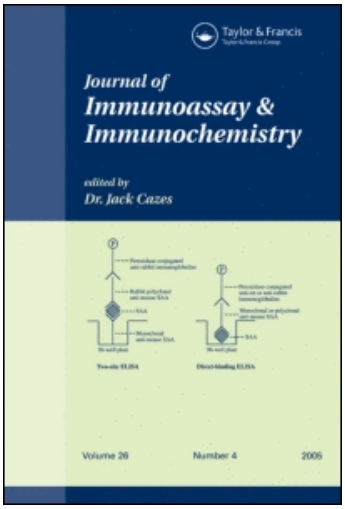

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A ONE-STEP IMMUNOTEST FOR RAPID DETECTION OF HEART-TYPE FATTY ACID-BINDING PROTEIN IN PATIENTS WITH ACUTE CORONARY SYNDROMES

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□ *Using heart-type fatty acid-binding protein (H-FABP) as an early cardiac marker for diagnosis of acute myocardial infarction (AMI) soon after the onset of symptoms requires a rapid assay. A one-step test called, CardioDetect®, is used for detection of H-FABP in whole blood sample.*

Thirty patients suspected of AMI presenting to the emergency department within 12 hours after onset were enrolled in this study. The diagnostic performance of CardioDetect® was compared with different cardiac markers. There were 59.1% of patients with positive H-FABP within 6 hours after onset, while there were only 18.2% with positive cardiac troponin I (cTnI). Results indicated the diagnostic power of H-FABP for AMI was significantly higher than that of cTnI. The sensitivity of H-FABP was 81.8%, which was higher than those of the other cardiac markers, while the specificity was comparable. The area under the receiver operating characteristic curve for H-FABP was 0.909, which was significantly larger than the others.

With this rapid and sensitive immunotest, H-FABP could be soon introduced in clinical practice in combination with well-established markers like troponins.

Keywords acute coronary syndromes, acute myocardial infarction, creatine kinase MB, heart-type fatty acid-binding protein, myoglobin, troponin

INTRODUCTION

In recent years, there have been many in-depth studies regarding acute coronary syndrome (ACS). Tremendous change and progress have appeared in risk stratification and treatment strategy, particularly in

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patients 3 hours after onset. An accurate diagnosis and a proper treatment have a direct impact on the clinical outcome. A rapid bedside cardiac-specific diagnosis is particularly important in ACS patients without a typical change in electrocardiogram (ECG). At present, cardiac troponin T or I, (cTnT or cTnI) and creatine kinase MB (CK-MB) are common cardiac markers, but they usually take 4–6 hours to reach a detectable level.

Heart-type fatty acid binding protein (H-FABP) has been demonstrated to be reliable in early diagnosis of acute myocardial infarction (AMI).[1–5] H-FABP with molecular weight about 15 kDa is rich in myocardial cells. The molecular structure and immunological characteristics of H-FABP are different from the other types of FABP (such as liver, intestine).[6] Its main functions are:[7] (1) to combine and transit of long-chain fatty acids to regulate the metabolism of fatty acids; (2) to probably protect or provide therapeutic effects for myocardial ischemia; (3) to be demonstrated its value on the early diagnosis of myocardial ischemia.

The aim of this study was mainly to evaluate the diagnostic value of H-FABP, myoglobin (MYO), CK-MB, and cTnI in early stage of AMI and to assess the diagnostic performance of a one-step H-FABP immunotest.

EXPERIMENTAL

Study Population

Thirty suspected ACS patients with acute cardiac chest pain within 12 hours presented to the Emergency Department of Tongde Hospital of Zhejiang Province from December 2007 to August 2008 were enrolled in this study. The average age of the patients was 60 years old in which 18 cases were male. They were examined under the 12-lead ECG series observation and qualitative analysis of three cardiac markers (cTnI, CK-MB, and MYO). According to the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction,[8] 18 cases were classified as ST-segment elevation myocardial infarction (STEMI), 4 cases as non-ST-segment elevation myocardial infarction (NSTEMI), and 8 cases as non-cardiac disease (NCD) with chest pain. Thirty healthy subjects from the medical center acted as a control group. They were free of cardiovascular disease, normal in ECG, and negative in three cardiac markers. Patients with renal failure and muscle endocrine disorder were excluded.

