Original Article

Elevated Serum Myeloperoxidase Activities are Significantly Associated with the Prevalence of ACS and High LDL-C Levels in CHD Patients

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***Aim*: Recent researches have shown that myeloperoxidase (MPO) is a potential inflammatory risk factor for coronary heart disease (CHD). In the present study, the possible associations of MPO with acute coronary syndrome (ACS), low density lipoprotein cholesterol (LDL-C) and other risk factors in CHD patients were investigated.**

***Methods*: Five hundred thirty-six CHD patients [363 ACS and 173 stable angina pectoris (SAP)] and 181 non-CHD patients confirmed by coronary angiography were enrolled in this study. The association study was performed by logistic regression analysis.**

***Results*: ACS patients had significantly higher MPO activities than SAP and non-CHD patients ($p<0.001$). The area under the receiver operating characteristic (ROC) curve of MPO for diagnosing ACS was 0.84 (95% CI: 0.80-0.88, $p<0.001$). The optimal cut-off value (sensitivity; specificity) of MPO was 164.77 ng/mL (79.1% and 82.1%). LDL-C (Ⅲ versus Ⅰ tertile, OR: 3.24, 95% CI: 1.67-6.29, $p=0.001$) and ACS (yes versus no, OR 2.74, 95% CI: 1.71-4.39, $p<0.001$) were significantly associated with elevated serum MPO activities, which had the highest odds ratio in quantitative and qualitative variables, respectively. There were significant increase trends in the prevalence of ACS and high LDL-C levels from Ⅰ to Ⅲ MPO tertile, which were 46.4% and 8.9% for Ⅰ, 68.0% and 31.5% for Ⅱ, 88.8% and 59.8% for Ⅲ tertile, respectively ($p<0.001$).**

***Conclusion*: The present study provides epidemiological evidence that elevated serum MPO activities are significantly associated with the prevalence of ACS and high LDL-C levels in CHD patients, and MPO may be a potential early warning marker for ACS.**

J Atheroscler Thromb, 2012; 19:435-443.

Key words; Myeloperoxidase, Inflammatory markers, Low density lipoprotein cholesterol, Coronary heart disease, Acute coronary syndrome

Introduction

Coronary heart disease (CHD) is expected to be the main cause of death within the next 15 years owing to its rapidly increasing prevalence in developing countries[1)]. Studies have demonstrated that high levels of low density lipoprotein cholesterol (LDL-C) are associated with CHD[2)]. Vulnerable plaque rupture is a major cause of CHD pathological events, including acute coronary syndromes (ACS) and sudden coronary death, and is morphologically characterized by an interruption in a thin fibrous cap overlying the lipid rich core. Often there are numerous inflammatory cells, including activated polymorphonuclear neutrophils (PMNs), macrophages, lymphocytes and other inflammatory cells focused on the fibrous cap of the vulnerable plaque shoulder, and high levels of inflammatory mediators in the atherosclerotic plaque

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Received: April 19, 2011
Accepted for publication: November 1, 2011

with increased blood levels of inflammatory cytokines and other acute-phase reactants[3-5].

More recently, myeloperoxidase (MPO), secreted directly by activated PMNs and macrophages, has been proposed as a useful risk biomarker of inflammation and as a diagnostic tool in ACS and in patients admitted to the Emergency Department for chest pain. In postmortem coronary specimens, luminal thrombi superimposed on eroded plaques had a higher density of MPO-positive cells and increased expression of MPO in PMNs and macrophages have been noted at the site of plaque disruption, suggesting a role of MPO in plaque rupture[6-8]. A better understanding of the association of MPO with ACS as well as LDL-C may be obtained by planning a new strategy for the early warning and intervention of ACS in CHD patients.

Aim

The present study was planned to evaluate the relationship of MPO with ACS, metabolic parameters, inflammatory markers, and traditional CHD risk factors, as well as lifestyle habits, and to explore the possible associations of MPO with ACS, LDL-C and other risk factors in patients with CHD.

