Diagnostic value of heart-type fatty acid binding protein determined by the rapid qualitative chromatographic immunoassay method for the detection of minor myocardial damage in patients presenting with non-ST elevation acute coronary syndrome

Kalitatif immünkromotografik yöntemle bakılan kalp tipi serbest yağ asidi bağlayıcı protein'in minör miyokardiyal hasarla seyreden ST yükselmesiz akut koroner sendromlardaki tanısal değeri

Yüksel Çavuşoğlu, Bülent Gök, Canan Demirüstü, Alparslan Birdane, Bülent Görenek, Necmi Ata*

From Departments of Cardiology and *Biostatistics, Faculty of Medicine, Eskisehir Osmangazi University, Eskişehir-*Turkey*

ABSTRACT

Objective: The aim of this prospective study was to evaluate the diagnostic value of heart-type fatty acid binding protein (H-FABP) determined by qualitative immunoassay method for the detection of minor myocardial damage (MMD) in patients with non-ST elevation acute coronary syndrome (NSTE-ACS).

Methods: The study consisted of 62 patients with NSTE-ACS. Cardiac troponin I (cTnI) and creatine kinase MB isoenzyme (CK-MB) values were measured at arrival. Myoglobin and H-FABP were obtained if cTnI level was found to be elevated. A control group included 20 subjects with normal cTnI and CK-MB values. H-FABP was determined by a rapid qualitative immunochromatographic test. Patients were classified as MMD-ACS group if they had abnormal cTnI and normal CK-MB (n=24) and as NSTEMI-ACS group if they had elevated both cTnI and CK-MB (n=38). The diagnostic accuracy of H-FABP for minor myocardial damage was determined using ROC analysis.

Results: The sensitivity of the H-FABP was significantly higher for NSTEMI-ACS than for MMD-ACS (44.7% vs 0%, p<0.001) and its specificity was 95% for both groups. The diagnostic efficacy rates for myoglobin and H-FABP were 75% and 43% for MMD-ACS, 74% and 62% for NSTEMI-ACS. Positive predictive value for H-FABP and myoglobin were found to be 0% and 80.8% in MMD-ACS, 94% and 87% in NSTEMI-ACS and negative predictive value was 44% and 69.5% in MMD-ACS, 47.5% and 59% in NSTEMI-ACS, respectively. AUC for myoglobin was significantly greater than that for H-FABP in MMD-ACS group (0.754 vs 0.525, p=0.027). The sensitivity of the H-FABP was significantly higher in patients with >3-fold increase in cTnI than those with <3-fold increase in cTnI (46.8% vs. 6.7%, p< 0.001). A positive correlation was found between the magnitude of cTnI rise and H-FABP results (r=0.45, p<0.001).

Conclusions: H-FABP determined by the rapid qualitative immunochromatographic test has almost similar diagnostic value to that of myoglobin for identifying NSTEMI-ACS, however, does not seem to represent diagnostic potential for the detection of MMD.

(Anadolu Kardiyol Derg 2012; 12: 584-90)

Key words: Heart-type fatty-acid binding protein, acute coronary syndrome, sensitivity, specificity, diagnostic accuracy

ÖZET

Amaç: Bu prospektif çalışmanın amacı, yatak başı kalitatif yöntemle belirlenen kalp tipi serbest yağ aside bağlayıcı proteinin (H-FABP), minör miyokardiyal hasarla (MMH) seyreden ST yükselmesiz akut koroner sendromlardaki (NSTE-ACS) tanısal değerini incelemekti.

Yöntemler: Çalışmaya NSTE-ACS'li 62 olgu alındı. Hastaneye kabulde kardiyak troponin I (cTnI) ve kreatin kinaz MB izoenzim (CK-MB) düzeyleri ölçüldü. cTnI yüksek bulunanlarda, H-FABP ve miyoglobin düzeyleri bakıldı. Ayrıca cTnI ve CK-MB düzeyleri normal olan 20 olguluk kontrol grubu oluşturuldu. H-FABP, yatak başı kalitatif immünkromotografik yöntemle bakıldı. On iki saatlik takipte cTnI ve CK-MB düzeyleri yüksek bulunanlar

Address for Correspondence/Yazışma Adresi: Dr. Yüksel Çavuşoğlu, Eskişehir Osmangazi Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalı, Eskişehir-*Türkiye* Phone: +90 222 239 29 79-3700 Fax: +90 222 239 90 11 E-mail: yukselc@ogu.edu.tr

Accepted Date/Kabul Tarihi: 25.04.2012 **Available Online Date/Çevrimiçi Yayın Tarihi:** 17.07.2012

doi:10.5152/akd.2012.185

NSTEMI-ACS (38 olgu) ve cTnI yüksek ancak CK-MB normal bulunanlar MMH-ACS (24 olgu) olarak tanımlandı. H-FABP'nın minör miyokart hasarı için tanısal doğruluğu ROC analizi ile değerlendirildi.

