

Multi-marker approach with the use of biochip cardiac array technology for early diagnosis in patients with acute coronary syndromes

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Abstract

Introduction: Diagnosis of acute coronary syndrome (ACS) is frequently a challenging task while immediate risk stratification remains crucial for the prompt implementation of appropriate therapy in this setting. The prolonged release pattern of both CK-MB mass and cardiac troponins makes it difficult to identify the origin of recent chest pain, thus a combination of early and later biomarkers might further facilitate the differential diagnosis. The study was designed to evaluate the efficacy of multi-marker approach using biochip array technology in identifying ACS shortly after the symptom onset.

Material and methods: The study group consisted of 42 patients suspected for ACS. Subjects were diagnosed as presenting with unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI) or ST-elevation myocardial infarction (STEMI). Biomarkers in the serum were determined twice: on admission (≤ 6 hours from the chest pain onset) and after next 6 hours. Cardiac troponin I was measured by routine sensitive automated assay (*STAT* cTnI) while other 6 cardiac markers (heart-fatty acid binding protein - H-FABP, myoglobin, glycogen phosphorylase BB, cTn I, CK-MB mass and carbonic anhydrase III) were assessed using biochip array technology.

Results: *STAT* cTnI concentrations within 6 hours from the symptom onset were elevated over the 99th percentile for reference population in 83.3% of subjects but none reached the cut-off value for myocardial infarction. Instead, H-FABP demonstrated a very good efficacy in early detection of ACS (90.5%), better than myoglobin and CK-MB mass. Sensitivity of H-FABP calculated for NSTEMI/STEMI subjects reached 100%. The diagnostic efficacy of troponin, myoglobin and CK-MB mass assay markedly increased within 12 hours. It was only

for the patients with UA that the cardiac panel was not efficient in the early stratification of risk.

Conclusions: A multi-marker strategy with H-FABP and highly sensitive troponin included enhances the early diagnosis and decision making process in patients with ACS. A new biochip cardiac array technology may serve as a powerful tool for ACS detection in the clinical practice.

Introduction

Diagnosis of acute coronary syndrome (ACS) is frequently a challenging task while immediate risk stratification remains crucial for the prompt implementation of appropriate therapy in this setting. Cardiac troponins are currently used as the markers of choice in making the critical identification of ACS. However, in patients with early presentation of chest pain, negative troponin or CK-MB mass, the other well established biomarker of myocardial necrosis, do not allow to rule out ACS regardless of ECG findings. The prolonged release pattern of both CK-MB mass and cardiac troponin makes it difficult to identify the origin of recent chest pain, thus a combination of early and later biomarkers might further facilitate both differential diagnosis and risk assessment.

Among numerous early markers of ACS those of cardiac ischemia (FFAu - free fatty acids unbound to albumin and IMA - ischemia modified albumin), inflammation and plaque instability (hsCRP-high sensitivity C-reactive protein, CD40 ligand, MPO - myeloperoxidase, MCP-1 - monocyte chemoattractant protein 1, choline, PAPP-A - pregnancy associated plasma protein A) and focal myocardial necrosis (H-FABP- heart fatty acid binding protein) have been evaluated the most extensively. On the basis of pathophysiological data multi-marker strategy seems to be advantageous over cardiac troponins alone for the high-risk ACS detection.

Myoglobin and H-FABP are released from cardiomyocytes rapidly after myocardial injury. Myoglobin is among the earliest markers released into circulation after the onset of ACS symptoms. However, its clinical value is considerably limited by low specificity for cardiac muscle. Previous studies have suggested that the ratio of myoglobin/carbonic anhydrase III (CA III), the enzyme found exclusively in skeletal muscles, correlates closely with the extent of myocardial damage (1,2). H-FABP is a low-molecular cytoplasmic protein that may offer several advantages over troponin. Due to its small size and high concentration in the cytoplasm of cardiomyocyte, H-FABP is released quickly into blood stream when membrane integrity is compromised in response to myocardial injury. In physiological

conditions H-FABP acts as a transport protein for the fatty acids and plays an important role in their oxidation (3).

The study was designed to evaluate the efficacy of multi-marker approach using biochip array technology in relation to cardiac troponin I measured by routine sensitive automated assay in identifying ACS shortly after the symptom onset.