Methods

Study Patients

This study included 363 ACS patients (248 male and 115 female) aged 36-85 (63.15 ± 14.98) years, 173 stable angina pectoris (SAP) patients (114 male and 59 female) aged 39-85 (60.45 ± 14.49) years, and 181 non-CHD patients (121 male and 60 female) aged 35-84 (62.10 ± 14.86) years from Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. All enrolled CHD patients had been confirmed by coronary angiography and diagnosed to be 363 ACS [157 acute myocardial infarction (AMI) and 206 unstable angina pectoris (UAP)] and 173 SAP according to 2007 ACC/AHA guidelines. One hundred eighty-one non-CHD patients with chest pain symptoms in the same period were confirmed by coronary angiography. Patients with the following diseases were excluded from this study: old myocardial infarction, acute and chronic infections, autoimmune disease, recent surgery, cancer, liver diseases, renal insufficiency, blood diseases, malnutrition, and thyroid dysfunction. All participating subjects were explained their participation rights and written informed consent was obtained. This study was carried out in accordance with the Helsinki Declaration and approved by the Regional Ethics Committee.

The data were collected consecutively from March 2010 to March 2011 (146 among 173 SAP patients from March 2010 to September 2010 were investigated for the incidence of ACS during a 6-month follow-up). Fasting blood was sampled in the morning within 24 h after the patients had been admitted to hospital. The blood must be collected before heparinization for coronary angiography. Moreover, AMI patients' blood was collected before percutaneous coronary intervention and thrombolytic treatment.

After blood was separated, fresh serum was used for the determinations of LDL-C, high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), fasting blood glucose (FBG), uric acid (UA) and homocysteine (Hcy). Another aliquot of serum was frozen and stored at −80℃ in a freezer. When sample collection was completed, all frozen serum was brought up to room temperature by gradual thawing, and centrifuged for 10 minutes at 3000 revolutions/min. The serum supernatants were used for the determination of MPO, interleukin-6 (IL-6), high sensitivity C-reactive protein (hs-CRP) and insulin (INS), simultaneously.

Markers of Inflammation

MPO was measured by the selective repression method with a Hitachi 7080 Automatic Biochemistry Analyzer (Hitachi High-Tech Instruments Co., Ltd., Japan; reagent and calibrator: Diazyme Laboratories, USA). IL-6 was quantified by the step sandwich method with the ACCESS 2 Immunoassay System and matched reagent and calibrator (Beckman Coulter, Inc., USA). Determination of the hs-CRP level was based on non-competitive near-infrared particle immunoassay with a matched high sensitivity CRP Kit (Immage 800 Immunochemistry System, Beckman Coulter, Inc., USA).

The reference interval of MPO was 19-160 ng/mL and the elevated serum MPO baseline level was defined as >164.77 ng/mL according to the optimal cut-off value of the MPO receiver operating characteristic (ROC) curve. The cut-off values for tertile distribution of each inflammatory marker considered in the study were MPO (ng/mL): Ⅰ <123.44, Ⅱ 123.44-234.55, Ⅲ >234.55; IL-6 (pg/mL): Ⅰ <6.45, Ⅱ 6.45-14.54, Ⅲ >14.54; hs-CRP (mg/L): Ⅰ <2.32, Ⅱ 2.32-7.25, Ⅲ >7.25, respectively.

Metabolic Parameters

Fasting INS was determined by a double-antibody, step sandwich method with the ACCESS 2

Immunoassay System and a matched reagent and calibrator (Beckman Coulter, Inc., USA). Other clinical chemistry parameters were measured on fresh serum samples with a Hitachi 7080 Automatic Biochemistry Analyzer (Hitachi High-Tech Instruments Co., Ltd., Japan). Commercial test kits (Beijing Leadman Biochemistry Technology Co., Ltd., China) were used to determine serum LDL-C, HDL-C, TC, TG, FBG, UA and Hcy concentrations, respectively.

