CLINICAL RESEARCH

Acute Myocardial Infarction

Plasma Concentrations of Myeloperoxidase Predict Mortality After Myocardial Infarction

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Objectives	This study investigated relationships between plasma myeloperoxidase (MPO), protein oxidation markers, and clinical outcome retrospectively in patients after acute myocardial infarction (MI).
Background	Reactive oxidants are implicated in cardiovascular disease, and elevated plasma MPO is reported to predict adverse outcome in acute coronary syndromes.
Methods	Detailed demographic information, radionuclide ventriculography, neurohormone measurements, and clinical history were obtained for 512 acute MI patients at hospital admission. Plasma levels of MPO and protein carbonyls were measured in patients and 156 heart-healthy control subjects. 3-Chlorotyrosine was measured in selected patients. Patient mortality was followed for 5 years.
Results	Plasma MPO and protein carbonyl concentrations were higher in MI patients 24 h to 96 h after admission than in control subjects (medians: MPO 55 ng/ml vs. 39 ng/ml, and protein carbonyls 48 pmol/mg vs. 17 pmol/mg protein, $p < 0.001$ for each). Both markers were significantly correlated with each other and with cardiovascular hormone levels. Chlorotyrosine was not elevated in patients with high MPO or carbonyl levels. Above-median levels of MPO but not protein carbonyls were independently predictive of mortality (odds ratio 1.8, 95% confidence interval 1.0 to 3.0, $p = 0.034$). Patients with above-median MPO levels in combination with above-median plasma amino-terminal pro-brain natriuretic peptide (NT-proBNP) or below-median left ventricular ejection fraction (LVEF) had significantly greater mortality compared with other patients.
Conclusions	Myeloperoxidase and protein carbonyl levels are elevated in plasma after acute MI, apparently via independent mechanisms. High MPO is a risk factor for long-term mortality and adds prognostic value to LVEF and plasma NT-proBNP measurements. (J Am Coll Cardiol 2007;49:1993–2000) © 2007 by the American College of Cardiology Foundation

Reactive oxygen species are important contributors to cardiovascular disease, including the events surrounding myocardial infarction (MI) (1,2). They have been implicated in numerous cardiovascular pathologies including endothelial dysfunction, plaque rupture, ventricular remodeling, and ischemia/reperfusion injury during MI (3,4). Injury can be the result of major insults such as lipid peroxidation or more subtle effects on signaling pathways (5,6). Reactive oxidants can modulate nitric oxide metabolism in endothelial cells (7,8), metalloprotease activation (9), and inactivation plasminogen activator inhibitor 1 (10).

There is increasing evidence that myeloperoxidase (MPO) contributes to cardiovascular disease (1,11,12). Myeloperoxidase is a neutrophil and monocyte enzyme that amplifies the reactivity of hydrogen peroxide through generation of hypochlorous acid, free radicals, and reactive nitrogen species (13). Myeloperoxidase and products of protein oxidation by hypochlorous acid have been detected in atheromatous lesions (14–17). High plasma MPO is reported to be a risk factor for early adverse cardiac events in patients with chest pain (18) or acute coronary syndromes (19) and to be associated with endothelial dysfunction (4,20). Elevations associated with heart failure (21,22)

**Abbreviations
and Acronyms**

ANP = atrial natriuretic
peptide

BNP = brain natriuretic
peptide

IQR = interquartile range

LVEDV = left ventricular
end-diastolic volume

LVEF = left ventricular
ejection fraction

LVESV = left ventricular
end-systolic volume

MI = myocardial infarction

MPO = myeloperoxidase

NT-proANP = amino-
terminal pro-atrial
natriuretic peptide

NT-proBNP = amino-
terminal pro-brain natriuretic
peptide

PTCA = percutaneous
transluminal coronary
angioplasty

might be useful for diagnosis in the community (23). Whether plasma MPO is a useful prognostic marker of long-term survival in MI patients has not been assessed.

We have compared plasma MPO levels in acute MI patients with matched control subjects and related them to markers of cardiac dysfunction (plasma amino-terminal pro-brain natriuretic peptide [NT-proBNP] and left ventricular ejection fraction [LVEF]) and subsequent 5-year mortality. We also investigated whether protein carbonyls, a widely used marker of protein oxidation (24), are increased in plasma after MI. To assess whether the presence of MPO in plasma resulted in hypochlorous acid formation and whether this could account for the carbonyl formation, 3-chlorotyrosine, a specific marker of hypochlorous acid reacting with tyrosine residues (25), was measured in a subgroup of patients.

