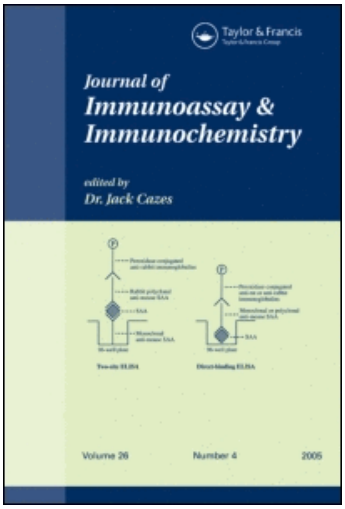

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Clinical Evaluation of Point-of-Care-Testing of Heart-Type Fatty Acid-Binding Protein (H-FABP) for the Diagnosis of Acute Myocardial Infarction

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Abstract: The present study was carried out for clinical evaluation of point-of-care-testing (POCT) of heart-type fatty acid-binding protein (H-FABP), Rapicheck H-FABP, for the diagnosis of acute myocardial infarction (AMI), in comparison with conventional cardiac biochemical markers such as myoglobin, creatine kinase isoenzyme MB (CK-MB), and cardiac troponin T.

Whole blood samples from patients with confirmed AMI (n = 53), patients with non-AMI cardiac diseases (n = 24), and patients with non-cardiac diseases with chest pain (n = 6) were used. When a test line appeared within 15 min after the addition of 150 μL of whole blood, it was designated to be positive for H-FABP. A control line indicates a proper use of the test. On the other hand, when no test line appeared, it was negative.

In the superacute phase of AMI within 3 hours, the diagnostic sensitivity of H-FABP was 93.1%, which was the highest of the four markers compared here. The

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diagnostic specificity in the phase for H-FABP was 64.3%, while it was 100% with cardiac troponin T.

The POCT of H-FABP is thought to be practical for the detection of cardiac damage and effective for the diagnosis of AMI in superacute phase within 3 hours and/or 6 hours.

Keywords: Rapicheck H-FABP, Fatty acid-binding protein, Immunochromatography, Panel test, Point-of-care-testing (POCT)

INTRODUCTION

To achieve an effective salvage of ischemic myocardium following a sudden or ongoing interruption of coronary flow requires reduction of door-to-needle time, i.e., immediate initiation of therapy. Revascularization therapy, such as percutaneous coronary intervention, including percutaneous transluminal coronary recanalization (PTCR) and/or percutaneous transluminal coronary angioplasty (PTCR), has been widely performed in an attempt to reduce infarct size and improve ventricular function. This medical procedure must be carried out as soon as possible, i.e., within 6 hours after the occlusion of a coronary artery. However, diagnosis in the early stage of AMI is sometimes difficult because of the delayed liberation of serum cardiac enzymes, such as creatine kinase isoenzyme MB (CK-MB) and equivocal early ECG changes. Thus, detection of a rapidly appearing serum biochemical marker specific for myocardial damage would facilitate a more appropriate diagnostic and therapeutic approach in patients with suspected AMI with chest pain.

Human heart-type fatty acid-binding protein (H-FABP) is an extremely abundant cytoplasmic protein in myocardial cells and is part of a multigene family of low molecular weight proteins (mol. wt. 14.9 kDa). H-FABP has been proposed as an early cardiac marker for the diagnosis of AMI and the estimation of myocardial infarct size using animal models[1–3] and clinical samples.[4–10] However, H-FABP has not yet been evaluated with respect to its diagnostic validity as a cardiac injury marker for AMI assessment. A point-of-care-testing (POCT) for detection of human H-FABP, named Rapicheck H-FABP (Figure 1), has recently been developed, which makes it possible to detect H-FABP in plasma fraction of whole blood.[11] This whole blood panel test is based on one-step immunochromatography using a combination of two distinct types of mouse anti-human H-FABP monoclonal antibodies.