Bulgular: H-FABP'nin duyarlılığı, NSTEMI-ACS'de MMH-ACS'den anlamlı yüksek (%44.7 ve %0, p<0.001), özgüllüğü ise her iki grup için %95 bulundu. Myoglobin ve H-FABP için tanısal doğruluk, MMH-ACS grubunda sırasıyla %75 ve %43, NSTEMI-ACS grubunda sırasıyla %74 ve %62 bulundu. Sırasıyla H-FABP ve myoglobinin pozitif prediktif değeri MMH-ACS'de %0 ve %80.8, NSTEMI-ACS'de %94 ve %87, negatif prediktif değeri ise MMH-ACS'de %44 ve %69.5, NSTEMI-ACS'de %47.5 ve %59 idi. MMH-ACS grubunda, miyoglobinin AUC değeri H-FABP'den anlamlı yüksekti (0.754 ve 0.525, p=0.027). cTnI <3 kat olanlarla karşılaştırıldığında, cTnI >3 kat olanlarda H-FABP duyarlılığı anlamlı yükselmekteydi (%6.7 ve %46.8, p< 0.001). cTnI yüksekliği ile H-FABP sonuçları arasında pozitif korelasyon saptandı (r=0.45, p<0.001).

Sonuç: Yatak başı kalitatif immünkromotografik yöntemle bakılan H-FABP, NSTEMI-ACS tanısında miyoglobin ile benzer tanısal özellikler gösterirken, MMH ile seyreden NSTE-ACS tanısındaki değeri düşük bulunmaktadır. *(Anadolu Kardiyol Derg 2012; 12: 584-90)*

Anahtar kelimeler: Kalp tipi serbest yağ aside bağlayıcı protein, akut koroner sendrom, duyarlılık, özgüllük, tanısal doğruluk

Introduction

Biochemical markers of myocardial injury are currently accepted as important determinants for the diagnosis of patients admitted to the hospital with chest pain suggestive of acute coronary syndromes (ACS) (1-4). Cardiac troponins (cTn) are the more sensitive and specific markers of myocardial injury (5). The value of troponins for the diagnosis (1-5), prognosis (6, 7) and risk stratification (8) of the patients with non-ST elevation-ACS (NSTE-ACS) is well established. However, cTn concentrations usually begin to rise 4 to 6 hours (h) after symptom onset (9). The MB isoenzyme of creatine kinase (CK-MB), which is currently regarded as the second standard marker for the diagnosis of myocardial infarction (MI) (1-4), release into plasma within 4 to 6 h after the onset of symptoms (10). Therefore these markers have little role within the first 4 to 6 h at initial presentation. Patients who have nonspecific symptoms and nondiagnostic electrocardiogram (ECG) with normal cTn and CK-MB in this period of time might be mistakenly discharged from the emergency department, whereas these patients have an increase in short-term mortality (11). Rapidly raised cardiac biomarkers are needed to enhance the rapidity of the detection of cardiac injury to either establish or exclude the diagnosis of MI. Myoglobin, the more sensitive but not specific marker of injury, can be detected within 2h after the onset of MI (12, 13).

Heart-type fatty-acid binding protein (H-FABP) is a new diagnostic marker of myocyte injury for the early diagnosis (within 2h after symptom onset) of ST elevation MI (14) and NSTE-ACS (15). There have been no detailed studies evaluating its usefulness in NSTE-ACS patients who have minor myocardial damage (MMD). The overall rate of death or MI is equally high among patients with abnormal cTn levels even if CK-MB is in the normal range (16).

The aim of this study was to evaluate the diagnostic value of qualitative immunochromatographic test-determined H-FABP for the detection of MMD in patients presenting with NSTE-ACS.

Methods

Study design

In this prospective study, the diagnostic validity of qualitatively-determined H-FABP has been investigated for the detection of MMD in patients presenting with NSTE-ACS and diagnostic performance of H-FABP was compared with myoglobin results. Patients presenting with NSTE-ACS to the emergency department who have elevated cTnI was assigned to NSTEMI-ACS if they had elevated both cTnI and CK-MB and to MMD-ACS if they had a minor elevation of cTnI in the absence of CK-MB elevation. In addition, a control group with normal cTnI and CK-MB was included in the study. The sensitivity, specificity, diagnostic efficacy, positive predictive value (PPV) and negative predictive value (NPV) of H-FABP were evaluated and the diagnostic accuracy was assessed by receiver operating characteristic (ROC) curve analysis in comparison with myoglobin.

