DOI: 10.1111/j.1365-2362.2010.02457.x

ORIGINAL ARTICLE

Diagnostic efficacy of myeloperoxidase for the detection of acute coronary syndromes

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ABSTRACT

Background Early diagnosis of acute coronary syndrome (ACS) is frequently a challenging task, while immediate risk stratification remains crucial for the prompt implementation of appropriate therapy in this setting. Employing markers that increase rapidly after the symptom onset may enhance triage and therapeutic decision-making in patients suspected for ACS. Myeloperoxidase (MPO) exerting proinflammatory and pro-oxidative properties is suggested as a reliable early marker for ACS associated with unfavourable clinical outcome. We assessed the diagnostic efficacy of plasma MPO alone or in combination with cardiac troponin I (cTnI) for detecting ACS in patients presenting with chest pain initiating within 6 h before the hospital admission.

Material and methods A study group consisted of 253 patients diagnosed with ACS and 47 subjects having other heart disease or unspecified chest pain. Clinically healthy volunteers (n = 124) served as controls. MPO concentration was measured in plasma (Abbott Diagnostics, USA), while serum was assayed for cTnI, creatine-kinase MB, lipids, glucose, creatinine, brain natriuretic peptide type B and C-reactive protein.

Results Both MPO and cTnI values were significantly lower in non-ACS subjects than in patients with ACS. At 97·5th percentile as cut-off, the superiority of MPO over cTnI was observed in patients with unstable angina and non-ACS subjects. Considerably higher MPO concentrations were demonstrated in the troponin-negative ACS patients on admission who became troponin-positive after 6 h. Combined evaluation of MPO and cTnI possessed remarkably higher sensitivity than assessment of cTnI alone in all patients with ACS.

Conclusions Myeloperoxidase substantially facilitates the early diagnosis of ACS.

Keywords Acute coronary syndrome, cardiac troponin I, chest pain, diagnostic efficacy, myeloperoxidase.

Eur J Clin Invest 2011; 41 (6): 667–671

Introduction

Early diagnosis of acute coronary syndrome (ACS) is frequently a challenging task, while immediate risk stratification remains crucial for the prompt implementation of appropriate therapy in this setting. However, the prolonged release pattern of troponins, well-established biomarkers of myocardial necrosis, and limited sensitivity of the routine troponin assay make it difficult to diagnose ACS at an early stage. Employing markers that increase rapidly after the symptom onset may enhance triage and therapeutic decision-making in patients suspected for ACS. Myeloperoxidase (MPO) possessing proinflammatory and pro-oxidative properties is suggested as a reliable early marker for ACS associated with unfavourable clinical outcome [1–6]. The purpose of this study was to assess the early diagnostic efficacy of plasma MPO alone or in combination with cardiac troponin I (cTnI) for detecting ACS in patients presenting with chest pain.

Subjects and methods

Study groups

The study group consisted of 300 patients with suspected ACS who were admitted to the Department of Cardiology and Internal Medicine. Of all hospitalized patients, 253 (94 women and 159 men, aged 64 ± 12) met clinical criteria for ACS. Electrocardiography examination was performed on admission and thereafter if clinically indicated. Echocardiography, stress tests

and cardiac catheterization were performed if needed. Patients with acute coronary syndrome were subsequently definitely diagnosed with unstable angina (UA $n = 100$), non-ST-elevation myocardial infarction (NSTEMI $n = 87$) or ST-elevation myocardial infarction (STEMI $n = 66$). Forty-seven patients were diagnosed with other heart disease or unspecified chest pain. Patients with heart failure, pulmonary embolism, chronic obstructive pulmonary disease, renal insufficiency and myocardial infarction within 6 weeks preceding the enrolment were excluded from the trial. Clinically healthy volunteers (40 women and 84 men, aged 49 ± 12 years) with no evidence of present renal, metabolic or inflammatory disease, heart failure and recent myocardial infarction served as controls.

