

THE USE OF BIOCHIP CARDIAC ARRAY TECHNOLOGY FOR EARLY DIAGNOSIS OF ACUTE CORONARY SYNDROMES

UPOTREBA TEHNOLOGIJE BIOČIPA ZA RANO DIJAGNOSTIKOVANJE AKUTNIH KORONARNIH SINDROMA

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Summary: Serum troponin is the best biomarker for the diagnosis of acute coronary syndrome, but it takes considerable time before a definitive diagnosis is available. The purpose of this study was to evaluate whether a multi-marker approach, using the biochip cardiac array, would facilitate the early diagnosis. Serum biomarkers were determined on admission (≤ 6 hrs) and after 6 hours in 42 patients suspected for ACS. Cardiac troponin I was measured by a sensitive assay (STATcTnI) and cardiac markers (H-FABP, myoglobin, cTnI, CK-MB mass, carbonic anhydrase III) were assayed with the use of Biochip Array Technology. STATcTnI concentrations, within the first 6 hours, were elevated $>99^{\text{th}}$ percentile for the reference population in 83.3% of subjects, but none reached the cut-off for AMI. On admission H-FABP was the only marker with 90.5% sensitivity in all ACS cases and 100% sensitivity in STEMI/NSTEMI patients. The sensitivity of myoglobin at presentation was 71.4% in ACS, however, combined sensitivity of myoglobin and H-FABP reached 95.2%. Lowering the cut-off for cTnI allowed early diagnosis (≤ 6 hrs) in only 26.2% of ACS patients and 95.2% after the next 6 hours. In unstable angina the cardiac panel was not sufficiently accurate for early risk stratification. In conclusion, testing for both markers, H-FABP and sensitive cardiac troponin, available with the cardiac array may facilitate the early detection of myocardial injury in clinical practice.

Keywords: acute coronary syndromes, cardiac biomarkers, troponin, H-FABP

Kratak sadržaj: Troponin u serumu je najbolji biomarker za dijagnostikovanje akutnog koronarnog sindroma, ali do postavljanja definitivne dijagnoze prolazi dosta vremena. Cilj studije je da se ispita da li pristup s više markera, pomoću biočip metode omogućava rano dijagnostikovanje. Biomarkeri u serumu određeni su prilikom prijema (≤ 6 časova) i posle 6 časova kod 42 pacijenata sa simptomima akutnog koronarnog sindroma. Srčani troponin I izmeren je osetljivim testom (STATcTnI) dok su srčani markeri (H-FABP, mioglobin, cTnI, maseni CK-MB, karbo-anhidraza III) određeni pomoću tehnologije »Biochip Array«. Koncentracije dobijene pomoću STATcTnI u prvih 6 časova bile su povišene >99 procenata u odnosu na referentnu populaciju kod 83,3% ispitanika, ali ni kod koga nisu prešle »cut-off« vrednost za akutni infarkt miokarda. Prilikom prijema samo je marker H-FABP imao senzitivnost od 90,5% u svim slučajevima akutnih koronarnih sindroma i senzitivnost od 100% kod pacijenata sa STEMI/NSTEMI. Osetljivost mioglobina na prijemu bila je 71,4% za AKS, međutim, osetljivost kombinacije mioglobina i H-FABP dostigla je 95,2%. Snižavanje »cut-off« vrednosti za cTnI omogućilo je rano dijagnostikovanje (≤ 6 časova) kod samo 26,2% pacijenata sa AKS i 95,2% posle sledećih 6 časova. Kod nestabilne angine srčani panel nije bio dovoljno precizan za ranu stratifikaciju rizika. Može se zaključiti da testiranje oba markera – H-FABP i senzitivnog srčanog troponina dostupno putem metode »Cardiac Array« – može olakšati ranu detekciju oštećenja miokarda u kliničkoj praksi.

Ključne reči: akutni koronarni sindromi, srčani biomarkeri, troponin, H-FABP

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List of abbreviations: acute coronary syndrome (ACS), cardiac troponin (cTn), creatine kinase MB (CK-MB), heart-type fatty acid binding protein (H-FABP), unstable angina (UA), acute myocardial infarction (AMI), ST-elevation myocardial infarction (STEMI), non-ST elevation acute coronary syndrome (NSTEMI) – meaning patients with diagnosis of unstable angina and non-ST elevation ACS, carbonic anhydrase III (CA III), myoglobin (MYO), area under the ROC curve (AUC).

