A Synergistic Role of Myeloperoxidase and High Sensitivity Troponin T in the Early Diagnosis of Acute Coronary Syndrome

Mihir D. Mehta · Simbita A. Marwah ·
S. Ghosh · Hitesh Shah · Amit Trivedi ·
N. Haridas

Received: 4 January 2015 / Accepted: 27 February 2015 / Published online: 14 March 2015
© Association of Clinical Biochemists of India 2015

Abstract Aim of this study was to evaluate the role of Myeloperoxidase (MPO) and high sensitive Troponin T in the early diagnosis of acute coronary syndrome (ACS). This was a cross sectional study that comprised of 120 individuals of which 75 were cases and 45 healthy controls. On the basis of clinical history and 12 lead electrocardiogram initial diagnosis of ACS was made in the cases. MPO and high sensitive Troponin T (hs-cTnT) was measured in all the individuals. Levels of MPO were significantly higher in patients of ACS as compared to those in control group [medians: 15.40 (95 % CI 11.06–20.84) vs 5.84 (95 % CI 5.50–6.44)]. By taking the cut off as >11.87 U/mL for MPO, its sensitivity was 87 % (95 % CI 73.7–95.1), specificity was 97.3 % (95 % CI 90.6–99.7), positive predictive value was 94.6 % and negative predictive value was 92.6 %. Positive likelihood ratio was 33.0 while negative likelihood ratio was 0.13, whereas the corresponding values in case of hs-cTnT were 95.6 % (95 % CI 85.2–99.5), 61.3 % (95 % CI 49.5–72.6), 59.7 %, 95.8 %, 2.47 and 0.07 by taking cut off as >14 pg/ml. The area under the ROC curves (AUC) of MPO and hscTnT at 0–6 h were 0.971 (95 % CI 0.92–0.99, $P < 0.001$) and 0.797 (95 % CI 0.71–0.86, $P < 0.001$) respectively. The logistic model combining the two markers yielded sensitivity, specificity, positive predictive value and negative predictive value of 95.7, 97.3, 98.2 and 93.7 % respectively. It was concluded that MPO and hs-cTnT may be useful tools for risk stratification of ACS and

can be used together with better accuracy in the early diagnosis of ACS.

Keywords Myeloperoxidase · High sensitivity Troponin T · Acute coronary syndrome

Abbreviations

MPO Myeloperoxidase
hs-cTnT High sensitivity Troponin T
ACS Acute coronary syndrome

Introduction

Acute coronary syndrome (ACS) encompasses acute myocardial infarction (MI) with and without ST-segment elevation and unstable angina. MI occurs as a result of myocardial necrosis which occurs due to myocardial ischemia. Now-a-days patient history, physical examination, and 12-lead ECG is used for the diagnosis along with biomarkers. Cardiac troponin has been defined as a standard marker for detection of myocardial ischemia in the third universal definition of MI [1]. Now-a-days high sensitivity troponin assays are available which measure cardiac troponin with adequate precision in majority of normal healthy subjects [2]. However, it takes several hours to detect serum levels of troponin in patients of acute MI because of its large size and the levels rise only when there is an evidence of myocardial necrosis [3]. If ischemia is relieved before necrosis occurs then the myocardium undergoes hibernation wherein the cells adapt to decrease energy supply and viability is maintained. Thus, biomarkers that are able to identify and differentiate the patients

M. D. Mehta (✉) · S. A. Marwah · S. Ghosh · H. Shah ·
A. Trivedi · N. Haridas

Department of Biochemistry, Pramukhswami Medical College
and Shree Krishna Hospital, Karamsad, Anand 388325, Gujarat,
India

e-mail: mihirac2006@gmail.com

with chest pain of aetiology other than myocardial ischemia, and those that could be used in place of troponin might serve an important role in a clinical setting [4, 5]. Patients with ACS are characterized by increased platelet activation and aggregation within the coronary circulation [6]. There is growing evidence that myocardial cell injury is not only related to platelet activation but also is preceded by recruitment and activation of polymorphonuclear neutrophils (PMNs). PMNs have been shown to increasingly undergo degranulation within the coronary circulation in ACS in spite of their apparent insignificance in coronary atherogenesis [7]. One of the principal mediators secreted on PMN activation is Myeloperoxidase (MPO), a hemoprotein traditionally viewed as a microbicidal enzyme [8]. Recently, it has been reported that plasma MPO is a useful prognostic marker of long-term survival in patients with MI and therefore been suggested that measurement of MPO may be useful for the early diagnosis of AMI and identifying short-term risk of recurrent ischemic events which are independent of clinical risk factors like cTnI and other novel biomarkers [9, 10].

