Atherosclerosis 280 (2019) 7–13

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

The combined utility of myeloperoxidase (MPO) and paraoxonase 1 (PON1) as two important HDL-associated enzymes in coronary artery disease: Which has a stronger predictive role?

Atena Variji[a,1], Yasaman Shokri[a,1], Samane Fallahpour[a], Mehryar Zargari[b], Babak Bagheri[c], Saeid Abediankenari[d], Ahad Alizadeh[e,**], Abdolkarim Mahrooz[b,d,*]

[a] Department of Clinical Biochemistry and Genetics, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[b] Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[c] Department of Cardiology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[d] Immunogenetics Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[e] Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

HIGHLIGHTS

- The diagnostic performance of serum paraoxonase 1 (PON1) alone was comparable to that of the myeloperoxidase (MPO)/PON1 ratio.
- MPO may increase the true positive rate in the MPO/PON1 ratio for CAD risk assessment.
- PON1 and MPO/PON1 had a stronger discriminatory power in the age range of ≥52 and < 60 years.
- A larger number of blocked vessels is associated with an increased predictive power for both PON1 and MPO/PON1.
- Recent data support the fact that PON1 and MPO may be appropriate therapeutic targets for preventing CVD.

ARTICLE INFO

Keywords:
Paraoxonase
Myeloperoxidase
Biomarker
Predictive value
Coronary artery disease

ABSTRACT

Background and aims: Serum paraoxonase 1 (PON1) and myeloperoxidase (MPO) are HDL-associated enzymes that contribute significantly to the formation of dysfunctional HDL. The present study thus seeks to comparatively analyze the predictive role of PON1, MPO and the MPO/PON1 ratio and to also evaluate which one has a stronger predictive role in their combined utility as an MPO/PON1 ratio in coronary artery disease (CAD).
Methods: PON1 activity and MPO concentrations were determined in patients with established CAD and those without significant CAD. Receiver operating characteristic (ROC) curves were drawn by plotting true positivity *versus* false positivity.
Results: The ROC curve analyses showed that PON1 (AUC = 61%, $p = 0.003$) and MPO/PON1 (AUC = 60%, $p = 0.01$) have a better diagnostic performance than MPO (AUC = 50%, $p = 0.42$) in detecting patients with CAD. PON1 and MPO/PON1 were found to have a significantly stronger discriminatory power for the age range ≥52 and < 60 years (AUC = 69%, $p = 0.008$ for PON1; AUC = 66%, $p = 0.022$ for MPO/PON1). The multivariate analysis revealed PON1 as an independent variable that was significantly associated with the multi-vessel disease [odds ratio (OR) = 0.98; $p = 0.017$]. At the cutoff point of 30 μmol/mL/min for PON1 and 1.85 for MPO/PON1, specificities were 97% and 73% and sensitivities 30% and 54% for discriminating patients with single-vessel disease from non-CAD subjects.
Conclusions: The diagnostic performance of PON1 alone was comparable to that of the MPO/PON1 ratio for CAD

* Corresponding author. Department of Clinical Biochemistry and Genetics, Faculty of Medicine, Mazandaran University of Medical Sciences, Km 17 Khazarabad Road, Sari, Iran.
** Corresponding author. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
E-mail addresses: a-alizadeh@razi.tums.ac.ir (A. Alizadeh), amahrooz@mazums.ac.ir (A. Mahrooz).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.atherosclerosis.2018.11.004
Received 29 August 2018; Received in revised form 17 October 2018; Accepted 7 November 2018
Available online 08 November 2018

risk assessment; however, MPO may increase the true positive rate. A larger number of blocked vessels seems to be associated with an increased predictive power for both PON1 and MPO/PON1. Recent data support the fact that PON1 and MPO may potentially be appropriate therapeutic targets for preventing CAD.

1. Introduction

According to reports, mortality from coronary heart disease and stroke has increased globally from around 10 million deaths in 1990 to 13 million in 2010, with more than 80% occurring in low- or middle-income countries [1]. Adverse cardiovascular events may occur in a significant number of people, even those with a relatively modest risk factor profile. Identifying new biomarkers for predicting cardiovascular outcomes is necessary alongside traditional risk factors such as low-density lipoprotein cholesterol (LDL-C), age, diabetes mellitus, smoking, and hypertension [2]. These new biomarkers can help develop appropriate therapeutic strategies for improving the patients' survival.

High-density lipoprotein (HDL) is a cardioprotective lipoprotein and its levels are inversely associated with the risk of cardiovascular disease (CVD). Nonetheless, due to its biological complexity, studies have recently proposed that the concentration of HDL cholesterol (HDL-C) alone may not always reflect the HDL function and may not have significant clinical benefits; rather, HDL proteins, particularly their enzyme component, may be more important [3,4]. According to studies, changes in HDL proteome affect the HDL function and are also associated with a risk of coronary artery disease (CAD) and their protein component may be a therapeutic target [5].

