

# Heart-type fatty acid binding protein – a reliable marker of myocardial necrosis in a heterogeneous group of patients with acute coronary syndrome without persistent ST elevation

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## Abstract

**Background:** Myocardial infarction (MI) is one of the most serious challenges of contemporary cardiology. Among biochemical markers, heart-type specific fatty acid binding protein (h-FABP) has a high potential as a marker for the early diagnosis of acute MI. The h-FABP is released early to the bloodstream and may be useful for both rapid confirmation and exclusion of infarction. As opposed to patients with ST segment elevation MI (STEMI), patients with unstable angina (UA)/non-ST segment elevation MI (NSTEMI) present a heterogeneous group in which the confirmation of MI often meets diagnostic difficulties. A rapid, qualitative immunoenzymatic 'point of care' type test, revealing h-FABP in blood, has recently been made available (CardioDetect med).

**Aim:** To evaluate diagnostic value of early measurements of h-FABP and other markers of necrosis (cTnT, CK-MB, CK-MB mass) in a group of 100 patients with an acute coronary syndrome (ACS) without persistent ST segment elevation (NSTEMI ACS).

**Methods:** We studied 100 consecutive patients (34 women, 66 men; mean age 61.6 years) with strong suspicion of NSTEMI ACS and chest pain lasting <24 h before admission. During admission and after 3 and 6 hours patients had measured a panel of conventional biomarkers as well as quantitative measurements of h-FABP (on admission and 3 hours later) using CardioDetect med. The ultimate diagnosis of infarction (NSTEMI) was confirmed in case of a second (6 h after admission) positive quantitative result of cardiac troponin. Non-ST segment elevation MI was finally diagnosed in 56 patients.

**Results:** The comparison of diagnostic utility of all analysed biomarkers of necrosis revealed that h-FABP was superior to other parameters, when measured on admission, and was characterised by 94.7% sensitivity, 100% specificity, 100% positive predictive value, 93.4% negative predictive value and 97% accuracy. Other biomarkers had on admission lower sensitivity – 70.1% for CK-MB mass, 66.7% for CK-MB, 64.9% for cTnT, whereas their specificity was 97.6% for CK-MB mass, 93% for CK-MB and 100% for cTnT.

**Conclusions:** Qualitative h-FABP test (CardioDetect med) showed excellent sensitivity, higher than measurements of CK-MB mass, CK-MB, and cTnT on hospital admission, and high specificity in the patient group with NSTEMI ACS. The h-FABP seems to be an excellent biochemical cardiac marker for diagnosing NSTEMI, especially in its early phase, allowing exclusion of myocardial necrosis.

**Key words:** acute coronary syndrome, myocardial infarction, markers of necrosis

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## Introduction

Biochemical diagnostics of acute coronary syndromes (ACS) constitutes one of the fastest growing fields of cardiology of the 21<sup>st</sup> century. Data gathered from the Silesian Registry confirm the tendency to rising incidence of non-ST elevation ACS (NSTEMI ACS), that is unstable angina (UA) and/or non-ST segment

elevation myocardial infarction (NSTEMI), as opposed to ACS with ST elevation (STEMI): about 50,000 (31.2%) patients per year hospitalised due to STEMI, 60,000 (42.25%) patients due to UA and 30,000 patients due to NSTEMI [1].

Patients with NSTEMI have just slightly better prognosis than patients with STEMI as regards

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in-hospital mortality and – as proved in the GRACE register – higher one-year mortality [2]. Thus, it is important to develop fast and appropriate patient risk stratification in order to provide the right and optimal therapy.

In spite of having a ‘gold standard’ at hand such as cardiac troponins (cTn) – markers of very high cardiac specificity – a reliable and at the same time early marker of necrosis having acceptably high sensitivity and specificity in the early stage of MI is lacking. Such a marker could be heart-type fatty acid binding protein (h-FABP). It belongs to the family of small (12-15 kDa) cytoplasmic proteins discovered by Ockner in 1972 while investigating intestinal absorption of fatty acids. These substances are present in tissues metabolising free fatty acids in many mammals, participating in their transportation in the body and at the same time having certain tissue specificity, with the greatest concentration found in the heart and liver. The h-FABP consists of 132 amino acids, and is present mainly in the cytoplasm of cardiomyocytes (amounting to about 0.5 mg/g of tissue) and skeletal myocytes (with levels about 10 times lower than in cardiomyocytes) [3].