Hypercholesterolemia, hypertriglyceridemia, and a high LDL-C level were defined as TC ≥6.21 mmol/L, TG ≥2.26 mmol/L, and LDL-C ≥4.13 mmol/L, respectively, according to NCEP/ATP Ⅲ. The cut-off values for tertile distribution of each clinical chemistry parameter considered in the study were LDL-C (mmol/L): Ⅰ <2.43, Ⅱ 2.43-4.12, Ⅲ >4.12; HDL-C (mmol/L): Ⅰ <0.99, Ⅱ 0.99-1.66, Ⅲ >1.66; TC (mmol/L): Ⅰ <4.91, Ⅱ 4.91-6.29, Ⅲ >6.29; TG (mmol/L): Ⅰ <1.36, Ⅱ 1.36-1.62, Ⅲ >1.62; FBG (mmol/L): Ⅰ <5.04, Ⅱ 5.04-5.86, Ⅲ >5.86; UA (μmol/L): Ⅰ <339.89, Ⅱ 339.89-446.84, Ⅲ >446.84; Hcy (μmol/L): Ⅰ <16.32, Ⅱ 16.32-23.79, Ⅲ >23.79; INS (pmol/L): Ⅰ <26.65, Ⅱ 26.65-56.93, Ⅲ >56.93, respectively.

Other Covariates

All patients were asked about their alcohol intake (yes or no, yes was defined as drinking at least once a week and drinking more than 200 mL of over 45% alcohol) and smoking habits (yes or no, non-smokers including never smoking and having stopped smoking for >1 year). Blood pressure, body weight and height were measured with standard techniques. Body mass index (BMI) was calculated as body weight (kg) divided by the height squared (m).

Hypertension was diagnosed when patients' systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg. The cut-off values for tertile distribution of each CHD risk factor were age (year): Ⅰ <48, Ⅱ 48-73, Ⅲ >73; SBP (mmHg): Ⅰ <128, Ⅱ 128-183, Ⅲ >183; DBP (mmHg): Ⅰ <88, Ⅱ 88-102, Ⅲ >102; BMI (kg/m^2): Ⅰ <23.54, Ⅱ 23.54-27.78, Ⅲ >27.78, respectively.

Statistical Analysis

All analyses were performed with SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed as the mean±SD or median (25th to 75th percentiles), according to normal or non-normal distribution, respectively. Means±SD of two samples were compared by the Independent-Sample t-Test, and means±SD of more than two samples were compared with one-way ANOVA, while the median (25th to 75th percentiles) of two independent samples was compared with the Mann-Whitney U rank-sum test. Categorical variables were expressed as a percentage and compared by the χ^2-test. ROC curve analysis for diagnosing ACS was performed to obtain the area under the ROC curve (AUC) of MPO and LDL-C. The optimal cut-off value was defined as a point on the ROC curve nearest to the point where both sensitivity and specificity were larger.

The correlation coefficients of MPO with each variable were calculated by Spearman's analysis because MPO was skewed. The associations between serum MPO activities with each of the metabolic parameters, inflammatory markers, traditional CHD risk factors as well as lifestyle habits (quantitative data expressed as tertiles, qualitative variables expressed as yes or no) were evaluated by univariate logistic regression analysis. Variables with a p value <0.25 in univariate analysis were retained for a further multivariate logistic regression analysis model. The variables included in the multivariate statistical model were as follows: LDL-C, HDL-C, TC, TG, Hcy, FBG, IL-6, hs-CRP, age, SBP, DBP, BMI, ACS, gender and smoking. In order to select the variables significantly associated with elevated MPO activities, the odds ratio (OR) and its 95% confidence intervals (95% CI) were calculated with multivariate logistic regression analysis (method: Stepwise Forward Wald).

Results

Comparison of Principal Characteristics in CHD and Non-CHD Patients

There were no significant differences between ACS, SAP and non-CHD groups not only in age (F=1.98, p=0.142) but also in gender (χ^2=0.34, p=0.843). The patients with ACS had significantly higher activities of MPO [210.63 (135.66-302.32)] than SAP and non-CHD patients (χ^2=124.45, p< 0.001); however, the levels of MPO between the SAP group [112.22 (76.24-170.24)] and non-CHD group [121.48 (89.65-149.85)] showed no significant difference (z=1.25, p=0.213). The principal characteristics of 536 CHD patients according to the activities of MPO are reported in **Table 1**. Compared to patients with normal-low MPO activities, patients with elevated MPO activities were characterized by ACS, male, smoking, older age, higher LDL-C, TC, TG, Hcy, FBG, SBP, DBP, BMI, hs-CRP and IL-6 value, and lower HDL-C levels. There were no significant differences in UA, INS concentration and alcohol intake between the two groups.