In the present study, we performed a clinical assessment of the POCT for H-FABP by using whole blood samples obtained from patients with suspected AMI, consisting of confirmed AMI and non-AMI with chest pain. In order to clarify the diagnostic utility of the POCT for H-FABP for early detection of myocardial damage, the diagnostic parameters such as sensitivity, specificity,

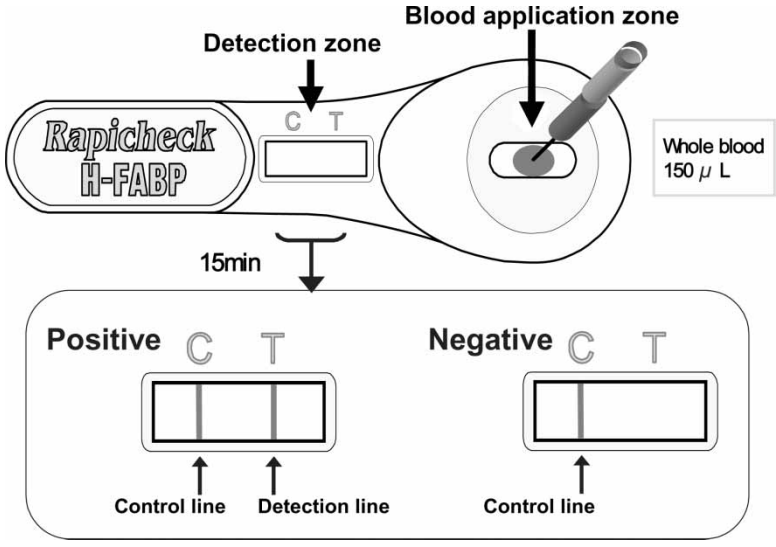

Figure 1. Point-of-care-testing for H-FABP.

efficacy, positive predictive value (PPV), and negative predictive value (NPV) of H-FABP were compared with those of three conventional rapid tests for the diagnosis of myocardial damage, i.e., myoglobin,[12,13] CK-MB,[14,15] and cardiac troponin T,[16,17] which are the most commonly and routinely utilized in clinical laboratories in Japan.

EXPERIMENTAL

Patients and Reference Cardiac Markers for Clinical Evaluation

The clinical validity of the POCT for H-FABP was compared with conventional rapid tests for myoglobin (Rapitex Mb, Dade Boehring, Deerfield, IL, USA), CK-MB (Merck Auto CK-MB, Kanto Kagaku, Tokyo, Japan), and cardiac troponin T (Trop T sensitive, Roche Diagnostics, Mannheim, Germany). In the H-FABP POCT, 6.2 ng/mL was used as a cutoff value for the diagnosis of AMI. On the other hand, 80 ng/mL was used for the myoglobin kit, and 0.1 ng/mL for troponin T kit. Whole blood samples were obtained from patients suspected of having AMI with chest pain, consisting of confirmed AMI patients (n = 53), patients with cardiac diseases who were afterward confirmed not to have AMI by a normal pattern of electrocardiography (ECG) and other clinical aspects (n = 24), and patients with non-cardiac diseases with chest pain (n = 6). Whole blood samples were also collected from normal healthy individuals (n = 30). In this study, normal,

healthy individuals were chosen on the basis of the following criteria, i.e., no abnormality with ECG aspects, blood pressure, glucose tolerance, and serum lipid levels (triglyceride, 150 mg/dL; total cholesterol, 240 mg/dL; HDL-cholesterol 40 mg/dL). The grouping of the clinical samples in this study is summarized in Table 1.

Diagnosis of AMI was based on WHO criteria[18] during the 24-hour monitoring period, i.e., 1) chest pain persisting for more than 30 min; 2) ECG evidence of ST segment elevation or depression of more than 0.2 mV or new Q waves in at least two leads; 3) elevation of serum CK-MB activity to more than twice the upper limit of normal range within 24 hours of admission, and confirmed by detection of obstruction in the culprit coronary artery by coronary angiography.