Hypertension was diagnosed if systolic blood pressure exceeded 140 mmHg and/or diastolic blood pressure was above 90 mmHg, whereas dyslipidaemia if even one of the lipid profile components was above/under the following values: total cholesterol > 5·2 mmol L^{-1}, triglycerides > 1·7 mmol L^{-1}, low density lipoprotein cholesterol > 3·37 mmol L^{-1}, HDL cholesterol < 1·3 mmol L^{-1} for women and < 1·0 mmol L^{-1} for men according to ESH/ESC recommendations. Detailed characteristics of study participants were presented in Table 1.

Table 1 Characteristics of ACS and control groups (percentages, medians and 25–75 percentiles)

Variables	Control group	ACS group	*P*
Clinical features			
n	**124**	**253**	
Age (years)	49 ± 12	64 ± 12	–
Women	40 (32%)	94 (37%)	–
Men	84 (68%)	159 (63%)	
Hypertension	2 (1·6%)	187 (74%)	–
Diabetes mellitus	(0%)	129 (51%)	–
Dyslipidaemia	37 (30%)	164 (65%)	–
Smoking	64 (52%)	157 (62%)	–
ACS family history	35 (28%)	124 (49%)	–
Biochemical markers			
cTnI ng mL^{-1}	**0·0** (0·0–0·0)	**0·118** (0·011–1·146)	$P < 0·05$
MPO pmol L^{-1}	**143** (88–258)	**630** (313–1241)	$P < 0·05$
hsCRP mg L^{-1}	**1·02** (0·36–2·77)	**3·14** (1·2–7·9)	$P < 0·05$
WBC × 10^6	**5·72** (5·15–6·86)	**8·33** (6·7–10·1)	$P< 0·05$
BNP (ng mL^{-1})	**21** (10–38·4)	**97·5** (38·6–271)	$P < 0·05$

ACS, acute coronary syndrome; BNP, brain natriuretic peptide; cTnI, cardiac troponin I; MPO, myeloperoxidase. Bold values indicate biochemical markers.

Methods

Venous blood samples were collected on hospital admission within 6 h of the chest pain onset. MPO concentration was measured in K2-EDTA (K2-ethylenediaminetetraacetic acid) plasma using fully automated chemiluminescent microparticle immunoassay with Architect ci8200 system; Abbott Diagnostics, Abbott Park, IL, USA. MPO method was validated by the manufacturer using C28-A2 National Committee for Clinical Laboratory Standards and EP5-A2 Clinical and Laboratory Standards Institute (CLSI) protocols. Evaluation of MPO assay precision was performed in our laboratory with the use of MPO control material low, medium and high (ref 8L11L, lot V23785L) according to the CLSI protocol. All within-run and between-run results were within ±1SD with coefficient of variation (CV) < 5%.

Otherwise, serum was assayed for cTnI, creatine-kinase MB (CK-MB) activity, lipid parameters, glucose, creatinine and brain natriuretic peptide type B (BNP) (Architect ci8200; Abbott Diagnostics). Additionally, in tested material, we determined C-reactive protein concentration (hsCRP) using high-sensitivity method (BNII; Siemens Diagnostics, Warsaw, Poland) and leucocyte count (WBC) (XE 2100; Sysmex, Kobe, Japan). MPO was assayed in plasma samples stored frozen at −20 °C not longer than 6 months. cTnI was assayed on admission and after 6 h. Any increase in cTnI above 0·032 ng mL^{-1} (the 99th percentile for the healthy population measured with 10% CV) was considered a positive result.

The study was approved by the Ethics Committee at Collegium Medicum, Nicolaus Copernicus University, Torun, Poland, and a written informed consent was obtained from all study participants.

Statistical analysis

Between-group analysis was made with Student's *t*-test, Mann–Whitney *U*-test, Kruskal–Wallis rank-sum test if appropriate. Reporting of the study conforms to STARD along with references to STARD and the broader EQUATOR guidelines. Clinical sensitivity, specificity, predictive values and area under the receiver operating characteristic (ROC) curve (AUC) were quantified. P value ≤ 0·05 was considered significant. Statistical analysis was performed with Statistica 8.0 and MedCalc 9.6.0.