Methods

Patients. Christchurch Hospital provides tertiary cardiac services to a New Zealand population of approximately 500,000. Patients admitted with acute MI between November 1994 and June 2001 were recruited to the Christchurch Cardioendocrine Post Myocardial Infarction study (26,27). A subgroup of 512 was randomly selected for this study. Inclusion criteria included age <80 years, absence of cardiogenic shock, and survival for at least 24 h after MI. Acute MI was defined by the presence of typical cardiac ischemic symptoms, ischemic change on the electrocardiogram in 2 or more contiguous leads, and peak elevation of plasma creatine kinase to at least twice normal (400 U/l). All patients were troponin T positive (≥ 0.17 μg/ml). Mortality was followed for 5 years. Five patients were lost to follow-up, although their data until withdrawal were included. Date of death was confirmed by searching the National Health Index linked to the National Register of Deaths.

The mean age of patients was 61.7 years (range 32 to 80 years), and 80% were male. Thirty-eight percent had dyslipidemia, 38% had documented hypertension, 13% had type 2 diabetes, 9% had heart failure, 37% had angina, and 18% had a previous history of MI. Current smokers represented 30%, and 37% had never smoked. The control population consisted of 149 volunteers (70% male) randomly selected from the Christchurch electoral roll, with a mean age of 64.7 years (range 42 to 83 years). The absence

of cardiovascular disease (heart failure, coronary artery disease, or stroke) in control subjects was determined by screening the National Health Information Database for previous hospital admissions and by a questionnaire confirming that subjects had no cardiovascular symptoms. Of the control subjects, 5% were current smokers and 51% had never smoked. The study was approved by the Canterbury Ethics Committee (ethics reference: CTY/94/08/783 for Post-MI Study, CTY/01/05/062 for Healthy Volunteer Study) and conformed to the principles outlined in the Declaration of Helsinki. All participants gave written, informed consent.

LV function. Left ventricular function, including LVEF and left ventricular end-systolic volume (LVESV), was assessed by radionuclide ventriculography within 24 h of blood sampling. A General Electric 400AC gamma camera interfaced to a 3000I computer system (General Electric Medical Systems, Milwaukee, Wisconsin) was used, after standard in vivo technetium-99m red blood cell labeling.

Biochemical and hormone assays. Blood samples were taken 24 to 96 h after admission to hospital and again at 4 months, in the morning, from an indwelling intravenous cannula placed at least 30 min before sampling and with the patient resting quietly while semi-recumbent. Where patients received thrombolytic agents or underwent percutaneous transluminal coronary angioplasty (PTCA), samples were taken subsequent to treatment. Most patients received heparin within a few hours of hospital admission. All blood samples were collected in chilled ethylenediaminetetraacetic acid (EDTA) tubes, placed immediately on ice, and centrifuged within 20 min at 4°C. Plasma samples were stored at $-80°C$.

Protein carbonyls were analyzed on the EDTA plasma by enzyme-linked immunosorbent assay (ELISA) after derivatization with 2,4-dinitrophenylhydrazine (28) with Zentech PC kits (BioCell, Auckland, New Zealand). We have found protein carbonyls to be stable for a number of years of storage at $-80°C$, and consistent with this, no relationship was seen between date of collection of patient sample and protein carbonyl level (r = 0.01, p = 0.76).

Myeloperoxidase was measured by sandwich ELISA on plasma diluted 1:10, with a monoclonal antibody (Abcam, Cambridge, United Kingdom) and a rabbit polyclonal antibody produced in-house. The detection range was 0.3 to 25 ng/ml, and the coefficient of variation was 13%. Control and patient samples were included on each plate. Results obtained with the in-house assay were linearly correlated (Pearson correlation coefficient 0.91, n = 53) but were 3.3-fold higher than those obtained with the Oxis MPO ELISA (Foster City, California). This difference is relevant when comparing studies. Median values of 120 and 198 pmol/l (18 and 30 ng/ml) reported by Brennan et al. (18) for control subjects and chest pain patients were obtained with the Oxis assay. The 10-fold higher median of 287 ng/ml reported by Baldus et al. (22) for acute coronary syndromes, measured with the Calbiochem ELISA, was obtained for

serum, which we have found to give higher MPO values than matched plasma samples.

Heparin administration to the patients was a possible complication, because it can mobilize MPO from the endothelium where it binds to negatively charged proteoglycans (20,29). Associations between heparin treatment and MPO were investigated retrospectively by reading hospital notes of the patients with the highest MPO levels (n = 20, mean MPO level = 185 ng/ml) and a random selection of other patients (n = 12, mean MPO level = 44 ng/ml). Approximately 60% of both groups were being treated with heparin at the time of sampling. There was also no association between MPO level and heparin treatment (median MPO, heparin treated: 145 [67 to 169], untreated: 149 [35 to 193] ng/ml, p = 0.93). In a preliminary kinetic study in 10 patients (M. Nallaratnam and T. Mocatta, personal communication, 2007), MPO was found to increase initially after heparin administration, in accordance with the findings of Baldus et al. (20), but was already declining at 2 h to 4 h and had returned to baseline within 24 h. The majority of the highest MPO patients had commenced heparin treatment at least 24 h before sampling. In light of these findings, analysis of hospital notes for additional patients was deemed unnecessary.