Table 1. Principal characteristics of 536 CHD patients aged 36-85 years according to levels of MPO

	Whole sample (n=536)	Normal-low MPO (n=239)	High MPO (n=297)	p value
LDL-C (mmol/L)	3.29 ± 1.20	2.76 ± 0.85	3.71 ± 1.26	< 0.001
HDL-C (mmol/L)	1.39 ± 0.52	1.56 ± 0.48	1.25 ± 0.52	< 0.001
TC (mmol/L)	5.73 ± 1.37	5.24 ± 1.09	6.12 ± 1.44	< 0.001
TG (mmol/L)	1.89 ± 1.10	1.66 ± 0.93	2.08 ± 1.18	< 0.001
Hcy (μmol/L)	20.69 ± 7.56	19.43 ± 6.69	21.71 ± 8.06	< 0.001
UA (μmol/L)	387.06 ± 88.39	391.12 ± 80.14	383.80 ± 94.52	0.332
FBG (mmol/L)	6.58 ± 2.84	6.26 ± 2.68	6.84 ± 2.95	0.018
Age (years)	62.29 ± 14.95	58.18 ± 15.28	65.59 ± 13.83	< 0.001
SBP (mmHg)	159.86 ± 31.73	153.51 ± 30.89	164.97 ± 31.52	< 0.001
DBP (mmHg)	94.70 ± 11.57	92.74 ± 11.60	96.28 ± 11.33	< 0.001
BMI (kg/m^2)	26.13 ± 3.30	25.48 ± 3.13	26.66 ± 3.35	< 0.001
hs-CRP (mg/L)	3.88 (1.69-9.22)	2.72 (1.25-6.21)	5.55 (2.35-16.62)	< 0.001
IL-6 (pg/mL)	8.86 (4.20-19.46)	7.09 (3.86-14.29)	12.35 (5.72-23.84)	< 0.001
INS (pmol/L)	37.10 (23.21-75.14)	37.29 (22.37-78.19)	36.03 (23.38-74.83)	0.985
ACS (%)	67.7	49.8	82.2	< 0.001
Gender (male %)	67.5	62.8	71.4	0.034
Smokers (%)	32.5	19.7	42.8	< 0.001
Alcohol intake (%)	42.7	43.1	42.4	0.876
Hypertension (%)	65.9	58.6	71.7	0.001
Diabetes mellitus (%)	29.5	21.8	35.7	< 0.001
Hypercholesterolemia (%)	35.3	18.0	49.2	< 0.001
Hypertriglyceridemia (%)	23.3	16.7	28.6	0.001

MPO: myeloperoxidase; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride; Hcy: homocysteine; UA: uric acid; FBG: fasting blood glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin-6; INS: insulin; ACS: acute coronary syndrome.

AUC and Cut-Off Value of MPO and LDL-C for Diagnosing ACS and the Correlations between MPO with Each Variable by Spearman's Analysis

The ROC curves of MPO and LDL-C for diagnosing ACS in 536 CHD patients are shown in **Fig. 1**. The AUCs of MPO and LDL-C were 0.84 (95% CI: 0.80-0.88, $p<0.001$) and 0.63 (95% CI: 0.58-0.68, $p<0.001$), respectively. The optimal cut-off value (sensitivity; specificity) of MPO was 164.77 ng/mL (79.1% and 82.1%). When the high LDL-C level was defined as 4.13 mmol/L, the sensitivity and specificity were 41.9% and 84.4%, respectively. The correlations between MPO activities with each of the metabolic parameters, inflammatory markers and anthropometrics are shown in **Table 2**. MPO was positively correlated with LDL-C, TC, TG, Hcy, FBG, IL-6, hs-CRP, age, SBP, DBP and BMI, and negatively correlated with HDL-C levels. In contrast, MPO was not significantly correlated with UA and INS.

