup of patients with CAD defined ≥ 50% of stenosis (Supplementary Table IIIB). Statistical analysis in the subgroup of individuals without CAD or with stable CAD (n = 2068, 311 cardiovascular deaths) revealed higher HRs compared to the entire study population (Supplementary Table IIIB). Both, HRs for total and cardiovascular mortality were only slightly attenuated by additional adjustment for TnT (Supplementary Table IV).

3.7. MPO, HDL and mortality

Next, a possible effect of MPO levels on the association of HDL-C and mortality was investigated. In the subgroup with low MPO levels, increasing concentrations of HDL-C were significantly associated with reduced risk for mortality (Supplementary Table V). In the subgroup with high MPO levels, the association of HDL-C with total and cardiovascular mortality was attenuated indicating a weaker protective effect of HDL. However, Cox proportional hazard model allowing for the interaction of MPO and HDL-C revealed that the interaction was not significant. Similar results were obtained when quartiles of apolipoprotein A-I were analysed in the two

subgroups (Supplementary Table V).

3.8. Reclassification analysis

We used ROC and reclassification analyses to assess the predictive value of MPO for total and cardiovascular mortality. The addition of MPO to a model consisting of established cardiovascular risk factors (age, sex, body mass index, hypertension, HDL-C, LDL-C, triglycerides, smoking, CAD, and diabetes mellitus) did not significantly increase the area under the ROC curve for total mortality (0.733 vs. 0.736) and cardiovascular mortality (0.714 vs. 0.716, respectively, $P = 0.093$, Supplementary Fig. III). However, reclassification analysis revealed improvement on addition of MPO. The net reclassification improvement for total mortality was 9.2% (95% CI: 7.4 to 17.6, $P = 0.033$), the integrated discrimination improvement was 0.0032 (95% CI: 0.0008 to 0.0057, $P = 0.011$). Similar results were obtained for cardiovascular death: net reclassification improvement was 10.1% (95% CI: 0.2 to 20.2, $P = 0.049$), integrated discrimination improvement was 0.0035 (95% CI: 0.0004 to 0.0065, $P = 0.028$).

3.9. MPO genotype and mortality

We analysed the association of the instrumental variable with total and cardiovascular mortality using Cox proportional hazard models with adjustment for established cardiovascular risk factors. HRs per 1 SD in the genotype category 1 was 1.01 (95% CI: 0.89–1.15, $P = 0.865$) compared to category 0/2 for death from all causes and 0.98 (95% CI: 0.83–1.16, $P = 0.841$) for death from cardiovascular causes, respectively.

To estimate the causal effect of MPO concentrations on total and cardiovascular mortality we calculated the causative HR based on the HR for total and cardiovascular mortality and on the mean difference of MPO concentration between the genotype categories. The causative HRs per 1 SD MPO were 1.07 (95% CI: 0.18–1.95) for total mortality and 0.86 (95% CI: 0.06–2.03) for cardiovascular mortality, respectively.

4. Discussion

This is the first study investigating associations between various MPO polymorphisms, MPO concentrations, and mortality in patients who underwent coronary angiography. High levels of MPO predicted total and cardiovascular mortality independent of established cardiovascular risk factors. The higher HRs for cardiovascular mortality compared to total mortality suggested that MPO is involved in the pathogenesis of cardiovascular diseases. We calculated higher HRs for total and cardiovascular mortality in a subgroup of patients without ACS compared to the entire study population suggesting that MPO is predictive for long-term outcome and that inclusion of

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ACS patients did not affect the association of MPO with mortality. Inclusion of MPO concentration in prognostic models for total and cardiovascular mortality slightly improved risk assessment. Finally, five out of eight MPO polymorphisms were associated with MPO levels but no significant association of MPO genotype with total or cardiovascular mortality was found.

In a prospective case–control study in apparently healthy individuals, MPO predicted future risk of CAD independent of other cardiovascular risk factors [21]. MPO concentrations were found to be elevated in patients with stable CAD [7], ACS [10,22], and acute MI [23,24]. MPO concentrations were further associated with the severity of CAD [11] and with complex lesion morphology on angiography [25]. Furthermore, MPO–DNA complexes were positively associated with thrombin generation and significantly elevated in patients with severe coronary atherosclerosis or extremely calcified coronary arteries [26]. However, in the present study MPO levels did not differ between CAD and non-CAD patients and levels of MPO were not associated with the severity of CAD as determined by the Friesinger coronary score [18].

Several studies addressed the prognostic value of MPO in patients with ACS [10,12,22,27–29]. In individuals with chest pain MPO concentrations at presentation of patients were predictive of major adverse coronary events after 30 days and 6 months [10,22]. In another study [29] the authors reported that MPO levels predicted short-term risk of major adverse coronary events even in patients with normal cardiac TnT levels.