3-Chlorotyrosine was measured in 4 groups of plasma samples from patients selected on the basis of whether they had high or low protein carbonyl and MPO levels and from a random selection of control subjects. Total plasma proteins were hydrolyzed with methane sulfonic acid with phenol added to minimize artifactual chlorination during sample preparation, as described previously (30). Tyrosine and 3-chlorotyrosine were derivatized and analyzed by isotope dilution gas chromatography/mass spectrometry with a Hewlett Packard 6890 gas chromatograph equipped with a ZB-50 capillary column interfaced to a Hewlett Packard 5973 mass spectrometer (Hewlett Packard, Palo Alto, California). Artifactual tyrosine chlorination, measured with a second isotopically labeled tyrosine, accounted for an average of 10 chlorotyrosine/million tyrosines and has been subtracted. Interassay and intra-assay variation was <5% for values >80 chlorotyrosine/million tyrosine, and 10 was the detection limit. Human serum albumin (Albumex, Commonwealth Serum Laboratories, Melbourne, Australia) or control plasma (40 mg/ml protein in phosphate-buffered saline pH 7.4) was treated with hypochlorous acid solution (Sarah Lee, Auckland, New Zealand), by rapidly mixing equal volumes. After 30 min, excess oxidant was quenched with 1 mmol/l methionine and aliquots of the protein were assayed for protein carbonyls and chlorotyrosine.

Atrial natriuretic peptide (ANP) (31), amino-terminal pro-atrial natriuretic peptide (NT-proANP) (31), brain natriuretic peptide (BNP) (31), NT-proBNP (32), norepinephrine (31), and adrenomedullin (33) were measured by radioimmunoassay. Creatine kinase and troponin T were measured with commercial ELISA kits (Boehringer Mannheim, Germany).

Statistical analysis. Levels of MPO and protein carbonyls in MI patients were compared with levels in control subjects with analysis of variance (ANOVA). Within the MI patient group, relationships between MPO and protein carbonyl levels and demographic data, treatment, ventricular function, and neurohormone levels were tested with chi-square, ANOVA, and Pearson's correlation analyses. Levels of MPO, protein carbonyls, and neurohormones displayed skewed distributions and were log transformed before analysis. Results are expressed as medians and interquartile ranges (IQR). Bivariate relationships with measures of cardiac function, including LVEF and LVESV, and levels of circulating hormones were explored with Pearson's correlation in MI patients. Kaplan-Meier curves of patients with above/below-median MPO and protein carbonyls were compared with log-rank tests. Multivariate analysis was performed with a Cox proportional hazards model to determine independent associations between MPO and established risk factors (age, gender, type 2 diabetes, LVEF, plasma NT-proBNP, and plasma endothelin levels) and survival. Survival in patients from risk groups (above/below median MPO in combination with either above/below median LVEF or above/below median plasma NT-proBNP) was compared with Kaplan-Meier curves and log-rank tests. Statistical analyses were performed with SPSS version 11 (SPSS Inc., Chicago, Illinois). A p value <0.05 was considered significant.

Results

Elevated MPO and protein carbonyl concentrations in MI plasma. The characteristics of the acute MI patients are given in Table 1. The MPO levels were elevated in plasma obtained from these patients 24 to 96 h after admission (median and IQR: 55 [38 to 75] ng/ml, n = 490) compared with control plasma (39 [29 to 49] ng/ml, n = 146, p < 0.001), as shown in Figure 1A. Female patients (61 [42 to 83] ng/ml, n = 97) had significantly higher MPO levels than male patients (53 [37 to 73] ng/ml, n = 393; p = 0.029).

The MI patients also had significantly elevated plasma concentrations of protein carbonyls 24 h to 96 h after admission (48 [23 to 73] pmol/mg protein, n = 508) compared with control subjects (17 [0 to 50] pmol/mg, n = 156, p < 0.001) (Fig. 1B). There was no significant difference between men and women (p = 0.239). There was a weak positive correlation between concentrations of protein carbonyls and MPO (r = 0.096, p = 0.033).

For the patient samples collected in the 1 to 4 days after hospital admission, levels of MPO or protein carbonyls were no higher in those closer to admission (r = 0.05, p = 0.47 and r = 0.03, p = 0.28, respectively, for the relationship between marker level and day of sampling, n = 421).