Paraoxonase 1 (PON1) is an esterase/lactonase mainly synthesized by the liver and secreted into the blood, where it physically binds to the HDL particles. This multifunctional enzyme is able to degrade oxidized phospholipids and hydrolyze lactones from lipoproteins [6,7]. The antioxidant property of HDL particles to prevent the oxidation of lipoproteins, particularly LDL, is attributed mostly to PON1 of all HDL-associated proteins [8]. Atherosclerotic lesions have been shown to decrease significantly in human PON1 transgenic mice, and the mice's HDL was more capable of protecting LDL oxidation [9]. In patients with CAD, the elevated formation of malondialdehyde in the endothelial HDL, which occurs due to decreased PON1 activity, can have a negative effect on endothelial HDL functionality [10].

Unlike PON1, myeloperoxidase (MPO) plays a role in the oxidative modification of lipoproteins. This enzyme is a hemeprotein expressed at high levels in neutrophils, monocytes, and certain tissue macrophages (such as in atherosclerotic plaques) [11–13]. The leukocyte-derived enzyme catalyzes the production of reactive oxidants. Although these products are important for the host's defense, oxidizing intermediates can also be potentially harmful to the inflamed tissue and are believed to also contribute to vascular inflammation and be a potential component in plaque formation [12,14].

The modification of the function of PON1 and MPO as HDL-associated enzymes contributes significantly to HDL functionality, which in turn plays a role in CVD [10,15]. MPO is a negative determinant of PON1 activity, which may itself be a potential mechanism affecting HDL dysfunction and leading to an increased risk of CVD [16–18]. PON1 binds to both MPO and HDL on the HDL lipid surface, and this binding helps PON1 inhibit the onset of lipid peroxidation, which is carried out by MPO [17].

PON1, MPO and HDL bind to one another, forming a ternary complex, and the interaction between PON1 and MPO within the HDL-PON1-MPO complex can have potential clinical significance in CVD. These two important enzymes reciprocally modulate each other's activity and their co-location in HDL can potentiate an inopportune interaction [16,19]. The combined use of these two important enzymes can therefore be valuable in predicting the risk of CAD. Nonetheless, to the researchers' knowledge, no comparative studies have yet been conducted to analyze which biomarker contributes more to the predictive power of the MPO/PON1 ratio in this disease. The present study thus seeks to comparatively analyze the predictive role of PON1, MPO and the MPO/PON1 ratio and to also evaluate which one has a stronger predictive role in their combined use as a MPO/PON1 ratio in CAD.

2. Materials and methods

2.1. Study population

This study examined 174 subjects who underwent coronary angiographic examination from February to September 2016 for showing clinical symptoms of CAD. Criteria for exclusion in the study were history of recent myocardial infarction within 1 month prior to angiography, previous history of ischemic or hemorrhagic stroke, cancer, infectious diseases, acute renal failure and autoimmune diseases. The participants underwent a physical examination and demographic characteristics, medical history, medication use, and personal habits were obtained from all patients using a questionnaire. Hypertension was defined as systolic blood pressure $\geq$140 mm Hg, diastolic blood pressure $\geq$90 mm Hg or treatment with antihypertensive medications. The patients with a physician-reported diabetes mellitus were included in the study based on their fasting glucose levels, the 2-h oral glucose tolerance test (according to the WHO criteria) or their use of antidiabetic medications. Table 1 presents the patients' medication intake including angiotensin-converting enzyme (ACE) inhibitor, calcium channel blocker, losartan, aspirin, metformin, and statin. The protocol study was planned in accordance with the ethical criteria detailed in the Declaration of Helsinki and was approved by the university local ethics committee. Written informed consent was received from all participants after explaining the purpose of the investigation. Fasting serum was

Table 1
Clinical and metabolic characteristics of patients.

Parameter	Non-CAD (n = 86)	CAD (n = 88)	*p* value
Age (year)	54.02 ± 11.53	57.61 ± 7.25	0.006
Women (%)	58.7	48.4	0.106
Hypertension (%)	60.9	60.4	0.945
Diabetes mellitus (%)	40.7	55.7	0.052
Current smoking (%)	17.9	21.4	0.570
Diastolic blood pressure (mmHg)	76.08 ± 8.17	77.87 ± 9.12	0.365
Systolic blood pressure (mmHg)	121.59 ± 15.73	125.47 ± 14.10	0.060
BMI (kg/m^2)	28.43 ± 4.43	28.45 ± 4.67	0.987
Fasting glucose (mmol/L)	7.03 ± 3.07	8.48 ± 4.09	0.008
Triglyceride (mmol/L)	1.71 ± 0.75	1.79 ± 0.81	0.544
Total cholesterol (mmol/L)	4.21 ± 1.04	4.26 ± 1.02	0.817
HDL-C (mmol/L)	0.99 ± 0.20	0.96 ± 0.21	0.389
LDL-C (mmol/L)	2.42 ± 0.86	2.46 ± 0.80	0.694
PON1 activity (μmol/mL/min)	57.65 ± 18.85	46.59 ± 25.44	0.007
MPO (ng/mL)	92.73 ± 53.24	95.37 ± 62.55	0.839
MPO/PON1	2.34 ± 4.13	4.02 ± 5.74	0.020
Medications (%)			
Beta blockers	45.7	48.4	0.702
ACE inhibitors	10.9	11.5	0.869
Calcium channel blockers	5.4	11.0	0.132
Diuretics	3.3	4.9	0.520
Aspirin	62.0	60.4	0.808
Statins	46.7	54.9	0.199
Fibrates	2.2	3.8	0.463
Metformin	17.4	24.7	0.168