Study design and patients

The study group consisted of 42 patients (10 women, 32 men, aged 44-83 years) admitted to the Department of Cardiology and Internal Medicine of the Collegium Medicum in Bydgoszcz with an initial diagnosis of ACS. The enrolment criteria included: typical anginal chest pain at rest, symptom onset less than 6 hours before the hospital admission and serum *STAT* cardiac troponin I level on admission below the cut-off value for acute myocardial infarction (AMI) of 0.30 ng/ml. Patients with chest pain of non-ischemic origin, heart failure (III or IV according to NYHA classification), pulmonary embolism, chronic obstructive pulmonary disease, pneumonia, renal insufficiency (serum creatinine >1.5 mg/dL), history of myocardial infarction within 6 weeks preceding the study recruitment were excluded from the trial. Serial ECG examinations were performed (at least four examinations: on admission, after next 6 hours, after coronary angioplasty, at discharge and each time when clinically indicated). All subjects underwent coronary angiography and coronary angioplasty with stenting if clinically indicated. The diameters of the heart chambers and indices of LV systolic and diastolic function were measured by transthoracic echocardiography. The investigated patients were discharged home with a final diagnosis of unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI) or ST-elevation myocardial infarction (STEMI). All participants provided informed written consent. The study protocol was approved by the Local Bioethics Committee.

Assessment of biomarkers

Peripheral venous blood samples were collected twice: on admission (≤ 6 hours from the chest pain onset) and after next 6 hours. Cardiac troponin I (*STAT* cTnI) was determined on the ARCHITECT ci8200 (Abbott Diagnostics) while other 6 cardiac biomarkers (H-FABP, myoglobin, glycogen phosphorylase BB, cTn I, CK-MB mass and carbonic anhydrase III) were assessed using Biochip Array Technology on the evidence investigator (RANDOX).

STAT cTnI is a CMIA assay characterized by $CV \leq 10\%$ for the samples with cTnI concentration ≥ 0.20 ng/ml. The lowest measured cTnI concentration with $CV=10\%$ is 0.032

ng/ml that also is over 99th percentile for the reference population. However, according to the manufacturer, the cut-off value of *STAT* cTnI for AMI is 0.30 ng/ml with the sensitivity of 60% and specificity of 95.4% at 0-6 hours and sensitivity of 78.6% and specificity of 94.6% at 6-12 hours after admission to the hospital.

Cardiac Array was developed as a simultaneous quick and quantitative detection of 6 ACS biomarkers (3 classic and 3 novel cardiac markers) with the use of biochip array technology (semi-automatic assay on RANDOX evidence investigator) in a serum sample of 60 µl. This technology is based upon ELISA principles. Multiple specific capture antibodies are attached at discrete test regions on the surface of the biochip. The light signal generated from each of the discrete test regions on the biochip is simultaneously detected. Unique image processing software is used to translate the light signal generated from the chemiluminescent reaction into an analyte concentration.

H-FABP was assessed with the diagnostic sensitivity of 0.15 ng/ml, while its range of detection and accepted cut-off value (95th percentile) were 0-100 ng/ml and 2.5 ng/ml, respectively. Myoglobin was evaluated with the diagnostic sensitivity, range of detection; accepted cutpoint for 95th percentile of 1.8 ng/ml, 0-700 ng/ml and 66.0 ng/ml, respectively. cTnI was detected with the diagnostic sensitivity of 0.18 ng/ml (imprecision $\leq 20\%$), while its range of detection and accepted cut-off values were 0-50 ng/ml, 0.48 ng/ml (95th percentile) and 0.56 ng/ml (99th percentile), respectively. The diagnostic sensitivity for CK-MB and CA III were 0.4 ng/ml and 0.2 ng/ml, respectively. The ranges of detection were 0-125 ng/ml for CK-MB and 0-200 ng/ml for CA III, while accepted cut-off values (95th percentile) were 1.92 ng/ml for CK-MB and 58.0 ng/ml for CA III, respectively. Concentrations of glycogen phosphorylase BB were nondetectable in all cases at first sampling. Therefore, they were not taken into account for further evaluation. Levels of biomarkers equal or above their cut-off values were regarded as positive results.