The h-FABP plasma concentration is relatively very low, ranging from 1 to 11.4 µg/l (median 1.5 µg/l) [4]. Elevated levels, above the established cut-off point, may be detected as soon as half an hour after the onset of angina (a low molecular weight protein localised near the cell membrane); maximum concentration is seen after about 4 hours in patients in whom reperfusion was successful [5], but after about 8 hours without reperfusion treatment [6]; normalisation of the h-FABP values is observed within 24 hours. Therefore, this marker could be useful to detect reinfarction. Levels of h-FABP in patients with MI may rise several times as compared to healthy individuals [7]. Recently, a qualitative immunochemical rapid test with ‘point of care’ analysis (CardioDetect med, Rennesens GmbH) has become available and among all those used in clinical practice this assay has the highest diagnostic sensitivity in the early stage of MI and may provide reliable detection of possible myocardial necrosis as soon as 30 minutes from the onset of pain, i.e. during the so-called ‘golden hour’ from the onset of MI [8]. The major advantage of h-FABP, despite a lack of absolute cardiospecificity, is its kinetics, which renders early detection of necrosis possible while other biomarkers, including cTn, are not yet detectable in the peripheral blood.

The aim of the study was to evaluate the diagnostic value of early h-FABP detection compared to other selected markers of myocardial necrosis such as CK MB mass, CK-MB and troponin T (cTnT) level. The h-FABP was measured using the qualitative test CardioDetect med in 100 patients with ACS without persistent ST elevation and pain duration less than 24 hours.

## Methods

### Study group

The study involved 101 patients with clinical diagnosis of NSTEMI ACS admitted to the 2<sup>nd</sup> Department of Cardiology of the Medical University in Łódź from March 2005 to September 2006. During hospitalisation one subject withdrew the previously given consent to participate in the study just after blood collection, and in fact the data of 100 patients were analysed. With regard to the study character (ACS subjects), no control group including patients without ACS was formed.

### Diagnosis of myocardial necrosis

Among patients finally enrolled into the study based on the second measurement of cTnT done 3 hours after admission, 43 were diagnosed with unstable angina, and 57 with NSTEMI. Troponin T level measured at 3 hours from hospital admission was assumed a ‘gold standard’ and a reference point for determination of all other markers (h-FABP, CK-MB mass, and CK-MB). In each subject, at the time of the second blood sample collection sufficient time had passed since the onset of pain to eliminate the possible ‘diagnostic window’ effect for cTnT, which was confirmed by the total quantitative convergence positive and negative cTn levels after 3, 6 and 12 hours from admission.

### Study inclusion criteria

1. Acute coronary syndrome without persistent ST segment elevation in patients <24 hours from the onset of chest pain or its evident worsening with electrocardiographic features implying acute ischaemia (ST segment depression, T wave pseudonormalisation, isolated T wave inversion) and without typical ischaemic changes.
2. Written informed consent for participation in the study by the patient.
3. Lack of exclusion criteria [STE ACS, renal failure (creatinine level >2.0 mg/dl), skeletal muscle disorders, known serious skeletal muscle disorders over the last few days before admission].

### Ethics

The study protocol was approved by the Bioethics Committee of the Medical University in Łódź.

### Statistical analysis

An analysis of statistical parameters was done on a PC using Windows XP and licensed software:

1. Statistical program MedCalc 8.0 for Windows (Frank Schoonjans, 1993-2005).
2. Statistica 6.0 PL software (StatSoft).

The results are expressed as means ± standard deviations or numbers and percentages. Analysis of

distribution of variables (continuous variables) using W Shapiro-Wilk test showed that the studied variables did not follow a normal distribution and so the Mann-Whitney test U was applied. In order to assess diagnostic and prognostic value as well as to determine optimal threshold levels of the quantitative variables, receiver operator characteristic curves (ROC) were computed. Area under ROC curve (AUC) is the measure of the test's ability to exactly differentiate between normal and abnormal results, thus allowing one to differentiate whether the given test is useful for group demarcation.

Test results with p values <0.05 were found statistically significant.

## Results

The study group of 100 subjects was divided into: patients with UA (n=43) and with NSTEMI (n=57). Demographic characteristics of both study groups are shown in Table I. There were no significant differences between groups with respect to age, body weight, body mass index or gender.

Prevalence of risk factors is presented in Table II. A relatively high incidence of smokers and patients with hypertension was found.

The original CardioDetect med test cards used in our study are shown in Figure 1; to increase the accuracy of results, serial initial tests were run and intensity of strip staining in the 'test result' field was compared to the pattern provided by the manufacturer.

