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Prognostic Value of Combination of Heart-Type Fatty Acid-Binding Protein and Ischemia-Modified Albumin in Patients With Acute Coronary Syndromes and Normal Troponin T Values

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Recent studies have suggested that heart-type fatty acid-binding protein (H-FABP) may detect ongoing myocardial damage involved in the progression of acute coronary syndromes (ACS). This study was prospectively designed to examine whether the combination of H-FABP, a marker for ongoing myocardial damage, and ischemia-modified albumin (IMA), a marker for myocardial ischemia, would effectively diagnose patients with ACS. H-FABP values above 1.5 μg/l can be correctly measured via an ELISA and 6 μg/l is the currently used cut-off value (1–3). We measured serum H-FABP and IMA of 108 patients on admission within 12 hr after onset of chest pain and normal troponin T. serum samples from ACS group ($n = 82$) had decreased capacity of ACB [64 (61–67) U/ml] compared with non-ACS ischemic chest pain group ($n = 26$) samples [75 (71–78) U/ml] ($P < 0.05$). The combination of IMA and H-FABP usually had better sensitivity [96.3% (92.2–100%)] ($P < 0.05$) and accuracy [92.6 (87.7–97.5%)] ($P < 0.05$) than when individually used. Thus, the combination of H-FABP and IMA measurements after initiation of chest pain may be highly effective for risk stratification in patients with ACS and normal cardiac troponin T. J. Clin. Lab. Anal. 23 : 14–18, 2009.

Key words: acute coronary syndrome; myocardial ischemia; fatty acid-binding proteins; biomarkers

INTRODUCTION

Biochemical diagnostics of acute coronary syndromes (ACS) constitutes one of the fastest growing fields of cardiology of the 21st century. Cardiac markers are an important tool in the diagnosis of acute myocardial infarction (AMI) (1–3). Cardiac troponins have become the preferred biochemical marker for ACS (4). Frequently, owing to their delayed appearance in serum, patients with suspected ACS have normal cardiac troponin T levels on admission. There is still a need for reliable early markers.

Recent research suggests that heart-type fatty acid-binding protein (H-FABP) might have potential as an early cardiac marker (5–8). It is a small cytosolic soluble protein that functions as the principal transporter of long-chain fatty acids in the cardiomyocyte (9–11). H-FABP is a powerful regulator of the mitochondrial β-oxidative system (12). It is present in abundance in the cytoplasm of myocardial cells and is released rapidly into the circulation in response to myocardial injury, but it is present in low concentrations in normal conditions (13). H-FABP represents 10% of the whole cardiac myocytes cytosolic protein (7). As such, there are data documenting the diagnostic utility of H-FABP and ischemia-modified albumin (IMA) as early markers of ACS (14–19). This study was prospectively designed to determine whether the combination of measuring H-FABP and IMA after onset of chest pain could effectively diagnose the patients with ACS and normal cardiac troponin T.

MATERIALS AND METHODS

Participants

This study was performed at Peking University Third Hospital, Beijing, China, and was approved by the local ethics committee. We recruited, on a prospective

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sequential basis, patients who arrived at the emergency departments (ED) within 12 hr after the onset of chest pain. The enrollment period was between November 2005 and October 2006. Pregnant women, patients with symptoms and signs suggestive of acute mesenteric ischemia, acute renal failure, peripheral vascular disease, or brain ischemia were not enrolled in the study. A total of 172 patients met these entry criteria, of whom 108 were included; 7 patients were excluded from the final analysis as a result of inaccuracy of timing for sample acquisition, 18 patients, because of incomplete biochemical characterization, and further 39 patients with positive cardiac troponin T results on ED admission. Therefore, the total study population comprised 108 patients, 56 male, and 52 female. Mean age was 58 years (range 37–79). The patients were divided into two groups: the ACS group and nonischemia chest pain (NICP) group.

Discharge Diagnosis

Coronary angiography was carried out on 108 patients. All angiographic images were reviewed by an experienced cardiologist blind to the patient's clinical characteristics and biochemistry results. A positive coronary angiography was defined as stenosis 70% diameter reduction in any major epicardial vessels. Final diagnosis for this study was based on ED discharge diagnosis for patients discharged from the ED, and hospital discharge diagnosis, for patients admitted to the hospital for further investigation and management. The ED consultant or medical consultant was responsible for assigning a final diagnosis on the basis of history, clinical examination, and data from medical records. These included results of ECG, treadmill exercise test, and coronary angiography, as available. Results of all investigations were reviewed blind to IMA and H-FABP results. Patients were classified as NICP when (1) a report noncardiac mechanism was confirmed as the cause of chest pain; (2) both of the following criteria were met: presence of normal ECGs, and absence of any current (lesions $<70\%$ diameter reduction in any major epicardial vessel) or previous evidence of CAD; or (3) a diagnosis of ACS was objectively excluded after admission to the coronary care unit.