Associations between MPO with Each of the Variables by Univariate Logistic Regression Analysis

Univariate logistic regression analysis revealed that elevated MPO activities were significantly associated with ACS (yes vs no, OR 4.64, 95% CI: 3.14-6.86, $p<0.001$), gender (male vs female, OR 1.48, 95% CI: 1.03-2.13, $p=0.035$), smoking (yes vs no, OR 3.05, 95% CI: 2.06-4.52, $p<0.001$), age (Ⅲ vs I tertile, OR 3.78, 95% CI: 2.44-5.86, $p<0.001$), SBP (Ⅲ vs I tertile, OR 1.95, 95% CI: 1.28-2.98, $p=0.002$), DBP (Ⅲ vs I tertile, OR 1.84, 95% CI: 1.21-2.80, $p=0.004$), BMI (Ⅲ vs I tertile, OR 2.34, 95% CI: 1.53-3.58, $p<0.001$), and were not significantly associated with alcohol intake (yes vs no, OR 0.97, 95% CI: 0.69-1.37, $p=0.876$). The associations between elevated MPO activities with each of the metabolic parameters and inflammatory markers are shown in **Table 3**. ACS had the highest odds ratio in the above qualitative variables, while LDL-C had the highest odds ratio (Ⅲ vs I, OR 7.38, 95% CI: 4.51-12.09, $p<0.001$) in all quantitative variables, respectively.

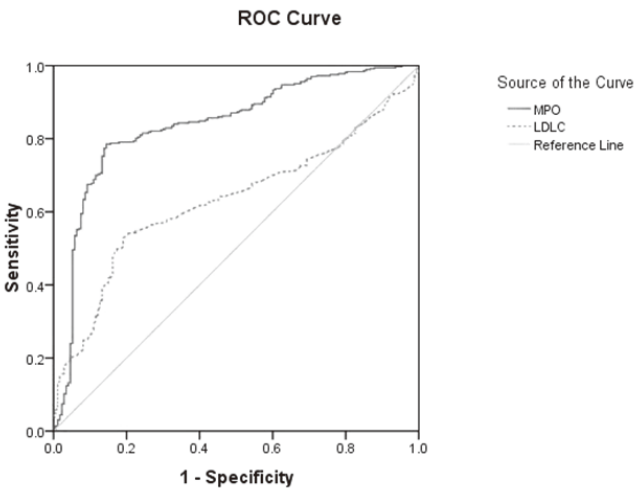

Fig. 1. Receiver operating characteristic curve of MPO and LDL-C for diagnosing ACS.

Table 2. Spearman's correlation coefficients between serum MPO activities with each of the metabolic parameters, inflammatory markers and CHD risk factors in CHD patients

Variable	MPO	*p* value
LDL-C	0.417	<0.001
HDL-C	−0.418	<0.001
TC	0.391	<0.001
TG	0.297	<0.001
Hcy	0.162	<0.001
UA	0.041	0.344
FBG	0.106	0.015
INS	0.021	0.627
IL-6	0.179	<0.001
hs-CRP	0.350	<0.001
Age	0.242	<0.001
SBP	0.213	<0.001
DBP	0.171	<0.001
BMI	0.138	0.001

Results of Stepwise Multivariate Logistic Regression Analysis

Table 4 shows the results of stepwise multivariate logistic regression analysis. LDL-C (Ⅲ vs Ⅰ), ACS (yes vs no), HDL-C (Ⅲ vs Ⅰ), TC (Ⅲ vs Ⅰ), hs-CRP (Ⅲ vs Ⅰ), age (Ⅲ vs Ⅰ), BMI (Ⅲ vs Ⅰ) and smoking (yes vs no) were significantly associated with elevated MPO activities ($p<0.05$). Among them, LDL-C (Ⅲ vs Ⅰ) and ACS (yes vs no) showed the highest odds ratio in quantitative and qualitative variables, respectively.