The onset of pain to hospital time is shown in Figure 2. It was shorter for NSTEMI patients than UA ones. In the greatest percentage of NSTEMI patients angina occurred between 6 and 12 hours prior to admission, and in the UA group between 12 and 24 hours.

Electrocardiographic characteristics of the study group are demonstrated in Figure 3. The most frequent abnormalities in the resting ECG in the NSTEMI group were ST depressions, whereas in patients with UA they were negative T waves.

In the first measurement h-FABP showed 94.7% sensitivity, 100% specificity, 100% positive predictive value, 93.4% negative predictive value and 97% accuracy in confirming myocardial necrosis. The characteristics of other markers are presented in Figures 4 and 5, and Table III. Following detailed data analysis regarding the onset of angina, cTnT level at 3 hours from admission was set as a reference marker for final diagnosis of UA or NSTEMI – another, third measurement of cTnT level was 100% consistent (with respect to the number of negative and positive results) with measurement after 3 hours. All patients with NSTEMI presented with typical biomarker changes (increase) over 12 hours.

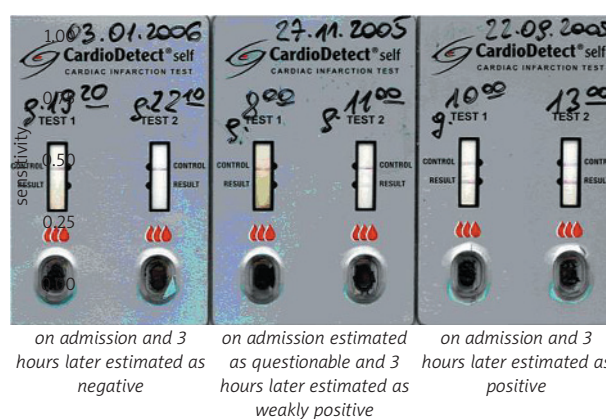
**Table I.** Basic demographic and clinical data of the study group

Parameter	UA	NSTEMI	p
Number of subjects	43	57	
Age [years]	63.0±10.5	62.0±10.8	NS
Body weight [kg]	78.0±12.6	80.0±11.7	NS
Body mass index [kg/m <sup>2</sup> ]	25.4±3.5	26.8±3.9	NS
Male gender [%]	15 (34.8%)	15 (33.3%)	NS

Abbreviations: UA – unstable angina, NSTEMI – non-ST segment elevation myocardial infarction

**Table II.** Major risk factors in the analysed group

Parameter	UA	NSTEMI	p
Smoking	16 (37.2%)	27 (47.3%)	NS
Hypertension	25 (60.0%)	34 (59.5%)	NS
Diabetes mellitus	9 (21%)	10 (17.6%)	NS
Obesity	20 (46.5%)	31 (52.4%)	NS
Positive family history	8 (19.5%)	9 (16.2%)	NS
Revascularisation			
percutaneous transluminal coronary angioplasty	11 (25.5%)	3 (5.2%)	NS
coronary artery bypass graft	1 (2.3%)	2 (3.5%)	



**Figure 1.** Original CardioDetect med tests used in the study and interpretation of their results

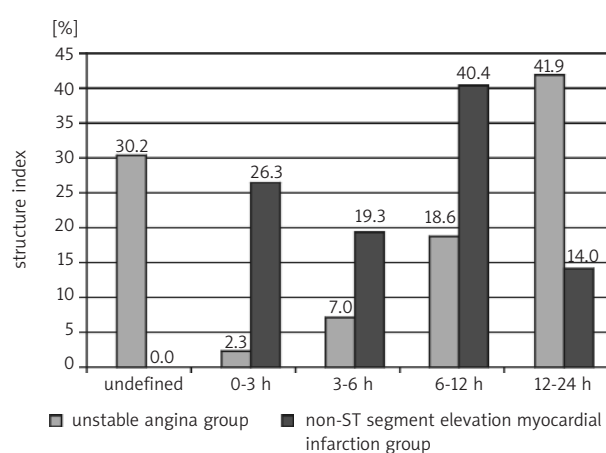
## Discussion

Taking into account the importance of the quickest possible diagnosis of myocardial ischaemia and necrosis in NSTEMI ACS patients, this study attempted to assess the diagnostic and prognostic value of a novel necrosis marker – h-FABP. It seems that optimisation of the diagnostic process, including the pre-hospital period, remains mandatory in this group of patients, in whom