Table 1	Characteristics of MI Patient Population
Demographic data	
Number (M/F)	512 (410/102)
Age (yrs)	61.7 ± 11.0
Age range (yrs)	32–80
Mortality over 5 yrs of follow-up	78 (15.3%)
Previous medical history	
Type 2 diabetes	63 (12.7%)
Previous MI	85 (17.5%)
Drug treatments/interventions	
Statin treatment before admission	13 (2.5%)
Thrombolytic treatment at admission	304 (62.6%)
PTCA	124 (24.8%)
ACE inhibitor treatment at discharge	210 (41.2%)
Beta-blocker treatment at discharge	363 (71.2%)
Cardiac function	
ST-segment elevation	415 (81.1%)
Plasma creatine kinase (U/l)	1,658 (923–2,790)
Troponin T (μg/l)	8.0 (3.5–15.6)
LVEF (%)	48 ± 12
LVEDV (ml)	159 ± 50
LVESV (ml)	86 ± 43
Neurohormone markers	
ANP (pmol/l)	28 (19–39)
NT-proANP (nmol/l)	1,230 (645–1,965)
BNP (pmol/l)	20 (13–29)
NT-proBNP (pmol/l)	110 (68–182)
cGMP (pmol/l)	7.0 (5.0–9.0)
Adrenomedullin (pmol/l)	9.3 (7.4–12.8)
Norepinephrine (pmol/l)	2,543 (1,840–3,521)

Age and ventriculography data are expressed as means and SD, plasma hormone and cardiac markers as medians and interquartile ranges.

ACE = angiotensin-converting enzyme; ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; cGMP = cyclic guanosine monophosphate; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; MI = myocardial infarction; NT-proANP = amino-terminal pro-atrial natriuretic peptide; NT-proBNP = amino-terminal pro-brain natriuretic peptide; PTCA = percutaneous transluminal coronary angioplasty.

Protein carbonyls also remained high at 4 months (59 [30 to 84] pmol/mg protein, n = 242, p = 0.047).

Associations with heart function, severity indexes, and treatment in MI patients. Neurohormonal levels and ventriculography indexes are established prognostic markers after MI (26,27). There were weak, statistically significant positive associations between plasma MPO levels and circulating levels of BNP, NT-proBNP, cyclic 3′,5′-guanosine monophosphate (cGMP), adrenomedullin, and norepinephrine, measured in patients at admission [r = 0.14 to 0.26, p < 0.05]. There was no correlation with ANP or NT-proANP. Protein carbonyl levels were positively correlated with ANP, NT-proANP, BNP, NT-proBNP, cGMP, and norepinephrine levels (r = 0.13 to 0.16, p < 0.05) but not adrenomedullin. Protein carbonyls were also correlated with ventriculography markers of cardiac dysfunction, LVESV (r = 0.10, p = 0.04) and LVEF (r = −0.10, p = 0.03).

There were no significant differences in either marker between patients who did and did not have PTCA (n = 124

vs. 377, MPO p = 0.61; protein carbonyls p = 0.55) or treatment with statins (n = 13 vs. 471, MPO p = 0.35; protein carbonyls p = 0.54) at the time of admission. Although MPO levels did not differ between patients who received thrombolytic therapy during admission and those who did not (n = 304 vs. 182, p = 0.79), protein carbonyl levels were significantly lower in those receiving treatment (treated: 41 [19 to 68], untreated: 59 [39 to 84] pmol/mg, p < 0.001). There were no significant differences in MPO levels between patients treated or not treated with angiotensin-converting enzyme inhibitors (n = 210 vs. 300, p = 0.44) or beta-blockers at discharge (n = 363 vs. 147, p = 0.35). Protein carbonyl levels did not differ in patients treated with angiotensin-converting enzyme inhibitors (p =
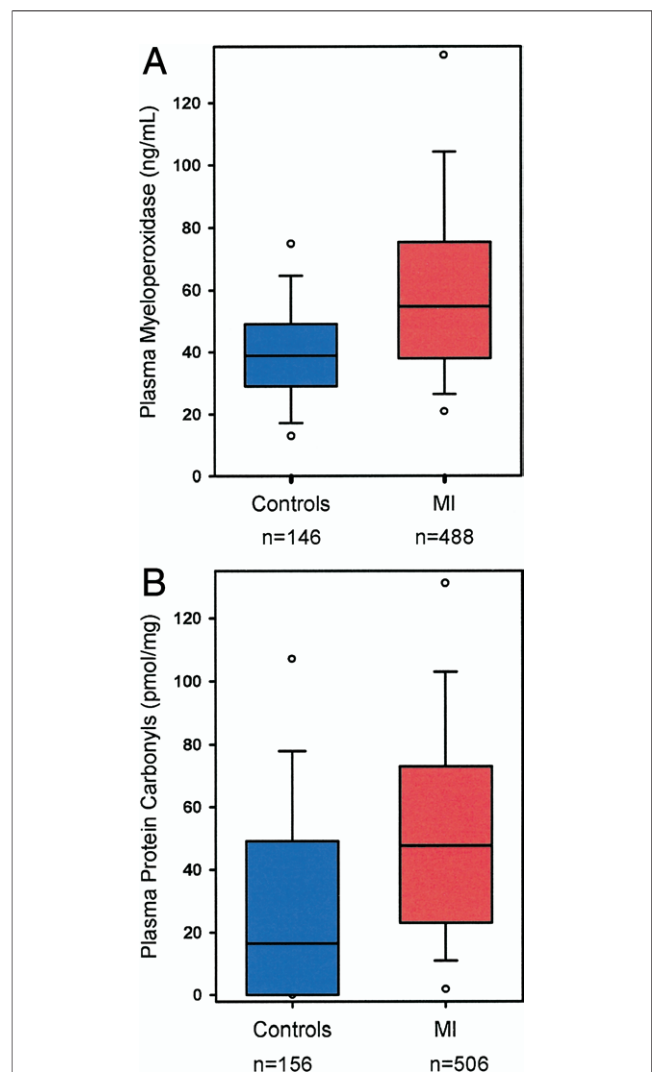