Values are presented as percent or mean ± SD.
ACE, angiotensin-converting enzyme.

obtained for different assays and stored to later perform the laboratory analyses.

The angiographic results were analyzed by two experienced cardiologists who were blinded to the study protocol. The patient group was defined as those having at least one epicardial stenosis ≥70%, and those with no significant coronary atherosclerosis (no major vessels with stenosis over 50%) were classified as the non-CAD subjects. The severity of CAD was classified by the number of coronary vessels showing obstructive CAD. The patients with obstructive CAD were therefore divided into the following categories: single-vessel disease or SVD (≥70% diameter stenosis in one of the major coronary arteries or their major branches) and multi-vessel disease or MVD (≥70% diameter stenosis in two or more coronary arteries or their major branches) [20–22].

2.2. Assay of laboratory parameters

Serum MPO levels were determined with an ELISA kit (BMS2038INST, eBioscience, Vienna, Austria) in which an anti-human MPO monoclonal antibody was used as capturing antibody. A biotinylated monoclonal antibody against human MPO binds to MPO captured by the coated antibody. Streptavidin-HRP then binds to the biotinylated antibody. Absorbance was determined at 450 nm.

Triglyceride (TG), total cholesterol (TC), HDL-C, fasting blood glucose, were measured using standard enzymatic methods. The LDL-C levels were obtained by the Friedewald formula.

For PON1 activity assay, the hydrolysis rate of the substrate phenylacetate was assayed spectrophotometrically in serum using a double-beam spectrophotometer (UV 1800, Shimadzu, Japan) at 270 nm. The assay cuvette contained 1 mM phenylacetate and 1 mM $CaCl_2$ in Tris/HCL buffer (100 mM, pH 8.0). The increase in absorbance at 270 nm was recorded after the addition of serum. The determined velocity is related to the initial velocity of substrate hydrolysis. Blank was used to correct for the nonenzymatic hydrolysis of the substrate. The molar extinction coefficient was 1310 $M^{-1}\,cm^{-1}$ for calculating the units of enzyme activity, which is expressed as micromoles of phenol formed per minute. The intra-assay and inter-assay coefficients of variation were 3.1% and 4.3%, respectively.

2.3. Statistics

The distributions of all variables were analyzed with the Lilliefors test. The differences of the parametric variables were analyzed using *t*-test and the nonparametric variables were tested by Mann–Whitney. Fisher exact and chi-square tests were used to evaluate the association of categorical variables. The results of the logistic regression models were presented using odds ratios (ORs) and 95% confidence intervals. Receiver operating characteristic (ROC) analyses were performed to compare the predictive performance of PON1, MPO and MPO/PON1 ratio in the study groups. ROC curves were drawn by plotting true positivity (sensitivity) *versus* false positivity (1–specificity) and were then used to calculate the cut-off points for optimal sensitivity and specificity. The area under the curve (AUC) was determined in order to estimate the overall discriminative capacity of the variables. The best cut-off value was defined as the point with the highest sum of sensitivity and specificity (Youden's index). Multivariate logistic regression analyses were used to evaluate the predictive role of the PON1, MPO and MPO/PON1 ratio adjusted for other confounders in CAD. Correlation analyses were performed using Spearman rank correlation and biserial correlation. A value of $p < 0.05$ (two-tailed) was accepted as statistically significant in all analyses. Statistical analyses were conducted using the softwares R (version 3.0.0) and SPSS (version 16.0).

3. Results

3.1. The clinical and metabolic features of patients with CAD compared to non-CAD subjects

Table 1 compares the clinical and metabolic characteristics of patients with CAD and non-CAD subjects. As shown, there were no significant differences between patients with CAD and the non-CAD group with respect to sex, smoking, systolic and diastolic blood pressure, BMI, TG, TC, LDL-C, HDL-C, and MPO levels. CAD patients had higher age and fasting glucose than the non-CAD group. Compared to non-CAD subjects, PON1 activity was significantly lower in CAD patients ($p = 0.007$). In addition, patients with CAD had a significantly higher MPO/PON1 ratio than non-CAD subjects ($p = 0.02$). There was no statistically significant difference between patients with CAD and non-CAD subjects with respect to medications such as beta blockers, ACE inhibitors, and statins.