Only a few studies investigated the association of MPO concentrations with long-term risk for major adverse coronary events. MPO concentrations were found to be elevated one to four days after acute MI and were predictive of mortality over a 5-year follow-up independent of other risk markers [23]. In one study with a follow-up of 3 years (1895 patients with stable CAD), MPO concentrations were associated with a 1.71-fold risk for major adverse coronary events [30]. Another group [31] failed to reveal an association of MPO levels with mortality in 382 patients with stable CAD. Heslop and coworkers [32] were the first to report an association of MPO levels with cardiovascular mortality (885 coronary angiography patients) with adjusted HRs slightly higher (2.06 vs. 1.48) than observed in the present study. The percentage of patients with severe CAD (stenosis ≥ 50%) was slightly higher as in the present study (93 vs. 87%). We observed even lower HRs in a subgroup of individuals with CAD (stenosis ≥ 50%). This could be due to a longer follow-up time period in the study by Heslop and coworkers [32] (13.5 vs. 7.5 years). However, the number of cases in the LURIC cohort (n = 3036) was considerably higher compared to all published studies.

Previous studies suggested a causal role of MPO in the development of endothelial dysfunction [33] by directly promoting catalytic consumption of nitric oxide [34,35]. We here found strong correlations between the levels of MPO and circulating adhesion molecules VCAM-1 and ICAM-1, which is indicative for increased endothelial activation in subjects with high MPO levels. Furthermore, we observed associations of MPO concentration with markers of systemic inflammation, i.e. IL-6, CRP, SAA, and fibrinogen, respectively.

This result is in line with previous studies reporting correlations of MPO with CRP and/or fibrinogen [33,36]. The association of MPO levels with inflammatory markers may be due to a reverse causation. Cytokines released from macrophages in atherosclerotic plaques trigger the synthesis of pro-inflammatory proteins and may favour recruitment and activation of MPO-containing leukocytes.

To elucidate a potential causal relationship between MPO and mortality, a Mendelian randomization approach was applied. We identified a genetic instrumental variable based on two SNPs that were associated with MPO concentrations. The genetic variable was not significantly associated with total and cardiovascular mortality. The effects of the genetic variable on HRs for both endpoints were weaker than predicted from the associations of the genetic variable with MPO levels and the MPO levels with mortality. Thus, the Mendelian randomization analysis provided no evidence for a causal role of MPO in the pathogenesis of CAD. The high concentrations of circulating MPO could be more likely a marker of the atherosclerotic burden than the primary initiator of atherosclerosis. However, a limitation of this analysis might be that the instrument variable explained only a small proportion of variance of the MPO concentration.

Lipid lowering drugs have recently been reported to down-regulate MPO-specific oxidation products [37] as well as systemic MPO levels in patients with ACS [38], findings that have been confirmed in patients undergoing coronary angiography (Table 2). Natural or synthetic statins strongly suppress MPO on mRNA level [39]. These authors [39] suggested that the suppression is mediated by a promoter element which encodes for the − 463G/A (c.− 653G>A according to the current manuscript) polymorphism upstream of human MPO. This is in line with our finding that statin treatment lowers plasma MPO concentrations.

Several studies addressed the association of MPO polymorphisms, in particular − 463G/A, with MPO levels [40–44]. In apparently healthy individuals, the A allele did not alter MPO concentrations [40]. However, it seemed to protect from symptomatic and angiographically proven CAD [41,45]. Two studies reported that the A allele was associated with a lower risk for cardiovascular events [42,45]. In all studies addressing the relationship between − 463G/A MPO polymorphism, MPO levels and cardiovascular events, the low number of patients participating is a limiting factor. This is the first report studying 8 different MPO polymorphisms (so far only followed in a cohort of 638 individuals from the Quebec Family study [20]) in a group of patients with different risks for cardiovascular disease and/or atherosclerosis including CAD. We demonstrate that the minor allele of five MPO polymorphisms (four polymorphisms are located in the non-coding region) increases plasma MPO levels, but has no effect on the prevalence of CAD as well as on total and cardiovascular mortality.

Recent progress in the therapeutic management has improved the survival rates of patients with acute coronary syndromes, once they reach in-hospital acute care units [46,47]. Therefore, the prevalence of patients with chronic CAD will increase in the future. While risk scores (e.g. TIMI and GRACE) for predicting short- and mid-term prognosis have been suggested, few such instruments exist for assessing long term prognosis [48,49], which is commonly considered only in yet asymptomatic persons [50,51]. The examination of

novel biomarkers associated with long-term prognosis of patients referred to coronary angiography — as conducted in the current study — may contribute to close this diagnostic gap.