Table 3. Univariate logistic regression analysis of the association between elevated serum MPO activities with each of the metabolic parameters and inflammatory markers in CHD patients

Variable	Odds ratio	95% CI	Wald	*p* value
LDL-C				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.11	0.73-1.69	0.23	0.635
Ⅲ tertile	7.38	4.51-12.09	64.14	<0.001
HDL-C				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	0.26	0.16-0.42	31.28	<0.001
Ⅲ tertile	0.13	0.08-0.20	71.25	<0.001
TC				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.67	1.10-2.54	5.69	0.017
Ⅲ tertile	5.67	3.57-9.02	53.88	<0.001
TG				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.74	1.14-2.65	6.69	0.010
Ⅲ tertile	3.83	2.46-5.97	35.43	<0.001
Hcy				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	0.85	0.56-1.28	0.63	0.427
Ⅲ tertile	1.83	1.20-2.81	7.75	0.005
UA				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	0.86	0.57-1.31	0.47	0.492
Ⅲ tertile	0.89	0.59-1.36	0.28	0.594
FBG				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	0.95	0.62-1.43	0.07	0.792
Ⅲ tertile	1.74	1.14-2.66	6.56	0.010
INS				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.06	0.70-1.61	0.08	0.785
Ⅲ tertile	0.96	0.63-1.45	0.05	0.832
IL-6				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.24	0.82-1.88	1.01	0.315
Ⅲ tertile	2.48	1.61-3.81	17.05	<0.001
hs-CRP				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.95	1.28-2.98	9.68	0.002
Ⅲ tertile	4.23	2.71-6.60	40.24	<0.001

Prevalence of ACS and High LDL-C Divided by Tertiles of MPO in CHD Patients

More than half of the CHD patients (50.2%) with elevated MPO activities had high LDL-C levels, 4 times higher than that (12.6%) in CHD patients

Table 4. Multivariate logistic regression (Stepwise Forward Wald) for elevated serum MPO activities in a sample of 536 CHD patients aged 36-85 years

Variable	Odds ratio	95% CI	Wald	*p* value
LDL-C				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.08	0.62-1.90	0.07	0.787
Ⅲ tertile	3.24	1.67-6.29	12.01	0.001
HDL-C				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	0.31	0.18-0.54	17.32	<0.001
Ⅲ tertile	0.19	0.11-0.34	30.91	<0.001
TC				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.57	0.88-2.80	2.34	0.126
Ⅲ tertile	2.94	1.55-5.58	10.83	0.001
hs-CRP				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.89	1.12-3.21	5.58	0.018
Ⅲ tertile	2.30	1.32-3.99	8.71	0.003
Age				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.54	0.90-2.65	2.48	0.115
Ⅲ tertile	2.37	1.36-4.13	9.31	0.002
BMI				
Ⅰ tertile	1	–	–	–
Ⅱ tertile	1.75	1.01-3.02	3.98	0.046
Ⅲ tertile	2.03	1.17-3.54	6.25	0.012
ACS	2.74	1.71-4.39	17.59	<0.001
Smoking	2.62	1.62-4.25	15.37	<0.001

According to the results of univariate analysis, fifteen variables included into the model were: LDL-C, HDL-C, TC, TG, Hcy, FBG, IL-6, hs-CRP, age, SBP, DBP, BMI, ACS, gender and smoking, and among them TG, Hcy, FBG, IL-6, SBP, DBP and gender were rejected by multivariate logistic regression analysis (method: Stepwise Forward Wald).

with normal-low MPO activities ($\chi^2=84.2$, $p<0.001$). The prevalence of ACS in the elevated MPO group was significantly higher than in the normal-low MPO group (82.2% vs 49.8%, $\chi^2=63.5$, $p<0.001$). The prevalence of ACS and high LDL-C levels in CHD patients divided by MPO tertiles is shown in **Fig. 2**. There was a significant trend toward an increase in the prevalence of ACS and high LDL-C levels from Ⅰ to Ⅲ MPO tertiles ($\chi^2=104.4$ and $\chi^2=73.8$, respectively, both $p<0.001$). The prevalence of ACS progressively increased from 46.4% in Ⅰ tertile to 68.0% in Ⅱ tertile, and to 88.8% in Ⅲ tertile of MPO distribution. Similarly, the prevalence of high LDL-C progressively increased from 8.9% in Ⅰ tertile to 31.5% in Ⅱ tertile, and to 59.8% in Ⅲ tertile of MPO distribution.