Figure 1 — **Plasma Concentrations of Myeloperoxidase and Protein Carbonyls in Patients After MI**

(A) Myeloperoxidase and (B) protein carbonyls, with patient data in **red** and control data in **blue**. **Boxes** show medians and interquartile ranges with **whiskers** and **symbols** representing the 10th/90th and 5th/95th percentiles, respectively. Both markers were significantly higher for patients than control subjects (p < 0.001). MI = myocardial infarction.

0.21) but were lower in patients prescribed beta-blockers (treated: 44 [20 to 70], untreated: 59 [35 to 86] pmol/mg, $p < 0.001$).

Relationships to MI patient survival. Of the MI patients, 78 died during the 5-year follow-up. Above-median levels of MPO were significantly associated with a nearly 2-fold increase in mortality (above-median MPO mortality: 21%, below-median MPO mortality: 10%, p = 0.001) (Fig. 2A). There was no significant association between levels of protein carbonyls and survival (p = 0.31) (Fig. 3B).

Cox proportional hazards analysis indicated that older age, pre-existing type 2 diabetes, below-median LVEF, and above-median levels of plasma NT-proBNP and plasma MPO were significantly predictive of mortality in this MI
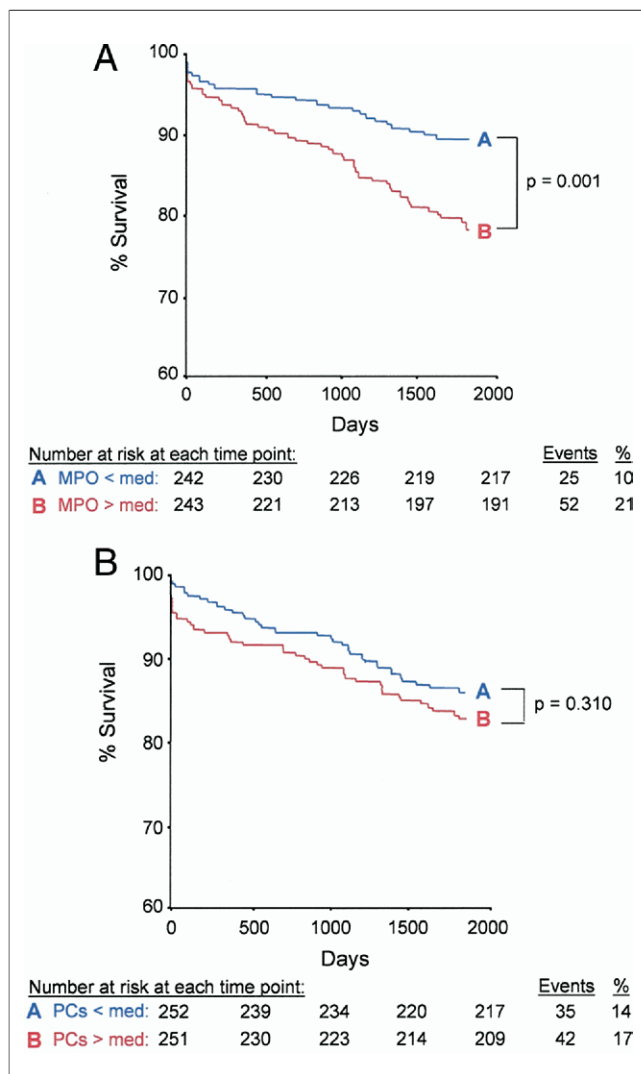