4.1. Limitations

Several limitations of the present study warrant consideration. (i) The participants are at intermediate or high cardiovascular risk and our results might not be applicable to patients at low risk. (ii) The study population consisted of selectively enrolled middle-aged to elderly Caucasians; therefore the results cannot be generalized to younger individuals or other races or ethnicities. (iii) As coronary angiography was indicated in each study participant a referral bias may occur. The definition of the coronary artery status, however, is at the same time the strength of the present study. The prevalence of clinically asymptomatic coronary atherosclerosis has been reported to be high at 50 years of age or older [52]. Hence, angiography-based recruitment prevents inadvertent allocation of individuals with significant, clinically unapparent CAD to the control group.

5. Conclusions

So far, this study is the largest to investigate the predictive value of MPO for cardiovascular mortality. The results obtained from the genetic analysis of the *MPO* locus do not support a causal role of MPO in the development of cardiovascular disease. The observed association of MPO concentration with total and cardiovascular mortality is therefore likely due to reverse causation. Yet, the determination of the MPO concentration may be a useful adjunct to the assessment of the risk of future cardiovascular events.

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Appendix A. Supplementary data

Supplementary materials.

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Figures and Tables

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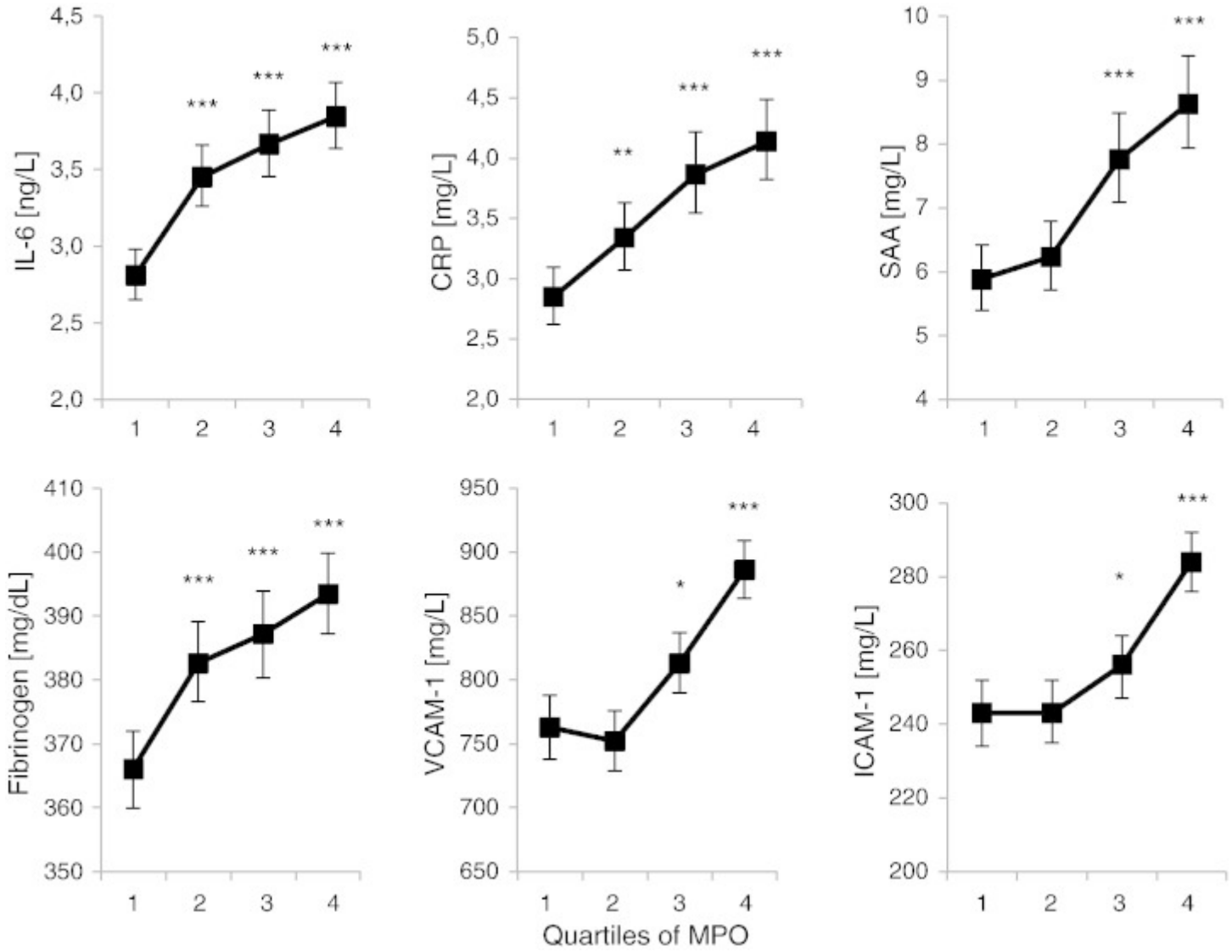
Fig. 1



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Association of MPO quartiles with markers of inflammation and vascular damage. MPO plasma concentrations were stratified into quartiles; values represent estimated marginal means and 95% confidence intervals obtained in a general linear model (ANOVA), adjusted for the use of lipid-lowering drugs and for each of the other factors, whereby age, body mass index, LDL-C, HDL-C, and triglycerides (log transformed), were included as continuous rather than categorial co-variables. Significance is indicated by *($P < 0.05$), **($P < 0.01$), and ***($P < 0.001$) compared to the first MPO quartile. (Interleukin-6, IL-6; C-reactive protein, CRP; serum amyloid A, SAA; vascular cellular adhesion molecule-1, VCAM-1; intercellular adhesion molecule-1, ICAM-1).

