Case Report

Serial Analyses of C-Reactive Protein and Myeloperoxidase in Acute Coronary Syndrome

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ABSTRACT

Background: C-reactive protein (CRP) and myeloperoxidase (MPO) are involved in the pathogenesis of atherosclerosis, mainly during periods of instabilization. This study aims to test the hypothesis that patients with acute coronary syndrome (ACS) maintain a persistent inflammatory state, and that this is associated with long-term mortality.

Hypothesis: We hypothesized that serum C-reactive protein and myeloperoxidase collected at the index event and later, could add to the prognostic information in patients with ACS.

Methods: In a prospective cohort of 115 consecutive patients with ACS, myeloperoxidase and C-reactive protein were measured at admission and 2 y later. Patients were followed-up for the occurrence of cardiac death and other major cardiac events.

Results: Levels of CRP decreased from 26±34 mg/L in the acute phase to 6±8 mg/L in the chronic phase ($p<0.001$), and MPO levels decreased from 86±43 pM to 27±32 pM ($p<0.001$). After 29±12 mo, 27% patients died, 39% had new episode of ACS, and 30% underwent revascularization procedures. Initial CRP levels above 10 mg/L were associated with higher long-term mortality (hazard ratio [HR]: 2.43; 95% confidence interval [CI]: 0.98 to 6.07; $p = 0.048$). MPO levels were not associated with death or other major events.

Conclusions Changes over time or absolute values in the chronic phase of both markers were not associated with clinical outcomes. CRP levels, but not MPO levels, in the index event were predictive of long-term cardiovascular mortality.:

Key words: inflammation, unstable angina, myocardial infarction, prognosis, markers

Introduction

Distinct inflammatory pathways have been implicated in atherogenesis, involving endothelial cells, blood, and molecular mediators through a process at local, myocardial, and systemic levels.[1–3] Much data exist on the prognostic capacity of C-reactive protein (CRP) determined in patients with acute coronary syndrome (ACS).[4] Some, but not all, studies have demonstrated an association between CRP levels and increased mortality.[5–8] A novel inflammatory marker, myeloperoxidase, released by neutrophils activation, has also shown potential for risk stratification in ACS patients. Myeloperoxidase (MPO) presents potent pro-inflammatory properties, contributing directly to endothelial injury.[9] It is plausible to speculate that elevation of circulating inflammatory markers during ACS may indicate intensification of such processes, therefore contributing to plaque vulnerability. Nonetheless, serial and long-term reassessment of myeloperoxidase in individuals that presented an episode of ACS has not been described and could add in the rational use of this marker in clinical practice. This study was thus conducted to test the hypothesis that patients who presented with ACS maintain a persistent inflammatory state with elevated serum markers, which may be associated with long-term prognosis.

Methods

This is an observational, prospective study, nested in a cohort of 740 consecutive patients with acute chest pain enrolled from the emergency department of a university hospital.[10] A subgroup of 115 patients who had a final diagnosis of ACS with blood samples collected at admission was enrolled. Clinical characteristics of this subgroup were not different from the total group of cases followed-up in the period.[10] The study was approved by the Research Ethical Committee of the institution and all participants gave written informed consent.

The diagnosis of myocardial infarction was made according to the World Health Organization's criteria. All patients were followed-up until hospital discharge or death. Major

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cardiac events and procedures were recorded during hospitalization. After 2 y, patients were contacted, an ambulatory interview was performed, and blood samples were collected in the stable period. After a mean follow-up period of 29±12 mo, patients were assessed for the occurrence of major cardiac events, which included new episodes of ACS, supraventricular or ventricular arrhythmias, revascularization procedures, heart failure, cardiovascular hospitalization, or death. The primary endpoint of the study was cardiovascular mortality.

Serum samples were collected within 12 h after patient arrival as well as during the follow-up interview and were stored at −80 °C. Determination of cardiac markers was performed blinded to patient's histories and status. MPO serum levels were measured by ELISA (MPO ELISA, Bioxytech, OXIS International, Inc, Portland, Oregon, USA). Readings were made in the ETI-max 3000 (DiaSorin, Saluggia, Italy). This assay provides a detection limit of 15 ng/mL. CRP levels were determined by high-sensitive nephelometry (BN II, Dade Behring, Marburg, Germany). In 17 cases, blood samples were refrozen and were excluded from analyses, resulting in 98 patients with available samples and follow-up data. In addition, blood samples from 30 blood donors were collected to determine reference values of CRP, and mean levels were 1.4±1.3 mg/dL.

