

# Heart-type Fatty Acid Binding Protein as an Adjunct to Cardiac Troponin-I for the Diagnosis of Myocardial Infarction

Kyung Su Kim<sup>1</sup>, Hui Jai Lee<sup>1</sup>,  
Kyuseok Kim<sup>2</sup>, You Hwan Jo<sup>2</sup>,  
Tae Yun Kim<sup>2</sup>, Jin Hee Lee<sup>2</sup>,  
Joong Eui Rhee<sup>2</sup>, Gil Joon Suh<sup>1</sup>,  
Mi Ran Kim<sup>3</sup>, Christopher C. Lee<sup>4</sup>,  
and Adam J. Singer<sup>4</sup>

<sup>1</sup>Department of Emergency Medicine, Seoul National University Hospital, Seoul; <sup>2</sup>Department of Emergency Medicine, Seoul National University Bundang Hospital, Seongnam; <sup>3</sup>Department of Emergency Medicine, Inje University College of Medicine, Goyang, Korea; <sup>4</sup>Department of Emergency Medicine, Stony Brook University Hospital, Stony Brook, New York, USA

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Address for Correspondence:

Kyuseok Kim, MD  
Department of Emergency Medicine, Seoul National University Bundang Hospital, 166 Gumi-ro, Bundang-gu, Seongnam 463-707, Korea  
Tel: +82.31-787-7572, Fax: +82.31-787-4055  
E-mail: dremkks@snuh.org

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We hypothesized that when used in combination with cardiac troponins, heart-type fatty acid binding protein (H-FABP) would have greater diagnostic value than conventional markers for the early diagnosis of myocardial infarction (MI). Patients with typical chest pain at a single emergency department were consecutively enrolled. Initial blood samples were drawn for H-FABP, myoglobin, creatine kinase isoenzyme MB (CK-MB), and cardiac troponin-I (cTnI) measurements. MI was defined by serial cTnI measurements. To evaluate the adjunctive role of biochemical markers, we derived and compared logistic regression models predicting MI in terms of their discrimination (area under the receiver operating characteristics curve, AUC) and overall fit (Bayesian information criterion, BIC). Seventy-six of 170 patients were diagnosed as having MI. The AUC of cTnI, H-FABP, myoglobin, and CK-MB were 0.863, 0.827, 0.784, and 0.772, respectively. A logistic regression model using cTnI ( $P = 0.001$ ) and H-FABP ( $P < 0.001$ ) had the biggest AUC (0.900) and the best fit determined by BIC. Sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of this model at 30% probability were 81.6%, 80.9%, 4.26, and 0.23, respectively. H-FABP has a better diagnostic value than both myoglobin and CK-MB as an adjunct to cTnI for the early diagnosis of MI.

**Key Words:** Myocardial Infarction; Fatty Acid-Binding Proteins; Point-of-Care Systems; Chest Pain

## INTRODUCTION

Biochemical markers, such as the highly sensitive and specific cardiac troponins, play a pivotal role in the diagnosis and management of patients with acute coronary syndrome (ACS) (1). With its higher sensitivity and virtually total specificity, cardiac troponin has become a critical determinant for the diagnosis of myocardial infarction (MI) (2).

Because cardiac troponins generally do not appear in the serum prior to 4-10 hr after symptom onset, early markers such as myoglobin have been used (3). However, as the sensitivity of troponin assays continues to improve and as the 99th percentile of the upper reference limit (URL) is used as a cutoff for the diagnosis of MI, the clinical value of such early markers is now doubtful (4-6).

Heart-type fatty acid binding protein (H-FABP) is one of the most abundant proteins in the heart and has performed better than myoglobin for the early diagnosis of MI (7-9). Rapid point-of-care tests (POCT) of H-FABP have also shown similar results (10, 11).

We hypothesized that when used in combination with cardiac troponins, H-FABP would have greater diagnostic value than other conventional markers for the early diagnosis of MI.

The aim of this study was to test this hypothesis by comparing the diagnostic performances of initial biochemical markers including H-FABP, myoglobin, and creatine kinase isoenzyme MB (CK-MB) along with cardiac troponin-I (cTnI).

