American Journal of Emergency Medicine 33 (2015) 326–330

Contents lists available at ScienceDirect

American Journal of Emergency Medicine

journal homepage: www.elsevier.com/locate/ajem

Original Contribution

Performances of the heart fatty acid protein assay for the rapid diagnosis of acute myocardial infarction in ED patients ☆

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ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 23 October 2014
Accepted 7 November 2014

ABSTRACT

Objective: We sought to evaluate the added value of heart fatty acid protein assay (HFABP) for rapid diagnosis of acute myocardial infarction in a prospective cohort of emergency department (ED) patients with acute chest pain.
Methods: High-sensitivity cardiac troponin T (hs-cTnT; Roche Diagnostics, Meylan, France) and HFABP (Randox, Mauguio, France) were blindly assayed from venous blood samples obtained at admission. Diagnosis was made by 2 ED physicians using all available data and serial cardiac troponin I as the biochemical standard. Diagnostic performances of HFABP combined with hs-cTnT were assessed using logistic regression. Analysis was conducted in all patients and in patients without ST-elevation myocardial infarction.
Results: A total of 181 patients were included (age, 61 ± 17 years; male sex, 66%). Acute myocardial infarction occurred in 47 (25.9%) patients, including non–ST-elevation myocardial infarction in 31 (17.1%). The receiver operating characteristic area under the curve was 0.893 for hs-cTnT levels at presentation (95% confidence interval, 0.812-0.974) and 0.908 (95% confidence interval, 0.839-0.977) for the combination of hs-cTnT and HFABP, with no significant ($P = .07$). Adding HFABP to hs-cTnT increased both sensitivity and negative predictive value (NPV) for non–ST-elevation myocardial infarction diagnosis, with about 13% and 3% increase, respectively, leading to a sensitivity of 97% and an NPV of 99%.
Conclusion: The assessment of HFABP at ED admission adds incremental value to initial hs-cTnT. The increase of sensitivity and NPV without sacrificing the specificity and positive predictive value in patients with chest pain with noncontributive electrocardiogram could potentially allow safe and early rule out of acute myocardial infarction without the need for further serial troponin testing.

1. Introduction

Identification and management of patients with suspected acute myocardial infarction (AMI) are common and difficult challenges for emergency physicians. It is clear that an early discrimination between AMI and non-AMI patients facilitates more rapid triage and improves treatment. Currently, the high-sensitive cardiac troponin (hs-cTn) I or T is the gold reference biomarker in the diagnosis of acute coronary syndrome (ACS), but its elevation occurs 6 to 9 hours after the onset of ischemia. Furthermore, numerous studies have highlighted that the high sensitivity of current sensitive cardiac troponins is at the expense of specificity [1]. In this context, the use of biomarkers in earlier stage than cTn is of great interest [2]. There is also growing evidence of the benefits of a multimarker strategy over the use of a single marker when evaluating patients with ACS.

Heart-type fatty acid binding protein (HFABP), known to be released from injured myocardium, is one of the promising plasma markers for AMI diagnosis. The HFABP is released very quickly in the circulation and could be detected as early as 1 hour after the onset of chest pain, reaching a peak at 4 hours and returning to baseline level within 24 hours [2, 3]. Until now, three manufacturers (Hycult Biotechnology, Dainippon Pharmaceutical, Randox laboratories) have released laboratory immunoassays with the first two based on enzyme-linked immunosorbent assay in format. Unlike the 2 other available tests that are based on enzyme-linked immunosorbent assay, the Randox HFABP assay is the only automated immunoturbidimetric assay enabling rapid testing for HFABP assay in clinical routine.

The aim of this study was to evaluate the analytical performances of the Randox HFABP assay on Roche Cobas8000 analyzer and the accuracy of this parameter alone or in combination with hs-cTnT for diagnosis of AMI in patients presenting with acute chest pain in the emergency department (ED).

☆ Conflicts of interest: The authors declare no financial or competing interests.

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http://dx.doi.org/10.1016/j.ajem.2014.11.012
0735-6757/

2. Subjects and methods

2.1. Study design

Adult patients with chest pain and onset within 12 hours of presentation to the ED were enrolled. Venous blood for investigational biomarker testing was drawn at presentation and collected into lithium heparin and EDTA-treated tubes. Blood samples were processed at the biochemistry laboratory, and plasma was stored at −80°C for later analysis. All diagnoses were reviewed at 1 month by 2 independent ED physicians using all available data, including serial cTnI results and cardiology reports, and blinded to investigational biomarker results (hs-cTnT and HFABP), as previously described [4,5]. Acute coronary syndrome and non-ACS diagnoses were distinguished and further categorized. Acute coronary syndrome, which refers to the constellation of symptoms manifesting as a result of acute myocardial ischemia (AMI), encompasses unstable angina (UA), ST-segment elevation myocardial infarction (STEMI), and non–ST-segment elevation myocardial infarction (NSTEMI). Patients with excluded ACS were categorized as having clinical symptoms of stable angina pectoris (group AP), nonischemic cardiac symptoms or noncardiac symptoms (group NCAD), and symptoms of unknown origin (group UO).

Sample collection was registered at the French Health Ministry (no. DC-2009-1052). All patients provided written informed consent. The study was performed according to the principles of the Declaration of Helsinki and was approved by the local ethics committee.

2.2. Hs-cTnT measurement

The Hs-cTnT assay was performed from frozen lithium heparin plasma samples. Samples were thawed just before analysis and run on the Cobas 8000/e602 analyzer (Roche Diagnostics, Meylan, France). The lowest concentration measurable at the 10% coefficient of variation (CV) level is 13 ng/L, and the 99th percentile among healthy individuals is 14 ng/L (confidence interval [CI], 12.7-24.9), as claimed by the manufacturer. The Limit of Detection (LoD) is 5.0 ng/L [6].