Introduction

Diagnosis of acute myocardial infarction in the emergency department, according to the recommendations published in 2007, is based on the increase of cardiac troponin above the 99th percentile reference value for healthy population, in patients with evidence of myocardial ischemia (1). The early risk stratification is crucial in the timing and treatment of patients with suspected ACS. However, in patients with chest pain lasting less than 6 hours, most current troponin assays do not have sufficient sensitivity for AMI to be rapidly ruled in or specificity for ruling it out. The prolonged release pattern of cTn and sensitivity of the assay makes it difficult to diagnose ACS early, thus adding of markers increasing rapidly after the symptoms, improves the identification of patients for more aggressive interventions. Among the early markers those of cardiac ischemia, inflammation and plaque instability and focal myocardial necrosis have been partially evaluated so far. Multimarker strategy seems to be more sensitive for myocardial infarction and ACS detection than cardiac troponin alone unless highly sensitive cTn assay is used and a concentration of this biomarker over the 99th percentile is accepted as indicative of ACS.

After myocardial injury myoglobin and H-FABP are released rapidly from the cardiomyocytes. Myoglobin is among the earliest markers released into the circulation after the onset of ACS symptoms, however, it has low specificity for the cardiac muscle. Earlier data have suggested that the ratio of myoglobin/carbonic anhydrase III, the enzyme found in the skeletal but not in the cardiac muscle, correlates closely to myocardial damage (2, 3).

H-FABP, a cytoplasmic protein, functions as a transport protein for the fatty acids and plays a role in their oxidation (4). H-FABP is quite specific for the cardiac muscle and has been suggested as an early marker of myocardial damage.

CK-MB is a cytosolic enzyme found predominantly in the cardiac muscle. CK-MB concentration in blood will increase within first 3–4 hours after the onset of chest pain, however it is not entirely cardio-specific as a very small proportion of less than 1% is found in the skeletal muscle. The purpose of this study was to evaluate the diagnostic efficacy of the multi-marker approach, using a biochip array technology, in identifying ACS in patients at presentation (≤ 6 hrs) and 6 hours later.

Materials and Methods

Forty-two patients suspected for ACS (10 women, 32 men, aged 44–83 yrs) were included in the study. The criteria for enrollment were the following: typical anginal chest pain at rest, symptom onset less than 6 hours before the hospital admission

and serum STATcTnI concentration at presentation below 0.3 ng/mL, the cut-off value for AMI. Patients with chest pain of non-ischemic origin, heart failure (NYHA III, IV) or pulmonary dyspnea, embolism, renal insufficiency, history of myocardial infarction during 6 weeks preceding hospital admission were excluded from the study. Serial ECG examinations were performed. All patients underwent coronary angiography and coronary angioplasty with stenting if clinically indicated. Transthoracic echocardiography was also performed. The investigated patients were discharged with a final diagnosis of unstable angina, non-ST-elevation myocardial infarction or ST-elevation myocardial infarction.

Venous blood samples were collected twice: at presentation (≤ 6 hours from the onset of chest pain) and after the next 6 hours. STATcTnI was assayed (ARCHITECT ci8200, Abbott Diagnostics) with the lowest measured concentration of 0.032 ng/mL (with CV=10%) that was $> 99^{\text{th}}$ percentile reference value for healthy population. However, the accepted cut-off value of STATcTnI for AMI was 0.30 ng/mL. Other cardiac biomarkers: cTnI, CK-MB mass, myoglobin, carbonic anhydrase III and H-FABP were determined simultaneously in a single serum sample of 60 μ L with the use of Biochip Array Technology on an evidence investigator (RANDOX). cTnI was detected with the diagnostic sensitivity of 0.18 ng/mL; range of detection 0–50 ng/mL; a cut-off value of 0.48 ng/mL (95th percentile) or 0.56 ng/mL (99th percentile) provided in assay package inserts. CK-MB mass was determined with the diagnostic sensitivity of 0.4 ng/mL; range of detection 0–125 ng/mL; a cut-off value of 1.92 ng/mL (95th percentile) suggested in assay package inserts. Myoglobin and CA III were assayed with the diagnostic sensitivity of 1.8 ng/mL and 0.2 ng/mL, respectively, range of detection 0–700 ng/mL and 0–200 ng/mL, a cut-off value of 66.0 ng/mL (95th percentile) for MYO and 58.0 ng/mL (95th percentile) for CA III. H-FABP was assayed with the diagnostic sensitivity of 0.15 ng/mL; range of 0–100 ng/mL, and a cut-off value of 2.5 ng/mL (95th percentile) provided by the manufacturer. Concentrations of biomarkers equal or above the cut-points were regarded as positive results.