Materials and Methods

This was a cross sectional study consisted of 120 individuals in the age groups 30–80 years, divided into 75 cases and 45 healthy controls. The case group included 75 patients admitted in emergency department and in intensive cardiac care unit within 6 h of clinical signs and symptoms of ACS. The patients had been diagnosed and classified as ischemic and non-ischemic depending upon the assessment as per the clinical symptoms such as chest pain with or without radiation, palpitations, epigastric pain, left arm pain, chest heaviness, shortness of breath, lower jaw pain and signs like hypotension and ECG changes suggestive of MI. Diagnosis of MI was done on the basis of third universal definition of MI. All the patients with sepsis, pulmonary embolism, peripheral vascular disease, cerebrovascular stroke and renal failure were excluded from study to avoid false positive results of MPO and hs-cTnT. A study protocol was set before undertaking this study and it was approved by institutional Human Research Ethical Committee. MPO was measured by continuous spectrophotometric rate determination using O-dianisidine hydrogen peroxide method. Whereas hs-cTnT was measured quantitatively using ECL (electro chemiluminescence immune assay) based on electro chemiluminescence technology (high sensitivity Troponin T, Cobas e411, Roche, Mannheim, Germany). Statistical analysis was performed using the commercially available statistical software SPSS—14.0 version, MedCalc version 12.5 and Microsoft excel. The P value of less than 0.05 was

considered statistically significant. ROC curve analysis and calculation of the area under the curve (AUC) was done for all parameters included in the study population according to the method of Hanley and McNeil. The optimum cutoff was used to dichotomously classify the positive or negative serum MPO and hs-cTnT levels, and it was also used for calculating the diagnostic sensitivity and the specificity.

Results

The present study comprised of 120 individuals between the age of 30 and 80 years (75 cases and 45 controls). 45 out of 75 patients who had acute chest pain and evidence of myocardial ischemia (their hs-cTnT being <100 pg/mL but had more than 14 pg/mL in initial 6 h of presentation and further rose by 100 % after 6–12 h of first estimation) were included in group 1 (ischemic chest pain), while 30 out of 75 patients who had acute chest pain, but no subsequent evidence of cardiac involvement (their hs-cTnT was <100 pg/mL but more than 14 pg/mL and did not have significant rise after 6–12 h of first estimation) were included in group 2 (non ischemic chest pain). As a control (group 3) 45 normal individuals coming for routine checkup were taken (their hs-cTnT was <14 pg/mL). The common age group of 120 individuals enrolled was between 30 and 80 years (divided into 45 patients of ischemic chest pain, 30 patients of non ischemic chest pain and 45 healthy individuals as controls) with a mean age of 58.08 ± 12.93 . Among those 120 individuals, 77 were males and 43 were females (Table 1).

Among 45 patients from ischemic chest pain group (26 patients with ST segment elevation, 19 patients without ST segment elevation which included patients with ST depression and LBBB) had a mean age of 61.29 ± 10.76 and among them 31 individuals were males. The 30 patients from the non ischemic chest pain group had a mean age of 60.07 ± 12.89 and among them 19 were males. The control group consisting 45 individuals had a mean age group of 53.49 ± 13.84 and among them 27 individuals were

Table 1 Demographic data of all individuals included in study groups (n = 120)

Age in years	Gender		Total (%)
	Female (%)	Male (%)	
31–40	3 (2.5)	8 (6.67)	11 (9.17)
41–50	11 (9.17)	15 (12.5)	26 (21.67)
51–60	11 (9.17)	19 (15.83)	30 (25)
61–70	8 (6.67)	20 (16.67)	28 (23.33)
71–80	10 (8.33)	15 (1.5)	25 (20.83)
Total (%)	43 (35.83)	77 (64.17)	120 (100)

males. The unpaired *t* test showed $t = -3.063$, degree of freedom = 118 and $P = 0.221$. This showed that there was no significant difference between these age groups.

