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Association of the serum myeloperoxidase/high-density lipoprotein particle ratio and incident cardiovascular events in a multi-ethnic population: Observations from the Dallas Heart Study

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ABSTRACT

Background and aims: Myeloperoxidase (MPO), a product of systemic inflammation, promotes oxidation of lipoproteins; whereas, high-density lipoprotein (HDL) exerts anti-oxidative effects in part via paraoxonase-1 (PON1). MPO induces dysfunctional HDL particles; however, the interaction of circulating levels of these measures in cardiovascular disease (CVD) has not been studied in humans. We tested whether serum levels of MPO indexed to HDL particle concentration (MPO/HDLp) are associated with increased CVD risk in a large multiethnic population sample, free of CVD at baseline.

Methods: Levels of MPO, HDL-C, and HDL particle concentration (HDLp) by NMR were measured at baseline in 2924 adults free of CVD. The associations of MPO/HDLp with incident ASCVD (first non-fatal myocardial infarction, non-fatal stroke, coronary revascularization, or CVD death) and total CVD were assessed in Cox proportional-hazards models adjusted for traditional risk factors. The median follow-up period was 9.4 years.

Results: Adjusted for sex and race/ethnicity, MPO/HDLp was associated directly with body mass index, smoking status, high-sensitivity C-reactive protein, and interleukin 18, and inversely with age, HDL-C levels, HDL size, and PON1 arylesterase activity, but not with cholesterol efflux. In fully adjusted models, the highest *versus* lowest quartile of MPO/HDLp was associated with a 74% increase in incident ASCVD (aHR, 1.74, 95% CI 1.12–2.70) and a 91% increase in total incident CVD (aHR, 1.91, 95% CI 1.27–2.85).

Conclusions: Increased MPO indexed to HDL particle concentration (MPO/HDLp) at baseline is associated with increased risk of incident CVD events in a population initially free of CVD over the 9.4 year period.

1. Introduction

Inflammation and oxidative stress play a key role in the progression of atherosclerotic plaques and development of cardiovascular disease. Myeloperoxidase (MPO), expressed in the azurophilic granules of leukocytes, is released during states of increased inflammation and catalyzes the formation of several reactive species. MPO is enriched within advanced atherosclerotic lesions in humans [1], suggesting a role in atherosclerosis. In animal models, MPO has been shown to catalyze initiation of lipid peroxidation at sites of inflammation *in vivo,* [2] and when incubated with low-density lipoprotein (LDL) *in vitro* [3]. MPO also oxidizes high-density lipoprotein (HDL), rendering it dysfunctional [4,5]. HDL-bound MPO retains its enzymatic activity, and MPO-dependent modification of HDL markedly increases the binding affinity of HDL for MPO, leading to a vicious cycle of MPO-dependent modifications at sites of chronic inflammation [6]. Paraoxonase1 (PON1), on the other hand, prevents oxidation of lipids in lipoproteins, allowing HDL to exert atheroprotective and anti-inflammatory actions [7]. PON1 promotes high-density lipoprotein (HDL)-mediated macrophage cholesterol efflux in *in vitro* studies [8,9]. In fact, MPO, PON1, and HDL have been shown to form a ternary complex, in which PON1 inhibits activity of MPO, and vice versa [10].

Many clinical studies have reported a direct association of MPO with acute coronary syndrome (ACS) and an inverse

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association of PON1 with ACS [11–17]. However, PON1 arylesterase activity and circulating levels of MPO have inconsistent associations with atherosclerotic cardiovascular disease (ASCVD) in those without ACS [16,18–22] Recently, the MPO/PON1 ratio was associated with atherosclerosis cross-sectionally [23]. In the present study, we sought to investigate whether MPO indexed to HDL particle concentration, as an indicator of the oxidative potential of HDL, would be associated with incident ASCVD, independent of PON1 arylesterase activity. This is the first study to report an association between MPO/HDL particle ratio and cardiovascular outcomes in a large cohort of humans without baseline cardiovascular disease.

2. Materials and methods

2.1. Study population

The Dallas Heart Study is a multiethnic, probability-based population-representative cohort study of Dallas County residents, including intentional oversampling of Blacks to make up 50% of the cohort [24]. Participants 30–65 years of age underwent fasting blood collection at baseline. We excluded individuals with a history of cardiovascular disease, defined as self-reported history of myocardial infarction, stroke, arterial revascularization, heart failure, or arrhythmia, those with niacin use, and those who died within 1 year of enrollment. We did not exclude participants with unknown subclinical disease. We included in our analysis 2924 out of a total 2971 enrolled participants.