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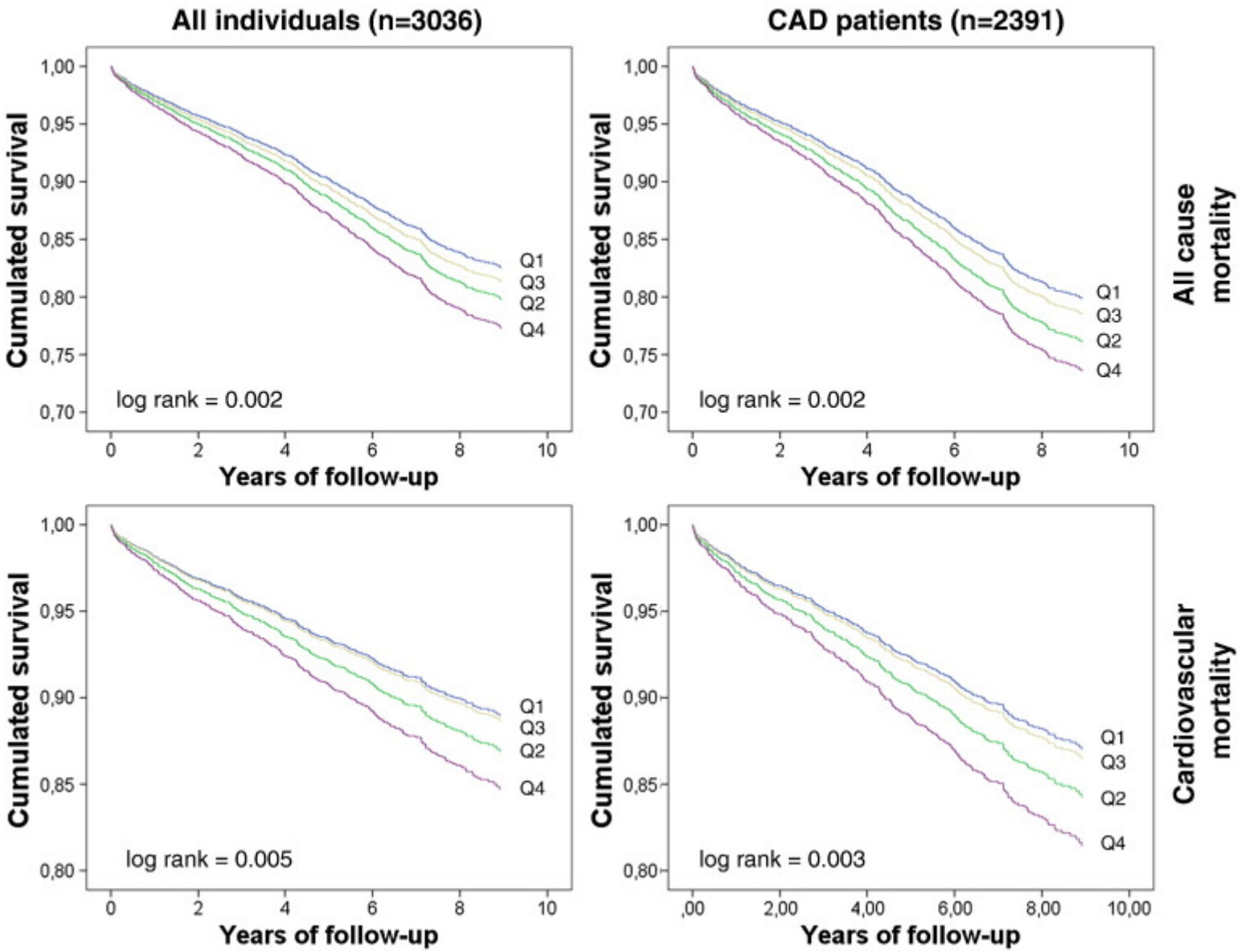
Fig. 2



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Cumulated survival functions for all cause and cardiovascular mortality. Cumulated survival functions for total (top panels) and cardiovascular mortality (bottom panels) according to quartiles (Q1–Q4) of MPO in all individuals (left panels) or in patients with angiographic CAD (right panels). Curves were estimated using a proportional hazard model adjusted for age, gender and cardiovascular risk factors. For HRs and confidence intervals, see Tables 4 and 5 (Model 3).

