Usefulness of Serum Myeloperoxidase in Prediction of Mortality in Patients with Severe Heart Failure

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Abstract

Background: Myeloperoxidase levels were shown to reflect endothelial dysfunction, inflammation, atherosclerosis and oxidative stress.

Objectives: To examine the role of circulating myeloperoxidase, a leukocyte-derived enzyme, as a predictor of mortality in patients with congestive heart failure.

Methods: Baseline serum MPO levels were measured in 285 consecutive CHF patients and 35 healthy volunteers. N-terminal pro-brain natriuretic peptide and high sensitivity C-reactive protein concentrations were also measured. The primary outcome endpoint was overall mortality.

Results: MPO levels were significantly elevated in patients with CHF compared to healthy volunteers (P = 0.01). During a mean follow-up of 40.9 ± 11.3 months there were 106 deaths. On a univariate Cox regression analysis MPO levels were of marginal value (P = 0.07) whereas NT-proBNP was of considerable value (P < 0.0001) in predicting all-cause mortality. By dividing our cohort according to NT-proBNP levels into high, intermediate and low risk groups a clear difference in mortality was shown. By further dividing the patient cohort according to MPO levels above or below the median (122.5 ng/ml), mortality prediction improved in the patients with intermediate NT-proBNP values.

Conclusions: MPO levels are elevated in CHF and correlate with disease severity. MPO has an additive predictive value on mortality in patients with intermediate NT-proBNP levels.

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Congestive heart failure is a common syndrome affecting 10% of subjects older than 65 years. Despite improved understanding of the underlying pathogenetic mechanisms, CHF carries a significant morbidity and mortality burden and is the leading cause of hospitalization among individuals above the age of 65. In order to improve patient control, continuous attempts are undertaken to define markers of disease severity and progression [1-3].

Myeloperoxidase, a member of the heme peroxidase superfamily, is contained in azurophilic granules of neutrophils and monocytes. It is released upon leukocyte activation, contributing to innate immunity. MPO alters the biological function of surrounding proteins and lipids by halogenation, nitration and oxidative cross-linking [4]. In the cardiovascular system MPO has been linked to the development of atherosclerotic disease and phenotypic transition of stable to vulnerable plaque [5,6]. It carries prognostic implication among patients admitted with chest pain to the emergency department [7]. Several studies suggest a direct role for the enzyme in endothelial dysfunction by limiting nitric oxide bioavailability and through direct consumption of NO [8-10]. Recently, it was demonstrated in an animal model that MPO contributes to adverse remodeling and left ventricular dilatation after acute myocardial infarction, and may thus contribute to the development of heart failure [11,12]. Moreover, oxidative stress fingerprints as anti-oxidized low density lipoprotein antibodies, which may mirror MPO-mediated LDL oxidation, have been shown to reflect clinical control in heart failure patients [13]. In view of its possible role in heart failure, we tested MPO serum levels in heart failure patients as a potential predictor of prognosis.

MPO = myeloperoxidase
CHF = congestive heart failure
NT-proBNP = N-terminal pro-brain natriuretic peptide
NO = nitric oxide
LDL = low density lipoprotein
HFPSF = heart failure and preserved systolic function

Patients and Methods

The study included 285 consecutive patients with clinically controlled CHF attending the outpatient clinic of the Tel Aviv Sourasky Medical Center. Patients with chronic heart failure according to New York Heart Association functional class II–IV were recruited. The clinical diagnosis of heart failure was based on the history of acute pulmonary edema or two of the following signs or symptoms not explained by other identifiable causes and improved with diuresis: exertional dyspnea or fatigue, paroxysmal nocturnal dyspnea, orthopnea, pleural effusion, and bilateral lower extremity edema. Patients with left ventricular ejection fraction > 45% were diagnosed as having HFPSF.

At baseline, patients answered a detailed questionnaire on medical history, performance status, atherosclerotic risk factors and medications. Thereafter, patients were examined and baseline serum was drawn and kept frozen at –80°C until performance of the assays. Control, healthy subjects (n=35) were also recruited and blood was drawn and preserved in a similar manner.

Follow-up

Patients were followed every 3–6 months or more frequently as required. The study end-point was all-cause mortality. Two patients were lost to follow-up after 18 months.

Determination of circulating MPO

Quantitative determination of MPO was performed using a sandwich enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (Immundiagnostik AG, Germany).

Determination of NT-proBNP levels

Measurements of serum NT-proBNP were performed by automated immunoassay (Elecsys proBNP, Roche Diagnostics, Germany). The test principle includes using two polyclonal antibodies directed against N-terminal pro-BNP; epitope 1: amino acid 1-21 and epitope 2: amino acid 39-50. The results are calibrated against a synthetic N-terminal pro-BNP (amino acid 1–76). Range of results is between 5 and 35,000 pg/ml.