## MATERIALS AND METHODS

### Patients

We performed this study at a single academic, urban emergency department (ED) with an annual census of approximately 65,000 from December 2006 to September 2007. Patients with chest pain who visited the ED between 9:00-17:00 on weekdays were screened upon the availability of a research nurse.

Patients were consecutively enrolled by senior residents or attending physicians of emergency medicine if their chest pain had satisfied any of the following criteria: 1) typically located in the substernal region, 2) sense of heaviness or squeezing nature,

3) caused by exercise, or 4) relieved by resting or nitroglycerin.

Exclusion criteria included age under 18 yr old, refusal to participate, and history of chronic renal insufficiency. Patients with increased levels of serum creatinine ( $\geq 1.5$  mg/dL [ $132 \mu\text{M/L}$ ]) were further excluded from the analysis to reduce false positive results, because elimination of H-FABP mostly depended on renal excretion (12). This study was approved by the institutional review board, and all participants gave informed consent (IRB No. B-0706/046-011).

### Measurement of biochemical markers

Immediately after enrollment, peripheral blood samples were collected for initial biochemical assays. cTnI, myoglobin, and CK-MB were measured at an emergency clinical laboratory using the Dimension<sup>®</sup> clinical chemistry system (Siemens, Newark, NJ, USA) with a one-step enzyme immunoassay based on the “sandwich” principle.

H-FABP was measured by the research nurse using CardioDetect<sup>®</sup> med (Rennesens GmbH, Berlin, Germany) and CardioDetect<sup>®</sup> quant (Rennesens GmbH). After placing three drops of whole blood onto a test strip of CardioDetect<sup>®</sup> med, H-FABP in the serum was bound to the monoclonal antibody resulting in a red line within 15 min. In order to avoid interpretation problems of qualitative measures of CardioDetect<sup>®</sup> med, CardioDetect<sup>®</sup> quant was further used to quantify the results (13). The medical staff responsible for patient care was blinded to the results of the assay.

The URLs and coefficients of variation (CV) of biochemical markers provided by the manufacturer at the specific limit were as follows: cTnI (99% URL = 0.07 ng/mL, CV = 15%), myoglobin (95% URL = 92 ng/mL, CV = 3.2%), CK-MB (95% URL = 3.6 ng/mL, CV = 9.7%), and H-FABP (99% URL = 7 ng/mL, CV = 15%).

### Outcomes

All patients underwent serial electrocardiography (ECG) and serial cTn-I measurements. Other diagnostic evaluations that were at the discretion of the attending physicians, included multi-detector row coronary angiographic computed tomography (MDCT), conventional coronary angiography (CAG), a treadmill test, or cardiac single photon emission computed tomography (SPECT).

Acute MI was defined as having a typical rise and fall in serial cTn-I with ischemic ECG changes or significant coronary lesions (2). ST segment elevation myocardial infarction (STEMI) was defined as having initial ECG changes indicative of a significant ST elevation ( $\geq 2$  continuous leads of  $\geq 0.1$  mV in limb leads or  $\geq 0.2$  mV in precordial leads) in MI patients. Non-ST segment elevation myocardial infarction (NSTEMI) was defined as having any initial ECG findings other than a significant ST elevation in MI patients. Angina was diagnosed in the presence of significant coronary lesions found on CAG or MDCT or with positive

test results on the treadmill test or SPECT in the absence of elevated cTn-I. Non-cardiac was diagnosed in the presence of negative results on CAG, MDCT, the treadmill test, or SPECT.

### Statistical analyses

Continuous variables are expressed as means  $\pm$  standard deviation (SD), and categorical data are presented as the percent frequency of occurrence. The Student t-test was used to compare continuous variables, and the chi-squared test was used to compare binomial variables.

The performances of initial biomarkers were compared using the area under the receiver operating characteristics curve (AUC).

To assess an adjunctive role of biochemical markers, we derived multivariate logistic regression models for the diagnosis of MI using variables composed of initial biochemical markers, i.e. Model 1 using cTnI only, Model 2 using cTnI and H-FABP, Model 3 using cTnI and myoglobin, and Model 4 using cTnI and CK-MB.