The MPO values for the control reference population were normally distributed. Values for the control reference population (Fig. 1; $n = 45$) are 1.56–8.51 U/mL (mean, 5.84 U/mL; median, 5.83 U/mL). The upper 95th percentile was 6.44 U/mL. To find out whether there was any correlation of serum MPO levels in between case and control group, unpaired '*t*' test was performed and *P* value was derived. The value $P \leq 0.001$ suggested significant difference in values of MPO between these two group. Similarly, when unpaired *t* test was performed between ischemic and non ischemic chest pain group the value $P \leq 0.001$ suggested that levels of MPO in patients of chest pain with ischemia was statistically significantly higher compared to patients of chest pain without ischemia (Tables 2, 3; Fig. 2).

ROC curves were constructed to test the performance of MPO and hs-cTnT for rule-in and rule-out of AMI and earlier prediction in patients with an initially less hs-cTnT result that turned positive within 6–24 h after admission. Sensitivities, specificities, negative predictive and positive predictive values were calculated from ROC curves. Statistics were used to compare the AUC of the ROC curves. The area under the ROC curve (AUC) for MPO at

Fig. 1 Distribution of MPO values for the control reference population ($n = 45$)

Table 2 Comparison of serum MPO levels between case and control group

Variables	Case group	Control group
Sample size	75	45
Median value (U/mL)	15.40	5.84
95 % Confidence interval	11.06–20.84	5.50–6.44
<i>t</i> value	–10.5	
Degree of freedom	118.0	
<i>P</i> value	$P \leq 0.001$	

the optimum cut-off value >11.87 U/mL was 0.971 (0.92–0.99; $P < 0.001$) and that of hs-cTnT at the optimum cut-off value >14 pg/mL was 0.797 (0.71–0.86; $P < 0.001$) (Fig. 3).

On considering the cut off points as >11.87 U/mL for MPO and >14 pg/mL for hs-cTnT which were obtained from ROC curve, the sensitivity (95.6 %) and negative predictive value (95.8 %) was higher for hs-cTnT. MPO, however, had higher specificity (97.3 %), positive likelihood ratio (33.0), and positive predictive value (94.6 %). By combining MPO with hs-cTnT, the sensitivity, specificity, PPV and NPV were 95.7, 97.3, 98.2 and 93.7 % respectively. The PLR was 6.43 and NLR was 0.05 (Table 4).

Discussion

In the present study when serum MPO levels were compared between group 1 (ischemic chest pain) and group 2 (non ischemic chest pain), it was found that median in group 1 was 27.70 U/mL with 95 % CI between 20.84 and 33.34 U/mL and that in group 2 was 9.69 U/mL with 95 % CI between 8.86 and 10.53 U/mL. The levels of MPO were significantly higher in ischemic chest pain group as compared to non ischemic chest pain group ($P \leq 0.001$). Similarly, *P* value was <0.001 on comparing levels between ischemic chest pain group and control group. The control group had median for MPO as 5.84 U/mL with 95 % CI between 5.50 and 6.44 U/mL.

Khan et al. [11] conducted a follow-up study of MPO and N-terminal pro-B-type natriuretic peptide (NT-BNP) in 384 post STEMI patients in which median MPO was raised in patients experiencing MI or who died than in survivors [median (range), 50.6 (15.3–124.1) ng/ml vs 33.5 (6.6–400.2) ng/ml, $P = 0.001$]. MPO had predictive power in both below and above median NT-BNP levels (log rank 5.60, $P = 0.020$ and log rank 5.12, $P = 0.024$, respectively). The receiver-operating curve for median NT-BNP yielded an AUC of 0.72 (95 % CI 0.65–0.79, $P < 0.001$); for median MPO, the AUC was 0.62 (95 % CI 0.55–0.69, $P = 0.001$). The logistic model combining the two markers yielded an AUC of 0.76 (95 % CI 0.69–0.82, $P < 0.001$). In the present study, MPO was used along with hs-cTnT and ROC curves were obtained. The AUC for MPO at cut off >11.87 was 0.971 (95 % CI 0.92–0.99; $P < 0.001$); and combination of MPO and hs-cTnT yielded an AUC of 0.904 (95 % CI 0.845–0.963, $P < 0.001$).