In this study, whole blood samples were obtained at the time of admission from the patients with suspected AMI within 12 hours after the onset of symptoms, then used for tests with these cardiac markers. Informed consent was obtained from all patients and normal healthy individuals, according to the standard procedure established by the internal review board of each of the hospitals that participated in the evaluation.

The diagnostic validity was evaluated by using the diagnostic sensitivity, specificity, efficacy, PPV and NPV as parameters. These parameters were calculated according to the following formula. The diagnostic sensitivity was assessed by calculating the percentage of patients with confirmed AMI whose results were positive. The specificity was assessed by calculating the percentage of patients with non-AMI, with chest pain, whose results were negative. The efficacy was calculated for the group of suspected AMI,

Table 1. Grouping of clinical samples in this study

			Age (y/o)	
Group	Gender	n	Mean	SD
AMI		57	64.9	11.0
	M	43	64.3	11.6
	F	14	66.7	8.9
Non-AMI (cardiac)		25	61.0	10.2
	M	20	59.9	10.7
	F	5	65.4	7.3
Non-cardiac		6	65.5	8.7
	M	4	64.0	10.7
	F	2	68.5	2.1
Healthy subjects		30	40.4	8.3
	M	23	41.6	8.8
	F	7	36.6	5.1

consisting of patients with confirmed AMI and non-AMI with chest pain as the percentage of patients with confirmed AMI whose results were positive plus patients with non-AMI whose results were negative in the group of suspected AMI. The PPV was calculated by the percentage of patients with confirmed AMI in all the patients whose results were positive. The NPV was calculated by the percentage of patients with non-AMI with chest pain in all the patients whose results were negative.

Assay Procedure for Rapicheck H-FABP and Other Reference Cardiac Markers

The principle of Rapicheck H-FABP (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) is based on one-step immunochromatography using two distinct types of mouse monoclonal antibodies specific for human H-FABP. One monoclonal antibody is used as a gold colloid-labeled antibody and the other is as a membrane-fixed antibody on the test (T) line. Whole blood (150 μL), including an anticoagulant such as heparin-Na or EDTA-2Na, was used as a sample for the Rapicheck H-FABP as a POCT of H-FABP. At 15 min after the addition of a blood sample, judgment was carried out macroscopically. As shown in Figure 1, two red lines at the control (C) line and T line came out, it was designated to be positive. On the other hand, only one red line at the C line was judged negative.

The assay procedures of the rapid tests for myoglobin, cardiac troponin T, and CK-MB were in accordance with the manufacturers' manuals. In the case of myoglobin, when an aggregation appeared, it was considered to be positive. No aggregation was judged negative. In the troponin T test, two red lines indicated positive; one red line indicated negative. The cutoff values for H-FABP, myoglobin, and troponin T used in these commercial kits were 6.2 ng/mL, 80 ng/mL, and 0.1 ng/mL, respectively. In the CK-MB assay, enzymatic activity of CK-MB was measured by an immunoinhibition assay. The cutoff value of CK-MB activity for the diagnosis of AMI was 25 U/L, which is most commonly utilized as a cutoff value for the diagnosis of AMI in Japan.

Positivity in Patients with Cardiac Diseases Including Confirmed AMI and Non-AMI and Normal Healthy Individuals

The positivity of H-FABP and reference cardiac markers were assessed using blood samples from patients with cardiac diseases with chest pain, including confirmed AMI and non-AMI. These patients were grouped by the time of onset of symptom of chest pain. In addition, the positivity in normal healthy subjects was also assessed. The positivity of H-FABP and other reference cardiac markers were calculated for each group.