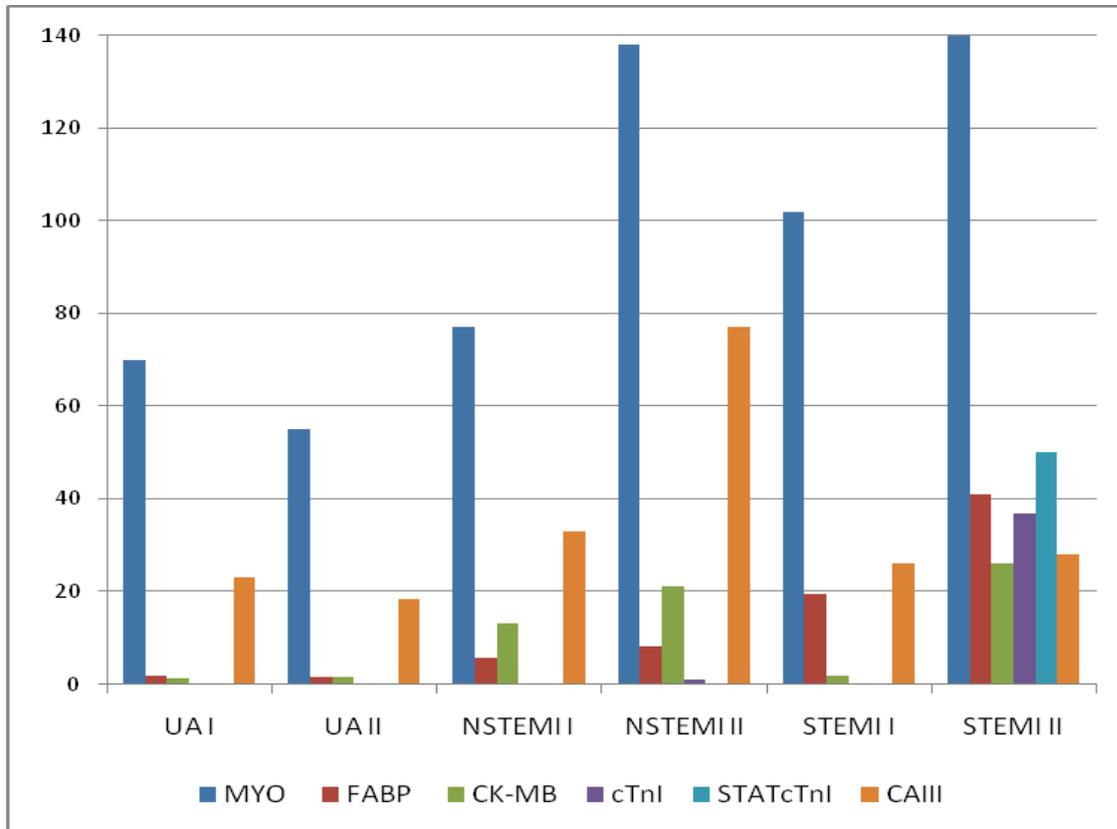
Statistical analysis

The use of the Shapiro-Wilk test demonstrated that the investigated 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. All computations were carried out with Statistica, version 6.0 (StatSoft).

Results

The median values of cardiac markers measured on admission (≤ 6 hours from the chest pain onset) and after next 6 hours (≤ 12 hours from the chest pain onset) are presented in **Fig. 1**.

Fig.1 Median values of cardiac markers at admission (< 6 hrs; I) and at ≤ 12 hrs (II).



Cut-off values for MYO (myoglobin RANDOX) was 66 ng/ml, H-FABP (RANDOX) -2,5 ng/ml, CK-MB (RANDOX) – 1,92 ng/ml, cTnI (RANDOX) – 0,48 ng/ml, *STAT* cTnI – 0,3 ng/ml, CA III (RANDOX) – 58 ng/ml.

STAT cTnI concentrations within 6 hours from the symptom onset were elevated over the 99th percentile for reference population in 83.3% of the study participants (4/5 UA subjects; 2/3 NSTEMI individuals; 29/34 STEMI cases) but none reached the cut-off value for myocardial infarction.

Myoglobin concentrations on admission were found to be increased over the respective cut-offs in some of UA (median 70; interquartile range 32-77 ng/ml) and most of NSTEMI (median 77; interquartile range 24-104 ng/ml) and STEMI (median 102; interquartile range

54-171 ng/ml) patients. Median CA III values were nearly the same and fairly constant in all subgroups and both measuring points with the exception of NSTEMI subjects, in whom at second blood sampling a simultaneous increase in both myoglobin and CA III indicated cardiac muscle damage was observed. We noted elevated calculated myoglobin/CA III ratio in STEMI subjects on admission (median 3.68; interquartile range 2.72-6.78), while it remained unchanged in both UA (median 1.59; interquartile range 1.48-2.26) and NSTEMI patients (median 1.4; interquartile range 0.96-5.48).

H-FABP demonstrated a very good efficacy in early detection of ACS (90.5%). Cardiac Array revealed positive results for H-FABP on admission in one of UA patients (median 1.8; interquartile range 1.75-2.5 ng/ml) and in all NSTEMI (median 5.6; 3.1-6.1 ng/ml) and STEMI (median 19.6; interquartile range 4.2-31.7 ng/ml) cases.