The study protocol was approved by the Bioethics Committee at Collegium Medicum and written informed consent has been obtained from all patients.

Statistics

The use of the Shapiro-Wilk test demonstrated that the variables were not normally distributed. Therefore, results were reported as median values and interquartile ranges. Qualitative variables were expressed as the number of patients presenting the given feature and the percentage of patients in the group analysed. Appropriate statistical tests were

applied. U-test, Mann-Whitney's and Wald-Wolfowitz test were used for comparisons. ROC curves were plotted and analyzed. Spearman and Pearson correlation tests were performed (5). All computations were carried out with Statistica, version 6.0.

Results

The concentrations of all cardiac biomarkers, excluding CA III, increased significantly within 12 hours from the symptom onset and varied in patient groups in relation to the degree of arterial stenosis (Figure 1). The median values of cTnI and myoglobin measured on admission (≤ 6 hrs) were significantly higher in three vessel disease than in one vessel disease group ($p < 0.04$; $p < 0.03$). After the next 6 hours troponin and CK-MB mass concentrations were higher in three vessel disease than in one vessel disease group ($p < 0.03$). Only H-FABP concentrations were not significantly associated with the degree of stenosis.

The levels of all markers were also compared in groups of patients in relation to the final diagnosis (Figure 2). In STEMI cases cTnI and H-FABP concentrations at presentation were significantly elevated ($p < 0.04$ and $p < 0.003$) and a trend to higher MYO concentrations ($p = 0.0512$) was observed in compa-

risson to NSTEMI patients. However, median STATcTnI values were nearly the same in NSTEMI and STEMI patients.

The concentrations of CK-MB mass and MYO in STEMI patients increased within 12 hours from the onset of clinical symptoms and were much higher than in NSTEMI cases, but the differences were not significant. On the contrary, both troponin and H-FABP concentrations were significantly higher in STEMI patients. Concentrations of CA III were nearly the same and fairly constant in both subgroups at both sampling points. Calculated MYO:CA III ratio was elevated only in STEMI patients on admission but remained unchanged in NSTEMI patients (results not shown).

We have found a very good positive correlation of H-FABP with MYO ($r = 0.84$; $p < 0.0001$) and CK-MB mass ($r = 0.58$; $p < 0.0001$) within the first 6 hours from the symptom onset and moderate relationship of CK-MB mass with MYO and a cTnI ($r = 0.56$ $p < 0.0001$; $r = 0.43$ $p < 0.005$). The correlation of H-FABP with myoglobin and CK-MB mass was even stronger after the next 6 hours (≤ 12 hrs) ($r = 0.91$ and $r = 0.83$; $p < 0.0005$). Additionally, a significant relationship was found between H-FABP and STATcTnI ($r = 0.72$; $p < 0.0005$) as well as CK-MB mass and STATcTnI or MYO ($r = 0.74$ and $r = 0.69$; $p < 0.0005$).