Qantification of H-FABP Concentration in Plasma by a Direct Sandwich ELISA

The plasma samples obtained by centrifuging a part of the whole blood samples were used for the quantification of H-FABP. The plasma concentration of H-FABP was quantified by a two-step direct sandwich ELISA kit using a combination of two distinct types of monoclonal antibodies specific for human H-FABP, named MARKIT-M H-FABP[19] (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). The standard assay procedure of the direct sandwich-ELISA is described below. In brief, the standard human H-FABP or test plasma sample was mixed with an equal volume of the dilution buffer of the kit. Then, 100 μL of the mixture was placed in a microtiter plate well coated with anti-human H-FABP monoclonal antibody. The plate wells were incubated for 30 min at 25°C to allow the H-FABP molecules to bind to the monoclonal antibody coating the well. Next, the content of each well was discarded and the wells were washed three times with the washing buffer of the kit to remove unbound H-FABP molecules. Horseradish peroxidase (HRP)-conjugated anti-human H-FABP monoclonal antibody (100 μL) was added to each microtiter well, followed by incubation for 30 min at 25°C. The content of each microtiter well was discarded, and the wells were washed three times with the washing buffer to remove unreacted HRP-conjugated monoclonal antibody. Then, the HRP enzyme assay was started by addition of 100 μL of color-developing reagent solution (one substrate tablet in 15 mL) of the substrate diluent buffer of the kit to each well. After incubation for 15 min at 25°C, the reaction was terminated by addition of 100 μL of the stop solution from the kit. The absorbance of each well at 492 nm was measured using a Multiskan Bichromatic plate reader (Labsystems, Helsinki, Finland) equipped with Delta Soft II (BioMetalics, Princeton, USA). Pure human H-FABP standards (50 μL of samples equivalent to 0, 5, 10, 25, 50, 100, and 250 ng/mL), mixed with an equal volume of the dilution buffer were placed in the microtiter plate wells coated with anti-human H-FABP monoclonal antibody, and the subsequent steps were performed as described above for the preparation of a standard curve. The concentration of H-FABP mass in plasma was calculated by reference to the standard curve prepared from the human H-FABP standards and expressed as ng of H-FABP protein per mL of plasma.

Statistical Analysis

Differences in diagnostic sensitivity, specificity, efficacy, PPV, and NPV, among the biochemical cardiac markers investigated in the present study, were evaluated by the sign test; $P < 0.05$ was considered to be statistically significant.

RESULTS

Diagnostic Validity of the POCT of H-FABP and other Reference Cardiac Markers

The diagnostic validity of the POCT for H-FABP was assessed by using the diagnostic sensitivity, specificity, efficacy, PPV, and NPV, in comparison with those of the rapid tests for myoglobin, troponin T, and CK-MB activity. Figure 2 through 6 present comparisons of these diagnostic parameters of H-FABP with those of myoglobin, troponin T, and CK-MB. Figure 2 shows the diagnostic sensitivity data for the confirmed AMI group, and it is seen that H-FABP showed the highest sensitivity (93.1% within 3 hours, 95.6% within 6 hours, 96.2% within 12 hours) of these four markers at all times after the onset of symptoms. The diagnostic sensitivity of CK-MB and cardiac troponin T at the time within 3 hours after the onset of symptoms was 13.8% ($P < 0.001$, vs H-FABP) and 24.1% ($P < 0.001$, vs H-FABP), respectively. The overall sensitivity of H-FABP within 12 hours after the onset of symptoms was 96.2%, compared with 52.8% for myoglobin ($P < 0.001$, vs H-FABP), 49.1% for cardiac troponin T ($P < 0.001$, vs H-FABP) and 34.0% for CK-MB ($P < 0.001$, vs H-FABP). The sensitivity of H-FABP at any time was significantly high when it was compared to those of others ($P < 0.001$). On the other hand, the diagnostic specificity data for the non-AMI with chest pain group are shown in Figure 3. The diagnostic specificity of H-FABP was almost of the same