3.2. A comparison of the accuracy of PON1, MPO and MPO/PON1 ratio in predicting CAD

Using the ROC curve analysis, the AUC was used to evaluate the accuracy of CAD diagnosis (Fig. 1). The ROC curves showed that decreased PON1 and increased MPO/PON1 ratios are associated with CAD with an AUC of 61% ($p = 0.003$) and 60% ($p = 0.01$), respectively.

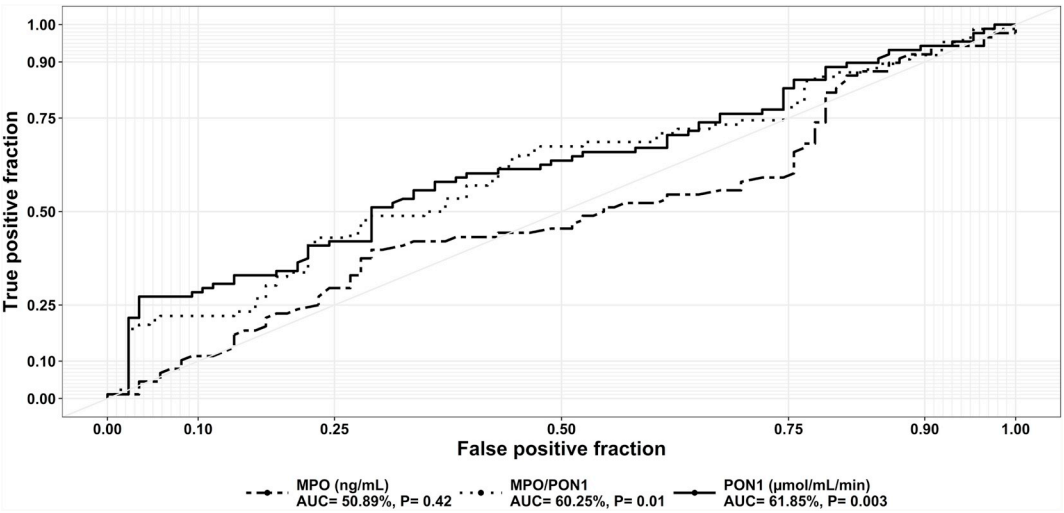

Fig. 1. Receiver operating characteristics (ROC) curves showing the discriminatory power of the three predictors for coronary artery disease. The corresponding value of the area under the curve (AUC) and *p*-values are reported for each biomarker.

Table 2
Univariate and multivariate logistic regression analyses for associations of the study parameters with CAD.

Parameter	Univariate analyses				Multivariate analyses					
					Model I		Model II		Model III	
	OR (95% CI)	*p* value	AUC (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
PON1	0.98(0.96,0.99)	0.002	0.62(0.62,0.70)	0.003	0.98(0.97,0.99)	0.024	–	–	–	–
MPO	1(0.995,1.001)	0.763	0.51(0.42,0.60)	0.420	–	–	1(0.99,1.01)	0.894	–	
MPO/PON1	1.08(1.01,1.09)	0.046	0.60(0.52,0.69)	0.010	–	–	–	–	1.06(0.99,1.17)	0.136
BMI	1(0.94,1.07)	0.976	0.5(0.41,0.59)	0.494	1.02(0.95,1.09)	0.646	1.02(0.95,1.09)	0.608	1.02(0.95,1.1)	0.580
Sex	1.59(0.88,2.91)	0.128	0.56(0.48,0.63)	0.064	1.66(0.87,3.23)	0.127	1.81(0.96,3.48)	0.069	1.68(0.88,3.25)	0.118
Age	1.04(1.01,1.08)	0.017	0.62(0.53,0.71)	0.003	1.03(1,1.07)	0.073	1.04(1.01,1.08)	0.018	1.03(1,1.07)	0.055
Hypertension	0.86(0.47,1.57)	0.621	0.52(0.45,0.59)	0.312	0.74(0.38,1.42)	0.369	0.8(0.41,1.54)	0.512	0.81(0.42,1.55)	0.522
Diabetes mellitus	1.83(1.01,3.36)	0.049	0.57(0.5,0.65)	0.024	1.84(0.96,3.57)	0.069	2.07(1.1,3.96)	0.025	2.08(1.1,4.03)	0.026

PON1 and the MPO/PON1 ratio had nearly similar AUC values and higher predictive powers compared to MPO (AUC = 50%, $p = 0.42$) for CAD characterization (Fig. 1).