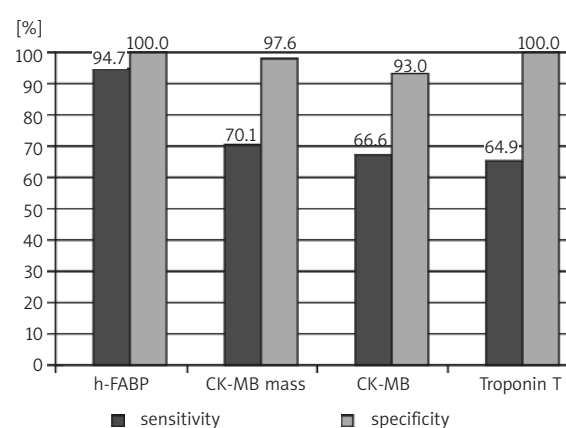
**Table III.** Summary of the analysed parameters of diagnostic usefulness of h-FABP compared to 'classical' markers of necrosis

	h-FABP		CK-MB mass		CK-MB		Troponin T	
	on admission	after 3 hours	on admission	after 3 hours	on admission	after 3 hours	on admission	after 3 hours
Sensitivity [%]	94.7	96.4	70.1	85.9	66.6	84.2	64.9	100
Specificity [%]	100	100	97.6	95.3	93.0	95.3	100	100
Positive predictive value [%]	100	100	97.5	96	92.6	96	100	100
Negative predictive value [%]	93.4	95.5	71.1	83.6	67.8	82	68.2	100
Accuracy [%]	97	98	82	90	78	89	80	100

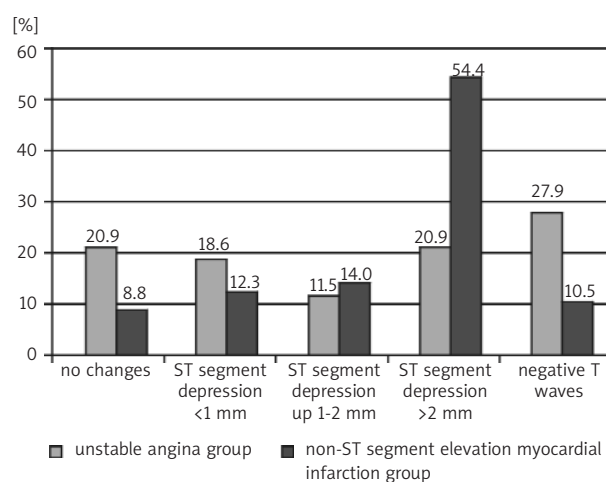
Abbreviations: h-FABP – heart-type fatty acid binding protein, CK-MB mass – creatine kinase MB fraction level, CK-MB – creatine kinase MB fraction activity



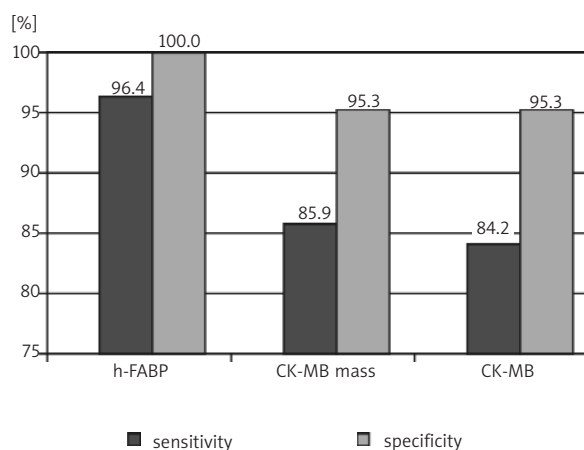
**Figure 2.** Pain to hospital time



**Figure 4.** Comparison of sensitivity and specificity of the analysed markers measured on admission



**Figure 3.** ECG characteristics (signs of ischaemia on ECG at rest)



**Figure 5.** Comparison of sensitivity and specificity of the analysed markers measured after 3 hours

misdiagnosis of MI in an ambulance or outpatient facilities is possible due to frequent ambiguity of clinical picture and ECG tracings.

Interesting results were obtained for the studied biochemical necrosis biomarkers regarding sensitivity, specificity, positive and negative predictive value and accuracy. H-FABP measured on admission showed as high as 94.7% sensitivity, 100% specificity, 100% positive predictive value, 93.4% negative predictive value and 97% accuracy. Comparison of all four markers measured on admission and h-FABP, CK-MB mass, CK-MB at 3 hours with the second reference measurement of cTn showed a significant advantage of h-FABP over the remaining necrosis markers, in particular with the first test.