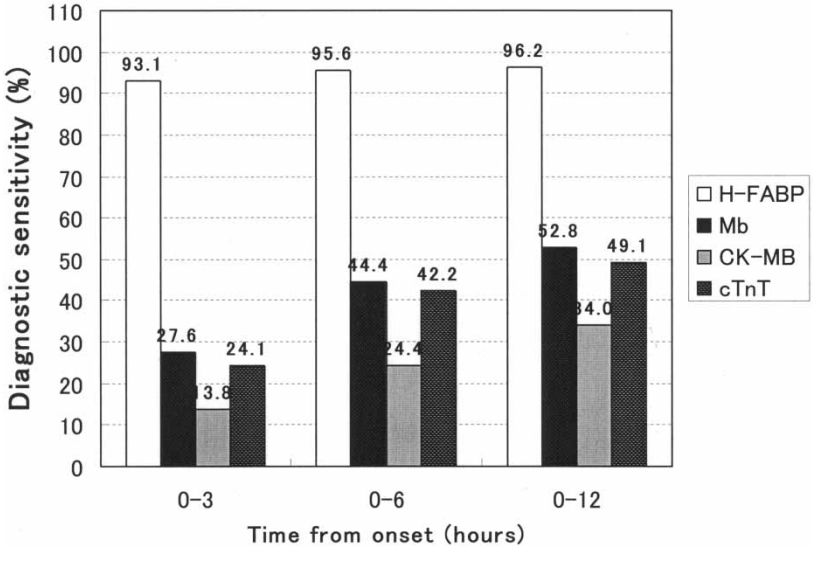

Figure 2. Diagnostic sensitivity of H-FABP and reference cardiac markers.

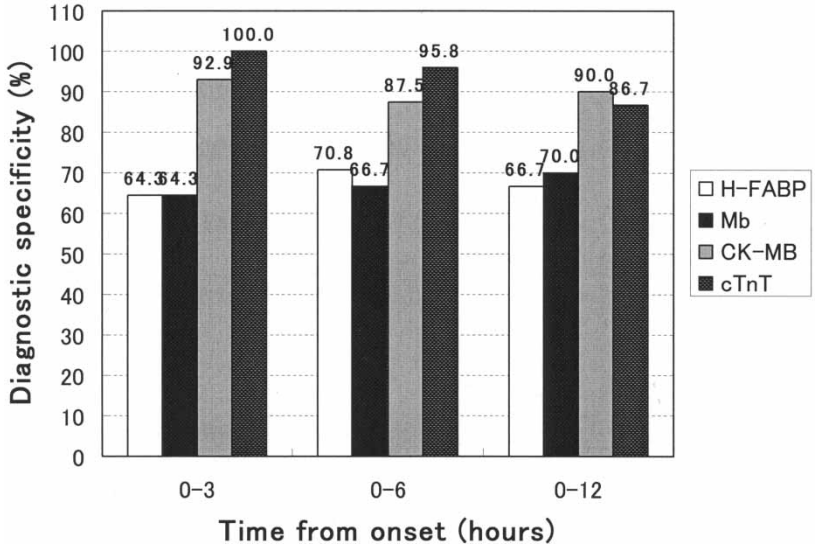

Figure 3. Diagnostic specificity of H-FABP and reference cardiac markers.

magnitude as that of myoglobin, but smaller than those of cardiac troponin T and CK-MB at all times after the onset of symptoms. The overall specificity of H-FABP within 12 hours after the onset of symptoms was 66.7%, compared with 70.0% for myoglobin ($P = 0.71$, vs H-FABP), 86.7% for cardiac troponin T ($P = 0.012$, vs H-FABP) and 90.0% for CK-MB ($P = 0.017$, vs H-FABP). Figure 4 presents the diagnostic efficacy data for H-FABP in comparison with myoglobin, cardiac troponin T, and CK-MB. H-FABP showed the highest diagnostic efficacy (83.7% within 3 hours, 87.0% within 6 hours, 85.5% within 12 hours) of these four biochemical cardiac markers at all times after the onset of symptoms. Within 3 hours, the efficacy of H-FABP was 83.7%, then cardiac troponin T and CK-MB showed 48.8% ($P < 0.001$, vs H-FABP) and 39.5% ($P < 0.001$, vs H-FABP), respectively. In Figures 5 and 6, the PPV and NPV of these four markers are shown. In the PPV, troponin T showed the highest value (100.0% within 3 hours, 95.0% within 6 hours, 86.7% within 12 hours) of these four biochemical cardiac markers at all times, while H-FABP showed the highest value (81.8% within 3 hours, 89.5% within 6 hours, 90.9% within 12 hours) in the NPV. The difference of NPV between H-FABP and others was statistically significant ($P < 0.001$).