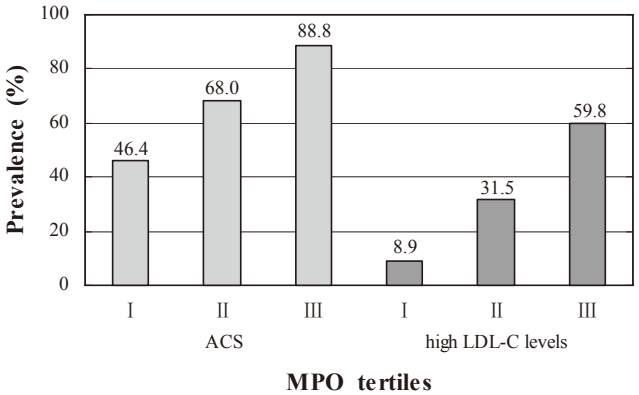

Fig. 2. Prevalence of ACS and high LDL-C levels divided by tertiles of MPO in 536 CHD patients aged 36-85 years.

Incidence of ACS During the Primary 6-Month Follow-Up in SAP Patients with Baseline MPO and LDL-C Levels

Results of a primary 6-month follow-up in 146 SAP patients aged 38-84 years revealed that elevated baseline MPO activities had a higher incidence of ACS (11/43) than that (9/103) of patients with normal-low baseline MPO activities (28.9% vs 8.3%, $\chi^2=10.10$, $p=0.001$); however, there was no significant different in the incidence of ACS between high baseline LDL-C and normal-low baseline LDL-C levels (17.6% vs 13.2%, $\chi^2=0.25$, $p=0.614$).

Discussion

Oxidative stress and inflammation play important roles in the pathogenesis of the destabilization of CHD leading to ACS. Infiltrating PMNs and macrophages participate in the transformation of stable coronary artery plaques to unstable lesions[6, 7]. Clinical and epidemiological studies have demonstrated that the deposition of LDL particles in the arterial intima precedes the formation of atherosclerotic lesions. In the extracellular matrix of arterial intima, LDL particles are oxidized to form oxidized LDL (ox-LDL). There is some evidence that lipoxygenase, peroxynitrite, oxygen-centered radicals and hypochlorous acid (HOCl) are involved in oxidative modification[9]. A likely source of HOCl in the arterial intima is MPO, a hemoprotein traditionally viewed as a microbicidal enzyme secreted by activated PMNs and macrophages, which is stored in azurophilic granules of these cells and released in the setting of the inflammatory process. MPO catalyzes the conversion of chloride and hydrogen peroxide to HOCl, resulting in LDL oxidation and conversion into high-uptake forms such as

ox-LDL for macrophages, leading to cholesterol deposition and foam cell formation *in vivo*[10-12].

Prospective-cohort, case-control and cross-sectional studies in a wide range of patients and populations have clearly indicated that MPO, in addition to traditional markers, is an important cardiovascular disease risk marker[13]. In the present study, ACS patients had significantly higher activities of MPO than SAP and non-CHD patients ($p<0.001$). The area under the ROC curve of MPO for diagnosing ACS was 0.84 (95% CI: 0.80-0.88). Multivariate logistic regression analysis revealed that LDL-C, ACS, HDL-C, TC, hs-CRP, age, BMI and smoking were significantly associated with elevated serum MPO activities. Among them, elevated MPO activities with LDL-C (Ⅲ versus I tertile, OR: 3.24, 95% CI: 1.67-6.29) and ACS (yes versus no, OR 2.74, 95% CI: 1.71-4.39) showed the highest odds ratio in quantitative and qualitative variables, respectively. The present study has provided valuable evidence that MPO is a good marker for diagnosing ACS and the elevated serum MPO activities are closely association with the prevalence of ACS and high LDL-C levels in CHD patients.

A case-control study revealed that elevated MPO levels in PMNs and whole blood was associated with the presence of coronary artery disease, which supported a potential role for MPO as an inflammatory marker in coronary artery disease and might have implications for atherosclerosis diagnosis and risk assessment[14]. Another study showed that MPO serum levels in patients with ACS powerfully predicted an increased risk for subsequent cardiovascular events, and extended the prognostic information[15].