3.3. The association of PON1, MPO and the MPO/PON1 ratio with CAD based on regression analysis

As shown in Table 2, univariate and multivariate logistic regression analyses were conducted to estimate the association of PON1, MPO, and MPO/PON1 with CAD. The univariate analysis showed a significant association between PON1 and MPO/PON1 with CAD. Other significant univariate predictors of CAD included age and diabetes mellitus. In the multivariate analyses, PON1, MPO, and MPO/PON1 were included in three separate models as independent variables (Table 2). After a multivariate adjustment for age, sex, BMI, hypertension, and diabetes mellitus, the increased odds of CAD was retained significantly for PON1. Accordingly, PON1 (model 1) was the most important independent predictor of CAD [adjusted OR = 0.98 (0.97–0.99); $p = 0.024$].

3.4. The role of age in the association between PON1 and CAD

Considering that some variables could interact with the effects of PON1, MPO and MPO/PON1 on an increased CAD risk, the interaction effects of age, sex, BMI, hypertension, and diabetes mellitus were evaluated in the present study using the multivariate logistic regression. According to these analyses, only a significant interaction was observed between age and PON1. The predictive behavior of PON1 in different age groups thus also had to be evaluated.

PON1 was found to be negatively correlated with age in total data ($r = -0.253$, $p = 0.001$). For the easier interpretation of the results, the age variable was classified into three strata at the thirty-third and sixty-sixth percentiles. The first stratum included subjects with an age less than 52 years, and 33% of the patients had an age less than this value. The second stratum consisted of patients with an age ≥52 and < 60 years, which comprised another 33% of all the patients. The last stratum consisted of patients aged 60 years and above. The biserial correlation coefficient showed significant correlations between PON1 and CAD in subjects aged < 52 ($r = -0.260$, $p = 0.036$) and with an age ≥52 and < 60 years ($r = -0.340$, $p = 0.010$); however, this correlation was not significant in the subjects older than age 60 ($r = -0.097$, $p = 0.491$). As shown in Fig. 2A, there was a decreasing trend in the incidence of CAD with an increase in PON1 levels, but there was a different slope and value for each specific level of PON1. Fig. 2B shows the accuracy of PON1 in the three age strata using ROC curves. According to the ROC curves, decreased PON1 is associated with the incidence CAD with an AUC of 62% ($p = 0.046$) in subjects aged < 52 and with an AUC of 69% ($p = 0.008$) in subjects aged ≥52 and < 60 years.

3.5. A comparison of the accuracy of PON1 and MPO/PON1 ratio in predicting SVD and MVD

As shown in Fig. 3, both PON1 and MPO/PON1 were appropriate biomarkers for the prediction of MVD *vs.* non-CAD, as shown in the AUC (AUC = 68%, $p = 0.001$ for PON1 and AUC = 62%, $p = 0.02$ for

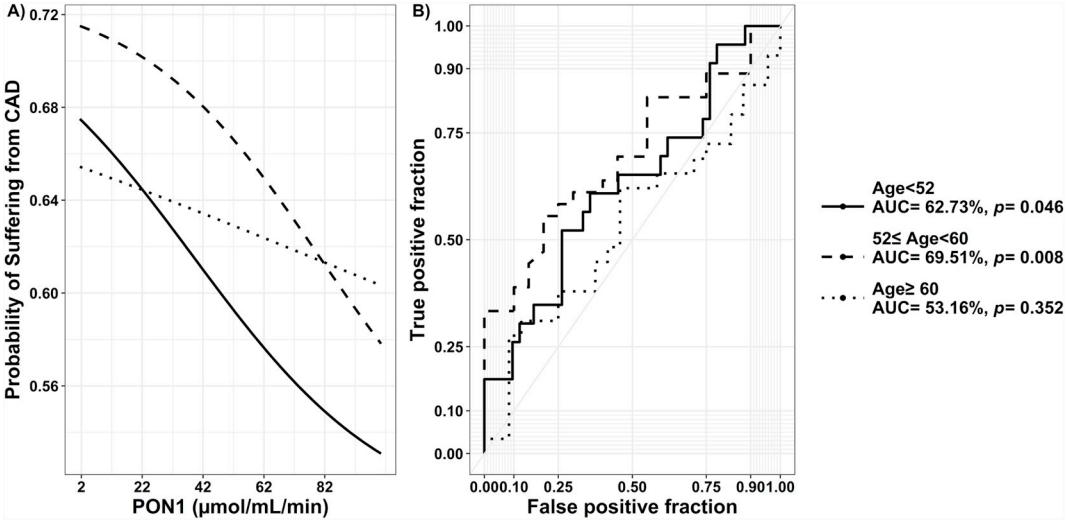

Fig. 2. (A) Probability of developing CAD against a decrease in PON1 based on the logistic regression. (B) Receiver operating characteristics (ROC) curve of PON1 in three strata of age.