Table 1

Clinical and biochemical characteristics of study participants at baseline.

	All individuals (n = 3036)	Without CAD (n = 645)	With CAD (n = 2391)	*P* [a]
Age (years) means ± SD	63 ± 11	58 ± 12	64 ± 10	< 0.001
Male sex (%)	70	52	75	< 0.001[b]
Body mass index (kg/m^2) means ± SD	27 ± 4	27 ± 4	28 ± 4	0.335
Diabetes mellitus (%)	40	27	44	< 0.001
Systemic hypertension (%)	73	63	76	0.001
Smoking				
Never(%)	36	52	32	
Past (%)	44	30	48	< 0.001
Current (%)	20	18	20	< 0.001
Unstable CAD, TnT- (%)	19	–	24	–
Unstable CAD, NSTEMI or STEMI (%)	12	–	16	–
Previous myocardial infarction (%)	42	–	53	–
Peripheral vascular disease (%)	10	2	12	< 0.001
Cerebrovascular disease (%)	8	5	9	0.023
Systolic blood pressure (mm Hg) means ± SD	141 ± 24	136 ± 22	143 ± 24	0.005[c]
Diastolic blood pressure (mm Hg) means ± SD	81 ± 11	80 ± 11	81 ± 12	0.510[c]
Semiquantitative left ventricular function				
Normal (%)	71	79	68	
Slightly impaired (%)	11	6	12	< 0.001
Moderately impaired (%)	12	9	13	0.043
Severely impaired (%)	7	6	7	0.669
Lipid-lowering drugs (%)	51	18	57	< 0.001
Beta-blockers, %	64	69	45	< 0.001

	All individuals (n = 3036)	Without CAD (n = 645)	With CAD (n = 2391)	*P* [a]
ACE inhibitors, %	54	58	36	< 0.001
AT1 receptor antagonists, %	4.6	4.3	4.7	0.809
Calcium channel blockers, %	16	13	17	0.501
Diuretics, %	29	26	30	0.852
Aspirin and/or other antiplatelet agents, %	72	43	80	< 0.001
Fasting blood glucose (mg/L) means ± SD	113 ± 36	105 ± 28	116 ± 37	< 0.001
eGFR, CKD-EPI (mL/min/1.73 m^2) means ± SD	84.6 ± 19.4	89.3 ± 17.3	83.3 ± 19.7	0.030
LDL-C (mg/dL) means ± SD	116 ± 34	119 ± 31	115 ± 34	0.005[d]
HDL-C (mg/dL) means ± SD	39 ± 11	43 ± 12	38 ± 10	< 0.001[d]
Triglycerides (mg/dL)	147	133	150	< 0.001[d, e]
median (25th and 75th percentile)	(110–200)	(97–194)	(113–202)	
NT-proBNP (ng/L)	297	158	336	< 0.001[e]
median (25th and 75th percentile)	(108–874)	(71–498)	(128–964)	
Myeloperoxidase (ng/mL)	30.3	30.3	30.3	0.731[e]
median (25th and 75th percentile)	(20.8–47.1)	(20.7–45.4)	(20.8–47.7)	

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ACE (angiotensin converting enzyme).

AT1 (angiotensin 1).

CKD-EPI (Chronic Kidney Disease Epidemiologic Collaboration).

eGFR (estimated Glomerular Filtration Rate).

NT-proBNP (N-terminal pro-B-type natriuretic peptide).

NSTEMI (non-ST elevation MI).

STEMI (ST elevation MI).

TNT- (Troponin-T not increased).

[a]Analysis of variance (ANOVA) or logistic regression, respectively, adjusted for age and gender; comparison between individuals with and without CAD.

[b]Logistic regression, adjusted for age only.

[c]Adjusted for use of beta blockers, ACE inhibitors, AT1 receptor antagonists, calcium channel blockers, diuretics and lipid-lowering agents.

[d]Adjusted for use of lipid-lowering agents.

[e]ANOVA of logarithmically transformed values.

Table 2

Association of MPO with cardiovascular risk factors and coronary artery disease.

	MPO (ng/mL)[a]	Difference (%)[b]	*P*[c]
Gender			
Men	34.3 (33.0–35.7)		
Women	32.3 (30.4–34.4)	− 5.7	0.132
Age (years)			
< 60	31.9 (30.1–33.9)		
60–70	34.1 (32.3–36.0)	+ 6.8	0.112
> 70	35.4 (33.1–37.5)	+ 11.0	0.017
Lipid-lowering drugs			
No	35.1 (33.5–36.8)		
Yes	32.3 (30.0–33.9)	− 8.1	0.018
Coronary artery disease			
None	33.5 (31.0–36.2)		
Stable CAD	33.8 (32.2–35.5)	+ 1.0	0.837
Unstable CAD (Troponin T-)	33.6 (31.2–36.2)	+ 0.3	0.969
Unstable CAD (Troponin T +)	33.5 (30.5–36.9)	+ 0.1	0.992
Friesinger score			
1st quartile (0–2)	32.4 (29.5–35.5)		
2nd quartile (3–5)	34.6 (32.3–37.1)	+ 6.9	0.241
3rd quartile (6–8)	33.4 (31.4–35.4)	+ 3.1	0.617
4th quartile (9–15)	34.3 (31.9–36.8)	+ 6.0	0.380
Left ventricular function			
Normal	33.0 (31.7–34.4)		
Slightly impaired	31.6 (28.4–35.1)	− 4.4	0.441