Results

In the control subjects, median MPO concentration was 143 pmol L^{-1} with the 95th percentile at 446 pmol L^{-1}, the 97·5th at 527 pmol L^{-1} and the 99th at 609 pmol L^{-1}. Any elevation of MPO above the 97·5th percentile was regarded as a positive result. Median and 97·5th percentile plasma MPO values among controls were higher in men than in women (145 vs. 94 pmol L^{-1} and 527 vs. 380 pmol L^{-1}, respectively),

but there were no age-dependent differences (data not presented).

Concentrations of biomarkers of necrosis (cTnI) and inflammation (hsCRP, MPO, WBC) were significantly higher in patients with ACS on admission than in control subjects (Table 1). Median MPO concentrations were considerably lower in the UA and NSTEMI patients when compared with the STEMI group (391 and 618 pmol L^{-1} vs. 1056 pmol L^{-1}, respectively). In the non-ACS subjects, MPO and cTnI were significantly lower than in the patients with ACS (MPO 280 pmol L^{-1}; $P < 0{\cdot}05$ and cTnI 0·002 ng mL^{-1}; $P < 0{\cdot}05$).

ROC analysis was performed for MPO, cTnI and MPO + cTnI (Fig. 1). At the 97·5th percentile for MPO, the calculated sensitivity and specificity were 55% (48–61; 95% CI) and 100% (96–100; 95% CI), respectively, with positive and negative predictive values of 100% (97–100; 95% CI) and 47% (40–54; 95% CI). For cTnI at 99th percentile cut-off, the sensitivity and specificity were 66% (60–72; 95% CI) and 100% (96–100; 95% CI), respectively, with positive and negative predictive values of 100% (98–100; 95% CI) and 54% (47–62; 95% CI). Both MPO and cTnI alone were characterized by similar, excellent diagnostic accuracy. The area under the ROC curve for MPO was 0·906 (0·870–0·934; 95% CI; $P = 0{\cdot}0001$), whereas for cTnI, it was 0·922 (0·889–0·948; 95% CI; $P = 0{\cdot}0001$). ROC analysis for two-marker strategy MPO + cTnI showed the AUC value of 0·931 (0·900–0·955; 95% CI; $P = 0{\cdot}0001$).

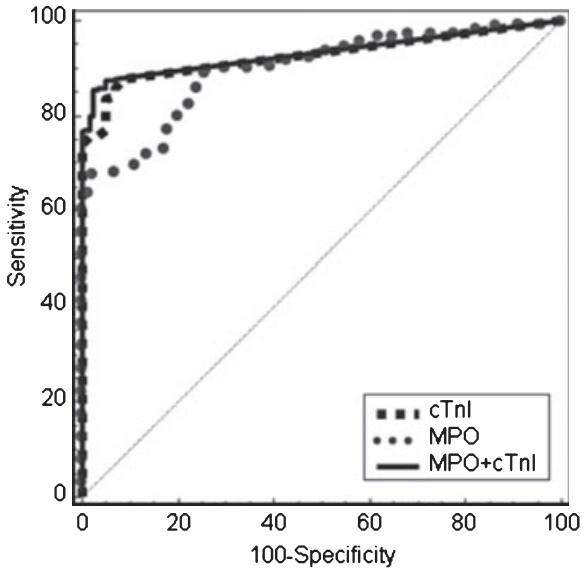

Figure 1 ROC analysis of myeloperoxidase (MPO), cardiac troponin I (cTnI) and MPO + cTnI in the discrimination of patients with acute coronary syndrome. AUC for cTnI = 0·922 (0·889–0·948; 95% CI; $P = 0{\cdot}0001$). AUC for MPO = 0·906 (0·870–0·934; 95% CI; $P = 0{\cdot}0001$). AUC for cTnI + MPO = 0·931 (0·900–0·955; 95% CI; $P = 0{\cdot}0001$).