High sensitivity C-reactive protein

The assay for hsCRP was performed by nephelometry using a Boering BN II Nephelometer (DADE Boering, Marburg, Germany).

Statistical analysis

Comparison between CHF patients and healthy controls regarding MPO levels was performed using two-sample Student's *t*-test. Correlations between MPO and other variables were examined by Spearman rank correlation coefficient. The relationship between common risk factors and MPO was examined using two-sample Student's *t*-test. Cox proportional hazard model was applied to the data to study the association of MPO and NT-proBNP levels with the risk of mortality. Univariate models included each parameter separately, followed by multivariate models that included both parameters and the interaction between them. Additional models included background variables as well. All statistical analyses were performed using SAS for Windows, version 9.1.

Results

Baseline clinical characteristics of our cohort are presented in Table 1. The cohort comprised 206 patients (72%) with systolic heart failure and 79 (28%) with HFPSF. The mean duration of patients' follow-up was 40.9 ± 11.3 months (18.3–53.5 month). During follow-up there were 106 deaths (26.8%).

MPO levels in the CHF cohort were significantly elevated compared to a group of healthy volunteers (205.7 ± 272.6 vs. 123 ± 170.5 ng/ml, respectively, $P = 0.01$). Among the CHF patients MPO levels correlated positively with NYHA score ($r = 0.12$, $P = 0.04$) and the higher the NYHA score the higher the serum MPO levels. We also found a positive and significant correlation between MPO and hsCRP levels ($r = 0.18$, $P = 0.004$). MPO levels did not correlate with other clinical and laboratory data including patients' age, LVEF, weight and NT-proBNP levels, nor was there a statistically significant difference between MPO levels and the presence or absence of ischemic heart disease, diabetes mellitus, smoking, hypertension, hyperlipidemia, gender, and renal failure.

In order to define the clinical significance of MPO levels and its relation to NT-proBNP a Cox regression model was used. On univariate analysis, time to mortality was predicted by NT-proBNP ($P < 0.0001$, hazard ratio = 1.009, 95% confidence interval 1.007–1.011) while a trend toward statistical significance was observed with MPO levels ($P = 0.07$, HR = 1.95% CI 1-1.001). However, when incorporating both into a single adjusted model a synergistic value of prediction was observed, and both NT-proBNP and MPO significantly predicted time to mortality (for NT-proBNP

Table I. Clinical characteristics of the CHF outpatient cohort (n=285)

Age (mean ± SD) (yrs)	71.2 ± 11.3
Males (%)	215 (75.4%)
NYHA (mean ± SD)	2.7 ± 0.6
LVEF (mean ± SD)	37.1 ± 14.1%
Hyperlipidemia	173 (60.7%)
Smoking	85 (29.8%)
Hypertension	173 (60.7%)
Diabetes mellitus	111 (38.9%)
Ischemic heart disease	211 (74%)
Chronic atrial fibrillation	69 (24.2%)
Transient ischemic attacks/ cerebrovascular accidents	38 (13.3%)
Percutaneous transluminal coronary angioplasty or coronary artery bypass surgery	137 (48%)
ACE inhibitors/ARBs	236 (82.8%)
Beta blockers	199 (69.8%)
Digoxin	68 (23.8%)
Aspirin	193 (67.7%)
Spironolactone	154 (54%)
Diuretics	234 (82.1%)
Statins	178 (62.4%)

NYHA = New York Heart Association, LVEF = left ventricular ejection fraction, ACE = angiotensin-converting enzyme, ARB = angiotensinogen receptor blockers

Table 2. Cox regression model: variables as predictors of mortality

Variable	HR	95% CI	*P*
Age (yrs)	1.046	1.02–1.072	< 0.001
Weight (kg)	0.997	0.98–1.013	0.68
Female gender	0.953	0.55–1.651	0.86
NYHA	1.373	0.911–2.071	0.13
LVEF	0.984	0.968–1.001	0.06
Hyperlipidemia	0.669	0.432–1.036	0.05
Smoking	1.286	0.804–2.057	0.29
Hypertension	0.679	0.44–1.046	0.07
Diabetes mellitus	1.874	1.214–2.892	0.004
Ischemic heart disease	0.761	0.437–1.324	0.33
NT-proBNP	1.006	1.004–1.009	< 0.0001
MPO	1.001	1–1.001	0.07

hsCRP = high sensitivity C-reactive protein
NYHA = New York Heart Association
LVEF = left ventricular ejection fraction
HR = hazard ratio
CI = confidence interval