2.2. Measurement of circulating markers

Blood was collected in EDTA tubes by venipuncture from all the participants at baseline, stored at 4 °C for less than 4 h, and centrifuged, and plasma was removed and stored at −70 °C. All biomarkers reported here have been previously measured and the analytical methods described [24], including MPO, plasma lipids, serum PON1 activity [25,26], cholesterol efflux [27], Interleukin 18 [28], high-sensitivity C-reactive protein (hs-CRP) [24,29], asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) [30]. MPO measurements were provided by Biosite, Inc. (now Alere, Inc. Waltham, MA) [31]. HDL particle sizes and concentrations were measured by NMR spectroscopy (LabCorp, formerly LipoScience, Inc. Raleigh, NC, USA) [32]. PON1 arylesterase activity was measured by cleavage of phenyl acetate resulting in phenol formation, measured in kU/L, based on the extinction coefficient of phenol [26]. Cholesterol efflux capacity was assessed by measuring the efflux of fluorescence-labeled cholesterol from J774 macrophages to apolipoprotein B–depleted plasma in study participants, expressed as a percentage of efflux in the sample, normalized to a reference sample [27]. All the analyzed data came from samples collected at baseline.

2.3. Clinical endpoints

The primary end point was a composite ASCVD outcome, defined as a first non-fatal myocardial infarction, non-fatal stroke, coronary revascularization (percutaneous coronary intervention or coronary-artery bypass grafting) or death from cardiovascular causes. A secondary end point, total cardiovascular disease, was defined as all the events included in the primary end point plus peripheral revascularization and hospitalization for heart failure or atrial fibrillation. All the events were adjudicated separately by two cardiologists from primary records, as described in our earlier study [33]. The median follow-up period was 9.4 years.

2.4. Statistical analysis

Demographic and clinical variables were compared across quartiles of MPO/HDLp with the use of the Jonckheere–Terpstra trend test [34]. Correlations with continuous markers were assessed with the use of nonparametric Spearman coefficients. Kaplan-Meier curves and Cox proportional-hazards models were used to assess the association between quartiles of the MPO/HDLp ratio and the time to a first event for both atherosclerotic cardiovascular disease (the primary end point) and total cardiovascular disease (the secondary end point). Multivariable models included age, sex, race, presence or absence of diabetes, presence or absence of hypertension, current smoking, body mass index, total cholesterol level, log-transformed triglyceride level, and history of statin use. Models were serially adjusted for HDL-C, PON1 arylesterase activity, hs-CRP, IL-18, and BMI. Two-sided p values of 0.05 or less were considered to indicate statistical significance. All statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute).

3. Results

The median age of the participants at study entry was 42 years. A total of 57% of the participants were women and 49% Black. The median MPO levels and the median HDLp levels by sex and race/ethnicity are summarized in Table 1.

3.1. Association of MPO/HDLp with risk factors, lipids, and biomarkers of inflammation and HDL function

There was a 2.6-fold increase in median MPO/HDLp ratio across its 4 quartiles (Table 2) and an approximately 4-fold increase from the 5th to 95th percentile. Increasing MPO/HDLp was driven primarily by increases in MPO, with a small contribution from decreases in HDLp (Table 2). Increasing quartiles of MPO/HDLp were associated inversely with age and directly with body mass index (BMI) and the rate of smoking, but were not associated with other traditional risk factors (Table 3). In terms of lipoprotein composition, increasing MPO/HDLp was associated with decreased HDL-C and modestly with HDL size, but not with LDL-C or triglyceride levels (Table 2). MPO/HDLp was directly associated with high-sensitivity C-reactive protein (hs-CRP) and interleukin 18 (IL-18). In sex- and race-adjusted models, MPO/HDLp was strongly associated inversely with PON1 arylesterase activity ($p<0.0001$, Table 4) but not associated with cholesterol efflux ($p=0.45$, Table 4).

3.2. Association of MPO/HDLp with cardiovascular events

There were 164 ASCVD and 205 total CVD first events. Increasing quartiles of MPO alone modestly trended towards increasing risk of ASCVD and CVD, but no individual quartile comparisons to quartile 1 were significant in unadjusted or adjusted analyses (Tables 5 and 6). Similar findings were seen for MPO indexed to PON1 (MPO/PON1) and MPO indexed to HDL-C (MPO/HDL-C, Tables 5 and 6).

In contrast, increasing quartile of MPO/HDLp monotonically associated with the unadjusted ASCVD event rate (5.1% in Q1, 5% in Q2, 7.4% in Q3, and 8.8% in Q4, log rank $p=0.038$, Fig. 1). The fourth *versus* first quartile of MPO/HDLp was also associated with an increased risk for ASCVD (HR 1.63, 95% CI 1.06–2.50, Fig. 1 and Table 5). Adjusting for the traditional risk factors did not attenuate this association (adjusted HR 1.74, 95% CI 1.12–2.70, Fig. 1 and Table 5). Similarly, there was a significant direct association between quartiles of MPO/HDLp and total CVD (Fig. 1 and Table 6). In adjusted analyses, the fourth *versus* first quartile of MPO/HDLp was associated with increased total CVD (adjusted HR 1.91, 95% CI