Evaluation of efficacy of heart-type fatty acid binding protein (H-FABP) for diagnosis of acute coronary syndromes

Diagnosis of acute coronary syndromes (ACS) is frequently a difficult task. The early risk stratification remains crucial for the prompt implementation of appropriate therapy in this setting. The delayed appearance of CK-MB mass and troponins in the circulation makes it difficult to diagnose the origin of recent chest pain, thus a combination of early and later biomarkers might further improve the differential diagnosis. Aim: The study was designed to evaluate the efficacy of H-FABP in comparison with other cardiac markers determined using biochip cardiac array technology in identifying ACS shortly after the onset of chest pain. Material and methods: Study group consisted of 42 subjects suspected for ACS with routinely measured serum sensitive cardiac troponin I level on admission below the cut-off value for acute myocardial infarction (AMI) of 0.30 ng/ml. Patients were diagnosed as presenting with unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI) or ST-elevation myocardial infarction (STEMI). A panel of 6 cardiac markers (H-FABP, myoglobin, glycogen phosphorylase BB, cTn I, CK-MB mass and carbonic anhydrase III) was assayed using biochip array technology. The biomarkers in the serum were determined twice: on admission (≤6 hrs from the chest pain onset) and after next 6 hours. Results: Cardiac troponin I concentrations over the 99th percentile for reference population, within 6 hours after the onset of symptoms, were elevated in 23,5% of subjects with STEMI but in none of UA or NSTEMI cases. Instead, H-FABP demonstrated a very good efficacy (90,5%) in early detection of ACS, better than myoglobin (83,3%) and CK-MB mass (45,2%). 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. Neither H-FABP nor any other biomarker of the cardiac panel was enough efficient in the early stratification of risk for the patients with UA. Conclusion: H-FABP seems to be an excellent cardiac biomarker that enhances the early diagnosis and decision making process in patients with ACS.

**Key words:** acute coronary syndromes, cardiac biomarkers, troponins

ANALIZA PRZYGODNOŚCI SERCOWEGO BIAŁKA WIĄZĄCEGO KWASY TLUSZCZOWE W DIAGNOSTYCE OSTREGO ZESPOŁU WIEŃCOWEGO

Właściwa diagnoza w ostrych zespołach wieńcowych (ozw.) nierzadko stanowi trudne wyzwanie, podczas gdy wczesna stratyfikacja ryzyka jest bardzo istotna dla wyboru odpowiedniej metody leczenia. Późne uwalnianie do krażenia CK-MB i troponin z uszkodzonych komórek mięśnia serca utrudnia właściwe zdignowanie przyczyny bólu w klatce piersiowej, zaś tem jednocześnie oznaczaniu wczesnych i późnych biomarkerów sercowych może znacznie ułatwić diagnostykę różnicową. Cel: Celem tej pracy było porównanie przydatności sercowego białka wiązającego kwasy tłuszczowe (H-FABP) w diagnostyce ozw, we wczesnym okresie po wystąpieniu bólu w klatce piersiowej, w odniesieniu do innych biomarkerów „panelu sercowego” oznaczanych z zastosowaniem technologii biochipów. Materiał i metody: Do badania włączono 42 pacjentów z podejrzeniem ozw, u których stwierdzono: niestabilną dławicę piersiową (UA), zawal bez uniesienia odcinka ST (NSTEMI) lub zawal z uniesieniem odcinka ST (STEMI), ze stężeniem troponiny I, oznaczonym rutynowo przy przyjęciu metodą o wysokiej czułości, wynoszącym poniżej wartości odciecia dla świętego zawalu mięśnia serca 0,30 ng/ml. Panel 6 biomarkerów sercowych oznaczanych z zastosowaniem technologii biochipów obejmował: H-FABP, mioglobwinę, fosforylazę glikogenu BB, sercową troponinę I (cTn I), CK-MB (masz) i anhydrzę węglanową III (CA III). Biomarkery w surowicy oznaczano dwukrotnie: przy przyjęciu pacjenta (<6 godz. od początku bólu) i po następnym 6 godzinach. Wyniki: Stężenie troponiny I powyżej 99 percentyla dla populacji osób zdrowych, w okresie pierwszych 6 godz. od początku bólu wieńcowego, było podwyższone u 23,5% pacjentów z STEMI, natomiast pozostawało poniżej u wszystkich pacjentów z UA i NSTEMI. Przeciwnie, stężenie H-FABP powyżej wartości odciecia stwierdzono u 90,5% pacjentów z ozw, co wskazuje na jego bardzo wysoką przydatność we wczesnej diagnostyce ozw, w porównaniu do mioglobyni i CK-MB. Czułość H-FABP obliczona dla pacjentów NSTEMI i STEMI wynosiła 100%. Przydatność diagnostyczna troponiny, mioglobiny i CK-MB wzrastała znacznie dopiero po 12 godzinach. Zarówno H-FABP, jak i żaden z pozostałych biomarkerów panelu sercowego nie cechował się wystarczającą przydatnością diagnostyczną we wczesnej stratyfikacji ryzyka u pacjentów z UA. Wnioski: H-FABP jako biomarker może być z powodzeniem stosowany dla celów wczesnej diagnostyki i wyboru właściwej metody leczenia u pacjentów z ostrymi zespołami wieńcowymi.
Diagnosis of acute coronary syndrome (ACS) is a difficult task. The early risk stratification is crucial for the prompt implementation of appropriate treatment. Cardiac troponins are currently used as the markers of choice in making the critical identification of ACS. However, in subjects with chest pain lasting less than 6 hours, negative troponin or CK-MB mass do not often allow to rule out ACS. The delayed appearance of CK-MB mass or troponins in the blood makes difficult the early identification of the origin of recent chest pain, thus a multi-marker approach might improve differential diagnosis and risk assessment. Among the early markers of ACS those of inflammation and plaque instability and focal myocardial necrosis (H-FABP – heart fatty acid binding protein) have been recently evaluated. Multi-marker strategy seems to be more efficient than cardiac troponins alone for ACS detection.