Figure 2   Kaplan-Meier Cumulative Survival Curves for MI Patients Show a Difference for MPO But Not PCs

(A) Comparison of patients with **(red)** above-median and **(blue)** below-median myeloperoxidase (MPO) concentrations (median 55 ng/ml; p = 0.001); and **(B)** patients with above- and below-median protein carbonyls (median 48 pmol/mg; p = 0.31), over a 5-year follow-up. MI = myocardial infarction; PC = protein carbonyl.
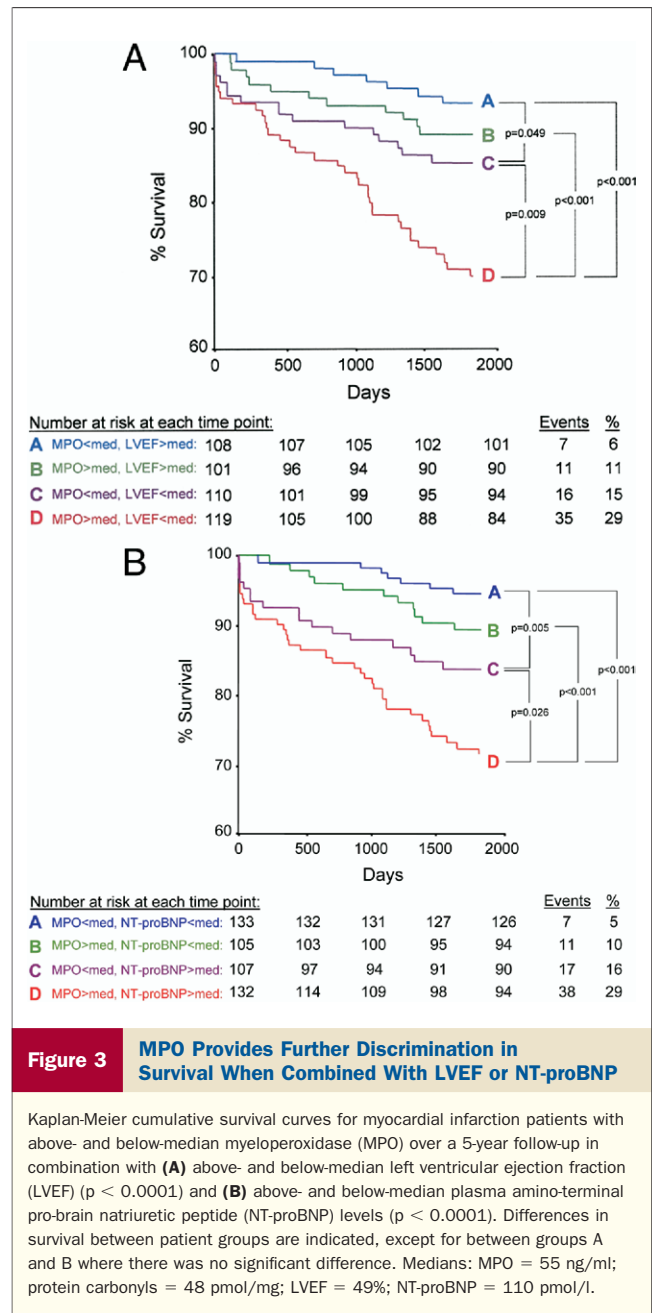

Figure 3   MPO Provides Further Discrimination in Survival When Combined With LVEF or NT-proBNP

Kaplan-Meier cumulative survival curves for myocardial infarction patients with above- and below-median myeloperoxidase (MPO) over a 5-year follow-up in combination with **(A)** above- and below-median left ventricular ejection fraction (LVEF) (p < 0.0001) and **(B)** above- and below-median plasma amino-terminal pro-brain natriuretic peptide (NT-proBNP) levels (p < 0.0001). Differences in survival between patient groups are indicated, except for between groups A and B where there was no significant difference. Medians: MPO = 55 ng/ml; protein carbonyls = 48 pmol/mg; LVEF = 49%; NT-proBNP = 110 pmol/l.

patient cohort. Above median MPO contributed to mortality independently of the other factors (p = 0.03, risk ratio = 1.8, 95% confidence interval 1.1 to 3.1) (Table 2). The effects of MPO and both LVEF and plasma NT-proBNP were additive in predicting post-MI mortality (Fig. 3). Patients with above-median MPO in combination with a below-median LVEF (Fig. 3A) had significantly worse survival than all the other groups, with 5-fold higher mortality than those with low MPO and high LVEF. Similarly, patients with above-median levels of MPO and plasma NT-proBNP (Fig. 3B) had significantly worse survival than other groups and 6-fold greater mortality than those with below-median levels of both markers.