Descriptive categorical data were listed as percentages with 95% confidence interval (CI) and continuous variables as mean ± SD. The comparisons between blood samples were made by Student *t* test and Wilcoxon rank-sum, when appropriate. Cut point levels were 10 mg/L for CRP and median levels for MPO.[11–14] Kaplan-Meier survival curves and Cox-proportional-hazards regression model were used to estimate the relative risk for mortality. A p value <0.05 (2-tailed) was considered statistically significant.

Table 1. Baseline characteristics of the patients (n = 115)

Clinical characteristics	n (%)
Male sex	61 (53)
Age, years, mean ± SD	64±12
Hypertension	102 (89)
Diabetes mellitus	36 (31)
Dyslipidemia	91 (79)
Cigarette smoking	21 (18)
Electrocardiogram	
T wave inversion	77 (67)
ST-segment depression	23 (20)
ST-segment elevation	21 (18)
Troponin T, median (IQR), ng/mL	0.41 (0.06–2.65)
Coronary angiography	58 (50)
Final diagnosis at the index event	
Unstable angina	72 (63)
Myocardial infarction	
Non ST elevation	22 (19)
ST elevation	21 (18)

Notes: IQR = interquartile range.

Results

Clinical and biochemical characteristics of patients are presented in Table 1. During hospitalization, half of patients were submitted for revascularization procedures. Most common complications were recurrent angina (18%) and development of heart failure (13%). Serial analyses of inflammatory markers are shown in Figure 1. On average

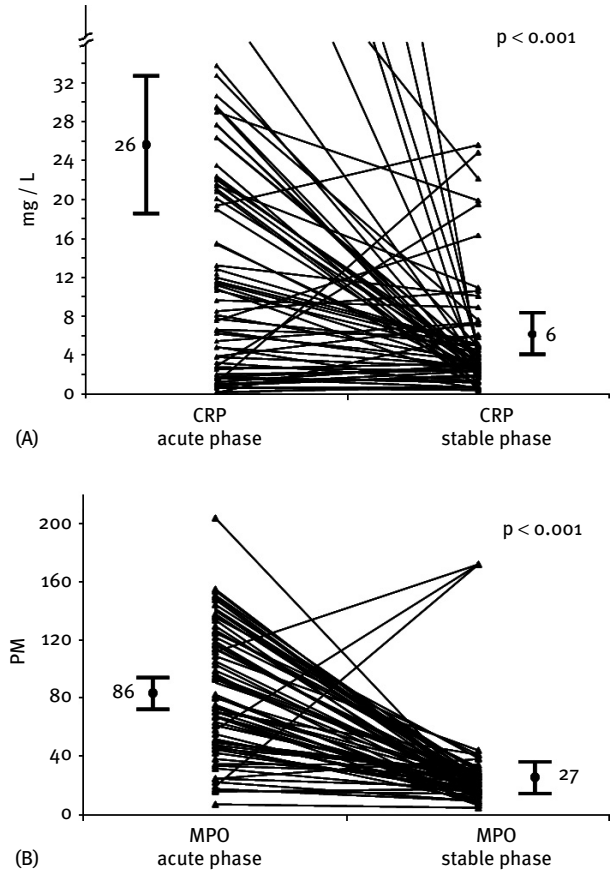

Figure 1. C-reactive protein (CRP, Panel A) and myeloperoxidase (MPO, Panel B) levels in the acute phase and in the stable phase during follow-up (n = 72). Data are presented as individual values, bars represent mean values and 95% confidence interval.