Myoglobin and H-FABP are released rapidly from the cardiomyocytes into circulation after myocardial injury. However, clinical value of myoglobin is limited by its low specificity for cardiac muscle [6, 7]. Earlier 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 [6, 7]. H-FABP a cytoplasmic protein of low-molecular mass offers several advantages over troponin. It is quite specific for cardiomyocytes, present abundantly in the cardiac muscle and is released quickly into blood stream in response to myocardial injury. H-FABP is a transport protein for the fatty acids and plays a role in their oxidation [1].

The present study was designed to evaluate the efficacy of H-FABP in comparison with other cardiac markers determined using biochip cardiac array technology in identifying ACS shortly after the onset of chest pain.

Study group consisted of 42 patients (10 women, 32 men, aged 44-83 yrs) admitted to the Department of Cardiology and Internal Medicine of the Collegium Medicum in Bydgoszcz with an initial diagnosis of ACS. The enrollment 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.
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(UA), non-ST-elevation myocardial infarction (NSTEMI) or ST-elevation myocardial infarction (STEMI).

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

Venous blood samples were collected twice: on admission (≤6 hours from the chest pain onset) and after next 6 hours. All measurement were performed in the serum. Cardiac troponin I was determined on the ARCHITECT ci8200 (Abbott Diagnostics) while other cardiac biomarkers (H-FABP, myoglobin, glycogen phosphorylase BB, cTnI, CK-MB mass and carbonic anhydrase III) were assayed simultaneously in a single serum sample of 60 μl using Biochip Array Technology on the evidence investigator (RANDOX).

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

Statistical methods

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

Calculated sensitivity and distribution of positive for AMI test results of cardiac markers at different sampling times is shown in Table I. In all patients with NSTEMI and STEMI only H-FABP values were positive at admission. At the second sampling when cTnI was over the cut-off for AMI the positive results of H-FABP were confirmed. In cases with UA, positive H-FABP result was found only in one out of five at both sampling times.

On the contrary, positive myoglobin results at admission were observed in two UA and most of NSTEMI and STEMI cases but increased to 100% only in NSTEMI and STEMI patients at the second sampling. Median CA III values were nearly the same and fairly constant in all subgroups at 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 (results not shown).
### Table 1.
Number of positive results and sensitivity (%) of cardiac panel markers (myoglobin, H-FABP, CK-MB mass, cTnl) at admission and at ≤12 h in subjects with ACS.