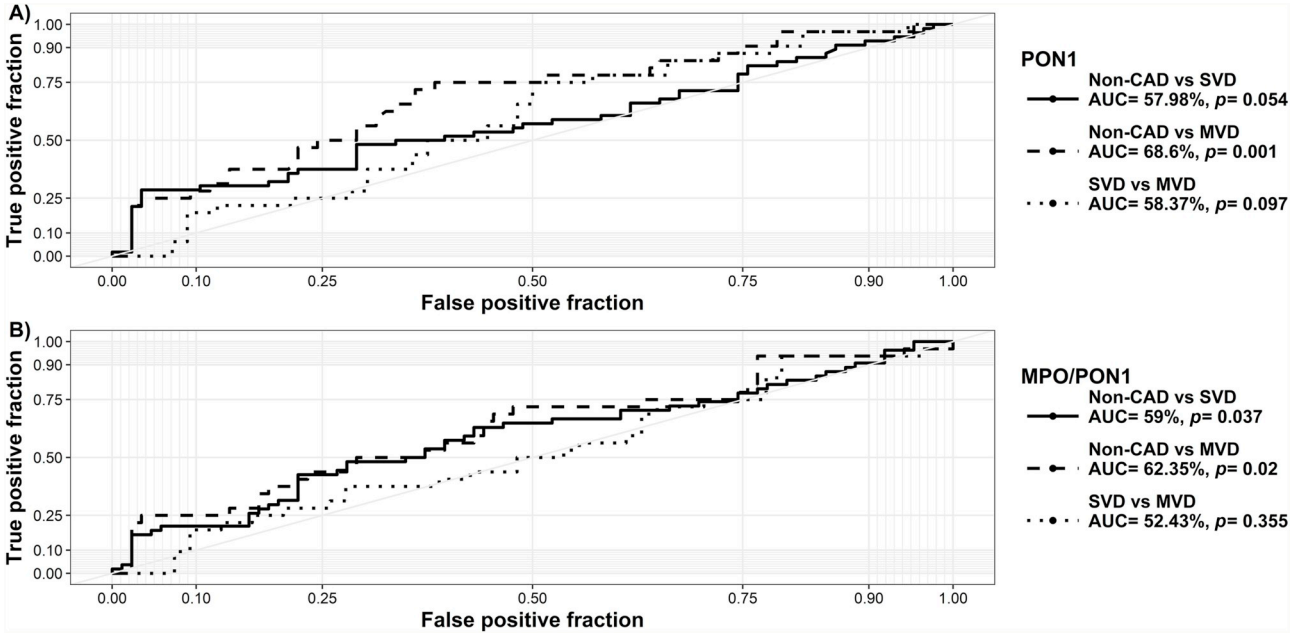

Fig. 3. Receiver operating characteristics (ROC) curves for PON1 (A) and MPO/PON1 (B) to discriminate patients with single-vessel disease (SVD) *vs.* non-CAD, multi-vessel disease (MVD) *vs.* non-CAD, and SVD *vs.* MVD.

MPO/PON1). Regarding discrimination between SVD and MVD, PON1 was more capable of differentiating patients with SVD from those with MVD compared to MPO/PON1 (AUC = 58%, $p = 0.097$ for PON1 and AUC = 52%, $p = 0.355$ for MPO/PON1).

3.6. The association of PON1 and the MPO/PON1 ratio with SVD and MVD based on regression analysis

Multivariate logistic regression analyses were performed to examine the independent association of PON1 and MPO/PON1 with SVD and MVD [see Supplementary Materials 1]. The same variables used for studying the association of PON1 and MPO/PON1 with CAD were entered into the model. As shown in Supplementary Materials 1, the multivariate regression analysis with adjustments for age, sex, BMI, hypertension, and diabetes mellitus showed that when PON1 and MPO/PON1 are analyzed in two separate models due to their multicollinearity, PON1 is an independent variable that has a significant association with MVD in the CAD group [adjusted OR = 0.98 (0.96–1); $p = 0.017$].

3.7. The optimal cut-off points of PON1 and the MPO/PON1 ratio for predicting CAD

The results showed that PON1 and the MPO/PON1 ratio are better predictors than MPO. These markers also had a better predictive ability than MPO in the subgroups of SVD and MVD. Based on Youden's index, the best cut-off points were estimated to predict CAD [see Supplementary Materials 2]. These values were calculated overall (i.e. for all the study subjects, including both the CAD and the non-CAD subjects) and also for the different age groups and the different combinations of non-CAD, SVD and MVD. The specificity, sensitivity and 95% confidence intervals were also calculated for each estimated threshold. The best overall cut-off point of serum PON1 for the prediction of CAD was 44 μmol/mL/min, with a specificity of 81% and a sensitivity of 45%. Also, the best overall cut-off point of the serum MPO/PON1 ratio for the prediction of CAD was 1.84, with a specificity of 73% and a sensitivity of 53%. Furthermore, the best cut-off point of PON1 and MPO/PON1 for the prediction of non-CAD *vs.* SVD was 30 μmol/mL/min (with a specificity of 97% and a sensitivity of 30%) and 1.85 (with a specificity of 73% and a sensitivity of 54%), respectively.