Table 2 Diagnostic efficacy of MPO in relation to cTnI in patients with ACS and non-ACS subjects on admission (≤ 6 h)

	Sensitivity (%)		
Patients	MPO> 446 pmol L^{-1} > 95th percentile	MPO> 527 pmol L^{-1} > 97·5th percentile	cTnI> 0·032 ng mL^{-1} > 99 percentile
ACS *n* = 253	62·8	54·5	66·3
UA *n* = 100	47·0	40·0	31·0
NSTEMI *n* = 87	62·1	52·9	86·2
STEMI *n* = 66	86·4	78·8	91·8
Non-ACS *n* = 47	17·0	0·0	12·8

ACS, acute coronary syndrome; cTnI, cardiac troponin I; MPO, myeloperoxidase; NSTEMI, non-ST-elevation myocardial infarction; UA, unstable angina.

The evaluation of diagnostic efficacy of plasma MPO in relation to cTnI was based upon the percentage of positive results in patients with ACS and non-ACS subjects on admission (Table 2).

If MPO values at 95th or 97·5th percentile were accepted as cut-off levels, the superiority of MPO over cTnI was observed only in patients with UA. However, the 97·5th percentile cut-off value allowed for the best discrimination between patients with ACS and non-ACS cases.

A trend towards higher MPO concentrations in the troponin-negative patients with ACS on admission who became troponin-positive after 6 h in comparison with those consistently troponin-negative was observed (782 pmol L^{-1} vs. 379 pmol L^{-1}; $P = 0{\cdot}09$).

Among the troponin-negative patients with UA, 25% of subjects were positive for MPO. Using the combination of early assessed MPO and cTnI, we have demonstrated sensitivity of 56% when compared to 31% for cTnI alone in patients with UA (Fig. 2). Corresponding sensitivity rates in NSTEMI and STEMI patients for combined MPO + cTnI evaluation of 92% and 100% were superior to values of 86·2% and 91·8% obtained on the basis of cTnI assessment alone.

Discussion

In our study, MPO concentration in plasma, determined shortly after the onset of chest pain, was significantly elevated in patients with the final diagnosis of ACS and provided diagnostic information independent from that derived from a well-established cardiac biomarker: sensitive cTnI. The superiority

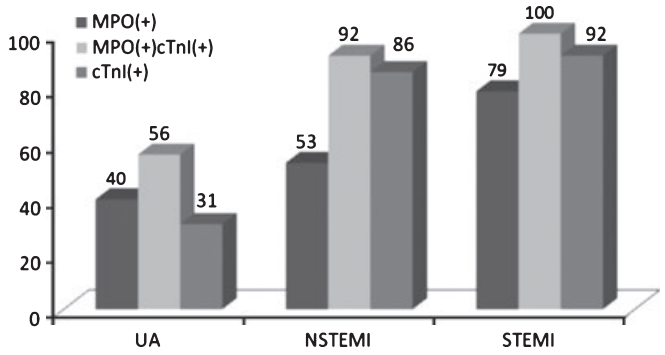

Figure 2 Diagnostic efficacy (%) of two-marker strategy in patients with acute coronary syndrome on admission.

of MPO was observed, above all, in patients with UA, in whom MPO sensitivity was higher than that of cTnI. Moreover, testing for MPO in troponin-negative ACS patients added improved early information in 14% of cases.

Cardiac troponins owing to their superior sensitivity and specificity for detecting myocardial necrosis are, so far, the best established biomarkers in cardiology. One of their limitations is insufficient sensitivity in the early stage of myocardial damage. Currently, multimarker strategy seems to be more effective for the diagnosis of ACS and identification of patients suitable for more aggressive treatment than cardiac troponin alone unless ultrasensitive assay is used [3,7–9]. The superiority of the two-marker strategy for early discrimination of patients with ACS was demonstrated by ROC analysis of MPO + cTnI (AUC 0·931 vs. 0·906 and 0·922 for MPO and cTnI alone).