Table 2	Cox Proportional Hazards Analysis of Factors Predictive of Death			
	Variable	Risk Ratio	95% CI	p Value
	LVEF (above- vs. below-median)	2.54	1.43–4.52	**0.002**
	Type 2 diabetes	2.24	1.25–4.03	**0.007**
	Plasma NT-proBNP (above- vs. below-median)	1.83	1.03–3.26	**0.039**
	Plasma MPO (above- vs. below-median)	1.81	1.07–3.05	**0.026**
	Gender (male vs. female)	1.30	0.71–2.38	0.395
	Age (yrs)	1.05	1.02–1.08	**0.001**
	Plasma protein carbonyls (above- vs. below-median)	0.85	0.52–1.39	0.513

Significant ($p < 0.05$) p values indicated in **bold type**. Medians: LVEF = 49%, NT-proBNP = 110 pmol/l, MPO = 55 ng/ml, protein carbonyls = 48 pmol/mg.

CI = confidence interval; MPO = myeloperoxidase; other abbreviations as in Table 1.

Relationships to 3-chlorotyrosine levels. To examine whether high MPO levels in MI plasma were active in producing hypochlorous acid and whether hypochlorous acid could be the source of the elevated protein carbonyls, chlorotyrosine was measured in total plasma protein from selected patients with upper or lower quartile levels of protein carbonyls, MPO, or both. In all the groups, chlorotyrosine was close to the level of detection and no higher than in control plasma (Table 3). There were no significant differences between groups that varied by 5-fold in mean MPO concentration or by 20-fold in protein carbonyls. If hypochlorous acid were the source of protein carbonyls in the patient samples, relative amounts of carbonyls and chlorotyrosine should be similar to those in purified human albumin or control plasma that had been treated with reagent hypochlorous acid. However, for both albumin and plasma, amounts of hypochlorous acid that gave protein carbonyls in the range seen for patient plasma generated 100 times more chlorotyrosine than measured in the patient samples (Fig. 4). These results imply that any hypochlorous acid generated by MPO in the plasma is insufficient to be detected by this sensitive technique and unlikely to be the source of protein carbonyls.

Table 3	3-Chlorotyrosine Levels in Total Plasma Protein From MI Patients With High or Low MPO and Protein Carbonyls		
Group	MPO (ng/ml)	Protein Carbonyls (pmol/mg)	Cl-Tyr/Million Tyr
1: high MPO/high PC	126 ± 39	113 ± 21	8 ± 6
2: high MPO/low PC	126 ± 73	15 ± 10	12 ± 10
3: low MPO/high PC	36 ± 9	93 ± 21	12 ± 9
4: low MPO/low PC	26 ± 8	4 ± 5	19 ± 21
Control subjects	52 ± 7	26 ± 28	15 ± 5

Group 1, protein carbonyls (PC) and myeloperoxidase (MPO) levels in the upper quartile; group 2, upper quartile MPO and lower quartile carbonyls; group 3, lower quartile MPO and higher quartile carbonyls; group 4, both markers in the lower quartile. Each group contained 12 to 14 samples except control subjects (n = 5); means ± SD are shown. There were no significant differences in chlorotyrosine (Cl-Tyr) levels between any of the groups.

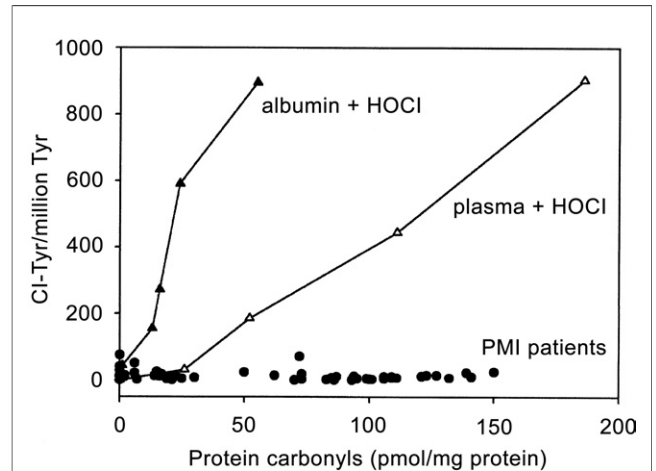MI = myocardial infarction; Tyr = tyrosine.

Figure 4	Relationships Between Protein Carbonyls and 3-Chlorotyrosine

The oxidative markers were generated in human serum albumin **(solid triangles)** and normal plasma **(open triangles)** by treatment with hypochlorous acid (HOCl) and compared with those for myocardial infarction plasmas **(circles)**. Cl-Tyr = chlorotyrosine; PMI = post-myocardial infarction; Tyr = tyrosine.