STAT cTnI concentration at the second blood sampling was over the cut-off value for AMI in some UA subjects and in all NSTEMI and STEMI patients. CK-MB mass levels were observed to be increased over the respective cutpoint in all of NSTEMI (median 5.44; interquartile range 3.21-100 ng/ml) and STEMI (median 26; interquartile range 7.4-100 ng/ml) subjects. However, CK-MB mass concentration did not reach the cutpoint in all UA patients. Similarly, concentrations of cTnI increased over the respective cut-off in two of UA cases (median 0.18; interquartile range 0.18-0.6 ng/ml) and in all of NSTEMI (median 1.08; interquartile range 0.6-3.54 ng/ml) and STEMI (median 36.8; interquartile range 6.4-50 ng/ml) subjects.

Distribution of cardiac markers results positive for AMI is shown in **Table 1**.

Table 1. Positive for AMI test results of cardiac markers in patients diagnosed with unstable angina, NSTEMI and STEMI at admission and at ≤ 12 h.

Positive for AMI test results of cardiac markers at admission (≤ 6 h)						
	STAT cTnI	STAT cTnI	Myoglobin	H-FABP	CK-MB mass	cTnI ≥ 0.56 ng/ml
Patients	> 0.032 and below <0.3 ng/ml	Positive ≥ 0.3 ng/ml	Positive	Positive	Positive	Positive
UA (n=5)	4 / 5	0 / 5	2 / 5	1 / 5	0 / 5	0 / 5
NSTEMI (n=3)	2 / 3	0 / 3	2 / 3	3 / 3	1 / 3	0 / 3
STEMI (n=34)	29 / 34	0 / 34	31 / 34	34 / 34	18 / 34	8 / 34

Positive for AMI test results of cardiac markers at ≤ 12 h						
	<i>STAT</i> cTnI	<i>STAT</i> cTnI	Myoglobin	H-FABP	CK-MB mass	cTnI ≥ 0.56 ng/ml
Patients	0.032 and below <0.3 ng/ml	Positive ≥ 0.3 ng/ml	Positive	Positive	Positive	Positive
UA (n=5)	3 /5	2 /5	2 /5	1 /5	0 /5	2 /5
NSTEMI (n=3)	0 /3	3 /3	3 /3	3 /3	3 /3	3 /3
STEMI (n=34)	0 /34	34 /34	34 /34	34 /34	34 /34	34 /34

It must be stressed that in all NSTEMI and STEMI patients only the H-FABP values were positive on admission. At the second blood sampling, when *STAT* cTnI was over the cut-off for AMI, the positive results of H-FABP were confirmed. A positive H-FABP result was found only in one out of five cases with UA at both sampling points. However, we should keep in mind that positive *STAT* cTnI results were noted only in two UA subjects at the second blood sampling.

Similarly to cTnI and CK-MB mass, the percentage of positive myoglobin results at the second blood sampling increased to 100% in NSTEMI and STEMI patients. Distribution of positive results of CK-MB mass and cTnI in relation to the sampling time indicates that positive results of both biomarkers on admission were found only in some NSTEMI and STEMI patients and nobody with UA. At the second sampling point, when *STAT* cTnI was over the cut-off for AMI in all NSTEMI and STEMI subjects, positive results of both CK-MB mass and cTnI were observed. The exception were UA cases in whom positive results of both troponins were observed merely in two out of five individuals.

To assess the performance of the Biochip Cardiac Array we calculated the sensitivity of cardiac markers assay and *STAT* cTnI at two different sampling points (**Table 2**).

Table 2. Sensitivity of cardiac panel markers (myoglobin, H-FABP, CK-MB mass, cTnI) in relation to *STAT* cTnI at admission and at ≤ 12 h in subjects with ACS.

Sensitivity (%)

Patients with ACS	Myoglobin ≥66ng/ml (95 th %)	H-FABP ≥2.5 ng/ml (95 th %)	CK-MB mass ≥1.92 ng/ml (95 th %)	cTnI ≥0.56 ng/ml (99 th %)	STAT cTnI >0.032 and <0,3 ng/ml (99 th %)	STAT cTnI ≥0.3 ng/ml (cut-off for MI)
Time from onset ≤ 6 h						
All patients (n=42)	83.3	90.5	45,2	19.0	83.3	0
UA (n=5)	40.0	20,0	0	0	80.0	0
NSTEMI (n=3)	66.7	100.0	33,3	0	66.7	0
STEMI (n=34)	91.2	100.0	52,9	23.5	85.3	0
Time from onset ≤ 12 h						
All patients (n=42)	92.8	90.5	88.1	92.8	-	92.8
UA (n=5)	40.0	20.0	0	40.0	60.0	40.0
NSTEMI (n=3)	100.0	100.0	100.0	100.0	-	100.0
STEMI (n=34)	100.0	100.0	100.0	100.0	-	100.0