4. Discussion

Studies have found that assessing HDL's proteome, particularly its enzymes, may have more significant clinical benefits for predicting cardiovascular events than simply assessing HDL-C concentrations. PON1 and MPO may be particularly helpful in this regard. The link between MPO and PON1 is a biochemical functional relationship, as they reciprocally regulate each other's activity. While PON1 inhibits MPO activity, MPO promotes site-specific oxidative modification, which can lead to the impairment of PON1 activity and also apoA1 function [4,19]. Such an interactive pattern on the HDL scaffold causes a decreased PON1 activity, and vice versa, an increased MPO activity which can be considered as a substitute biomarker for dysfunctional HDL [19,23]. HDL dysfunction is considered a prominent marker and a therapeutic target in coronary disease, and low PON1 and high MPO can be important determinants of this dysfunctionality [10,16,23].

The evaluation and comparison of the clinical performance of PON1, MPO and their ratio using ROC curves showed that MPO alone may not be an appropriate biomarker for the prediction of CAD. Such non-significance was also observed for MPO in the univariate and multivariate analyses. Some studies have shown that the MPO concentration is associated with an increased risk of CAD, although there are inconsistencies in these findings [14,24]. According to the study by Scirica et al. [24], MPO does not have a significant prognostic value when assessed along with cardiac markers such as cardiac troponin I. Also, Schaub et al. also found no significant relationship between MPO concentrations and an increased risk of cardiovascular death [14].

Unlike MPO, PON1 activity was a significant predictor of CAD, and the risk of CAD remained significantly increased for PON1 even after multivariate adjustment for age, sex, BMI, hypertension, and diabetes mellitus, as supported by the results of two meta-analyses [25,26]. In 2012, Zhao et al. [25] reviewed 43 studies conducted on a total of 20,629 people to investigate the association between PON1 activity and the susceptibility for CHD. Their meta-analysis showed that a decreased PON1 activity is a risk factor for an increased CHD susceptibility. A more recent meta-analysis by Kunutsor et al. [26] on six prospective

studies reached similar findings and showed an inverse association between PON1 activity and CVD risk. Szentpéteri et al. also reported that PON1 activity predicts MPO concentrations negatively and independently in patients with vascular complications [27]. High concentrations of PON1 have also been reported to improve the prognosis after a coronary artery bypass [28], and a prospective cohort study found that the arylesterase activity of PON1 is associated with adverse outcomes in stable outpatients with heart failure [29].

Due to the significant interaction observed between age and PON1, the results were analyzed after categorizing the age variable into three strata. According to these strata, the probability of developing a CAD trend to decrease with an increase in the PON1 activity. They have a different slope and value for each specific concentration of PON1, thereby suggesting that it is better to evaluate the performance and accuracy of PON1 as a medical diagnostic test separately in different age strata. PON1 appears to have a stronger discriminatory power, particularly for the age range of ≥ 52 and < 60 years. The analysis of MPO/PON1 also showed that this ratio has a stronger diagnostic power in the same age range.

In the analyses carried out for detecting SVD and MVD in patients with CAD, both PON1 and MPO/PON1 were found to be better biomarkers for predicting cases of non-CAD *vs.* MVD than cases of non-CAD *vs.* SVD. For discriminating SVD from MVD, PON1 had a stronger discriminatory power than MPO/PON1. In addition, PON1 was significantly associated with MVD in the CAD group as an independent variable, but the same was not true for the MPO/PON1 ratio. No studies were found with which to compare these results; however, it appears that a larger number of obstructed vessels is associated with an increased predictive power for both PON1 and MPO/PON1.

According to the present findings, the combination of PON1 and MPO has a better discriminatory power for the risk of CAD compared to MPO alone that is in agreement with Haraguchi et al. [23]. But how about PON1 alone? Previous studies have not answered this question. The present findings showed that the predictive role of PON1 alone in assessing the risk of CAD is comparable to that of the MPO/PON1 ratio. It is worth noting, however, that MPO may increase sensitivity (true positive rate), since (the overall) sensitivity increased from 45% for PON1 to 53% for MPO/PON1. This effect was more evident in the discrimination between non-CAD *versus* SVD, as sensitivity increased from 30% for PON1 to 54% for MPO/PON1. Altogether, it seems that the combined use of PON1 and MPO can be a better predictor for CAD discrimination than MPO or PON1 alone. The present findings are supported by previous studies, which introduced the MPO/PON1 ratio as a useful biomarker in CAD [23,30]. Sun et al. [30] reported a positive correlation between the MPO/PON1 ratio and the atherosclerotic lesion size. Additionally, this ratio is inversely correlated with the HDL particle size, which can be in turn associated with alterations in the antioxidative and antiatherogenic activities of HDL [31,32]. Based on the present findings, it should be noted that the predictive value of MPO/PON1 in CAD may be greatly attributed to PON1. In the other hand, PON1 appears to play a key role in the MPO/PON1 ratio as a biomarker for identifying CAD. The importance of MPO/PON1 and the significant contribution of PON1 become more apparent when noting that this ratio may be an appropriate alternative biomarker for dysfunctional HDL.