Study population

From November 2004 to September 2005, we enrolled 62 patients with a diagnosis of NSTE-ACS on the basis of an acute chest pain episode and electrocardiographic changes manifested by ST depressions or T wave inversions within 12 h after the onset of symptoms at the Eskişehir Osmangazi University hospital. A control group included 20 subjects without ACS. All patients gave written informed consent. The study was approved by the local ethics committee.

Study protocol

cTnI and CK-MB were measured at the time of arrival in the emergency department. A single test for myoglobin and H-FABP were obtained if cTnI level was found to be elevated. A non-ACS group included 20 subjects who had atypical chest pain with normal cTnI and normal CK-MB levels. Myoglobin and immunochromatographic test-determined H-FABP was also obtained in these non-ACS subjects if cTnI and CK-MB were in the normal range. ECG examination is repeated at intervals considered appropriate on the basis of the evolution of symptoms. Additional measurements of cTnI and CK-MB were serially obtained at 6 h and 12 h of admission in patients with NSTE-ACS and non-ACS. Any values higher than the upper limit of normal either at initial presentation or serially within the first 12 h of admission was considered abnormal (>1 ng/mL for cTnI, >24 U/l for CK-MB and >76 ng/mL for myoglobin). Patients with impaired renal function (serum creatinine level >1.6 mg/dL), left ventricular dysfunction (ejection fraction < 0.45), traumatic injury, recent cardiac or noncardiac surgery, suspected myocarditis, skeletal muscle myopathy, suspected acute pulmonary embolism, chronic obstructive pulmonary disease were excluded from the study.

In NSTE-ACS group, patients were classified as MMD-ACS group if they had minor elevation of cTnI but normal CK-MB levels (n=24) and as NSTEMI- ACS group if they had elevated both cTnI and CK-MB levels (n=38) in 12 h follow-up according to the NSTE-ACS guideline (2, 17, 18).

Patients with abnormal cTnI were admitted to the coronary care unit and treated with standard medical treatment of NSTE-ACSs including aspirin, clopidogrel, low molecular weight hepa-

rin, beta-blocker and nitrate. All patients underwent coronary angiography after clinical stabilization before the hospital discharge. Coronary angiography revealed that there were 11 patients with single vessel disease, 18 patients with two-vessel disease and 33 patients with three-vessel disease with a coronary lesion ≥70% diameter stenosis at major coronary arteries among NSTE-ACS patients. In non-ACS patients, subsequently performed coronary angiography based on the results of non-invasive stress testing showed single vessel disease in 6 patients, two-vessel disease in 7 patients and three-vessel disease in 6 patients with a ≥50% diameter stenosis and in one patient, coronary angiography was not indicated.

Study variables

The clinical variables include age, gender, cardiovascular risk factors for coronary artery disease, ejection fraction, cTnI, CK-MB, hemoglobin, fibrinogen, C-reactive protein and serum creatinine. The outcome variables of study were H-FABP and myoglobin. The sensitivity, specificity, diagnostic efficacy, PPV, NPV and diagnostic accuracy were assessed as primary outcome variables.

Measurement of biochemical cardiac markers

H-FABP was determined by a rapid chromatographic immunoassay method designed for qualitative determination of H-FABP in whole blood samples (CardioDetect^{R} med, Rennesens GmbH, Berlin, Germany). It was performed at emergency department. After application of three drops of blood to the test field on the card, the result is available within 15 minutes. The test card has two line, H-FABP line and control line. If the concentration of H-FABP is below the threshold value, only control line becomes visible. Control line always appears when the test has worked properly. If the concentration of H-FABP is above the threshold value, both H-FABP and control line become visible. The lower detection limit of this method is 7 ng/mL. A positive result means the concentration of H-FABP in the blood sample is above the threshold value of 7 ng/mL.