Because AUC ignores the predicted probability values and the goodness-of-fit of the model, the performances of these models were compared in terms of discrimination and overall fit (14). Discrimination was assessed using AUC, and overall fit was assessed using Bayesian information criterion (BIC). When using maximum likelihood estimation, it is possible to increase the likelihood by adding variables. BIC is a criterion for model selection that penalizes the number of variables to avoid overfitting (15). Unlike a likelihood ratio (LR) test, BIC can be used to compare the fit of two models which are not nested. A model is better than another if it has a smaller BIC value (16).

Since AUC weighs sensitivity and specificity equally, AUC cannot precisely estimate the performance of predictive models under the conditions in which sensitivity is clinically more important. Therefore, the diagnostic performances of initial biochemical markers at URL and of logistic regression models at 30% probability were examined using sensitivity, specificity, positive LR, and negative LR with each 95% confidence intervals.

All statistical procedures were performed using Stata version 10.1 (Stata corp., College Station, TX, USA), and a two-tailed *P* value  $< 0.05$  was considered as statistically significant.

## RESULTS

A total of 186 patients were enrolled during the study period. After excluding 16 patients with elevated serum creatinine, 170 patients were included in the final analysis.

Among 76 patients (44.7%) with MI, 54 had STEMI, and 22 had NSTEMI. Among 94 patients (55.3%) with non-MI, 41 had angina, and 53 had non-cardiac chest pain. The clinical characteristics of patients are described in Table 1.

Receiver operating characteristics (ROC) curves of initial bio-

chemical markers are presented in Fig. 1. AUCs of myoglobin and CK-MB were 0.784 (95% confidence intervals [CI], 0.711-0.858) and 0.772 (95% CI, 0.697-0.847), respectively. Those were significantly lower than that of cTnI, which was 0.863 (95% CI, 0.808-0.919). AUC of H-FABP was 0.827 (95% CI, 0.761-0.893).

Logistic regression models using initial biochemical markers were derived and assessed in terms of discrimination and overall fit and are presented in Table 2. Only H-FABP had an independent predictive value when combined with cTnI in the model. The logistic regression model including cTnI and H-FABP (Model 2) had the best discrimination ability measured by AUC. However, it was not statistically different from that of Model 1 (cTnI only). Model 2 also had the best overall fit measured by BIC (i.e., the smallest BIC). A difference in BIC over 20 means a very de-

cisive support for the selection of model with lower BIC (16).

ROC curves of Model 1 and Model 2 were presented in Fig. 2.

Sensitivity, specificity, positive LR, and negative LR of initial biochemical markers at URL and at the 30% predictive probability of logistic regression models are described in Table 3. In contrast to Model 2, neither Model 3 nor Model 4 had any improvement of diagnostic performances compared with Model 1 in terms of sensitivity.

## DISCUSSION

In this study, only H-FABP had an adjunctive diagnostic value to initial cTnI for the diagnosis of MI. The discrimination power of cTnI was best among initial biochemical markers, and other