	MPO (ng/mL)[a]	Difference (%)[b]	*P*[c]
Moderately impaired	36.7 (33.3–40.5)	+ 11.2	0.050
Severely impaired	39,2 (34.3–45.1)	+ 19.0	0.017
Body mass index (kg/m^2)			
< 26 or 27[3]	34.1 (32.5–35.8)		
> 26 or 27[3]	33.2 (31.8–34.8)	− 2.6	0.448
Metabolic syndrome/diabetes			
None	31.9 (29.9–34.1)		
Metabolic syndrome	32.6 (30.5–34.7)	+ 2.0	0.699
Diabetes mellitus	36.0 (34.1–37.9)	+ 12.7	0.010
Hypertension			
No	33.5 (31.4–35.8)		
Yes	33.7 (32.4–35.0)	+ 0.5	0.898
Smoking			
Never	32.8 (31.0–34.7)		
Former	33.0 (31.4–34.7)	+ 0.6	0.874
Current	37.2 (34.3–40.2)	+ 13.3	0.015
eGFR (mL/min/1.73 m^2)			
≥ 90	32.3 (30.7–34.0)		
60–89	33.6 (31.9–35.3)	+ 4.0	0.319
30–59	38.5 (34.6–42.8)	+ 14.6	< 0.001
< 30	34.4 (25.4–46.5)	+ 6.5	0.689
NT-proBNP (ng/L)			
1st quartile (< 109)	30.9 (28.8–33.1)		

	MPO (ng/mL)[a]	Difference (%)[b]	*P*[c]
2nd quartile (109–297)	33.1 (31.0–35.4)	+ 7.3	0.144
3rd quartile (298–874)	33.1 (31.0–35.3)	+ 7.1	0.163
4th quartile (> 874)	37.8 (35.3–40.4)	+ 22.3	< 0.001
LDL cholesterol (mg/dL)			
1st quartile (< 101)	33.6 (31.7–35.6)		
2nd quartile (101–119)	33.7 (31.6–36.1)	+ 0.3	0.936
3rd quartile (120–141)	33.6 (31.4–36.0)	+ 0.1	0.978
4th quartile (≥ 142)	33.7 (31.4–36.2)	+ 0.2	0.975
HDL cholesterol (mg/dL)			
1st quartile (< 36)	35.5 (32.6–37.3)		
2nd quartile (36–41)	33.2 (30.9–35.8)	− 6.3	0.191
3rd quartile (42–49)	33.0 (30.8–35.3)	− 7.0	0.103
4th quartile (≥ 50)	30.8 (28.1–33.6)	− 13.3	0.010
Apolipoprotein B (mg/dL)			
1st quartile (< 87)	33.6 (31.3–36.0)		
2nd quartile (87–102)	33.0 (31.0–35.3)	− 1.6	0.744
3rd quartile (103–119)	33.2 (31.2–35.4)	− 1.1	0.830
4th quartile (> 119)	34.9 (32.6–37.3)	+ 3.9	0.467
Apolipoprotein A-I (mg/dL)			
1st quartile (< 123)	32.5 (28.1–37.6)		
2nd quartile (123–137)	32.1 (27.9–36.9)	− 1.3	0.895
3rd quartile (138–157)	30.6 (26.5–35.4)	− 5.8	0.224
4th quartile (> 157)	28.6 (24.8–33.0)	− 12.0	0.048

	MPO (ng/mL)[a]	Difference (%)[b]	*P*[c]
Triglycerides (mg/dL)			
1st quartile (< 98)	33.3 (29.4–37.9)		
2nd quartile (99–132)	31.7 (29.4–34.3)	− 4.9	0.408
3rd quartile (133–194)	34.5 (32.6–36.6)	+ 3.6	0.640
4th quartile (≥ 195)	34.7 (31.0–38.8)	+ 4.1	0.720

[a]Estimated marginal means and 95% confidence intervals obtained in a general linear model (ANOVA), adjusted for the use of lipid-lowering drugs and for each of the other cardiovascular risk factors, whereby age, body mass index, LDL cholesterol, HDL cholesterol, eGFR (CKD-EPI), and triglycerides (log transformed), were included as continuous rather than categorial covariables.