2.3. Analytical performances of the Randox HFABP assay on Cobas8000

The HFABP levels were determined using an immunoturbidimetric method from Randox applied on the c502/Cobas8000. Serum protein calibrator (ref. FB 3134), controls 2 levels (ref. FB 4026 et FB 4027), and system reagent for HFABP (ref. FB 4025) were from Randox. Reagent and calibrator were used according to the manufacturer's recommendations with analytic range from 0.747 to 120 ng/mL. Calibration was performed once a month and with change of lot.

Analytical performances, including linearity, imprecision, limit of quantification, and LoD, were assessed according to Clinical Laboratory and Standards Institute guidelines [7,8]. Three different lithium heparin plasma pools with HFABP concentrations greater than 50 ng/mL were diluted in distilled water down to the following final percentage: 100%, 50%, 31%, 20%, 10%, 5%, and 2%. Specimens were analyzed in triplicate, and recoveries were calculated. An average recovery within 10% of expected values was considered acceptable. Imprecision values (CVs) were assessed using 3 plasma pool samples and 1 level of the quality control from Randox ranging from 2.5 to 26.1 ng/mL and were determined through 20 replicated analyses. Plasma pool samples were divided into aliquots on 20 consecutive days. Aliquots were thawed just before analysis and assayed in duplicate at 2 separate times per day on the basis of a single calibration. The CVs of 10% and 20% were obtained by extrapolation from the imprecision data. The LoD was determined using 10 replicates of both the distilled water and low-concentration samples. Low-concentration samples were made using plasma pools diluted down to 3 and 4 times the assay's sensitivity claimed by the manufacturer. The LoD was calculated as $\mathrm{LoD} = \mathrm{LoA} + 1.645\,\sigma S$, where LoA is the value of 10 replicates of the A sample used as analyte free sample, σS is the standard deviation of the low-concentration sample measurements.

2.4. Statistical analysis

Continuous variables were presented either as mean and standard deviation or as median and interquartile range. Patient groups were compared using Mann-Whitney *U* test for continuous variables. Logistic regression was used to assess the performance characteristics of the hs-cTnT and HFABP assays and their combination for diagnosis of AMI. Overall diagnostic values were quantified by calculating the area under the receiver operating characteristic (ROC) curve (AUC) using the trapezoidal method, with 95% CI computed by using the Delong method. Comparison between AUCs was performed through the Delong test for correlated ROC curves. Optimal hs-cTnT and HFABP thresholds were determined using the Youden index. A bootstrap analysis (2000 bootstraps) was performed to determine 95% CIs for optimal thresholds. Sensitivity, specificity, and predictive values were calculated for each threshold, along with 95% CI based on binomial distribution. Statistical analysis was conducted in all patients with chest pain as well as in a subgroup of patients with the exclusion of STEMI. The significance level was set at 5% for all tests. Statistical analysis was performed by NK using R 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Analytical performances of the HFABP assay on Cobas8000

The linearity was tested in the range of 1 to 50 ng/mL according to the LoD claimed by the manufacturer (<1 ng/mL). The linear equation of linearity was $y = 1.01x - 0.99$, $r^2 = 0.99$, with a mean recovery (SD) percentage of 89 (8)%. Within and total run precision ranged from 1.5% to 15% and 5% to 20%, respectively. The functional sensitivity at a total imprecision of 20% (which corresponds to the limit of quantification) was 1.85 ng/mL. The lowest concentration giving CV of 10% was 6 ng/mL. The LoD was 1.29 ng/mL in our conditions, close to the LoD claimed by the manufacturer.

3.2. Characteristics of study participants

A total of 181 patients were analyzed; and 47 (26%) patients were diagnosed with AMI, including 31 (17%) with NSTEMI. The mean (± SD) age of AMI patients was 61 (±17) years; 66% were male. The patients' characteristics are shown in Table 1. At admission, hs-cTnT and HFABP concentrations were higher in patients with AMI compared with all other diagnoses. Median hscTnT level was 49 (interquartile range [IQR], 18-128.5 ng/L) in AMI vs 6.5 (IQR, 1.5-10.1 ng/L) in all other diagnoses ($P < .001$). Median HFABP level was 10.2 (IQR,4.9-22.0 ng/L) in AMI vs 3.9 (IQR, 2.8-5.7 ng/mL) in all other diagnoses ($P < .001$). The Hs-cTnT and HFABP levels according to final diagnosis are

Table 1
Baseline characteristics of patients at admission

	All	HFABP positive[c]	Hs-cTnT positive[c]
Patient number[a]	181	65 (35.9)	61 (33.7)
Male sex[a]	120 (66)	46 (70.8)	44 (72.1)
Age, y[b]	61 (17)	68 (18)	70 (17)
Time onset, h[b]	4 (3)	4 (3)	4 (3)
CKD-EPI, ml/min/1.73 m^2[b]	85 (26)	72 (32)	67 (29)
Group ACS[a]			
STEMI [a]	16 (8.8)	9 (56.3)	10 (62.5)
NSTEMI [a]	31 (17.1)	25 (80.6)	26 (83.9)
UA [a]	24 (13.3)	5 (20.8)	5 (20.8)
Group non-ACS [a]			
AP [a]	9 (5)	2 (22.2)	1 (11.1)
NCAD [a]	101 (55.8)	24 (23.8)	19 (18.8)

[a] Values are expressed as number of patients (percentage).
[b] Values are expressed as means (SD).
[c] Positive and negative values were determined using cutoff concentrations of 5.8 ng/mL for HFABP and 14 ng/L for hs-cTnT on Cobas8000 instrument.