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Table 2. Univariate Cox Proportional-hazard Model for long-term mortality

Variable	Hazard Ratio	95% CI	p
Male Sex	0.8	0.4 a 1.8	0.65
Age>63 years	1.1	1.0 a 1.1	0.002
Risk factors			
Hypertension	3.6	0.5 a 26.8	0.2
Diabetes mellitus	0.9	0.4 a 2.2	0.92
Dyslipidemia	2.1	0.9 a 4.7	0.07
Cigarette smoking	1.5	0.6 a 3.8	0.35
Inflammatory markers at admission (n = 98)			
Troponin T>0.01 ng/mL	2.9	1.1 a 7.8	0.03
C-Reactive Protein>10 mg/L	2.4	0.98 a 6.1	0.048
Myeloperoxidase, pM	0.9	0.9 a 1.0	0.65
Events during hospitalization			
ICP with stent	3.1	1.1 a 9.0	0.04
CABG	2.2	0.8 a 6.6	0.13
Congestive heart failure	1.5	0.5 a 4.3	0.47
Recurrent angina	0.8	0.3 a 2.2	0.63
Arrhythmias	1.1	0.3 a 3.5	0.92
Events on long term follow-up			
New episode of ACS	3.9	1.1 a 14.3	0.03
Hospital readmission	4.7	1.1 a 20.8	0.04
ICP or CABG	0.3	0.1 a 1.2	0.08
Congestive heart failure	4.1	1.2 a 14.2	0.02
Arrhythmias	4.5	1.2 a 16.8	0.02

Notes: ACS = acute coronary syndrome, CABG = coronary artery bypass graft surgery, ICP = interventional coronary procedure, CI = confidence interval.

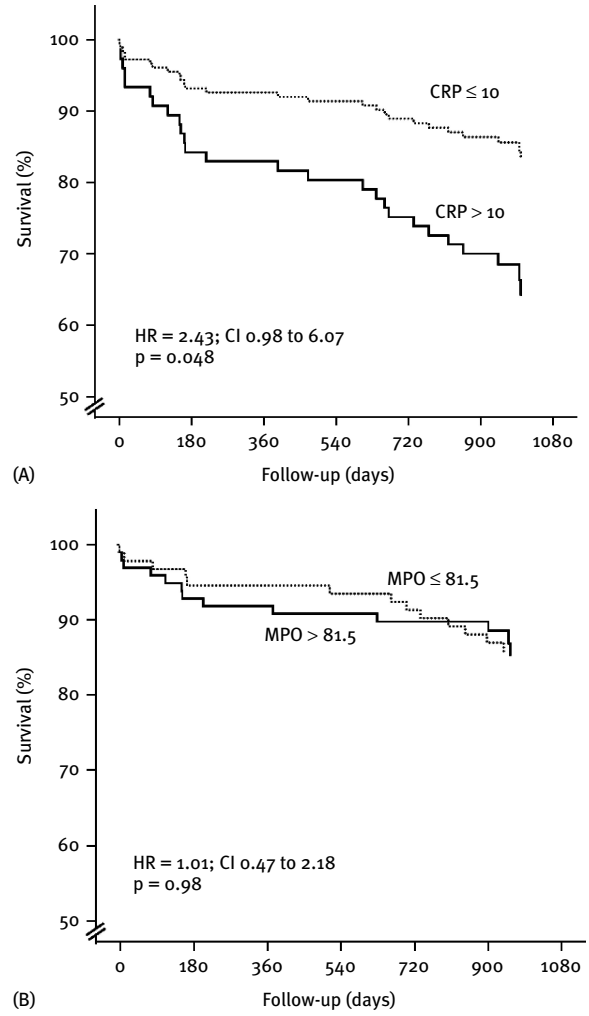

Figure 2. Kaplan-Meier survival curves in patients with acute coronary syndrome stratified by C-reactive protein (CRP, Panel A) and myeloperoxidase (MPO, Panel B) levels in the index event.

MPO and CRP levels decreased significantly after the acute event. Levels of CPR decreased from 26 mg/L (minimum 0.16; maximum 181) in the acute phase to 6 mg/L (minimum 0.3; maximum 46) during the chronic phase ($p<0.001$). Levels of MPO decreased from 86 pM (minimum 7.0; maximum 204) to 27 pM (minimum 4.7; maximum 172; $p<0.001$).