[b]Compared to the first category of each variable.

[c]Thresholds of 26 and 27 kg/m^2 apply to males and females, respectively.

Table 3A

The association of MPO polymorphisms with plasma MPO concentrations (dominant model).

Sequence variation	Position	Patients (n)	MPO[a] (ng/mL)	Difference[b] (%)	*P*	Minor allele frequency
c.− 822C>A (rs2243827)	Non-coding					
CC		2135	32.9 (31.7–34.1)			
CA or AA		901	37.2 (35.2–39.4)	+ 13.3	< 0.00	0.16
c.− 765T>C (rs2243828)	Non-coding					
TT		1914	32.9 (31.7–34.2)			
TC or CC		1122	36.2 (34.3–38.1)	+ 10.0	0.004	0.21
c.-653G>A (rs2333227)	Non-coding					
GG		1942	33.0 (31.7–34.4)			
GA or AA		1094	36.2 (34.4–38.1)	+ 10.0	0.004	0.20
g.287C>T (rs2856857)	Non-coding					
CC		2134	32.9 (31.7–34.1)			
CT or TT		902	37.2 (35.2–39.4)	+ 13.3	< 0.001	0.16
g.5237G>A (rs11575868)	Non-coding					
GG		2593	34.3 (33.2–35.5)			
GA or AA		442	32.7 (30.1–35.4)	− 4.8	0.276	0.08
g.9890A>C (rs2071409)	Non-coding					
AA		2161	34.2 (33.0–35.5)			
AC or CC		875	33.8 (31.9–35.8)	− 1.1	0.761	0.16
c.157G>T (rs7208693)	Val53Phe					
GG		2596	34.3 (33.2–35.5)			
GT or TT		456	32.8 (30.8–35.6)	− 4.6	0.301	0.08
c.2149T>C (rs2759)	Ile717Val					
TT		2855	33.8 (32.7–34.8)			

Sequence variation	Position	Patients (n)	MPO[a] (ng/mL)	Difference[b] (%)	*P*	Minor allele frequency
TC or CC		181	40.1 (35.3–45.6)	+ 18.9	0.009	0.03

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[a]Means and 95% confidence intervals.

[b]Compared to the first category of each variable.

Table 3B

The association of MPO polymorphisms with plasma MPO concentrations (co-dominant model).

Sequence variation	Position	Patients (n)	MPO[a] (ng/mL)	Difference[b] (%)	*P*	Minor allele frequency
c.− 822C>A (rs2243827)	Non-coding					
CC		2135	32.9 (37.7–34.1)			
CA		813	37.3 (35.1–39.6)	+ 13.5	< 0.001	
AA		88	36.6 (30.5–43.9)	+ 11.4	0.255	0.16
c.− 765T>C (rs2243828)	Non-coding					
TT		1914	32.9 (31.7–34.2)			
TC		986	36.2 (34.2–38.2)	+ 9.9	0.006	
CC		136	36.5 (31.5–42.2)	+ 10.9	0.183	0.21
c.− 653G>A (rs2333227)	Non-coding					
GG		1942	33.0 (31.7–34.4)			
GA		962	36.2 (34.2–38.2)	+ 10.0	0.007	
AA		132	36.7 (31.6–42.6)	+ 11.3	0.171	0.20
c.287C>T (rs2856857)	Non-coding					
CC		2134	32.9 (31.7–34.1)			
CT		814	37.3 (35.1–39.6)	+ 13.4	< 0.001	
TT		88	36.6 (30.5–43.9)	+ 11.4	0.255	0.16
g.5237G>A (rs11575868)	Non-coding					
GG		2593	34.3 (33.2–35.5)			
GA		427	32.5 (29.9–35.3)	− 5.3	0.235	
AA		16	37.6 (24.5–57.7)	+ 9.6	0.675	0.08
g.9890A>C (rs2071409)	Non-coding					
AA		2161	34.2 (33.0–35.5)			
AC		796	33.7 (31.8–35.8)	− 1.4	0.708	

Sequence variation	Position	Patients (n)	MPO[a] (ng/mL)	Difference[b] (%)	*P*	Minor allele frequency
CC		79	34.8 (28.7–42.2)	+ 1.9	0.852	0.16
c.157G>T (rs7208693)	Val53Phe					
GG		2596	34.3 (33.2–35.5)			
GT		424	32.4 (29.8–35.2)	− 5.5	0.212	
TT		16	43.3 (38.2–66.4)	+ 26.1	0.289	0.08
c.2149T>C (rs2759)	Ile717Val					
TT		2855	33.8 (32.7–34.8)			
TC		178	40.0 (35.2–45.4)	+ 18.4	0.012	
CC		3	51.4 (19.2–137.7)	+ 52.2	0.401	0.03

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[a]Means and 95% confidence intervals.

[b]Compared to the first category of each variable.