After early onset of chest pain, both inflammatory and necrosis markers were highly elevated in our patients with ACS when compared with the control and non-ACS subjects. It confirms earlier reports indicating markedly elevated plasma concentrations of MPO within 2 h of symptom onset in patients with myocardial infarction [10] or in patients with ACS presenting within 3–12 h of their last episode of chest pain [1]. Higher plasma MPO concentrations in patients with myocardial infarction compared with patients with UA have also been suggested in other studies [6,11].

In different reports, the cut-off points for MPO in healthy population are defined as the 75th, 97·5th or 99th percentiles [6,12,13]. We have accepted the 97·5th percentile as the upper reference value for MPO, which resulted in excellent specificity and fairly good sensitivity in all patients with ACS and much better diagnostic efficacy in patients with UA and non-ACS subjects in comparison with cTnI. The findings of Apple *et al.* [13], who used the 99th percentile limit for healthy population, did not show better diagnostic accuracy of MPO over cTnI in patients presenting with symptoms suggestive of ACS although the sensitivity for MPO was higher.

In the recent report, Eggers *et al.* [6] argued that MPO provides no clinically relevant information in unselected patients with chest pain presenting within 8 h. They applied the same MPO assay as in this study, but their results are not consistent with ours. In contrast to our data, no significant differences in median MPO concentrations between patients with UA or myocardial infarction and those with other heart disease or non-cardiac disease were found. We used the same decision limit, but MPO concentrations in our non-ACS subjects were found to be far below the cut-off value. Our study was performed in one emergency department, whereas the other study was conducted in three different medical centres, which might have influenced the selection of chest pain patients. Moreover, the median MPO value and the 97·5th percentile in our control group were much higher than those reported by Eggers *et al.* (143 vs.78·9 pmol L^{-1} and 527 vs. 208 pmol L^{-1}, respectively). This is quite difficult to explain whether we take into consideration the available data of others which, similar to us, have shown the 95th–99th percentile values for middle-aged healthy population to vary from 285 to 862 pmol L^{-1}, depending on the method used [2,12–14].

The impact of collection tube and preanalytical handling on MPO measurement is a very important aspect as it may lead to falsely increased results. This has been reported in details by Shih *et al.* [15]. They have concluded that for determination of MPO concentration, the preferred sample type and the most stable during short storage at room temperature is EDTA plasma, whereas serum or heparin plasma samples gave consistently higher values because of MPO leakage from leucocytes. However, this was not the case in our study in which MPO was measured in EDTA plasma samples frozen within 2 h after blood collection. Unfortunately, in several studies evaluating the diagnostic value of MPO in patients with chest pain, serum was used as an assay material [1,5,14,16] and different ELISA methods were applied [16–18]. When EDTA plasma was used for the determination of MPO concentration, a very good correlation (R^2 = 0·9204) was shown between ELISA and the same automated method we have applied [18].

In our study, gender differences in MPO concentrations were found in the control group which have not been observed by others [17]. We do not know the reason for this discrepancy; however, it is worth to note that in the manufacturer's leaflet, plasma MPO concentrations at 95th percentile were given as 246·6 and 354·3 pmol L^{-1} for women and men, respectively.

Testing for MPO in the troponin-negative ACS patients added improved early information. In patients who were consistently troponin-negative within 12 h, median MPO concentration was lower than in patients who became cTnI-positive. The prognostic value of initial MPO measurement in the troponin-negative patients with chest pain was also reported earlier [2].

In conclusion, MPO, reflecting inflammatory and oxidative processes distinct from those detected by markers of necrosis, substantially facilitates the early diagnosis of ACS. Single plasma MPO measurement obtained within 6 h of the chest pain onset provides important diagnostic information, particularly in initially troponin-negative patients. The diagnostic efficacy of MPO, especially in patients with UA, may be further enhanced by combined testing for MPO and cTnI.

Acknowledgements

This study was in part supported by Abbott Diagnostics Poland (MPO and BNP assay kits); however, the sponsoring organization played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

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Received 14 June 2010; accepted 2 December 2010

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