During the follow-up period, 57 (49%) were readmitted for cardiovascular reasons, 45 (39%) presented new episodes of ACS, 34 (30%) were submitted for revascularization procedures, and 31 (27%) died. Predictors of cardiovascular mortality are described in Table 2. Levels of MPO were not related to mortality (HR: 1.01; 95% CI: 0.47 to 2.18) or with any other outcome in long term follow-up (Figure 2). During initial ACS admission, 69 patients (60%) presented high levels of troponin T. Patients with troponin T >0.01 ng/mL had higher cardiovascular mortality compared to patients with negative troponin (HR: 2.93; 95% CI: 1.10 to 7.78; $p = 0.031$). Similarly, patients with CRP >10 mg/L in the acute phase had higher cardiovascular mortality than those with low CRP (HR: 2.43; 95% CI: 0.98 to 6.07; $p = 0.048$; Figure 2). In the Cox-proportional-hazards multivariate analysis, the 2 variables that most influenced the outcome were age (HR: 1.24; 95% CI: 1.10 to 1.39; $p<0.001$) and CRP on admission (HR: 4.9; 95% CI: 1.05 to 22.86; $p = 0.04$). Levels of CRP were not related to any other outcome

besides mortality in long-term follow-up. Changes over time or absolute values of both markers in the chronic phase were not related to clinical outcomes.

Discussion

The present study confirms previous observations that levels of CRP and MPO are elevated during ACS,[4,6,7,9] suggesting the participation of these proteins in the pathophysiologic process. After 2.4 y of follow-up, these markers significantly decreased with the stabilization of the disease. Based on other studies, it was expected that patients with an activated inflammatory profile during an acute event that persists over time would have worse prognosis with multiple clinical events. Nonetheless, our study does not support this hypothesis, since only CRP levels in the acute phase were associated with increased mortality. Release of MPO, through activation of polymorphonuclear neutrophils, does not seem to confer the same risk observed with other inflammatory markers.

Although several studies have described the value of CRP on the atherosclerotic process, recent attention has focused on the importance of neutrophils activation. Few clinical studies have described the prognostic value of MPO in patients with ACS. In the CAPTURE Trial, (C7E3 Fab antiplatelet therapy in unstable refractory angina) patients with high levels of MPO had an increased risk (adjusted HR: 2.25; 95% CI: 1.32 to 3.82) of death and infarction in 6 mo.[9] Brennan et al.[15] demonstrated that levels of MPO on admission in 604 patients with acute chest pain were predictors of major coronary events in 30 d and 6 mo of follow-up after adjustment for troponin T and CRP. Similar findings were observed by Esporcatte et al.[16] in a heterogeneous group of chest pain patients. Mocatta et al.[17] showed a significant relationship between above-median levels of MPO after acute myocardial infarction and increased 5 y mortality. Nonetheless, we were unable to show an association of MPO with cardiovascular events in long-term follow-up. Our cohort differs from those of the aforementioned studies because it included real-world patients with a definite diagnosis of ACS who had a higher risk, as demonstrated by the incidence of events. Therefore, it is possible that in this higher risk population MPO levels may not contribute to risk stratification. Another potential explanation for this finding relates to the technical difficulties in the dosage and interpretation of this protein, since there are no standardized commercial assays.[13,18] Several variables seem to interfere with the results provided from commercially available kits, such as temperature, coating antibody affinity, type of material analyzed, among others.[19] In addition, there are no definitive data establishing the cut point value of MPO from which it is a predictor of cardiovascular risk.

This study confirms CRP as a marker of cardiovascular risk in the long-term, suggesting that it contributes in the risk stratification process. CRP was a mortality predictor however, not capable of early identification of a high risk subgroup independently of troponin, as suggested in previous studies.[6,11,19] It seems that markers of necrosis are more related to the extension of myocardial damage and early risk, and inflammatory markers probably identify a profile at higher risk of plaque vulnerability and late events.

It is important to recognize some caveats present in this study. The reduced number of patients could be responsible for the lack of association between MPO and cardiovascular events. However, we believe that the large number of events observed in the follow-up minimized this fact. In addition, some demographic variables, such as obesity and smoking, well-known risk factors for ischemic heart disease, were not appropriately assessed in the emergency department and were not considered in the analysis.

Patients with ACS present high levels CRP and MPO in the acute phase, and although they significantly decrease thereafter, they remain elevated compared to healthy populations. Levels of CRP in ACS are predictors of cardiovascular mortality in long-term follow-up. Measurement of CRP, in the follow-up of patients with ACS, can be clinically useful for the identification of patients with persistent arterial inflammatory states, and on-going clinical trials will show whether these patients could benefit from more aggressive therapeutic strategies.[20,21] Additional studies are necessary to define parameters of MPO and to establish its prognostic value in ACS and in the follow-up of patients with coronary artery disease.

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