The serum concentration of cTnI and myoglobin were measured with a commercially available immunometric assay kit (IMMULITE^{R} Turbo Troponin I and IMMULITE^{R} Turbo Myoglobin, Diagnostic Products Corporation, Los Angeles, USA). Serum CK-MB activity was determined with a commercial immunoinhibition assay kit (Roche Diagnostics GmbH, Mannheim, Germany) with an automated clinical chemical analyzer (Roche/Hitachi analyzer, Roche Diagnostics GmbH, Mannheim, Germany). The assay procedures for cTnI, myoglobin and CK-MB using these kits were in accordance with the manufacturer's manuals. The cutoff levels for serum myoglobin concentration, CK-MB activity and cTnI levels were 76 ng/mL, 24 U/l and 1 ng/mL, respectively, according to the manufacturer's manuals.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences software 15.0 (SPSS 15.0, SPSS Inc, Chicago, US), Sigma Stat 3.5 (Systat Software Inc., California, US). The variables were expressed as mean±standard deviation and median (25.-75th percentiles). Proportions were compared by exact Chi-square test. The variables were first tested for normal distribution by normality test of Kolmogorov-Smirnov Test and then analyzed by Kruskal-Wallis test for non-normally distributed variables, one way ANOVA test for normally distributed variables for the comparison between groups and thereafter multiple comparisons among groups were performed by Dunn's and Tukey's multiple comparison tests. Pearson Correlation analysis was used for the correlation of variables. The performance of H-FABP test was compared with myoglobin results. The diagnostic validity was evaluated in terms of sensitivity, specificity, diagnostic efficacy, PPV, NPV. The sensitivity was assessed by calculating the percentage of the patients with NSTE-ACS confirmed by elevated cTnI levels whose H-FABP or myoglobin results were above the cutoff level. The specificity was assessed by calculating the percentage of the patients with non-ACS confirmed by normal cTnI and CK-MB levels whose H-FABP or myoglobin results were below the cutoff level. The diagnostic efficacy was calculated as the group of NSTE-ACS plus non-ACS as the percentage of patients with NSTE-ACS whose results were above the cutoff level plus patients with non-ACS whose results were below the cutoff level. The diagnostic accuracy of H-FABP was assessed by ROC curve analysis (MedCalc 11.3 - MedCalc Software bvba, Mariakerke, Belgium) in comparison with myoglobin. In ROC curve analysis, H-FABP was used as binary variable and myoglobin as a continuous variable. P values <0.05 were considered as statistically significant.

Results

Clinical characteristics and laboratory findings obtained at admission are shown in Table 1. MMD-ACS group, NSTEMI-ACS group and non-ACS group did not differ significantly in cardiovascular risk factors. Mean time from symptom onset to hospital arrival was similar among three groups. There was no statistically significant difference in cTnI levels between MMD-ACS and NSTEMI-ACS groups. C-reactive protein and fibrinogen levels did not differ between MMD-ACS and NSTEMI-ACS groups, but higher in patients with ACSs than those with non-ACS as expected. Although left ventricular ejection fraction was lower in NSTEMI-ACS compared to MMD-ACS patients, it was still >50%.

Diagnostic value of H-FABP in comparison with myoglobin in ACSs

In MMD-ACS group, 17 of the 24 patients had elevated myoglobin levels, but no patient showed positive test result of H-FABP. In NSTEMI-ACS group, myoglobin levels were elevated in 27 of the 38 patients, while H-FABP test was found to be positive in 17 of the 38 patients. Myoglobin in 4 patients and H-FABP in 1 patient were found to be elevated in non-ACS group. Thus, the sensitivity of myoglobin within 12 h after symptom onset was 70.8%, while it was 0% with H-FABP test for MMD-ACS. However, the sensitivity for NSTEMI-ACS was 71% with myoglobin and 44.7% with H-FABP test (Table 2). Therefore, the sensitivity of the immunochromatographic test of H-FABP was significantly higher for NSTEMI-ACS than for MMD-ACS ($p<0.001$), but myoglobin has had similar sensitivity in both NSTEMI-ACS and MMD-ACS ($p>0.05$). The specificity of test for H-FABP within 12 h after the onset of symptoms was 95%, com-

pared with 80% for myoglobin for both MMD-ACS and NSTEMI-ACS. The diagnostic efficacy rates for myoglobin and H-FABP test were 75% and 43% for MMD-ACS, 74% and 62% for NSTEMI-ACS (Table 2). PPV for H-FABP and myoglobin were found to be 0% and 80.8% in MMD-ACS, 94% and 87% in NSTEMI-ACS and NPV were 44% and 69.5% in MMD-ACS, 47.5% and 59% in NSTEMI-ACS, respectively.

The area under the ROC curve (AUC) for myoglobin was significantly greater than that H-FABP test in MMD-ACS group (0.754 vs 0.525, p=0.027) (Fig. 1), whereas there was no significant difference in the AUC between the two markers in NSTEMI-ACS (0.755 vs 0.699, p=0.44) (Fig. 2).