**Table 1.** Clinical characteristics of patients

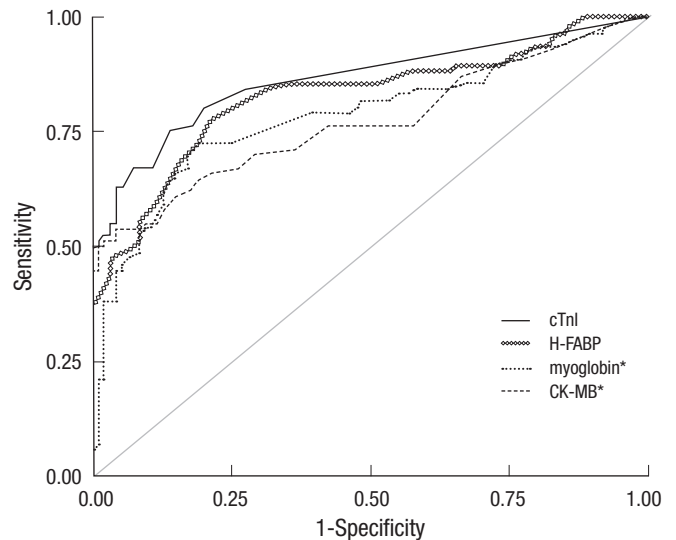
Parameters	MI	Non-MI	P value
Patients, No.	76	94	
Age (yr) (means $\pm$ SD)	60.0 $\pm$ 11.8	58.6 $\pm$ 15.6	0.491
Male Sex, No. (%)	53 (69.7%)	62 (66.0%)	0.600
Diabetes, No. (%)	14 (18.4%)	15 (16.0%)	0.671
Hypertension, No. (%)	34 (44.7%)	39 (41.5%)	0.671
Dyslipidemia, No. (%)	13 (17.1%)	25 (26.6%)	0.140
Ischemic heart disease, No. (%)	12 (15.8%)	19 (20.2%)	0.458
Current smoker, No. (%)	41 (54.0%)	35 (37.2%)	0.029
Symptom duration, No. (%)			
$\leq$ 3 hr	40 (55.6%)	43 (45.5%)	-
3-6 hr	21 (22.2%)	14 (40.9%)	
> 6 hr	12 (18.5%)	33 (9.1%)	
unclear*	3 (3.7%)	4 (4.5%)	
Diagnostic evaluations, No. (%) <sup>†</sup>			
CAG	69 (90.8%)	40 (42.6%)	-
MDCT	3 (3.9%)	40 (42.6%)	
Treadmill test	- <sup>‡</sup>	13 (13.8%)	
SPECT	-	6 (6.4%)	

\*Cases with missing data of symptom duration; <sup>†</sup>One can have more than one evaluation; <sup>‡</sup>Evaluations are not indicated in these cases. MI, myocardial infarction; No., number; SD, standard deviation; CAG, coronary angiography; MDCT, multi-detector row coronary angiographic computed tomography; SPECT, single photon emission computed tomography.

**Table 2.** Performances of logistic regression models for diagnosis of MI

Variables	Model 1	Model 2	Model 3	Model 4
Variable 1	cTnI	cTnI	cTnI	cTnI
OR (95% CI)*	185.8 (10.9-3171.6)	117.1 (6.5-2126.2)	127.0 (6.6-2436.9)	82.1 (2.4-2833.0)
P value	< 0.001	0.001	0.001	0.015
Variable 2	-	H-FABP	Myoglobin	CK-MB
OR (95% CI)*	-	1.2 (1.1-1.3)	1.0 (1.0-1.0)	1.1 (0.8-1.4)
P value	-	< 0.001	0.426	0.506
Discrimination				
AUC (95% CI)	0.863 (0.808-0.919)	0.900 (0.853-0.948)	0.871 (0.813-0.928)	0.845 (0.783-0.908)
P value <sup>†</sup>	-	0.121	0.593	0.237
Overall fit				
Log (likelihood)	-76.368	-63.760	-76.043	-76.141
BIC	-75.888	-95.970	-71.402	-71.206
$\Delta$ BIC <sup>‡</sup>	-	-20.082	4.486	4.682

\*OR per 1 unit increment of each biochemical marker; <sup>†</sup>When compared with AUC of Model 1; <sup>‡</sup>When compared with BIC of Model 1. MI, myocardial infarction; cTnI, cardiac troponin-I; H-FABP, heart-type fatty acid binding protein; CK-MB, creatine kinase isoenzyme MB; OR, odds ratio; CI, confidence intervals; AUC, area under the receiver operating characteristics curve; BIC, Bayesian information criteria.