Peacock et al. [12] carried out a study of 1018 patients out of which 288 (23 %) patients had ACS and 788 (77 %) had noncardiac chest pain (NCCP). Of the patients with ACS, 94 (9.2 %) had MI at presentation (69 non-ST-elevation MI, 25 ST-elevation MI), and 136 had unstable

Table 3 Comparison of serum MPO levels between ischemic chest pain and non ischemic chest pain groups

Variables	Ischemic chest pain group	Non ischemic chest pain group
Sample size	45	30
Median value (U/mL)	27.70	9.69
95 % Confidence interval	20.84–33.34	8.86–10.53
t value	−7.02	
Degree of freedom	73.0	
P value	$P \leq 0.001$	

angina. Using a cut-point of 210 ng/mL, MPO had a specificity of 90 %; sensitivity of 18 %; negative predictive value of 69 %; positive predictive value of 47 %; negative likelihood ratio of 0.91; and a positive likelihood ratio of 1.83 to differentiate ACS and NCCP. The values of sensitivity, specificity, PPV, NPV, PLR and NLR are higher in the present study. This probably was because the use of MPO cut-off >11.87 U/mL was a balance between maximizing sensitivity and the tradeoff of increasing false-positive results. Similar studies were conducted by Mocatta et al. [9], Meuwese et al. [13], Cavusoglu et al. [14] and Li et al. [15]; showing utility of MPO in ACS. In our study, Levels of MPO were significantly higher in case of ACS group as compared to non ACS or control group, which were in agreement with studies of Mocatta et al., Meuwese et al., Cavusoglu et al. and Li et al.

Till date there has been no study reported showing the utility of MPO and hs-cTnT together in the early diagnosis of ACS. The current scenario is changing to high sensitive troponin assays which have 10–100 folds lower detection limits than conventional assays. The gain in sensitivity may be particularly important in patients with a short duration

Fig. 3 Comparison of ROC curves of MPO and hs-cTnT

from symptom onset to admission. A negative hs-cTnT test has a high negative predictive value, and may thus serve as an exclusionary test early in the diagnostic process. It was the main advantage of choosing hs-cTnT instead of conventional cTnT. However, with a decrease in the diagnostic cutoff by implementing more sensitive and precise assays, sensitivity is further increased but specificity is decreased owing to detection of more acute, subacute, and chronic cardiac diseases not related to ACS [2, 16].

MPO is an early marker of ACS and having high specificity and positive predictive value as compared to hs-cTnT which will help in the diagnosis of MI instead of waiting for the rise in troponin levels and postponing the

Fig. 2 Box and whisker plot showing comparison of MPO levels between different study groups

Table 4 Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR) and area under the curve (AUC) of

MPO and hs-cTnT during initial 6 h of presentation at the optimum cut-off value obtained from the ROC taking significant rise in hs-cTnT after 6–12 h as standard marker

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR (%)	NLR (%)	AUC
MPO (U/mL)	87.0	97.3	94.6	92.6	33.0	0.13	0.971
Hs-cTnT (pg/mL)	95.6	61.3	59.7	95.8	2.47	0.07	0.797
MPO + hs-cTnT	95.7	97.3	98.2	93.7	6.43	0.05	0.904

treatment. Hence, during early hours when the results of troponin are confusing you can use this marker to support in the diagnosis of myocardial ischemia. As multiple cardiac and non-cardiac conditions are associated with mild-to-moderate hs-cTnT elevations serial testing will become increasingly important for identifying those patients with true ACS. By the time hs-cTnT levels rises on serial dilution irreversible injury occurs in the myocardium. So, an added value by early and specific markers such as MPO would help in the decision making power of myocardial ischemia and can help in early intervention. If treatment of AMI is done within 1 h (the golden hour), mortality can be reduced from 9 to 3 %, if delay of 3–4 h, mortality could be five times higher [17]. MPO levels are significantly elevated within 2 h after the onset of chest pain. This has significant advantages over hs-cTnT which takes 3–6 h to rise to measurable circulating levels after MI, suggesting MPO may be useful in a triage. Furthermore, MPO can be a useful marker of unstable angina preceding MI—a predictor of vulnerable plaque and may assume a role as a marker of instability and not simply a marker of oxidative stress and damage [7]. Thus, MPO and hs-cTnT may comprise an excellent combination in the early diagnosis of ACS.