The relationship between cTnI levels and H-FABP positivity

In NSTE-ACS patients, there were 32 patients who have cTnI rise greater than 3 times (3x) upper limit of normal (ULN) and 30 patients who have cTnI rise less than 3x ULN. In patients with cTnI >3x ULN, 15 of the 32 (46.8%) patients showed positive result of H-FABP test, while H-FABP test was found to be positive in 2 of the 30 (6.7%) patients with cTnI levels <3x ULN. Myoglobin was found to be elevated in 24 (75%) and 20 (66.7%) patients with cTnI >3x ULN and with cTnI rise <3x ULN, respectively. Therefore, the sensitivity of the immunochromatographic H-FABP test for NSTE-ACS was significantly higher in patients with cTnI elevations >3x ULN as compared to those with cTnI levels <3x ULN (p<0.001), but myoglobin has had similar sensitivity in both subgroups (p>0.05). A positive correlation was found between the degree of cTnI rise and H-FABP results (r=0.45, p<0.001).

Discussion

The results of this study suggest that qualitative immunochromatographic test-determined H-FABP and myoglobin have almost similar diagnostic value for identifying NSTEMI-ACS patients. However, H-FABP test does not appear to represent diagnostic potential for the detection of MMD in patients presenting with ACS, and myoglobin is more sensitive and has better diagnostic accuracy than H-FABP in ACS patients who have only MMD. Furthermore, our results suggest an association between the degree of cTnI rise and H-FABP results.

The diagnosis of NSTEMI is currently based on the elevation of cTn and/or CK-MB according to the ACCF/AHA and the ESC guidelines (1, 2). Elevations of cTn and/or CK-MB reflect an irreversible loss of cardiac myocytes (cellular necrosis) which is considered as MI according to the consensus document of the ESC and ACCF/AHA (17). In order to demonstrate or rule out myocardial damage, serial biomarker testing is recommended 6 to 12 h after admission if the initial values are indeterminate, clinical suspicion remains high, and the ECG changes are equivocal or absent (1, 2). Cardiac troponins are the preferred markers of myocardial damage, because they have superior diagnostic sensitivity and specificity over CK-MB (17). The aim of this study was not to compare diagnostic performance of H-FABP test versus myoglobin, cTn or CK-MB for earlier detection of myocardial injury. Therefore, NSTE-ACS patients with myocardial damage confirmed by the elevation of cTnI based on the current guidelines criteria were included in the study.

Table 1. Clinical characteristics of study patients

Variables	MMD-ACS (n=24)	NSTEMI-ACS (n=38)	Non-ACS (n=20)	p
Age, years	66±10	65±11	65±11	0.892(1)
Male, n (%)	15 (62)	16 (42)	13 (65)	0.147(3)
Hypertension, n (%)	12 (50)	24 (63)	10 (50)	0.488(3)
Diabetes, n (%)	9 (37)	13 (34)	6 (30)	0.872(3)
Hypercholesterolemia, n (%)	9 (37)	10 (26)	9 (45)	0.332(3)
Smoking, n (%)	9 (37)	10 (26)	7 (35)	0.612(3)
Family history of CAD, n (%)	8 (33)	11 (29)	4 (20)	0.610(3)
Previous MI, n (%)	4 (16)	14 (36)	2 (10)	0.045(3)
Symptom onset, hours	5.7±2.1	5.2±1.8	4.6±0.6	0.115(1)
Hemoglobin, gr/dL	13±2.0	13±1.8	13±1.3	0.374(1)
Fibrinogen, mg/dL	393±188	448±152	229±121**†	0.001(1)
C-reactive protein, mg/dL	3.1 (1.5-8.6)	2.7 (1.4-5.8)	0.2 (0.1-0.5)*#	0.001(2)
Serum creatinine, mg/dL	1.04±0.29	1.11±0.28	1.01±0.18	0.352(1)
LV ejection fraction, %	59.7±8.8	52.6±9.1**	60.7±9.0†	0.01(1)
Troponin I, ng/mL	1.8 (1.6-3.6)	4.0 (1.6-8.5)	0.5 (0.3-0.6)*#	0.001(2)
Creatine kinase MB, U/l	18.5 (15-23)	48 (36-59)*	16.5 (11-20)#	0.001(2)
Myoglobin, ng/mL	126±120	210±224	54±64†	0.005(1)
Myoglobin positive, n (%)	17 (70.8)	27 (71)	4 (20)	0.001(3)
H-FABP positive, n (%)	0 (0)	17 (44.7)	1 (5)	0.001(3)