PON1 and MPO are enzymes that play a role in both oxidative stress and inflammation, which are important features that can markedly affect the development of atherosclerotic plaques and cardiovascular events [33]. Decreased PON1 and increased MPO can be considered important determinants of HDL dysfunctionality, which is a feature of atherosclerosis [10,16,30]. Haraguchi et al. [23] evaluated patients who had previously undergone percutaneous coronary interventions and who had been hospitalized for coronary re-angiography. Based on their results, the HDL isolated from the patients with a low MPO/PON1 ratio had a greater inhibitory effect on vascular cell adhesion molecule-1 (VCAM-1) expression compared to that isolated from patients with a high MPO/PON1 ratio, which reveals decreased anti-inflammatory properties in patients with a high MPO/PON1. In addition, Szentpéteri et al. showed that PON1 activity correlates negatively with soluble intercellular adhesion molecule-1 (sICAM-1) concentrations, while MPO levels showed a positive correlation with sICAM-1 in untreated hyperlipidemic patients [27]. The researchers concluded that the HDL-associated pro- and antioxidant enzymes can play an important role in the development of endothelial dysfunction and atherogenesis. MPO/PON1 was also shown to be positively correlated with the HDL inflammatory index [30]. The induction of MPO and reduction of PON1 by reactive oxygen species and advanced glycation products together with an inflammation can result in the formation of dysfunctional HDL, which can in turn lead to cholesterol accumulation within the macrophages entrapped in the subendothelial space [8]. Additionally, Srivastava et al. [34] used the cholesterol efflux capacity assay as a measure of HDL functionality in a number of animal models and demonstrated that HDL functionality is more beneficial than HDL-C measurement. This information is in line with the results of studies which have shown that the HDL composition and function provide more beneficial information than HDL-cholesterol concentrations [35].

Just as in most previous studies, the present study used the MPO mass rather than its activity. The reason may be the strong correlation between the MPO mass and its activity; additionally, the ex-vivo assay of serum MPO activity may not always reflect its in-vivo activity since it is measured under saturated concentrations of the substrate [23].

There are two common functional polymorphisms in the coding region of PON1 (Q192R and L55M) and one common promoter polymorphism (-108T/C), and they are associated with different serum concentrations and thereby activities of PON1 [36,37]. The research groups should therefore be matching in terms of these polymorphisms so as to enable more accurate analyses of PON1 levels. In the present study, the frequency of these polymorphisms was not significantly different among the study groups (results not shown). As a result, these polymorphisms did not significantly affect the present analyses. The present study also used arylesterase activity of PON1, which, according to some studies, is not significantly affected by polymorphisms such as Q192R [36].

The limitations of this study include the small sample size; the researchers recommend large prospective studies on this subject to help clarify and confirm these findings. A second limitation is that previous studies had revealed the cholesterol efflux capacity as a measure of HDL functionality that correlated inversely with CHD events [34,38]; the study of the relationship between this capacity and PON1 and MPO can provide a better understanding of their roles in HDL function. PON1 and MPO, however, are enzymes that mainly play a role in antioxidative and oxidative processes. The last limitation is that blood serum samples were used in this study for measuring MPO; however, some studies have used plasma for assaying this enzyme, and it should be noted that PON1 is a calcium-dependent enzyme and samples containing EDTA (a calcium chelator) are not appropriate for assaying its activity, and it is therefore necessary to use serum for samples.

To conclude, the diagnostic performance of PON1 alone was comparable to that of the MPO/PON1 ratio for CAD risk assessment; however, MPO may increase the true positive rate. Both PON1 and MPO/PON1 had a stronger discriminatory power, particularly in the age range of ≥ 52 and < 60 years. A larger number of blocked vessels seems to be associated with an increased predictive power for both PON1 and MPO/PON1. Generally, recent data highlight the importance of the HDL enzyme component in the diagnosis of CVD and support the fact that PON1 and MPO may potentially be appropriate therapeutic targets for preventing CVD.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Acknowledgements

This study was funded by Mazandaran University of Medical Sciences (grants 95-2613 and 95-2617). The authors thank all investigators and patients for participating in the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.11.004.

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