The use of h-FABP as a method of early detection of myocardial necrosis has been shown recently, however relatively limited data confirming its clinical usefulness in the very first hours of ACS with and without ST segment elevation has been published. In particular, Japanese investigators have been using h-FABP in clinical practice. Okamoto et al. [9] evaluated clinical reliability and diagnostic usefulness of h-FABP in almost 200 patients with chest pain lasting up to 12 hours. This was a retrospective analysis, considering clinical condition and conventional enzymatic markers such as myoglobin and CK-MB. In 140 patients MI diagnosis was confirmed whereas 49 were classified as 'non-infarction' patients. Total sensitivity of h-FABP tests up to 12 hours from the onset of pain was 92.9%, while it reached 88.6% for myoglobin and only 18.6% for CK-MB. Specificity was 67.3% for h-FABP, 57.1% for myoglobin and 98% for CK-MB. The h-FABP proved to be more sensitive than myoglobin and CK-MB for detection of myocardial necrosis in patients with chest discomfort lasting less than 12 hours.

Suzuki et al. [10] compared sensitivity and specificity of qualitative assays for h-FABP and cTnT in a group of 130 patients (median age 68 years) admitted to hospital due to chest pain (37 diagnosed with STEMI, 39 with NSTEMI and 54 with UA). The sensitivity of tests was 59.1% for h-FABP and 15.4% for cTnT, if performed within 3 hours from the onset of pain, and 65.2% and 56.4%, respectively, if carried out beyond 3 hours. Very high sensitivity (100%) and high, almost 100% negative predictive value in all time intervals make h-FABP a very promising marker also to possibly exclude the early stage of infarction. The issue of usefulness of h-FABP especially to exclude MI was also investigated by Chan et al. [11]. In patients with chest pain suspected of MI they determined h-FABP (as well as cTnI and CK) twice, on admission and after one hour of hospitalisation. Myocardial infarction was confirmed in 94 out of 218 patients. Two sequential measurements of h-FABP allowed reliable diagnosis of MI in 100% of

patients; of importance two negative tests in this population showed virtually 0% false negative results. The results of measurements of two other markers, in particular cTnI, also showed high sensitivity, but it was reached only 7 hours later. Similar results of high 95-99% negative predictive value of normal h-FABP levels in patients with chest pain were observed by Pagani et al. [12]. Determination of h-FABP allowed exclusion of MI earlier and with higher certainty than using myoglobin. Also Pelters et al. [13] documented higher sensitivity of h-FABP than myoglobin in patients with ACS. Haastrup et al. [14] compared the usefulness of h-FABP tests, myoglobin, CK-MB and cTn in the early diagnosis of NSTEMI. The sensitivity of h-FABP up to 6 hours from the onset of chest pain was the highest and reached 95%, with 94% specificity.

Our experience with the use of qualitative measurements of h-FABP, presented in the abstract form also confirmed high accuracy of h-FABP, especially in the early stages of ACS [15]. It is worth mentioning that sensitivity and specificity of CK-MB mass and CK-MB are compared to the 'gold standard', which is cTn. The first measurements carried out on admission showed their sensitivity and specificity to be higher than of those cTn (according to the kinetics of markers in the bloodstream) – 70.1 and 97.67% respectively for CK-MB mass and 66.6 and 93%, respectively for CK-MB. After 3 hours diagnostic values of CK-MB and CK-MB mass increased (85.9 and 95.3%, respectively for CK-MB mass and 84.2 and 95.3% for CK-MB), but their sensitivity was not comparable to that of cTn (h-FABP was 96.4% sensitive and 100% specific in the same time interval). At the same time the second measurement of CK-MB mass revealed no necrosis in over 14% of NSTEMI patients, while CK-MB revealed no necrosis in over 15%. These results are consistent with the available published data and guidelines of cardiology societies regarding the advantage of cTn over CK-MB/CK-MB mass measurements.

## Conclusions

Quantitative determination of h-FABP using the CardioDetect med strip test proved to be a reliable diagnostic marker of NSTEMI, having 94.7% sensitivity and 100% specificity in the first measurement, thus showing the highest accuracy among other necrosis markers determined on admission (CK-MB mass, CK-MB, cTnT). Therefore, this test may be a very useful tool for both diagnosis and exclusion of NSTEMI, in particular at the early infarction phase, also including patients with normal or 'discrete' ECG changes.