Cardiac Markers

Blood samples were taken from every patient at the hospital presentation, together with routine blood sampling. Five mL of blood was taken from veins of all enrolled subjects after physical examination. Four mL of

blood was sent to the laboratory for analysis of three cardiac markers. 9 to 10 drops of blood were added to the well of a three-in-one rapid diagnostic test which was produced by Cortez in the United States and distributed by Shanghai Ling-Yi Ltd. A red line appeared on a test zone after 20 minutes for a positive result. In addition, 100–120 μL of blood was used for a qualitative detection of H-FABP (CardioDetect®)[9–12] in which a sandwich immunoassay was applied. The medical staffs were trained in performing and reading the CardioDetect®. They were blinded to the standard diagnosis. A red line was observed on the test zone after 15 minutes if a sample with the H-FABP concentration exceeding the threshold level (7 μg/L). Another red line was shown on the control zone for verification of the test. Two red lines were observed for a positive result and only one control line was shown for a negative result. If no line appeared on the control zone, the test was invalid and re-testing was required. CardioDetect® H-FABP rapid tests were provided by Shenzhen Kang Sheng Bao Biotechnology Limited and invented by the Hong Kong University of Science and Technology.

Statistical Analysis

Data were presented as means ± standard deviation (SD). Diagnostic parameters including sensitivity, specificity, negative and positive predictive values were determined by using MedCalc (Belgium MedCalc Software; 7.0 version). SPSS 16.0 software was used for plotting the receiver operating characteristic (ROC) curves. The level of significance was set at $P < 0.05$.

RESULTS

Positive Rates of H-FABP Test in Different Groups

Thirty patients were enrolled in this study from December 2007 to August 2008. The average age was 60 ± 18 years old (20 to 87) in which 18 cases were male (60%). 22 cases (73.3%) were diagnosed as AMI in which 18 cases (81.8%) were classified as STEMI, 4 cases as NSTEMI, and 8 cases as NCD. The positive rate of H-FABP in different groups is shown in Table 1. None of the NCD patients and healthy subjects showed positive H-FABP. Most of the patients with ST-segment elevation were finally diagnosed as AMI (81.8%). In this group of patients, 88.9% of them showed positive H-FABP while only 44.4% showed positive cTnI.

Diagnostic Performance of H-FABP and cTnI at Different Periods of Time

Figure 1 shows the resultant distribution of H-FABP and cTnI in AMI patients at different periods of time. Among 22 AMI cases, 5 cases

TABLE 1 Comparison of the Positive Rates of H-FABP in Different Groups

Group	Number of Cases	Mean Age	Sex (M/F)	Positive Rate of H-FABP (%)
Control	30	58	17/13	0.0
STEMI[a]	18	62	12/6	88.9
NSTEMI[a]	4	74	1/3	50.0
NCD[b,c]	8	53	5/3	0.0

STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST-evaluation myocardial infarction; NCD: non-cardiac disease with chest pain; M: male; F: female.

[a]Compared to control group with $P < 0.0001$.

[b]Compared to STEMI group with $P < 0.0001$.

[c]Compared with NSTEMI group with $P < 0.05$.

(22.7%) showed positive H-FABP within 3 hours after onset, but none showed positive cTnI. 13 cases (59.1%) showed positive H-FABP within 6 hours after onset while only 4 cases (18.2%) showed positive cTnI. This indicates that the true positive rate of H-FABP was significantly higher than that of cTnI within 6 hours after onset. Therefore, H-FABP is an earlier cardiac marker than cTnI for diagnosis of AMI.

Diagnostic Performance of Different Cardiac Markers

The sensitivities and specificities of different cardiac markers are shown in Table 2. The sensitivity and specificity of H-FABP for early diagnosis of AMI were 81.8% and 100.0%, respectively. Its sensitivity was superior to the other cardiac markers and its specificity was higher than that of CK-MB, but not much different in specificity when compared with MYO and cTnI.