The sensitivity was also calculated separately for UA, NSTEMI and STEMI subjects. The highest sensitivity on admission in all ACS patients was shown for H-FABP (90.5%), whereas determination of both myoglobin and CK-MB mass were characterized by lower sensitivity. In patients with unstable angina both early biomarkers (H-FABP and myoglobin) possess low sensitivity (20 and 40%, respectively) when evaluated on admission, whereas in STEMI subjects their sensitivity was very high (100 and 91.2%, respectively). At the second blood sampling (≤12 hours from the chest pain onset) the sensitivity for all myoglobin, H-FABP, CK-MB mass and c TnI was excellent in NSTEMI and STEMI patients, whereas in UA cases it was still unsatisfactory.

Discussion

Biomarkers play an increasingly important role in the evaluation and management of patients with suspected ACS. To date, cardiac troponins due to their high sensitivity and specificity for detecting myocardial necrosis remain the best established biomarkers in ACS for both diagnosis and risk assessment. Moreover, cardiac troponins sufficiently identify the patients who benefit most from particular treatment strategies, including glycoprotein IIb/IIIa inhibitors, low molecular-weight heparins and routine coronary angiography. However, troponin release is usually delayed for several hours after the onset of ischemic injury. It is also increasingly recognized that a substantial proportion of elevated troponin levels are

caused by other than ACS conditions involving myocardial damage. Having in mind a large number of ACS patients without typical symptomatology or electrocardiographic changes, novel biomarkers effectively facilitating the early diagnosis of ACS and enhancing the risk stratification process are eagerly awaited.

In our study we applied a new biochip array technology and evaluated a panel of both early and late cardiac biomarkers. In this preliminary evaluation of the innovative technology we accepted the cut-off values for all biomarkers provided by the manufacturer.

STAT cTnI concentrations within 6 hours from the symptom onset were elevated over the 99th percentile for reference population in 83.3% of subjects but none reached the cut-off value for myocardial infarction. On the contrary, positive cTnI results ($\geq 99^{\text{th}}$ percentile) with the biochip array technology were observed only in 19% of patients. A recent study, in which the same biochip assay was applied, has shown much higher sensitivity for cTnI (81.8%) but the cut-off value used for calculation was considerably lower than that utilized in our trial (0.32 ng/ml vs. 0.56 ng/ml) (4). The cut-off level used by Zaninotto et al is similar to the *STAT* cTnI cut-off for AMI used in our laboratory.

Instead, H-FABP demonstrated a very good efficacy in early detection of ACS (90.5%), better than myoglobin and CK-MB mass. Sensitivity of H-FABP calculated for NSTEMI and STEMI subjects reached 100%. It corroborates observations of other authors indicating H-FABP as an early and specific biomarker of cardiac damage (5-8). H-FABP is the only biomarker included in the cardiac panel that enhances the early diagnosis in patients suspected for ACS and may identify those subjects who are candidates for aggressive treatment strategies. The diagnostic efficacy of troponin, myoglobin and CK-MB mass assay markedly increased within 12 hours from the chest pain onset while that of H-FABP did not change.

Earlier studies with the use of the biochip cardiac array technology demonstrated similar sensitivity for H-FABP (98.7%) and myoglobin (81.8%) but higher for CK-MB mass (95.5%) within 6 hours from the chest pain onset (4,8). However, as previously discussed the cutpoints for AMI applied to calculate the sensitivity were much higher than that accepted in our study. Average sensitivity of H-FABP for the early diagnosis of AMI (≤ 6 h from the chest pain onset) in the available literature reaches 90% independently of the assay, with AMI cutpoint of 5-6 ng/ml (5-7,9).

UA patients were the only group in our material in which the cardiac panel failed to improve the early risk stratification. This observation may be affected by a small sample size that along with a lack of clinical follow-up remain the major limitations of our study. It also seems that cut-off value for cTnI should be lower than that suggested by the biochip cardiac

array technology manufacturer (4). Considering underlying pathophysiology of UA, we hypothesize that a combined analysis of plaque instability markers (myeloperoxidase, matrix metalloproteinases) and necrosis indicators by highly sensitive assays may add both diagnostic value and prognostic information in this scenario.

In conclusion, a multi-marker strategy with H-FABP and highly sensitive troponin included enhances the early diagnosis and decision making process in patients with ACS. A new biochip cardiac array technology may serve as a powerful tool for ACS detection in the clinical practice.

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