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Table 4

Hazard ratios for death from all causes according to MPO concentrations.

MPO (ng/mL)	Deaths n (%)	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*	Model 3 OR (95% CI)	*P*
All individuals (n = 3036)							
1st quartile (< 21)	136 (18)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (21–30)	170 (23)	1.28 (1.02–1.60)	0.033	1.26 (1.00–1.58)	0.046	1.18 (0.93–1.49)	0.178
3rd quartile (31–45)	160 (22)	1.27 (1.01–1.60)	0.038	1.20 (0.96–1.51)	0.113	1.08 (0.85–1.37)	0.547
4th quartile (> 45)	242 (30)	1.76 (1.42–2.17)	< 0.001	1.60 (1.30–1.97)	< 0.001	1.34 (1.09–1.67)	0.009
Angiographic CAD (n = 2391)							
1st quartile (< 21)	122 (21)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (21–30)	154 (26)	1.30 (1.03–1.65)	0.030	1.28 (1.01–1.62)	0.042	1.21 (0.95–1.56)	0.121
3rd quartile (31–45)	139 (25)	1.26 (0.99–1.60)	0.065	1.19 (0.93–1.51)	0.172	1.14 (0.83–139)	0.576
4th quartile (≥ 45)	212 (32)	1.70 (1.36–2.12)	< 0.001	1.58 (1.26–1.97)	< 0.001	1.48 (1.08–1.72)	0.009
None CAD (n = 645)							
1st quartile (< 21)	14 (9)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (21–30)	16 (10)	1.14 (0.56–2.33)	0.723	1.12 (0.55–2.29)	0.764	1.18 (0.57–2.47)	0.660
3rd quartile (31–45)	21 (13)	1.52 (0.77–3.00)	0.223	1.45 (0.74–286)	0.279	1.37 (0.67–2.79)	0.388
4th quartile (≥ 45)	30 (19)	2.21 (1.18–4.18)	0.014	1.88 (0.97–3.56)	0.051	1.66 (0.84–3.28)	0.143

Model 1: unadjusted.

Model 2: adjusted for age and gender.

Model 3: in addition adjusted for coronary artery disease (none, stable CAD, unstable CAD, NSTEMI, STEMI), the use of lipid-lowering drugs, body mass index, type 2 diabetes, hypertension, smoking status, eGFR (CKD-EPI), LDL-C, HDL-C, and triglycerides.

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Table 5

Hazard ratios for death from cardiovascular causes according to MPO concentrations.

MPO (ng/mL)	Deaths n (%)	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*	Model 3 OR (95% CI)	*P*
All individuals (n = 3013)							
1st quartile (< 21)	82 (11)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (21–30)	108 (13)	1.35 (1.01–1.80)	0.043	1.33 (1.00–1.77)	0.055	1.20 (0.89–1.62)	0.237
3rd quartile (31–45)	94 (14)	1.24 (0.93–1.67)	0.148	1.18 (0.88–1.59)	0.276	1.03 (0.76–1.40)	0.760
4th quartile (> 45)	158 (18)	1.90 (1.45–2.48)	< 0.001	1.73 (1.32–2.25)	< 0.001	1.42 (1.07–1.88)	0.010
Angiographic CAD (n = 2368)							
1st quartile (< 21)	75 (13)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (21–30)	98 (16)	1.35 (1.00–1.82)	0.053	1.33 (0.98–1.80)	0.066	1.23 (0.90–1.69)	0.197
3rd quartile (31–45)	83 (14)	1.23 (0.90–1.67)	0.203	1.16 (0.85–1.59)	0.353	1.05 (0.75–1.45)	0.688
4th quartile (≥ 45)	143 (20)	1.86 (1.40–2.45)	< 0.001	1.73 (1.31–2.28)	< 0.001	148.(1.10–1.98)	0.009
None CAD (n = 645)							
1st quartile (< 21)	7 (4)	1.0[reference]		1.0[reference]		1.0[reference]	
2nd quartile (21–30)	10 (6)	1.42 (0.54–3.72)	0.480	1.39 (0.53–3.65)	0.504	1.30 (0.49–3.43)	0.600
3rd quartile (31–45)	11 (7)	1.59 (0.62–4.10)	0.338	1.51 (0.59–3.91)	0.391	1.18 (0.44–3.15)	0.742
4th quartile (≥ 45)	15 (9)	2.19 (0.89–5.37)	0.087	1.86 (0.76–4.58)	0.176	1.47 (0.58–3.73)	0.415

Model 1: unadjusted.

Model 2: adjusted for age and gender.

Model 3: in addition adjusted for coronary artery disease (none, stable CAD, unstable CAD, NSTEMI, STEMI), the use of lipid-lowering drugs, body mass index, type 2 diabetes, hypertension, smoking status, eGFR (CKD-EPI), LDL-C, HDL-C, and triglycerides.