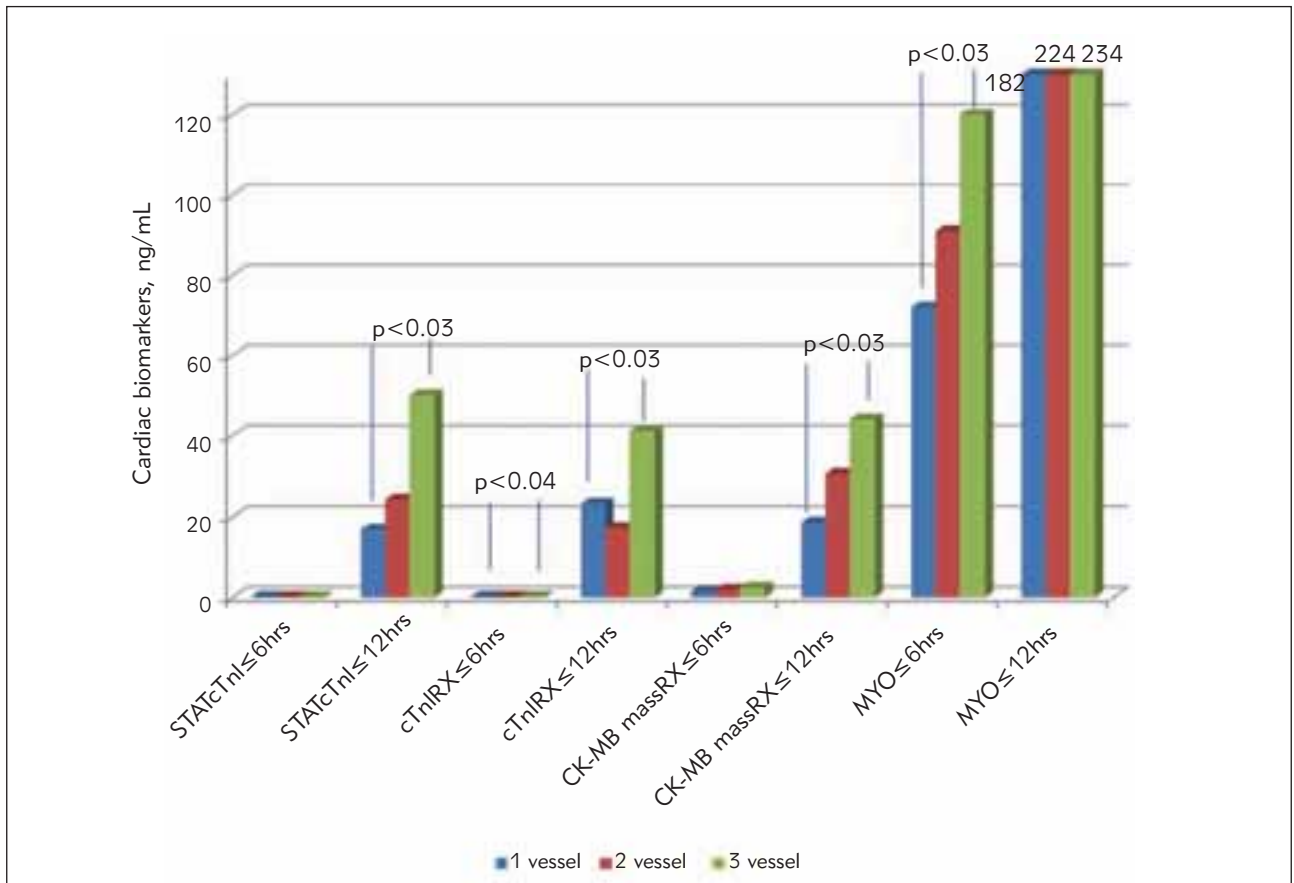


Figure 1 The concentrations of cardiac biomarkers in relation to degree of arterial stenosis.