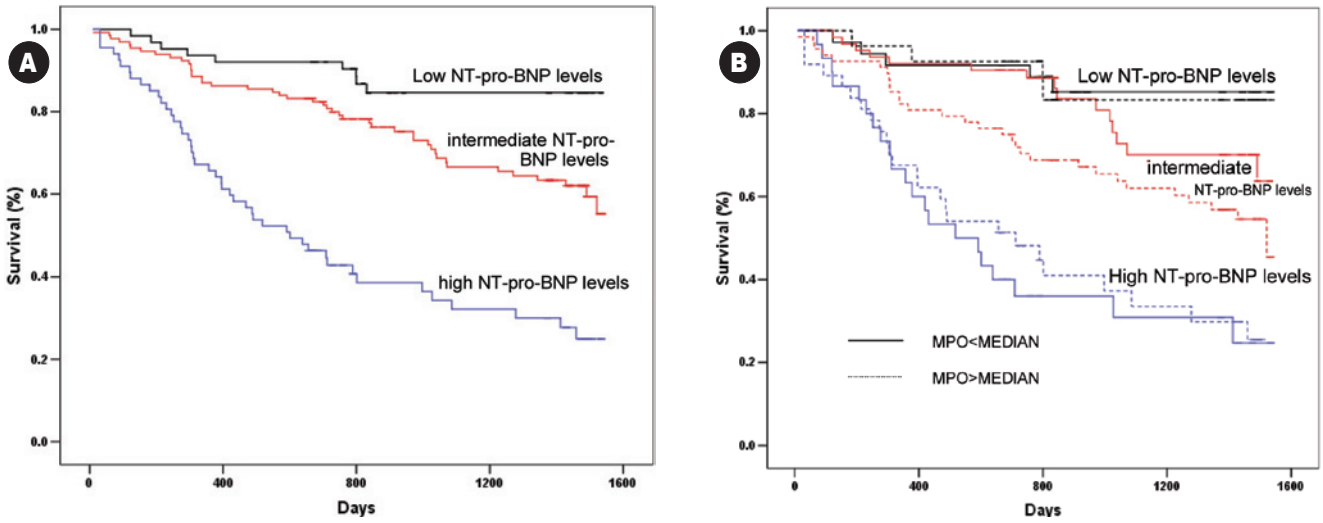

Figure 1. NT-proBNP and MPO in prediction of mortality in CHF patients. Patients were divided into three groups: Low risk with NT-proBNP levels below the 25th percentile (656 pg/ml); high risk with levels above the 75th percentile (4350 pg/ml), and intermediate risk group with levels in between. **[A]** Kaplan-Meier survival curve according to division into three groups ($P < 0.0001$. **[B]** Kaplan-Meier survival with further dividing the patients' cohort to MPO levels above (dotted line) or below (solid line) the median. $P < 0.0001$, for the intermediate risk group only $P = 0.04$.

$P < 0.0001$, HR 1.009, 95% CI 1.007–1.011; and for MPO $P = 0.02$, HR 1.001, 95% CI 1–1.001).

Results of multivariate Cox regression model are presented in Table 2. Advanced age, diabetes mellitus and NT-proBNP all independently predicted time to death, while MPO was borderline significant ($P = 0.07$).

To explore the additive value of MPO to NT-proBNP in predicting mortality, we divided our cohort into three groups according to NT-proBNP level: a low risk group with NT-proBNP level below the 25th percentile (NT-proBNP < 656 pg/ml), a high risk group with NT-proBNP level above the 75th percentile (NT-proBNP > 4350 pg/ml) and intermediate risk group with levels between these two groups. Using the Kaplan-Meier survival curve, a significant difference was demonstrated between the three groups; the best outcome was evident in the low risk group [Figure 1A]. Further dividing the patients according to MPO level above or below the median (122.5 ng/ml) improved mortality prediction in the intermediate risk group [Figure 1B]. The percentage of deaths in each subgroup is presented in Figure 2.

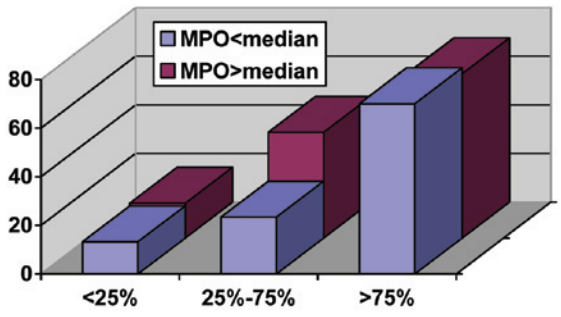

Figure 2. Percentage of death in each subdivision of patients' cohort into NT-proBNP levels belowthe 25th percentile, above the 75th percentile and in between and MPO levels above or below the median.