Laboratory Methods

Blood samples for the IMA and H-FABP tests were taken from every patient at hospital presentation. Blood was collected in serum separator vacutainer tubes as quickly as possible. Specimens were allowed to clot and then centrifuged for 10 min at $1000 \times g$. Serum was harvested and stored at $-80^{\circ}C$ until testing. Frozen samples were mixed thoroughly after thawing and re-centrifuged before analysis. Serum IMA was measured by ACB test (20) on the Hitachi analyzer 7170. H-FABP was measured by immunochromatography assay (Kang Sheng Bao Bioscience Corporation, Shenzhen, China). H-FABP plasma values below 5 μg/l cannot be detected because of the insensitivity of the applied immunological lateral flow assay.

Statistical Analysis

Diagnostic assay data for normally distributed continuous variables are expressed as mean ± standard deviation (SD) and continuous variables with nonnormal distribution are presented as median and 25–75% percentile. Categorical data are shown as number (percentage). The cutoffs for IMA were experimentally determined by receiver–operator characteristic (ROC) analysis. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy (defined as the sum of true positive and true negatives divided by the total number of patients) were calculated. The 95% confidence intervals of sensibilities, specificities, and accuracies were based on binomial distribution. Comparisons of sensibilities, specificities, and accuracies were made by exact McNemar tests. Statistical significance was established at the 0.05 confidence level. Analyses were performed with the SPSS software package 10.0 (SPSS, Chicago, IL).

RESULTS

Of the 108 enrolled patients, 82 were discharged with a final diagnosis of ACS and 26 with the diagnosis of NICP. Table 1 shows demographic and baseline clinical characteristics of the study patients. Among the ACS patients, 43 had AMI and 39 had unstable angina. In the AMI group, 36 patients had $\geq 70\%$ stenosis on coronary angiography and 7 had 50% stenosis. In the unstable angina group, a clinical diagnosis was made in 11 cases and 28 diagnoses were based upon objective testing (21 with $\geq 70\%$ stenosis on coronary angiography and 7 with a positive treadmill exercise test). A clear noncardiac cause of chest pain was identified in 14 NICP patients (7 had a final diagnosis of gastroesophageal reflux disease, 3 musculoskeletal chest pain, and 4 neuralgia). Among the remaining 12 NICP patients, 7 underwent coronary angiography (normal coronary arteries) and 5 patients had a negative treadmill exercise test.

At the basal levels the two groups were compared in terms of age, sex, smoking, hypertension, hypercholesterolemia, family history of CAD, and diabetes mellitus ($P<0.05$). Statistically, there was no significant difference in the case choice between the two groups. However serum samples from ACS group ($n=82$) had decreased capacity of ACB [64 (61–67) U/ml] compared

TABLE 1. Clinical Characteristics of the Study Group

	Nonischemia chest pain ($n = 26$)	Acute coronary syndrome ($n = 82$)	P
Ages (years)	59±8	58±11	NS
Male	10 (38.5%)	46 (56.1%)	NS
Smoking	4 (15.4%)	16 (19.5%)	NS
Hypertension	8 (30.8%)	39 (47.6%)	NS
Hypercholesterolemia	8 (30.8%)	43 (52.4%)	NS
Family history of CAD	6 (23.1%)	28 (34.1%)	NS
Diabetes mellitus	3 (11.5%)	7 (8.5%)	NS
ACB(U/ml)*	75 (71–78)	64 (61–67)	0.000
H-FABP	5 (19.2%)	68 (82.9%)	0.000

Data are expressed as mean±standard deviation, median (interquartile range)* or number (%). NS, not significant; CAD, coronary artery disease; IMA, ischemia-modified albumin; H-FABP, heart-type fatty acid binding protein.

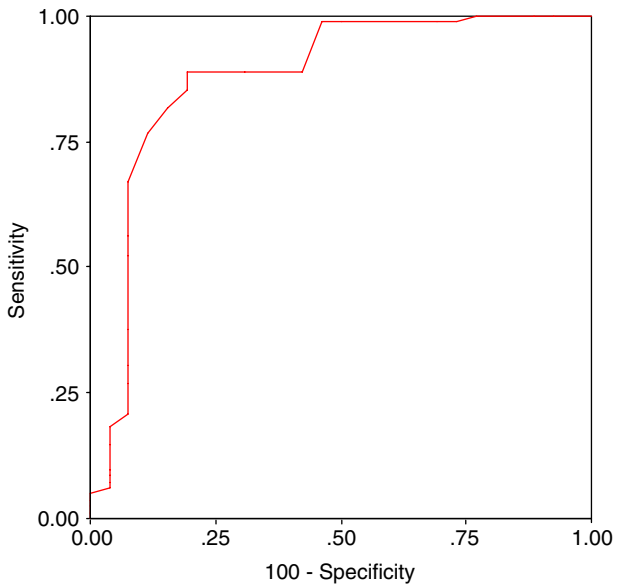

Fig. 1. Receiver–operator characteristic curves of IMA for diagnosis of acute coronary syndrome.

with NICP group ($n = 26$) samples [75 (71–78) U/ml] ($P < 0.05$).