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# Sercowe białko wiążące kwasy tłuszczowe – wiarygodny marker martwicy mięśnia sercowego w heterogennej grupie chorych z ostrym zespołem wieńcowym bez przetrwałego uniesienia odcinka ST

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## Streszczenie

**Wstęp:** Zawał mięśnia sercowego (MI) z jego powikłaniami, w tym zwłaszcza z nagłym zgonem sercowym, jest jednym z najważniejszych wyzwań współczesnej kardiologii klinicznej. Szybka diagnoza MI, a następnie wdrożenie odpowiedniego leczenia reperfuzyjnego (pierwotna koronaroplastyka, tromboliza) prowadzą do zmniejszenia śmiertelności wewnątrzszpitalnej i polepszenia rokowania długoterminowego w tej grupie chorych. Białko wiążące wolne kwasy tłuszczowe (h-FABP), które jest małym cytozolemowym białkiem zaangażowanym w transport i metabolizm kwasów tłuszczowych i obficie występuje w komórkach miokardium, może być obiecującym bardzo wczesnym markerem ostrego MI. Białko h-FABP jest uwalniane z martwiczo zmienionych kardiomiocytów bardzo szybko (ok. 50% czułość już w pierwszej godzinie po rozpoczęciu objawów) i może być bardzo użyteczne zarówno do wczesnego potwierdzania, jak i wykluczania MI. W przeciwieństwie do chorych z MI z uniesieniem odcinka ST (STEMI), chorzy z ostrymi zespołami wieńcowymi (ACS) bez uniesienia odcinka ST (NSTEMI) stanowią heterogenną grupę (zwłaszcza co do czasu rozpoczęcia dolegliwości i rodzaju zmian w EKG), w której potwierdzenie MI, a w ślad za tym szybkie wdrożenie terapii reperfuzyjnej, napotyka często diagnostyczne trudności. Szybki jakościowy test typu *point of care* do oznaczania stężenia h-FABP we krwi obwodowej (CardioDetect med) jest dostępny do użytku klinicznego.

**Cel:** Głównym celem badania była ocena wartości diagnostycznej wczesnych oznaczeń h-FABP wobec innych wybranych biomarkerów martwicy (cTnT, CK-MB, CK-MB mass) u chorych z NSTEMI.

**Metodyka:** Do badania włączono 100 chorych (34 kobiety, 66 mężczyzn; średnia wieku 61,6 roku) z klinicznym rozpoznaniem NSTEMI. Przy przyjęciu oraz 3 i 6 godz. po przyjęciu chorzy mieli oznaczane stężenie standardowych markerów martwicy, w tym dwukrotnie (przy przyjęciu i 3 godz. później) wykonywano jakościowe oznaczenie h-FABP przy użyciu CardioDetect med. Ostatecznie diagnozę MI bez uniesienia odcinka ST (NSTEMI) potwierdzano w wypadku dodatniego wyniku ilościowego troponiny T (cTnT) w 6 godz. od przyjęcia. Większość chorych poddano diagnostycznemu cewnikowaniu serca i w razie potrzeby przeszła rewaskularyzacji. Zawał serca bez uniesienia odcinka ST ostatecznie rozpoznano u 56 chorych.

**Wyniki:** Porównanie typowych parametrów określających wartość diagnostyczną testu (czułość, swoistość, wartość predykcyjną, dokładność) dla h-FABP oraz pozostałych, „klasycznych” markerów martwicy wykazało znaczącą przewagę badanego markera w pierwszym pomiarze – h-FABP charakteryzował się 94,7% czułością, 100% swoistością, 100% wartością predykcyjną dodatnią, 93,4% wartością predykcyjną ujemną oraz 97% dokładnością. Pozostałe markery charakteryzowały się przy pierwszym pomiarze czułością odpowiednio: 70,1% dla CK-MB mass, 66,7% dla CK-MB, 64,9% dla cTnT oraz swoistością: 97,6% dla CK-MB mass, 93,3% dla CK-MB oraz 100% dla cTnT.

**Wnioski:** Jakościowy test oznaczający h-FABP (CardioDetect med) wykonany przy przyjęciu wykazał znakomitą czułość, wyższą niż „klasyczne” markery martwicy, takie jak CK-MB mass, CK-MB, cTnT, jak również wysoką specyficzność w heterogennej grupie chorych z NSTEMI. Wydaje się, że h-FABP może być doskonałym markerem zarówno dla potwierdzania, jak i wykluczania zawału typu NSTEMI, zwłaszcza w jego wczesnej fazie.

**Słowa kluczowe:** ostry zespół wieńcowy, zawał serca, markery martwicy

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