Data are presented as mean±SD, median (25^{th}-75^{th} percentiles) and number (percentage)
(1)One-way ANOVA, (2)Kruskal Wallis test, and (3)exact Chi-square test.
Posthoc and posttest analyses: *p<0.05 and **p<0.01 versus MMD-ACS, #p<0.05 and †p<0.001 versus NSTEMI-ACS.
ACS - acute coronary syndrome, CAD - coronary artery disease, H-FABP - heart-type fatty acid binding protein, LV - left ventricular, MI - myocardial infarction, MMD - minor myocardial damage, NSTEMI - non-ST elevation myocardial infarction

Table 2. Diagnostic performance of H-FABP and myoglobin in diagnosis of MMD and NSTEMI

	MMD-ACS		NSTEMI-ACS	
	H-FABP	Myoglobin	H-FABP	Myoglobin
Sensitivity, %	0	70.8	44.7	71
Specificity, %	95	80	95	80
Diagnostic efficacy, %	43	75	62	74
PPV, %	0	80.9	94	87
NPV, %	44	69.5	47.5	59

ACS - acute coronary syndrome, H-FABP-heart - type fatty acid binding protein, MMD - minor myocardial damage, NPV - negative predictive value, NSTEMI - non-ST elevation myocardial infarction, PPV - positive predictive value

Cardiac troponins and CK-MB are often measured concurrently and rises simultaneously in most patients with NSTE-ACS. However, even minor elevations in cTn without CK-MB elevation still suggest the presence of focal cell necrosis in the myocardium because

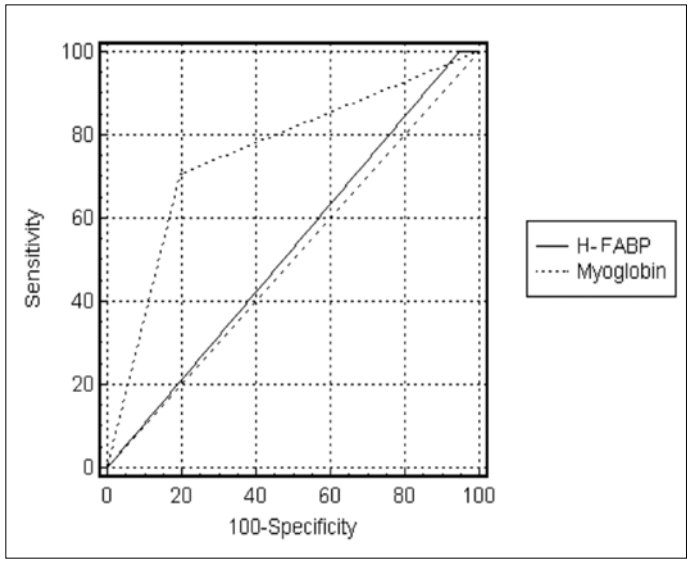

Figure 1. ROC curves of H-FABP and myoglobin in MMD-ACS group. ROC curves were constructed by plotting the sensitivity for MMD-ACS group on the y-axis and 100-specificity for the non-ACS group on the x-axis. The AUCs are 0.525 for H-FABP and 0.754 for myoglobin (p=0.027)

ACS - acute coronary syndrome, AUC - area under the ROC curve, H-FABP-heart-type fatty acid binding protein, MMD - minor myocardial damage, ROC - receiver operator characteristics

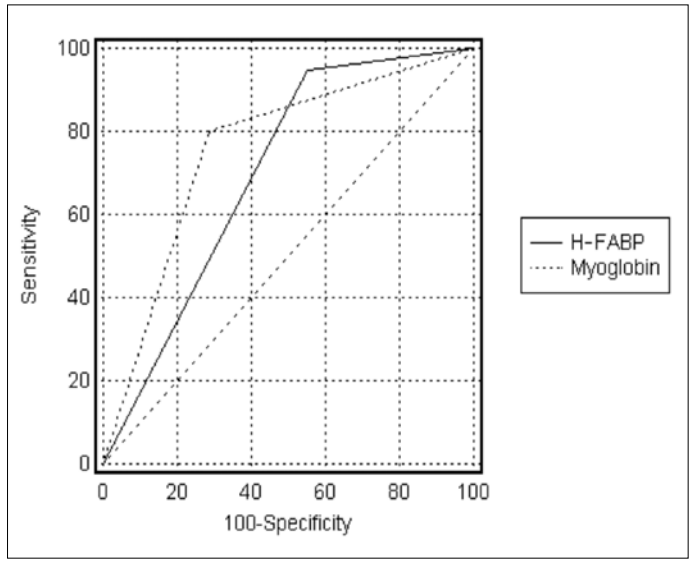

Figure 2. ROC curves of H-FABP and myoglobin in NSTEMI-ACS group. ROC curves were constructed by plotting the sensitivity for NSTEMI-ACS group on the y-axis and 100-specificity for the non-ACS group on the x-axis. The AUCs are 0.699 for H-FABP and 0.755 for myoglobin (p=0.44)

ACS - acute coronary syndrome, AUC - area under the ROC curve, H-FABP-heart-type fatty acid binding protein, NSTEMI-non-ST elevation myocardial infarction, ROC - receiver operator characteristics

small amounts of necrosis may not be detected by CK-MB measurements which remain within the normal range. Elevated levels of cTn in the absence of CK-MB elevation have been labeled as 'minimal myocardial damage', although it is not a distinct entity from NSTEMI and rather is a type of NSTEMI (16-19).