Positivity of the POCT for H-FABP and Other Reference Cardiac Markers

The positivity of the POCT for H-FABP and other reference cardiac markers in the patients with cardiac diseases, including confirmed AMI and non-AMI,

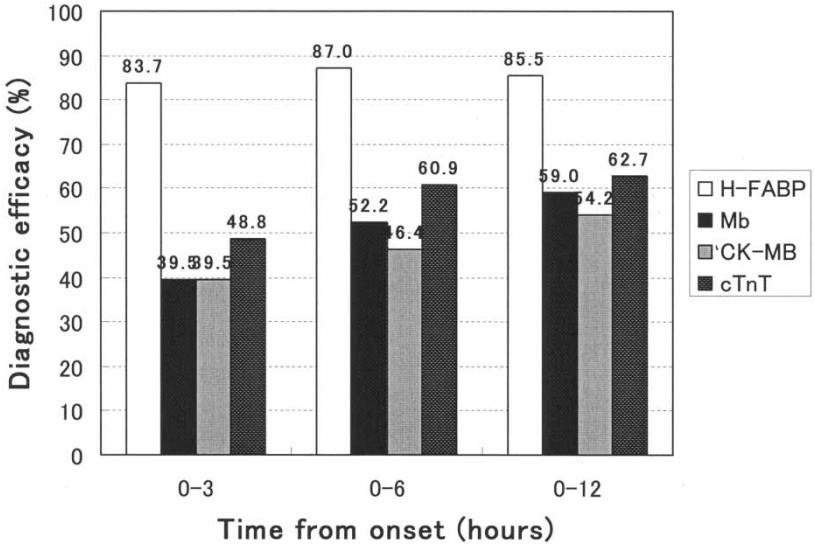

Figure 4. Diagnostic efficacy of H-FABP and reference cardiac markers.

such as unstable angina pectoris, cardiomyopathy, endocarditis, etc., is shown in Figure 7. H-FABP showed the highest positivity at all times after the onset of symptoms when it was compared to those of other markers ($P < 0.001$). Namely, it was 79.5% within 3 hours, 77.8% within 6 hours and 77.9%

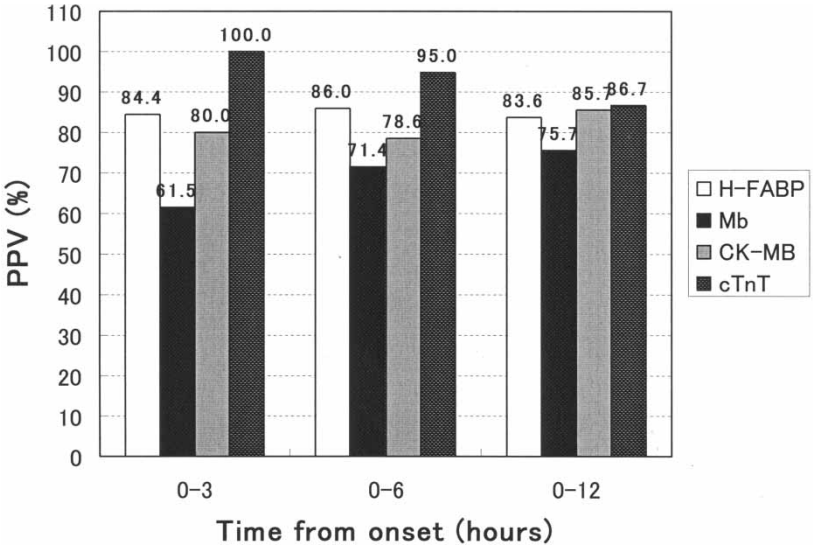

Figure 5. PPV of H-FABP and reference cardiac markers.