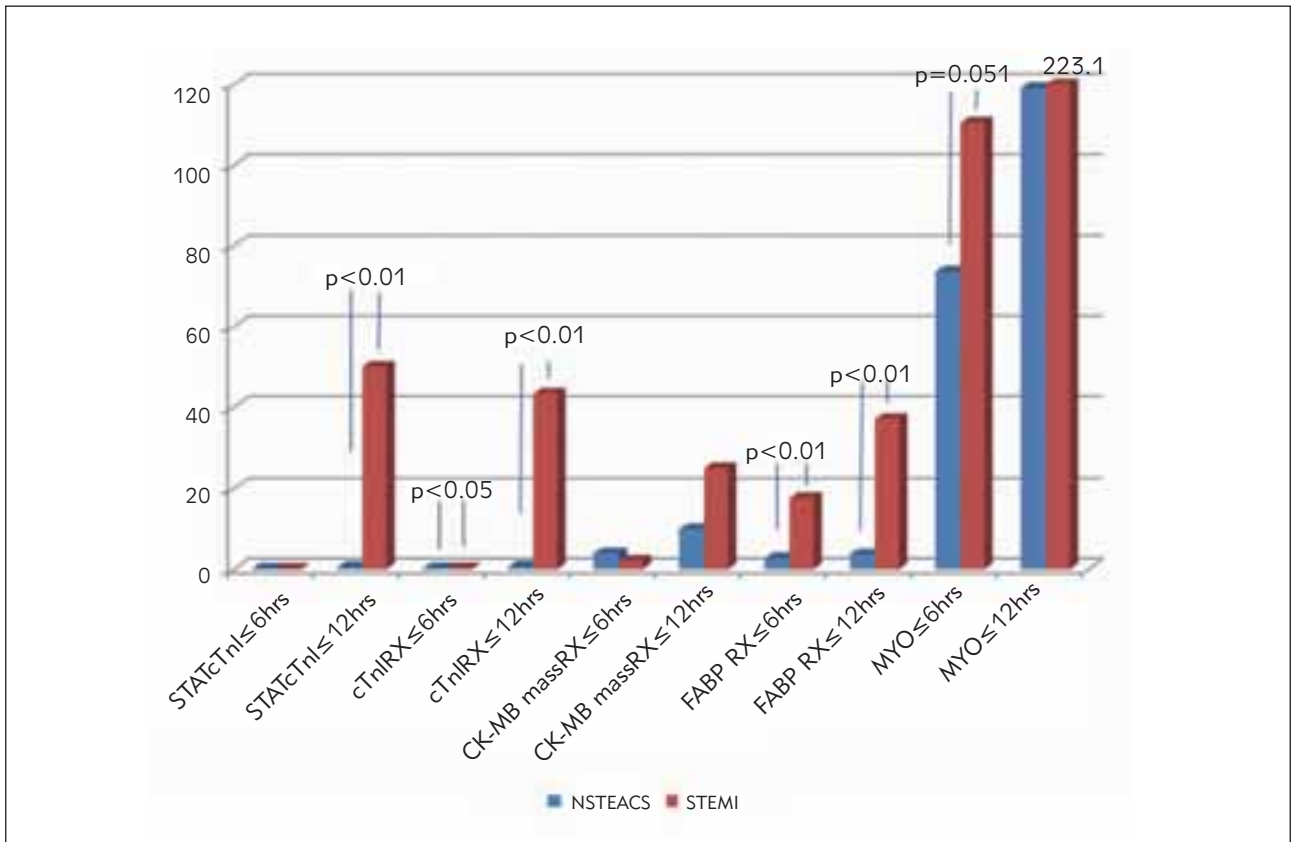


Figure 2 The concentrations of cardiac markers in groups of patients in relation to the final diagnosis.