More recently, several studies have investigated the value of MPO in predicting long-term outcomes of CHD. Li *et al*.'s study[16] during 6-month follow-up found that the ACS rate (36.2%) in the fourth quartile of the MPO level was 6 times higher than that (5.2%) in the first quartile of the MPO level ($p<0.01$). Cavusoglu *et al*.[17] investigated the long-term prognostic significance of baseline MPO levels in a well-characterized cohort of 193 men with ACS, and concluded that baseline MPO levels independently predicted myocardial infarction onset at 2 years in patients with ACS. In addition, Tang *et al*.'s prospective study[18] revealed that after adjusting for traditional cardiac risk factors, increased MPO was significantly associated with the incidence of major adverse cardiovascular events (MACEs) over the ensuing 3-year period.

We found that in addition to HDL-C, TC, TG, Hcy, FBG, IL-6, hs-CRP, age, SBP DBP and BMI, the serum MPO activities were mainly correlated with LDL-C levels in CHD patients. CHD patients with elevated MPO activities had higher LDL-C levels than CHD patients with normal-low MPO activities. The prevalence of ACS in the elevated MPO group was significantly higher than in the normal-low MPO group. There was a significant trend toward an increase in the prevalence of ACS as well as high LDL-C levels from I to Ⅲ MPO tertiles. Furthermore, the present primary prospective study demonstrated that elevated baseline MPO activities had a higher incidence of ACS than patients with normal-low baseline MPO activities. Although the primary 6-month follow-up study may not predict the long-term outcome, this finding suggests that MPO is a potential early warning marker for ACS in CHD patients.

Several factors cause high levels of LDL-C to contribute to the elevation of MPO activity in CHD, especially in ACS patients. Firstly, excess LDL infiltrates the artery and is retained in the arterial intima. Oxidative and enzymatic modifications lead to the release of inflammatory lipids that induce endothelial cells to express various types of leukocyte adhesion molecules, which cause blood cells including PMNs, mononuclear phagocytes, T-lymphocytes and mast cells on the vascular surface to adhere at the site of activation, with migration into and retention in arterial intima[19, 20]. Once resident in the intima, these cells undergo degranulation, releasing preformed MPO, tumor-necrosis factor-α (TNF-α), IL-6, interferon-γ, interleukin-1 and so on into the coronary circulation[21, 22]. Secondly, adipokines in adipose tissue, including leptin, adiponectin, and resistin, may also influence inflammatory responses throughout the organism[22, 23]. Thirdly, molecules generated during lipid peroxidation in CHD patients can induce protective as well as inflammatory reactions: for instance, by binding to nuclear receptors that control inflammatory genes. These inflammatory responses could cause increased MPO activity in the blood circulation[24].

On the other hand, MPO together with other cytokines might also influence LDL-C levels and promote ACS pathological events by PMNs recruitment. Enriched within arterial intima and atherosclerotic plaque, MPO serves as an enzymatic source of eicosanoids and bioactive lipids and generates atherogenic forms of both LDL and HDL. MPO has emerged as an enzymatic catalyst for LDL oxidation and carbamylation and conversion into more atherogenic high-uptake forms (oxidized-LDL and carbamylated-LDL) within the artery wall *in vivo*[25, 26]. In addition, MPO-

generated products promote impairment of the ability of apolipoprotein A1 (apoA-1) via its site-specific modification and dysfunction of HDL, which results in the reduction of cholesterol efflux, and the increase of lipid accumulation and foam cell formation[27]. Furthermore, MPO accelerates tissue damage of the atherosclerotic artery and affects the transformation from stable coronary artery plaques to unstable lesions, resulting in ACS by oxidative stress[28]. Therefore, the coexistence of high levels of MPO activity and LDL-C would synergistically prompt the progression of atherosclerosis and the development from SAP to ACS.

Limitations

Since the present study was only a single site study, and the follow-up period was short and the number of events was too small to perform multivariate analysis, a larger sample number in a multicenter study and longer prospective investigation are necessary to further confirm the value of elevated MPO activity as an early warning marker of ACS and other events in CHD patients.

Conclusion

The present study provides epidemiological evidence that elevated serum MPO activities are significantly associated with the prevalence of ACS and high LDL-C levels in CHD patients, and MPO may be a potential early warning marker for ACS.

Acknowledgements

This study was supported by the Sichuan Provincial Health Department Research Foundation of the People's Republic of China (No. 070080).

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