Conclusion

From this study it can be concluded that hs-cTnT assay is a useful diagnostic test that can be performed to rule out ACS in patients with chest pain. However, by employing hs-cTnT the chances of detecting false positive cases increases as various non cardiac ailments contribute to the rise in the levels of hs-cTnT. Hence serial sampling becomes important for making confirmatory diagnosis, especially in early presenters. Since there is a substantial delay before the factor is expressed after cardiac damage, and many ischemic episodes do not lead to increased troponin levels, results of hs-cTnT may be less confirmatory in patients who present to the emergency department soon after the onset of chest pain. Under these circumstances early cardiac biomarker such as MPO can be used which would help in the decision making power by increasing

diagnostic efficacy of MI and early treatment can be initiated to prevent mortality.

Conflict of interest The authors do not have any conflict of interest.

References

1. Thygesen K, Alpert JS, White HD, Jaffe AS, Apple FS, Galvani M, et al. Joint ESC/ACCF/AHA/WHF Task Force for universal definition of myocardial infarction. Third universal definition of myocardial infarction. *JACC*. 2012;60(16):1581–98.
2. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. *Clin Chem*. 2009;55:1303–6.
3. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, et al. ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction—summary article: a report of the American College of Cardiology/American Heart Association task force on practice guidelines (Committee on the Management of Patients With Unstable Angina). *J Am Coll Cardiol*. 2002;40(7):1366–74.
4. Chen C, Liu J, Hua D, Ma L, Lai T, Fallon JT, et al. Impact of delayed reperfusion of myocardial hibernation on myocardial ultrastructure and function and their recoveries after reperfusion in a pig model of myocardial hibernation. *Cardiovasc Pathol*. 2000;9(2):67–84.
5. Apple F, Jaffe A. Cardiac functions: chapter 44. In: Burtis CA, Ashwood ER, Bruns DE, editors. *Tietz textbook of clinical chemistry and molecular diagnostics*. 4th ed. St.Louis: WB Saunders, Elsevier Inc. 2006. p. 1461–6.
6. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135–43.
7. Buffon A, Biasucci LM, Liuzzo G, D'onofrio G, Crea F, Maseri A. Widespread coronary inflammation in unstable angina. *N Engl J Med*. 2002;347:5–12.
8. Klebanoff SJ. Myeloperoxidase. *Proc Assoc Am Physicians*. 1999;111:383–9.
9. Mocatta TJ, Pilbrow AP, Cameron VA, Senthilmohan R, Frampton C, Richards M, et al. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. *J Am Coll Cardiol*. 2007;49:1993–2000.
10. Morrow DA, Sabatine MS, Brennan ML, de Lemos JA, Murphy SA, Ruff CT, et al. Concurrent evaluation of novel cardiac biomarkers in acute coronary syndrome: myeloperoxidase and soluble CD40 ligand and the risk of recurrent ischaemic events in TACTICS-TIMI 18. *Eur Heart J*. 2008;29(9):1096–102.
11. Khan SQ, Kelly D, Quinn P, Davies JE, Ng LL. Myeloperoxidase aids prognostication together with N-terminal pro-B-type natriuretic peptide in high-risk patients with acute ST elevation myocardial infarction. *Heart*. 2007;93(7):826–31.

12. Peacock WF, Nagurney J, Birkhahn R, Singer A, Shapiro N, Hollander J, et al. Myeloperoxidase in the diagnosis of acute coronary syndromes: the importance of spectrum. *Am Heart J*. 2011;162(5):893–9.
13. Meuwese MC, Stroes SG, Hazen SL, Van Miert JN, Kuivenhoven JA, Schaub RG, et al. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals. *J Am Coll Cardiol*. 2007;50(2):159–67.
14. Cavusoglu E, Ruwende C, Eng C, Chopra V, Yanamadala S, Clark LT, et al. Usefulness of baseline plasma myeloperoxidase levels as an independent predictor of myocardial infarction at two years in patients presenting with acute coronary syndrome. *Am J Cardiol*. 2007;99(10):1364–8.
15. Li SH, Xing YW, Li ZZ, Bai SG, Wang J. Clinical implications of relationship between myeloperoxidase and acute coronary syndromes. *Zhonghua xin xue Guan Bing Za Zhi*. 2007;35(3):241–4.
16. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med*. 2009;361:858–67.
17. Alhashemi JA. Diagnostic accuracy of a bedside qualitative immunochromatographic test for acute myocardial infarction. *Am J Emerg Med*. 2006;24:149–55.