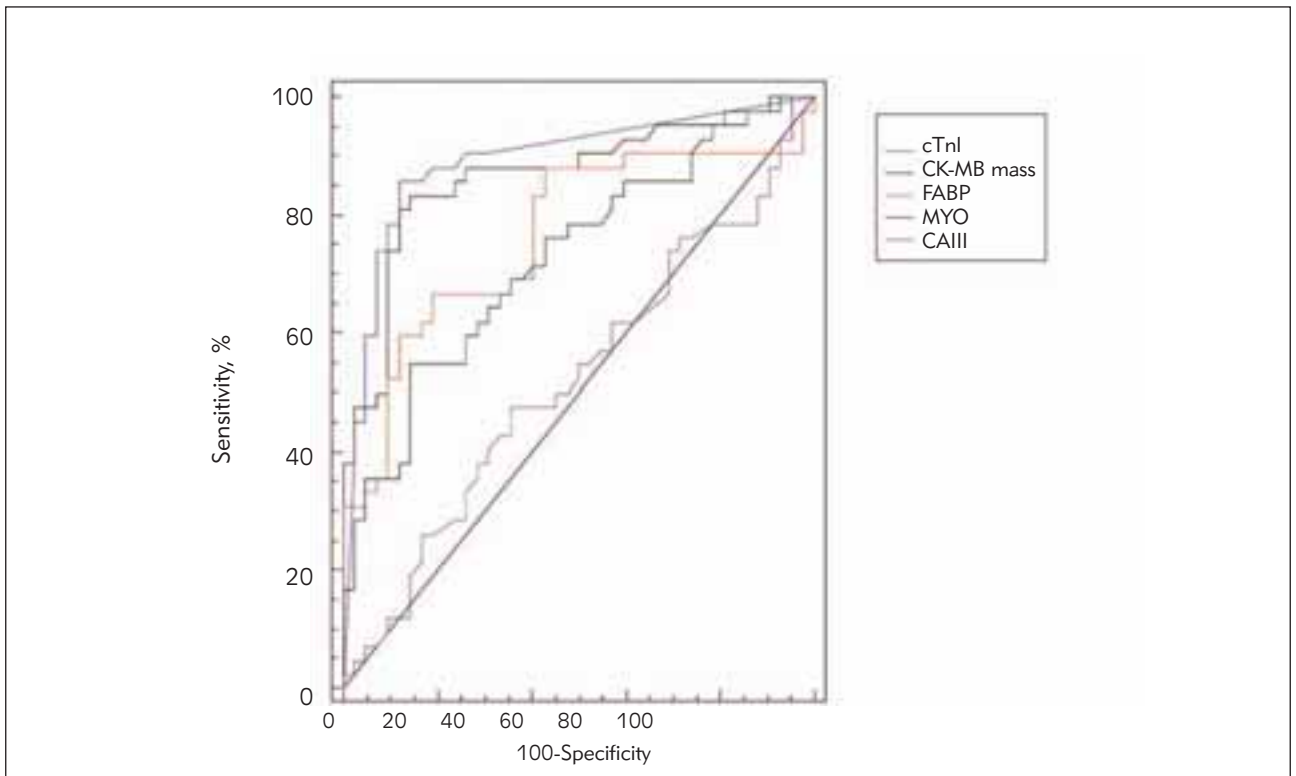


Figure 3 ROC curves plots for cardiac markers.

The diagnostic efficacy of cardiac biomarkers was evaluated based upon the ROC curves analysis. The troponin assays were characterized by very high diagnostic value, AUC for STATcTnl and cTnl were 0.965 and 0.891, respectively (Table I). With the use of cTnl assay and the value of >0.45 ng/mL as cut-off, the sensitivity for diagnosing ACS and specificity (with 95% CI) were the best, 88% (74–96) and 81% (66–91) comparing to the 99th percentile reference value of 0.56 ng/mL. The lower cut-off allowed early diagnosis of 11 instead of 8 patients presenting with STEMI already on admission.

ROC curve analysis has shown that the 95th percentile reference value as cut-off for H-FABP (≥2.5 ng/mL) was the best to identify cases presen-

ting with ACS on admission (Figure 3, Table I). Similarly, the concentrations very close to the 95th percentile reference value as cut-off for CK-MB mass and myoglobin have shown the best results to identify, especially patients with STEMI within the first 6 hours from the onset of chest pain.

It must be stressed that on admission only H-FABP concentrations were positive in all STEMI patients (Table II). After the next 6 hours, when STATcTnl was over the cut-off for AMI, the positive results of H-FABP were confirmed. Moreover, the positive H-FABP results were found in four out of eight NSTEMI cases (3 NSTEMI and 1 UA) at both sampling points. We should keep in mind that positive

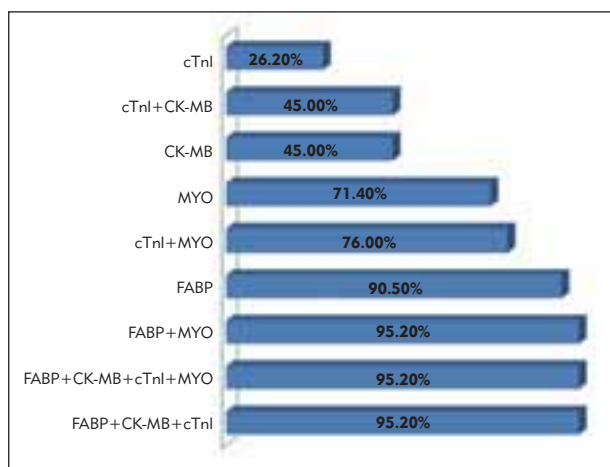


Figure 4 Combined sensitivity of cardiac markers within 6 hrs from the chest pain onset.