Discussion

We found that plasma concentrations of both MPO and protein carbonyls measured after hospital admission for acute MI patients were significantly higher than in a matched control population. Levels of MPO were independently predictive of 5-year survival and might provide additional prognostic information if used in combination with the established markers, LVEF and NT-proBNP.

MPO and adverse clinical outcomes. Our observed 1.4-fold higher median MPO concentration in MI patients than in control subjects is comparable to the difference reported for patients with chest pain (18), 23% of whom had MI at evaluation. Our results are consistent with the MPO content of neutrophils being low in MI patients (34) and the source of the MPO being neutrophil degranulation. The markers did not appear acutely elevated in samples collected closest to the onset of symptoms. This agrees with low neutrophil content of MPO persisting for 72 h (34) and implies that elevation is not a transient event. Although release of MPO into plasma from the endothelium owing to heparin (20,29) was a potential confounder, we found no evidence that the extent or timing of heparin administration was responsible for the high MPO levels. However, the impact of heparin administration on MPO levels in heart patient needs further, more direct investigation.

A striking finding in previous studies of patients with chest pain (18) and acute coronary syndromes (19) was that plasma MPO concentration at enrolment was a strong predictor of adverse outcomes (MI or death) that occurred within hours or a few days, especially in patients with low troponin T or C-reactive protein. Those studies suggested that MPO measurements might have clinical utility for identifying risk of imminent cardiac events in a mixed

cohort of acute cardiac and non-cardiac patients where MI is not yet confirmed. Our study focused on a different patient population, with a confirmed MI diagnosis who had already survived 24 h to 96 h after MI. This study design would have excluded many of the early events. In this group, plasma MPO levels were independently prognostic of mortality over a 5-year follow-up. Furthermore, MPO in combination with LVEF or NT-proBNP provided more discrimination than any 1 measure. Mortality was 6-fold higher for patients with both MPO and NT-proBNP above the group median than for those with both below the median, and significant differences were also seen between above- and below-median MPO and LVEF groups. These novel findings suggest that measurement of MPO after MI could give long-term prognostic information and improve risk stratification, particularly when used in combination with the established markers, LVEF and plasma NT-proBNP. As analyzed by Ng et al. (23) for screening for heart failure, inclusion of MPO might also provide a cost benefit.

Although MPO levels were positively correlated with a range of neurohormone markers of severity, these relationships were weak and the adverse association between MPO and survival was independent of LVEF or NT-proBNP. This suggests that MPO levels contribute to mortality independently of the degree of cardiac remodelling or neurohormonal activation after MI.

Acute treatment with statins has been reported to downregulate MPO expression in macrophages (35). We saw no influence on plasma levels of MPO by prior treatment with statins, but the treated numbers are small and this needs substantiating with a larger study. The MPO levels were also not affected by PTCA or thrombolytic drugs.

Oxidized proteins, MPO, and acute MI. Protein carbonyls are elevated in heart failure (21) but have not been measured previously in coronary heart disease. The higher plasma concentrations in MI patients than control subjects provide evidence of oxidative stress and protein oxidation in these patients. These findings complement other reports of raised levels of lipid peroxidation products such as 8-isoprostane (36,37) and another putative oxidative marker, advanced oxidation protein products (38). Transient increases in isoprostanes have also been detected in coronary sinus after angioplasty and reperfusion (37,39). High protein carbonyls were not associated with increased mortality. However, they were weakly correlated with circulating levels of prognostic hormone markers and with indexes of poor cardiac function.

Although plasma MPO was elevated in MI patients, we found no corresponding increase in chlorotyrosine. Therefore, it is highly unlikely that MPO activity is the source of protein carbonyls in MI patient plasma. Other oxidants formed during ischemia and reperfusion or by activation of vascular nicotinamide adenine dinucleotide (phosphate) oxidases (2,5,6) are possible alternative sources. The continued elevation in protein carbonyls at 4 months suggests

that levels might be chronically raised in association with the underlying vascular disease.

Proteins from atherosclerotic lesions contain more chlorotyrosine than control aorta (16), and high-density lipoprotein has been shown to be preferentially chlorinated (17,40). Our observations imply that any chlorinated proteins released from the lesion are insufficient to elevate total plasma levels. The low chlorotyrosine levels in plasma with high MPO suggests that circulating MPO might have low chlorinating ability. Possible explanations include the enzyme being inactive or limited by the availability of hydrogen peroxide.

Conclusions

Plasma MPO and protein carbonyl levels were higher in patients after MI than in control subjects. High levels of MPO were independently prognostic of mortality over a 5-year follow-up and, in combination with low LVEF or high plasma NT-proBNP, were associated with an even greater mortality than any of these risk factors alone. Myeloperoxidase shows promise as a prognostic marker of long-term mortality in patients with a confirmed MI diagnosis, particularly when used in combination with the other established markers.

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