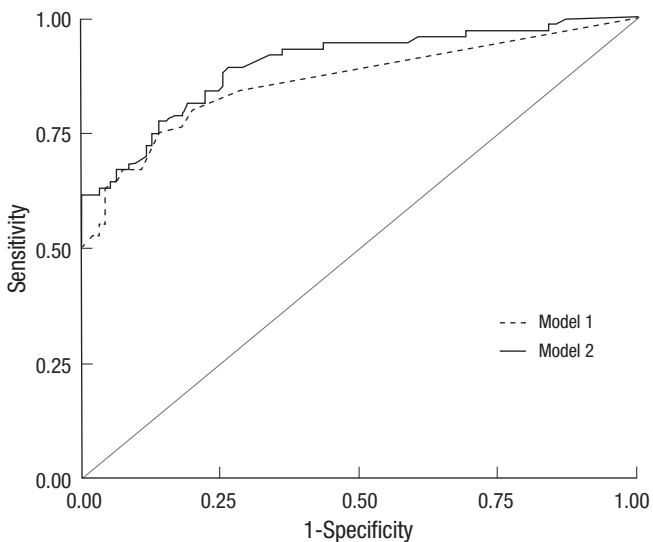


**Fig. 1.** Receiver operating characteristics curves of initial biochemical markers for the diagnosis of myocardial infarction. cTnI indicates cardiac troponin-I; H-FABP, heart-type fatty acid binding protein; CK-MB, creatine kinase isoenzyme MB. \*P value < 0.05 when compared with AUC of cTnI.

**Table 3.** Diagnostic performances of initial biochemical markers and logistic regression models for the diagnosis of MI\*

Markers/Models	Cutoff	Sensitivity	Specificity	Positive LR	Negative LR
cTnI	0.07 ng/mL	67.1 (55.3-77.2)	91.5 (83.4-96.0)	7.88 (3.99-15.58)	0.36 (0.26-0.50)
H-FABP	7 ng/mL	100 (94.0-100)	10.6 (5.5-19.1)	1.12 (1.04-1.20)	0 (NA)
H-FABP	10 ng/mL	85.5 (75.2-92.2)	62.8 (52.1-72.3)	2.30 (1.74-3.03)	0.23 (0.13-0.40)
Myoglobin	92 ng/mL	46.1 (34.7-57.8)	94.7 (87.5-98.0)	8.66 (3.57-21.02)	0.57 (0.46-0.70)
CK-MB	3.6 ng/mL	51.3 (39.7-62.8)	95.7 (88.8-98.6)	12.06 (4.51-32.25)	0.51 (0.40-0.64)
Model 1	0.3 <sup>†</sup>	67.1 (55.4-77.5)	89.4 (81.3-94.8)	6.31 (3.44-11.60)	0.37 (0.27-0.51)
Model 2	0.3 <sup>†</sup>	81.6 (71.0-89.5)	80.9 (71.4-88.2)	4.26 (2.77-6.54)	0.23 (0.14-0.37)
Model 3	0.3 <sup>†</sup>	68.4 (56.7-78.6)	89.4 (81.3-94.8)	6.43 (3.51-11.80)	0.35 (0.25-0.50)
Model 4	0.3 <sup>†</sup>	68.4 (56.7-78.6)	89.4 (81.3-94.8)	6.43 (3.51-11.80)	0.35 (0.25-0.50)

\*Data are shown percentages or ratio with 95% confidence intervals; <sup>†</sup>Predicted probability of 0.3 was used for a cutoff. MI, myocardial infarction; LR, likelihood ratio; cTnI, cardiac troponin-I; H-FABP, heart-type fatty acid binding protein; CK-MB, creatine kinase isoenzyme MB; NA, not applicable.



**Fig. 2.** Receiver operating characteristics curves of logistic regression model for the diagnosis of myocardial infarction. Model 1 was derived using cTnI only as a predictive variable and model 2 was derived using both cTnI and H-FABP. cTnI indicates cardiac troponin-I; H-FABP, heart-type fatty acid binding protein.

markers other than H-FABP had a significantly lower AUC than that of cTnI. A logistic regression model including cTnI and H-FABP had the highest AUC. The overall fit determined by BIC was best in the model that included both cTnI and H-FABP. Combining cTnI and H-FABP in the logistic regression model has resulted in increased sensitivity.

Clinical decisions for the management of MI are guided by various clinical and laboratory findings such as demographics, history, ECG, physical examination, and biochemical markers. Early identification of MI may enable physicians to initiate aggressive medical treatments or early invasive management, especially in patients with high risk (17). Myoglobin is considered useful in the early detection of MI because of its high sensitivity (17). However, our results suggest that H-FABP may play a greater role in the early detection of MI.