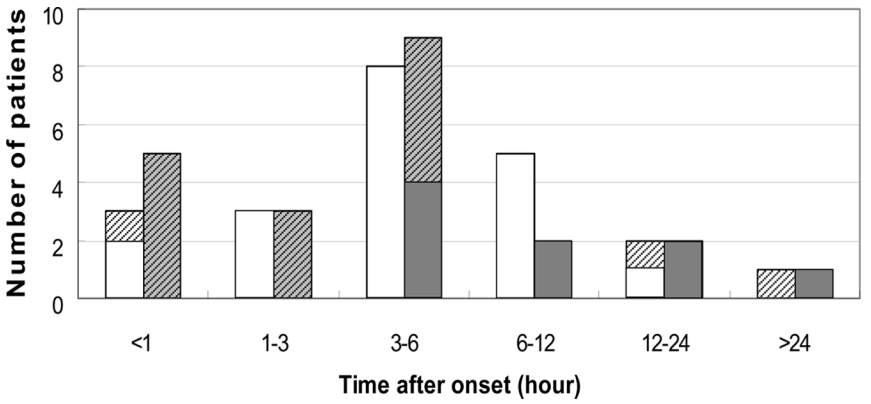

FIGURE 1 Result distribution of H-FABP and cTnI in 22 AMI patients at certain period of time (□: H-FABP positive; ▨: H-FABP negative; ■: cTnI positive; ▨: cTnI: negative).

TABLE 2 Sensitivities, Specificities, and Predictive Values of Different Cardiac Markers

Cardiac Marker	H-FABP	MYO	CK-MB	cTnI
Cutoff value	7 μg/L	70 μg/L	5 μg/L	1 μg/L
Sensitivity	81.8%	54.6%	40.9%	40.9%
Specificity	100.0%	100.0%	75.0%	100.0%
Positive predictive value	100.0%	100.0%	81.8%	100.0%
Negative predictive value	66.7%	44.4%	31.6%	38.1%

H-FABP: heart-type fatty acid-binding protein; cTnI: cardiac troponin I; CK-MB: creatine kinase MB; MYO: myoglobin.

Receiver Operating Characteristic Curves for Different Cardiac Markers

The areas under the ROC curves to distinguish AMI from non-AMI were 0.909 (95%; CI = 0.804–1.000; SE = 0.054; $P = 0.001$) for H-FABP, 0.773 (95%; CI = 0.608–0.937; SE = 0.084; $P = 0.024$) for myoglobin, 0.648 (95%; CI = 0.428–0.868; SE = 0.112; $P = 0.223$) for CK-MB, and 0.705 (95%; CI = 0.518–0.891; SE = 0.095; $P = 0.091$) for cTnI. The area under the curve for H-FABP was significantly greater than those of the others. Thus, H-FABP has great potential as an excellent cardiac marker for diagnosis of AMI in the early phase.

DISCUSSION

Acute cardiac chest pain is the most common symptom in the emergency department and the cardiology department. If ACS patients can be diagnosed within 3 hours after onset, together with a proper treatment strategy, the death rate can be dramatically reduced. In addition, accurate discharge of non-AMI patients in the early stage can conserve the economic benefits for both patients and the hospital.

At present, the most common biomarkers for diagnosis of AMI are troponins, CK-MB, and MYO. In fact, cTnI and CK-MB has been widely used in AMI diagnosis and risk stratification. Although their specificities are high, their sensitivities have been poor within 6 hours after onset. H-FABP was proved to be more sensitive than the other cardiac markers in the early diagnosis of AMI.[1–5] In this study, the true positive rate of H-FABP in the STEMI group was 88.9% 12 hours after onset, while only 44.4% for cTnI. A British prospective study demonstrated that the sensitivity of H-FABP for diagnosis of AMI was significantly higher than cTnI (73.0% vs. 55.0%, $P = 0.043$) 4 hours after onset. The combination of H-FABP and cTnI for diagnosis can further improve the sensitivity to 85.0%.[13]