Stratifying our cohort according to the presence or absence of ischemic heart disease or diastolic versus systolic heart failure did not influence the predictive value of MPO.

Discussion

Heart failure continues to be a prevalent syndrome with substantial morbidity and mortality [1,2]. Ongoing research aims to define markers of disease severity in order to guide clinical management. NT-proBNP is a wildly accepted prognostic factor in heart failure [14,15]. However, the pathogenesis of heart failure is multifactorial, involving activation and dysregulation of multiple axes, many of which do not involve activation of nerurohormonal pathways and BNP. Therefore development of additional complimentary predictors is warranted.

In the current study we demonstrated that levels of circulating MPO are elevated in CHF patients and correlate with disease severity as reflected by the positive correlation with NYHA functional class. In our cohort, levels of MPO were not affected by the type of heart failure, i.e., systolic or heart failure with preserve systolic function and ischemic or non-ischemic cardiomyopathy, implying that the principal determinant of circulating MPO levels is severity of CHF. Also, MPO levels were not affected by common atherosclerotic risk factors.

On a Cox regression analysis, MPO together with NT-proBNP had a synergistic effect in predicting all-cause mortality. More importantly, we found that the main additive value of MPO was in the intermediate risk patient group, i.e., patients with NT-proBNP above 656 pg/ml and below 4350 pg/ml. This group of patients comprised approximately half of the total study cohort. This finding is of clinical relevance in further stratifying a large cohort of patients with ambiguous differential outcome.

In a study conducted in patients with LVEF < 35%, Tang et al. [16] demonstrated that MPO levels are related to right ventricular systolic dysfunction as well as to diastolic dysfunction mainly in

patients with LVEF < 20%. They also demonstrated a clear relationship between MPO level and prognosis. The difference from our results may be partially explained by the different patient population studied, i.e., our cohort includes also patients with heart failure and HFPSF and the mean LVEF is higher (37%). However, we could not demonstrate different MPO levels between patients with systolic or HFPSF. Larger multicenter trials may resolve this issue. In accordance with Tang et al. [16], MPO levels were not affected by the specific etiological factor of heart failure or its type.

Several observations led us to test MPO levels as a prognostic marker among heart failure patients. In humans, MPO levels were found to be associated with endothelial dysfunction [8]. I*n vitro* [9,17] histological [10] and animal models demonstrated a direct catalytic effect of nitric oxide by the enzyme as well as inhibition of its bioavailability. Endothelial dysfunction among heart failure patients was shown to be an independent prognostic factor. Potential mechanisms include loss of NO-dependent vasodilatation that contributes to impaired myocardial coronary flow, as well as augmented afterload and myocardial work. Indeed, impaired myocardial perfusion in patient with LV dysfunction adversely affects prognosis [18,19].

Recently, it was found in an animal model of acute myocardial infarction that MPO released from leukocytes infiltrating the necrotic zone might contribute to adverse ventricular remodeling through its inhibition of plasminogen activator inhibitor 1 causing an increased tissue plasminogen activity [11]. Also, several authors [20,21] demonstrated that inhibition of NO production impedes myocardial oxygen consumption and leads to adverse remodeling and ventricular dysfunction. Although not tested, these mechanisms may be operative in chronic CHF and influence the outcome of the patients.

Several lines of evidence implicate the role of MPO in oxidant generation in the cardiovascular system [22,23]. MPO is secreted upon leukocyte activation and resides in and around endothelial cells [24]; the enzyme and its oxidative products are abundant within human atheromas [4]. Moreover, studies in MPO knockout mice and humans with MPO deficiency [25] demonstrated a central role for MPO in lipid peroxidation. Assessment of oxidative stress in humans is complex and not standardized; nevertheless, markers of oxidative stress like anti-oxidized LDL antibodies did correlate with clinical control in patients with CHF [13]. MPO through its pivotal role in oxidant generation may thus not only stand as a marker, but could also affect prognosis in heart failure patients.

The fact that MPO and NT-proBNP do not correlate and have synergistic prognostic value may be related to their reflection of different aspects in the pathogenesis of heart failure. While NT-proBNP is related to activation of neurohormonal pathways and reflects ventricular wall stretch, MPO may mirror pathways such as inflammatory, endothelial dysfunction and oxidative stress. MPO could stand as a possible marker of these derangements or alternatively be an active participant in their pathogenesis.

In conclusion, we found that circulating MPO levels are elevated in heart failure patients and correlate with disease severity. MPO has an additive prognostic value over NT-proBNP testing and its principal prognostic value is in patients with intermediate NT-proBNP levels.

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