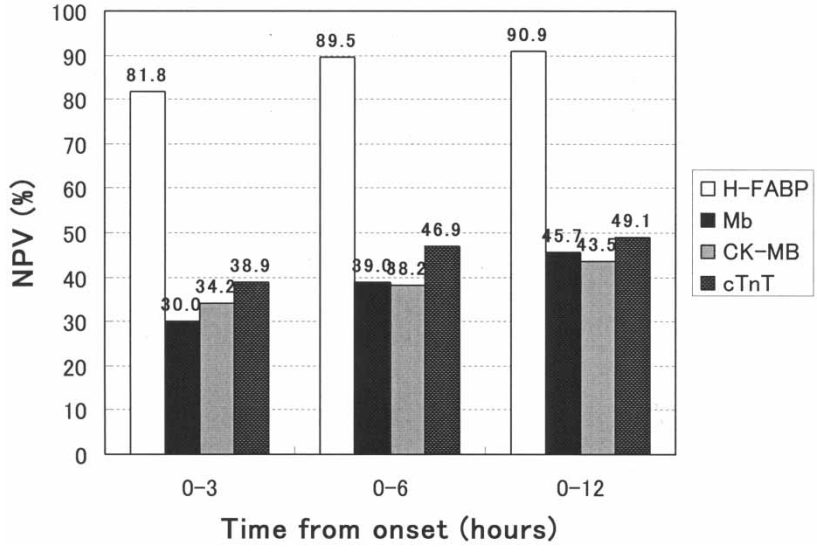

Figure 6. NPV of H-FABP and reference cardiac markers.

within 12 hours, respectively. On the other hand, the positivity of cardiac troponin T was 17.9%, 31.7%, and 39.0%, respectively. In the case of CK-MB, the positivity was lower than that of cardiac troponin T at any time windows. In the group of normal healthy individuals, no case with positive

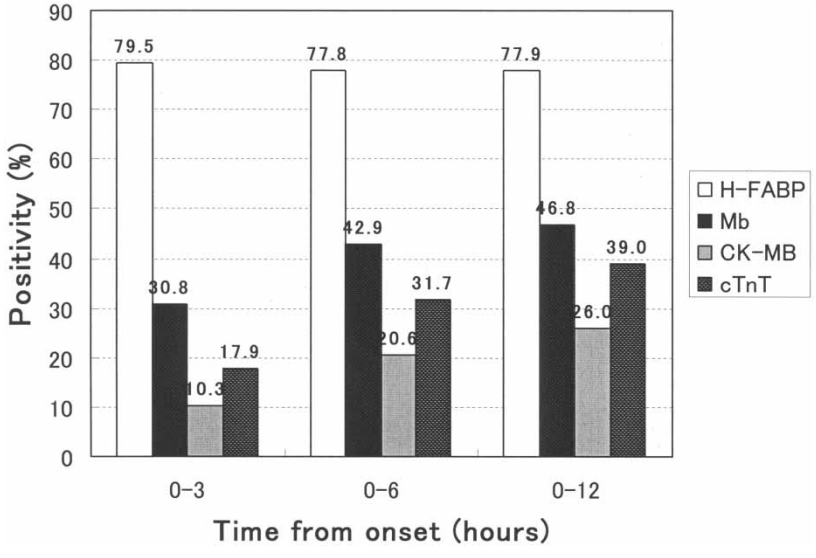

Figure 7. Positivity of H-FABP and reference cardiac markers in cardiac diseases.

judgment was seen with H-FABP. Then, the corresponding plasma concentrations of H-FABP in the normal healthy individuals were 2.7 ± 0.96 ng/mL (mean $\pm$ SD, $n = 30$) for all, 2.8 ± 1.0 ng/mL for male ($n = 23$), and 2.1 ± 0.37 ng/mL for female ($n = 7$), which were much less than the cutoff value (6.2 ng/mL) for the diagnosis of AMI.[8]