Table I Diagnostic value of biomarkers assayed with the use of biochip cardiac array in relation to STATcTnl in patients suspected for ACS referring to the hospital.

Marker	AUC	Best cut-off	Sensitivity % (with 95% CI)	Specificity % (with 95% CI)
STATcTnl (ng/mL)	0.965	> 0.307	93 (81–98)	100 (92–100)
cTnl (ng/mL)	0.891	> 0.45	88 (74–96)	81 (66–91)
CK-MB mass (ng/mL)	0.867	> 1.91	88 (74–96)	57 (41–72)
H-FABP (ng/mL)	0.765	> 2.51	90 (77–97)	14 (6–29)
MYO (ng/mL)	0.728	> 64	86 (71–94)	29 (16–45)

Table II Sensitivity of cardiac panel markers (myoglobin, H-FABP, CK-MB mass, cTnl) in relation to STAT cTnl on admission and at ≤12 h in patients with ACS.

Patients with ACS	Sensitivity (%)					
	Myoglobin >64 ng/mL	H-FABP >2.51 ng/mL	CK-MB mass >1.91 ng/mL	cTnl >0.45 ng/mL	STAT cTnl >0.032 <0.3 ng/mL (99th%)	STATcTnl cut-off for MI ≤0.3 ng/mL
Time from onset ≤ 6 h						
ACS patients n=42	71.4	90.5	45.2	26.2	83.3	0
NSTEMI n=8	50.0 (4/8)	50.0 (4/8)	12.5 (1/8)	0 (0/8)	75.0 (6/8)	0
STEMI n=34	76.5 (26/34)	100.0 (34/34)	52.9 (18/34)	32.3 (11/34)	85.3 (29/34)	0
Time from onset ≤ 12h						
ACS patients n=42	85.7	90.5	90.5	95.2	7.1	92.9
NSTEMI n=8	62.5 (5/8)	50.0 (4/8)	50.0 (4/8)	75.0 (6/8)	37.5 (3/8)	62.5 (5/8)
STEMI n=34	91.2 (31/34)	100.0 (34/34)	100.0 (34/34)	100.0 (34/34)	0 (0/34)	100.0 (34/34)

STATcTnI results were found in five NSTEMI cases (3 NSTEMI and 2 UA) within 12 hours.

Similarly to cTnI, the percentage of positive CK-MB mass results increased by up to 100% in STEMI patients and up to 50% or 75% in NSTEMI cases within 12 hours. Among NSTEMI cases, only one UA patient was positive for CK-MB mass and three UA were positive for cTnI at the second sampling point. Distribution of positive results of myoglobin increased mainly in STEMI patients from 76.5 to 91.2% within 12 hours.

The highest sensitivity at presentation in all ACS and in STEMI patients was shown for H-FABP (90.5 and 100%). The sensitivity for myoglobin was lower (71.4 and 76.5%, respectively) and for CK-MB mass the lowest (45.2 and 52.9%). However, combined sensitivity of myoglobin and H-FABP reached 95.2% for all ACS subjects (*Figure 4*).

At the second blood sampling (≤ 12 hours) the sensitivity for all cardiac biomarkers but MYO was excellent in STEMI cases (100%) and very good in all ACS (90.5–95.2%). Lower cut-off for cTnI increased the sensitivity of the assay by up to 75% in NSTEMI cases, however the sensitivity of other biomarkers was still unsatisfactory in this group. Again, myoglobin determination was characterized by lower sensitivity in STEMI (91.2%) and in all ACS patients (85.7%).