<table>
<thead>
<tr>
<th></th>
<th>Patients with ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myoglobin (95th %)</td>
</tr>
<tr>
<td><strong>Patients with ACS n=42</strong></td>
<td></td>
</tr>
<tr>
<td>Positive results</td>
<td>83,3 (35/42)</td>
</tr>
<tr>
<td><strong>At admission</strong></td>
<td></td>
</tr>
<tr>
<td>UA n=5</td>
<td>40,0 (2/5)</td>
</tr>
<tr>
<td>Positive results</td>
<td>66,7 (2/3)</td>
</tr>
<tr>
<td><strong>NSTEMI n=3</strong></td>
<td>91,2 (31/34)</td>
</tr>
<tr>
<td>Positive results</td>
<td>92,8 (39/42)</td>
</tr>
<tr>
<td><strong>At ≤12 h</strong></td>
<td></td>
</tr>
<tr>
<td>Positive results</td>
<td>40,0 (2/5)</td>
</tr>
<tr>
<td>UA n=5</td>
<td>100,0 (3/3)</td>
</tr>
<tr>
<td>Positive results</td>
<td>100,0 (34/34)</td>
</tr>
</tbody>
</table>

Distribution of positive results of CK-MB mass and cTnl in relation to the sampling time shows 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 all positive results of both CK-MB mass and cTnl were observed. The exception were cases with UA in which positive results were found only for troponin in two out of five individuals.

The sensitivity was calculated for all ACS patients and separately for UA, NSTEMI and subjects. The highest sensitivity on admission in all ACS patients was shown for H-FABP (90,5%) whereas this for other biomarkers was lower. In patients with unstable angina both early markers (H-FABP and myoglobin) possess low sensitivity (20 and 40%) whereas in STEMI subjects their sensitivity was very high (100 and 91,2%). At the second sampling (≤12 hrs from the chest pain onset) the sensitivity was excellent for all measured cardiac markers in NSTEMI and STEMI patients, whereas in UA was still unsatisfactory.

Cardiac troponins due to their superior sensitivity and specificity for detecting myocardial necrosis remain the best established biomarkers in ACS for diagnosis and risk assessment. However, troponin release is usually delayed for several hours after the onset of ischemic injury. Having in mind a large

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number of ACS patients without typical symptomatology or electrocardiographic changes, novel biomarkers improving the early diagnosis of ACS and enhancing the risk stratification process are eagerly awaited.

We have applied a new biochip array technology to evaluate the panel of early and delayed cardiac biomarkers. In this preliminary evaluation study we accepted the cutpoints for all biomarkers provided by the manufacturer.

At admission positive cTnl results (≥ 99th percentile) were found in 19% of subjects. Recent study, in which the same biochip assay was applied has shown much higher sensitivity for cTnl (81,8%) but the cutpoint used for calculation was much lower than that used in our study (0,32 ng/ml vs 0,56 ng/ml) [9].

H-FABP demonstrated a very good efficacy in early detection of ACS (90,5%) in comparison with myoglobin and CK-MB mass. Sensitivity of H-FABP calculated for NSTEMI and STEMI subjects reached 100%. It is in agreement with the findings of other authors indicating H-FABP as an early and specific biomarker of cardiac damage [2, 3, 4, 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 symptoms onset while that of H-FABP did not change.

Recently published data with the use of the same 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 h from the onset of chest pain [9, 8]. However, the cut-off values for AMI used to calculate the sensitivity were much higher than those accepted in this trial. Average sensitivity of H-FABP for the early AMI diagnosis (≤ 6h), reported by others, was shown to be over 90%, independently of the assay, with AMI cut-off between 5-6 ng/ml [2-5].

UA patients were the only group in our study in which the cardiac panel failed to facilitate the early stratification of risk. 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 cutpoint for cTnl should be lower than that suggested by the biochip cardiac assay manufacturer [9]. We may assume that in patients with UA a combined analysis of plaque instability markers and indicators of necrosis may add both diagnostic value and prognostic information.

Conclusion

In conclusion, a multi-marker strategy with H-FABP 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.

References


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