The optimum diagnostic cut-off point for ACB levels in this study population was found to be 70.5 U/ml by ROC analysis (Fig. 1). ACB levels <71.0 U/ml demonstrated a sensitivity of 89.0% and a specificity of 80.8% (area under ROC curve = 0.876 [95% CI 0.783–0.970]) for the diagnosis of ACS. The positive predictive value and negative predictive value were 93.6 and 70.0%, respectively.

The sensitivity and specificity of H-FABP were 82.9% [74.8–91.0%] and 80.8% [65.7–95.9%], respectively. In addition, the diagnostic accuracy was 82.4% [75.2–89.6%].

Patients' presentation according to initial ECG and biological markers and clinical characters are summarized in Table 2. The sensitivity and specificity of combination of IMA and H-FABP were 96.3% [96.2–100.0%] and 80.8% [65.7–95.9%], respectively. In addition, the diagnostic accuracy was 92.6% [87.7–97.5%]. Overall, the combination of IMA and H-FABP was found to have a significant better sensitivity and diagnostic accuracy than all the other biomarker assays ($P < 0.05$). In addition, the positive predictive value and negative predictive value were 94.0 and 87.5%, respectively.

DISCUSSION

Patients without ST-elevation on the initial ECG and with normal troponin but with suspected acute ischemic chest pain are the most difficult to handle in terms of diagnosis. Indeed, they represent two-third of the whole population and one-third of them have a final diagnosis of nonST-elevation myocardial infarction (NSTEMI). H-FABP was found to be very efficient to exclude NSTEMI in this group of patients (21). This test may be particularly valuable to manage patients with atypical symptoms or silent MI or in those with noncontributive ECG (left bundle branch block, LBBB, pacemaker) and in whom the need for urgent reperfusion is uncertain.

In this study, IMA value was significantly higher in patients with ACS compared with NICP. Our observation also supports the study by others (22–24), because an ischemia event may cause as much or more damage to serum albumin and the surrounding tissue as ischemia itself.

This study has shown for the first time that the combination of IMA and H-FABP is an independent sensitive method for the identification of ACS in patients presenting to the ED with acute chest pain and negative cTnT. In our patients, IMA and H-FABP could diagnose ACS with adequately high sensitivity and accuracy, but when combined, it usually had better sensitivity and accuracy than used individually, particularly IMA alone. Measuring the combination of

TABLE 2. Sensitivity, Specificity and Diagnostic Accuracy of all Assays in the Whole Population

	Positive test (*n*)	Sensitivity	Specificity	Diagnostic accuracy
IMA	78	89.0% [82.2–95.8%]	80.8% [65.7–95.9%]	87.0% [80.7–93.3%]
H-FABP	73	82.9% [74.8–91.0%]	80.8% [65.7–95.9%]	82.4% [75.2–89.6%]
CK	41	40.2% [29.6–50.8%]	69.2% [51.5–86.9%]	47.2% [37.8–56.6%]
CK-MB	40	37.8% [27.3–48.3%]	73.1% [56.1–90.1%]	46.3% [36.9–55.7%]
IMA+H-FABP	84	96.3% [92.2–100%][a]	80.8% [65.7–95.9%]	92.6% [87.7–97.5%][a]

[a]There was significant difference compared with the four biomarkers.

admission concentrations of IMA and H-FABP represents a novel method for the early risk stratification of patients with acute chest pain and may be a useful addition to the routine clinical evaluation of patients admitted for chest pain.

H-FABP is a useful biomarker for detection of cardiac injury in ACS. Limitations include a lack of complete cardiac specificity, a relatively small diagnostic window of 24–30 hr after the acute event, and the probability of falsely increased values in patients with renal insufficiency. In this study, the positive rate is significantly higher in patients with ACS compared with NICP. H-FABP secretion into the interstitial space may be mediated by increased permeability of the myocardial cell membrane associated with severe ischemia. Because of its small size, H-FABP is released quickly into the circulation when membrane integrity is compromised in response to cardiac ischemia. Levels of H-FABP are detectable as early as 2–3 hr after injury, with a return to baseline levels typically within 12–24 hours of the initial insult (25,26). Nakata and colleagues (27) found that elevated H-FABP at presentation was associated with an increased need for emergent hospitalization, coronary angiography, and interventional therapy in 133 patients with suspected ACS.

In this study, there was a relatively small number of AMI ($n = 43$) and UA ($n = 39$). Thus, additional studies should be carried out in a larger ACS population to confirm our results. Cut-off values derived from the receiver–operator characteristic (ROC) curves are highly dependent on the study population and might have been different in another set of patients. Thus, the threshold value for IMA that was used in this study should be confirmed in larger follow-up studies.

In conclusion, the combination of IMA and H-FABP measurements after initiation of treatment may be highly effective for risk stratification in patients with ACS and normal cardiac troponin T. In combination with H-FABP, IMA may be useful to cover the complete diagnostic window of patients presenting with ACS in the emergency department, along with ACS in the emergency department, along with the electrocardiographic and clinical symptoms.

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