DISCUSSION

In the present study, the detection of H-FABP in whole blood was performed by a POCT, using a combination of two distinct types of anti-human H-FABP monoclonal antibodies which has been commercially available, named as Rapicheck H-FABP.[11] The diagnostic validity of the POCT for H-FABP was compared with those of rapid tests for myoglobin, cardiac troponin T, and CK-MB. The diagnostic sensitivity of H-FABP for confirmed AMI in the group of suspected AMI was higher than those of myoglobin, cardiac troponin T, and CK-MB, whereas the specificity for non-AMI in the group of patients with suspected AMI was also higher than that of myoglobin, but lower than that of cardiac troponin T and CK-MB. These results are thought to be caused by the molecular size and location of these cardiac markers. H-FABP, myoglobin, CK-MB, and a part of cardiac troponin T are located in cytoplasm of myocardium. On the other hand, most of the cardiac troponin T is localized in the fibrous structure of myocardial cells. On top of that, the results would be also caused by the fact that CK-MB was used as one of the WHO criteria for the confirmation of AMI. In addition, the low diagnostic sensitivity of cardiac troponin T and CK-MB may be caused by the fact that a large number of patients with confirmed AMI were in the acute phase shortly after the onset of symptoms in this study. In the diagnostic efficacy, calculated using the data for sensitivity and specificity, H-FABP was superior to the other three markers, and the difference in the diagnostic efficacy between H-FABP and CK-MB, as well as cardiac troponin T, was especially remarkable. Myoglobin is not very specific for myocardial damage because of its abundance in skeletal muscle, such as in the myocardium,[20,21] which also explains the lower diagnostic specificity in patients with chest pain. These findings indicate that H-FABP is more sensitive and specific than myoglobin for the detection of AMI within 12 hours, especially within 3 hours, after the onset of chest pain.[22] Recently, it has been reported that elevated levels of H-FABP were also found in stroke and certain neurodegenerative diseases.[23,24] On the other hand, cardiac troponin T and CK-MB were less sensitive for AMI in the acute phase within 6 hours; therefore, they would not be used for the early detection of myocardial injury, but for the confirmation of AMI after 6 hours or more from the onset of symptoms. Recently, Seino et al. reported the superiority of the POCT for H-FABP, in comparison with cardiac troponin T, for the diagnostic sensitivity in the early evaluation of patients who present with acute chest

pain.[25,26] In addition, they compared the diagnostic accuracy of H-FABP and myoglobin by ROC curve analysis based on quantitative measurements of both markers, and indicated the predominance of H-FABP to myoglobin. In the present study, H-FABP showed the highest positivity in cardiac diseases at any time windows, which would reflect minor myocardial damage in these cardiac diseases.

Lately, therapeutic interventions, such as PTCA and PTCR, designed to interrupt the ongoing process of myocardial necrosis, have been established as medical treatments for patients with AMI; it has been ascertained that the prognosis of AMI after such interventions depends on how early this kind of medical care is started. Accordingly, rapid detection of H-FABP would enable the diagnosis of AMI soon after the onset of symptoms and make it possible to achieve coronary reperfusion in the early phase of AMI. Ishii et al. reported the utility of H-FABP as a parameter for early detection of successful coronary reperfusion, based on serum concentration of H-FABP.[27] Furthermore, the diagnostic and prognostic utility of H-FABP in acute coronary syndrome, including AMI, was discussed recently.[28]

In the present study, we compared the diagnostic utility of the POCT for H-FABP and three conventional cardiac markers using patients with suspected AMI, with chest pain within 12 hours after the onset of symptoms, and the results show the superior diagnostic validity of H-FABP, especially in terms of the diagnostic sensitivity, efficacy, and NPV, compared to those of myoglobin, cardiac troponin T, and CK-MB.

In conclusion, the POCT for H-FABP seems to have a potent diagnostic utility for the detection of myocardial damage, and its detection should be valuable and practical for the early diagnosis of AMI in the superacute phase within 3 hours and/or 6 hours.

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