There are several possible reasons why H-FABP may be superior to myoglobin as an early adjunctive marker of MI. One possibility is that the levels of H-FABP in cardiac muscles are 2

to 10 folds higher than the levels in skeletal muscle, while the levels of myoglobin in cardiac muscles are 2 folds lower than the levels in skeletal muscles. A second possibility is that normal baseline concentrations of H-FABP are considerably lower than myoglobin. These two facts suggest that H-FABP may have higher sensitivity and specificity for myocardial injury than myoglobin. While H-FABP was superior to myoglobin in this study, the specificity of H-FABP at 7 ng/mL was only 10.6%. This low specificity is discordant with previous studies using CardioDetect<sup>®</sup> med (10, 11, 18). One possible cause of this discrepancy is the use of a new definition of MI. Another is the high prevalence of MI or myocardial ischemia in this study. H-FABP is a very sensitive marker, which can be elevated in patients with ischemia or even congestive heart failure (19). However, when a cutoff value of 10 ng/mL was used, H-FABP demonstrated improved specificity while retaining high sensitivity (Table 3).

CK-MB is highly specific to myocardial injury. However, the initial CK-MB has low sensitivity because CK-MB takes a similar amount of time as cTnI to appear in serum. In fact, the usefulness of CK-MB resides mainly in the diagnosis of re-infarction because of its short half-life (1).

Unlike older studies using enzyme-linked immunosorbent assays (ELISA), rapid POCT of H-FABP was used in this study (7, 8). Furthermore, CardioDetect<sup>®</sup> quant has enabled quantitative measurements of this rapid POCT, eliminating the interpretation problem raised by Mad et al. (18). The whole assay time was less than 20 min. It is worth mentioning that rapid POCT of H-FABP was superior to conventional ELISA of myoglobin. Rapid POCT has several merits, especially in emergency situations. It would shorten the time lag for the diagnosis and does not require a specific laboratory facility. Quantitative tests have some advantages over qualitative tests, too. For instance, higher levels generally indicate a greater probability of myocardial injury or a larger size of myocardial infarction. Thus, along with other biomarkers or clinical findings, quantitative measures of H-FABP can be used for diagnosis, risk stratification, and prognosis of patients with MI (19, 20).

Due to the outstanding diagnostic performance of cardiac

troponins, H-FABP is likely to play a role only in the early presentation after symptom onset. That is the reason why we have only evaluated initial biomarkers.

Subgroup analyses performed in patients with different symptom durations did not demonstrate any results worth mentioning. Furthermore, the relatively modest patient number has limited this kind of analysis.

Several limitations apply to this study. First is that we did not exclude patients with STEMI. Because early biochemical markers are not helpful in these patients, it might have been better to have excluded these patients at the enrollment step.

Second is that the prevalence of MI was high (46.0%). This is probably due to the fact that we included patients with typical angina pain who require diagnostic evaluations to confirm diagnosis. Thus the results of this study might not reflect the usual population suspected of acute coronary syndrome. However, our patients are representative of the patients with a high risk and prevalence of MI.

In conclusion, the initial H-FABP measured by quantitative POCT has a better diagnostic value than initial myoglobin or initial CK-MB as an adjunct to the initial cardiac troponins for the early diagnosis of MI. H-FABP deserves further investigation for the early diagnosis of MI, especially in patients presenting early after symptom onset.

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**AUTHOR SUMMARY****Heart-type Fatty Acid Binding Protein as an Adjunct to Cardiac Troponin-I for the Diagnosis of Myocardial Infarction**

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We tested whether the heart-type fatty acid binding protein (H-FABP) would have greater diagnostic value than conventional markers for the early diagnosis of myocardial infarction (MI), when used in combination with cardiac troponins-I (cTnI). Initial H-FABP, myoglobin, creatine kinase isoenzyme MB (CK-MB), and cTnI were measured in consecutive patients with typical chest pain at a single emergency department. Seventy-six of 170 patients had MI. Area under the curve (AUC) of cTnI, H-FABP, myoglobin, and CK-MB were 0.863, 0.827, 0.784, and 0.772, respectively. A logistic regression model using cTnI ( $P = 0.001$ ) and H-FABP ( $P < 0.001$ ) had the biggest AUC (0.900) and the best fit. H-FABP has a better diagnostic value than both myoglobin and CK-MB as an adjunct to cTnI for the early diagnosis of MI.