Discussion

With their high sensitivity and specificity cardiac troponins remain the markers of choice for the diagnosis and risk assessment in ACS as well as for identifying the patients who benefit from particular treatment strategies. However, the use of troponin, a marker of myocardial necrosis, has its limitations. It should be mentioned that troponin determination does not allow diagnosis of ACS shortly after the onset of symptoms and other than ACS conditions involving myocardial damage lead to increased blood troponin concentrations. The newly proposed early biomarkers of myocardial injury that may add improved information in ACS diagnosis and enhance the stratification of risk seem very promising. Before implementation in clinical practice their diagnostic performance should be carefully assessed. We have applied the protein biochip array technology in the search for an early marker of high sensitivity and specificity for risk stratification. The panel of both early cardiac biomarkers and those with prolonged release pattern was evaluated.

In our study the diagnostic performance of cardiac markers was assessed based on the ROC curve analysis and calculation of sensitivity. This analysis has shown that for H-FABP and CK-MB mass the 95th percentile reference values, provided by the manufacturer, were the best cutpoint, whereas for myoglobin and cTnI the values very close to the 95th

percentile were the best cut-off to identify patients presenting with ACS at both sampling points.

On admission H-FABP was the only marker with 100% sensitivity in STEMI patients and 90.5% sensitivity in all ACS cases. This was confirmed after the next 6 hours. Taking into account that H-FABP was characterized by excellent sensitivity also in NSTEMI patients at both sampling points it may be concluded that from all cardiac biomarkers included in the panel only H-FABP seems to improve the early risk stratification. Other authors indicated H-FABP as an early and specific marker of myocardial injury (6–9). Very good correlations of H-FABP with myoglobin reflected similar release pattern of both small proteins after myocardial injury. Interestingly, H-FABP concentrations were not significantly associated with the degree of stenosis whereas in the case of myoglobin such association was found at first sampling. One earlier study with the use of the same technology demonstrated a 100% sensitivity for H-FABP and higher AUC (0.998) at cut-point of 5.08 ng/mL within 6 hours from the onset of chest pain (10). On average, the sensitivity of H-FABP assays for the early diagnosis of AMI reaches 90% at 5–6 ng/mL as cut-off value (6–8, 11).

In this study the best cut-point for STATcTnI was nearly the same as provided by the manufacturer (>0.307 ng/mL). At first sampling point in 83.3% of all patients STATcTnI concentrations were elevated over the 99th percentile of a reference population but none reached the cut-off for AMI. On the contrary, the accepted lower cut-off for cTnI allowed early diagnosis of 32.3% of STEMI patients already on admission, and 95.2% of all ACS patients after the next 6 hours. Others have reported higher sensitivity (81.8%) within the first 6 hours, at a considerably lower cut-point of 0.324 ng/mL with the biochip array technology (10).

Similar sensitivity for the early diagnosis of ACS was demonstrated, with the same assay, for myoglobin, but much higher for CK-MB mass (9). As previously discussed, the best cut-off values applied by us for calculations of sensitivity were considerably lower than reported by Zaninotto et al. (9). Nevertheless, the sensitivity of myoglobin for the diagnosis of ACS reached 71.4% and only 45.2% for CK-MB mass on admission. On the contrary, after the next 6 hours the clinical performance of CK-MB mass was nearly the same as those of troponins or H-FABP and better than that of myoglobin.

Our experience with the cardiac panel demonstrated that only in some NSTEMI patients, specifically with diagnosis of UA, it failed to add any improved early information (12). This observation may be affected by small sample size which remained the major limitation in this study. However, lower cut-point, particularly for cTnI, was shown to be beneficial for the diagnosis of patients with unstable angina within 12 hours from the onset of chest pain.

Very recent data indicated that a multiple-biomarker approach including markers of plaque instability, inflammation and necrosis but no H-FABP for the early diagnosis of myocardial infarction did not show better clinical accuracy than the use of sensitive cTnl assay based on the 99th percentile (>0.1

ng/mL) (1). We may conclude that testing for both markers, H-FABP and sensitive cardiac troponin, available with the biochip cardiac array may facilitate the early detection of myocardial injury in the routine clinical practice.

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