In this study, the effectiveness of a one-step bedside H-FABP immunotest using specific monoclonal H-FABP antibodies was evaluated. The H-FABP immunotest gave higher sensitivity, specificity, positive and negative predictive values than those of the other cardiac markers at the early stage. Its diagnostic performance was similar to that of Rapicheck H-FABP (Dainippon Pharmaceutical Co., Ltd. Osaka, Japan) with a slightly lower cutoff value (6.2 μg/L) described by Tanaka et al.[14] Although MYO usually gives a positive result within 3 hours after onset, the damage of cardiac and skeletal muscles cannot be easily distinguished. The myocardial content of H-FABP (0.57 mg/g wet weight) is four- to five-fold lower than that of myoglobin (2.7 mg/g wet weight), yet the plasma reference concentration of H-FABP (1.8 μg/L) is 19-fold lower than that of myoglobin (34 μg/L). This means that, after injury, the tissue to plasma gradient is almost five-fold steeper for H-FABP than for myoglobin, making plasma H-FABP rise above its upper reference concentration at an earlier point after AMI onset, thus permitting an earlier diagnosis of AMI.[1,15]

The area under the ROC curve for H-FABP was the greatest among cTnI, CK-MB and MYO within 12 hours after the onset of chest pain. The other studies also showed that H-FABP was superior to the other cardiac markers in the early diagnosis of myocardial infarction.[1–5] This characteristic can be explained by the small molecular size of H-FABP. It can be released rapidly from the cells into the circulation when myocardial ischemic cells are damaged. The H-FABP concentration increased sharply within 3 hours after onset. It reached maximum in 4–8 hours and returned to normal level after 24–30 hours.[3]

H-FABP is cardiac specific, together with immuno-recognition function. These properties provide its ability in diagnosis of acute phase of myocardial damage, confirmation of AMI, and as a key indicator in revascularization for ACS patients.

Limitations of H-FABP Immunotest

A false positive result of H-FABP immunotest may be shown in non-AMI patients. This can be explained by the low molecular weight of H-FABP which is quickly released into the blood stream and is rapidly eliminated by the kidneys. Gorski et al.[16] reported that H-FABP was higher in non-AMI patients with chronic renal failure than that in healthy subjects. Cardiac troponins also have this limitation. In fact, the concentrations of cTnI and cTnT were also relatively high in renal deficient non-AMI patients.[17]

Since a small amount of H-FABP also presents in skeletal muscle, a person with severe muscle injury may show a false positive result on the

H-FABP immunotest.[18] However, the concentration of H-FABP in the heart muscle is much higher than that in skeletal muscle[19] and, thus, gives high specificity in the diagnosis of AMI.

Improper use of the H-FABP immunotest, such as applying less than 3 drops (less than 100 μL) of blood to the test or using the tests in less than 20 minutes after chest pain, may cause a false negative result.

Study Limitations

This study is based at a single centre and so the generalisability of our findings to other settings requires further study. Secondly, this is a hospital based rather than community based study, and, so, the application of our findings to the family physician or outpatient clinic setting is unclear. Furthermore, this study was limited by the number of patients and a continuous measurement of H-FABP was omitted after confirmation as a negative case. Chan et al. proved that the sensitivity and the negative predictive value of H-FABP reached to 100.0% in 1 hour after admission.[3] Whether the re-measurement of H-FABP negative samples after 1 hour can improve the diagnostic accuracy should be further investigated.

CONCLUSION

This study showed that H-FABP is able to diagnose AMI within 1 hour. Its sensitivity is superior to the other cardiac markers, especially within 3 hours after onset. Its specificity was comparable to the others. A non-AMI patient may be safely ruled out with a negative H-FABP result. In the past, H-FABP could only be quantified by using enzyme-linked immunosorbent assay, which took at least 1 hour to obtain the result and the operation procedure was complicated. Also, special equipment was required. Therefore, it cannot be used as an on-site test for rapid diagnosis. In this study, a one-step H-FABP immunotest based on a sandwich principle was evaluated. It is very easy to be operated and, thus, is favorable to be used in an ambulance, in the emergency department, and in any emergency situation.

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