The concept of minimal or minor myocardial damage is of clinical importance, because it is associated with short and long-term adverse clinical outcomes similar to that of NSTEMI, and substantial proportion of patients with NSTE-ACS has the marker status of MMD-ACS at the emergency department. Data from 29.357 NSTE-ACS patients in the Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes with Early Implementation of the ACC/AHA Guidelines (CRUSADE) Quality Improvement Initiative (18) showed that nearly one-fifth of the patients with NSTE-ACS (18.8%) had elevated cTn with normal CK-MB result, while the majority of patients (59.7%) had both cTn and CK-MB elevations. Importantly, any degree of cTn elevation without CK-MB elevation has been reported to be associated with higher in-hospital mortality rate (4.5%) than isolated CK-MB elevation, for which mortality (3%) was similar to that with both markers negative (2.7%). Furthermore, the data from the Canadian Acute Coronary Syndromes Registry (16) showed that one-year mortality rate was similar in patients with isolated cTn elevation (12.5%) and in patients with elevated both cTn and CK-MB (11.7%), whereas CK-MB status did not provide incremental prognostic value. Similarly, Rao et al. (19) also showed that cTn elevation with or without CK-MB elevation was associated with increased risk of death or MI at 30 days, and patients with CK-MB elevation without cTn rise had 30-day risk that was similar to patients with both markers negative. The association of cTn with adverse clinical outcomes reflects the enhanced sensitivity and specificity of cTn in the detection of MMD that predict to subsequent major clinical events. In a large prospective observational study, H-FABP has also been shown to predict all-cause 1-year mortality after ACSs and identify high-risk patients across the range of cTn values. However, its predictive value in specifically MMD-ACS is not clear (20).

Unstable angina, MMD and NSTEMI comprise part of the spectrum of NSTE-ACS. Patients with MMD-ACS have an increase in short term mortality as well as the patients with NSTEMI (16-19). Therefore, patients presenting with chest pain suggestive of NSTE-ACS should be rapidly evaluated for the detection of myocardial damage in the emergency department. MMD and NSTEMI are distinguished from unstable angina by the presence of elevated cardiac biomarkers. However, 2 to 10 percent of the patients which are eventually diagnosed with MI are misdiagnosed and inappropriately discharged from the emergency department (11) because an elevation in cTn and CK-MB are usually not detectable for 4 to 6h after onset of chest pain. The detection of rapidly appearing cardiac biomarker that are sensitive and specific for myocardial damage would facilitate a more appropriate diagnostic and therapeutic approach in patients with suspected ACS. Thus, newer markers that rise earlier than cTn and CK-MB can be helpful in the early triage and therapeutic decision-making in ACS patients presenting to the emergency department during the first 4-6h after onset of chest pain.

Myoglobin is relatively early marker because elevations occur more rapidly (12). It is released from damaged tissue within 90 minutes (min) after symptom onset, peaks around 6 h and return to normal baseline level within 24 h. Because of the high sensitivity of myoglobin, it is reported to be superior to cTn and CK-MB for ruling out MI within the period of 3 to 6 h at initial presentation (21). However, main limitation of myoglobin is the lack of specificity for the heart. H-FABP has recently been introduced as a potential novel marker for the early detection of

MI (22). In contrast to myoglobin, H-FABP is abundant in cardiac myocytes as compared with the skeletal muscle content, making this a potentially more cardiospecific marker. After myocardial cell damage, it is released into the circulation within 1to 3 h and peaks around 4 to 6 h and return to normal values in 24 to 36 h. The most quantitative immunochemical assays for H-FABP take an analysis time of 45 min or more and requires the complicated assay procedures, and so have limited use for routine clinical practice in emergency situations. A newly developed whole blood rapid panel test for qualitative determination of H-FABP, which is completed in 15 min and is meant for point-of-care testing, has been shown to have similar diagnostic potential at a cut-off value 6.2 or 7 ng/ml as compared to the quantitative assays for the detection of ST elevation MI (23-25).

H-FABP has been reported to have > 80 % sensitivity for the diagnosis of ACSs within the first 6 h after symptom onset (14, 22, 26), while the sensitivity of other cardiac markers such as cTn and CK-MB has been reported to be around 64% within the first 6 h (27). However, the sensitivity, specificity and diagnostic efficacy depend on the cutoff levels used in the studies and selection criteria of the patient population. The plasma concentration of H-FABP under normal conditions is reported to be <5 ng/mL (24). A cut-off level 6.2 to 12 ng/ml of H-FABP is generally used for the diagnosis of MI in different studies. Haastrup et al. (15) reported 90-95 % sensitivity and 81-94% specificity by H-FABP at a cut-off level 8-12 ng/ml in patients with NSTEMI within 6 h of onset of symptoms. Okamoto et al. (14) reported 6.2 ng/ml of the cut-off level as the highest diagnostic efficacy. In several studies, the areas under the ROC curves were found significantly greater for H-FABP than for myoglobin. In contrast, in the multicenter study by Ghani et al. (28) the area under the ROC curve of cTnI was reported to be significantly larger than that of either H-FABP or myoglobin. The results of the present study showed that the area under the ROC curve and sensitivity of immunochromatographic H-FABP test (0.699 and 44.7%) were almost statistically similar as compared with those of myoglobin (0.755 and 71%) in NSTEMI-ACS, while H-FABP test showed very limited diagnostic potential when compared to myoglobin in the detection of MMD-ACS. The characteristics of the release of H-FABP from injured myocardium and its plasma kinetics closely resemble of myoglobin. However, the cardiac muscle content of myoglobin is approximately 2.7 mg/g wet weight of tissue, whereas the myocardial content of H-FABP is 0.57 mg/g (29, 30). In addition, the range between ULN concentration and diagnostic cutt-off value of H-FABP in MI is relatively narrow and diagnostic cut-off levels of H-FABP have not been widely evaluated in various type of NSTE-ACS and requires further standardization. It can be speculated that in most patients with MMD-ACS, plasma release curve of H-FABP may not reach the values above the discriminator value of 7 ng/ml and remain within or just above the upper limit of normal or it may exceed the cut-off limit for a very short period of time. Also some studies suggest that removal of H-FABP from plasma occurs quickly by the kidneys and therefore detection of small increases of this marker may not be observed in exercise-induced ischemia (31). The other important observation in this present study was an association between H-FABP results and the degree of cTnI elevation with the higher sensitivity of H-FABP in patients with cTnI >3x ULN. This observation further suggests that small amounts of myocardial necrosis may not be detected by H-FABP with 7 ng/ml of the cut-off level that is currently used for the diagnosis of STEMI and NSTEMI.

Study limitations

A relatively small patient population is one of the important limitations of this present study. In addition, there is currently no clear consensus in defining MMD in spectrum of NSTE-ACSs. As has been discussed above, elevated cardiac troponins in the absence of CK-MB elevation is referred as 'minimal myocardial damage', therefore an increased level of cTn without CK-MB elevation has been accepted as a criterion of MMD for our study. Moreover, qualitative determination of H-FABP was the other limitation for this study. However, the lower detection limit of this method is 7 ng/mL and the result is available within 15 minutes and it has been previously shown to have similar diagnostic potential at this cut-off value as compared to the quantitative assays (23-25). Therefore, this study, at least, pointed out that in patients with NSTE-ACSs, H-FABP with this cut-off level may not have potential for the detection of MMD.

Conclusions

The results of this study suggest that rapid immunochromatographic test of H-FABP displays a diagnostic potential for the detection of myocardial injury that is almost similar to that of myoglobin in patients with NSTEMI-ACS who have elevated cTn levels. However, this bedside qualitative of H-FABP, at least with currently used cut-off levels, does not appear to be a sensitive marker able to detect MMD even in the elevation of cTn. H-FABP as an early marker of myocardial necrosis still requires further evaluation and standardization for diagnostic performance in various subgroups of patients within the spectrum of NSTE-ACS.

Conflict of interest: None declared.

Authorship contributions: Concept - Y.Ç. ; Design - Y.Ç., B.G.; Supervision - Y.Ç., N.A., A.B.; Resource - B. Gök., B. Görenek, N.A.; Materials - B. Gök., A.B., N.A.; Data collection&/or Processing - Y. Ç., B. Gök, C.D.; Analysis &/or interpretation - C.D., Y.Ç.; Literature search - B. Gök., B. Görenek; Writing - Y.Ç.; Critical review - A.B., B. Görenek; Others - C.D., N.A.

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