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ARTYKUŁ ORYGINALNY / *ORIGINAL ARTICLE*

Early kinetics of heart-type fatty acid binding protein in patients undergoing dipyridamole stress echocardiography and relationship with high-sensitivity troponin

Giuseppe Lippi[1], Mariella Dipalo[1], Rosalia Aloe[1], Fiorenza Robuschi[2], Francesco Scioscioli[2], Livia Ruffini[3], Gianfranco Cervellin[2]

[1]Laboratory of Clinical Chemistry and Haematology, Academic Hospital of Parma, Parma, Italy
[2]Emergency Department, Academic Hospital of Parma, Parma, Italy
[3]Department of Radiology, Academic Hospital of Parma, Parma, Italy

Abstract

Background: The assessment of cardiospecific troponins is the mainstay for diagnosing myocardial injury, although their diagnostic sensitivity remains suboptimal at patient admission to the emergency department (ED), thus paving the way for translational research to identify early and complementary biomarkers which may help improve the diagnostic sensitivity of high-sensitivity troponin immunoassays at patient presentation to the ED.

Aim: To investigate whether heart-type fatty acid binding protein (H-FABP) provides distinctive and/or adjunctive information over high-sensitivity troponin I (HS-TnI) in ED patients undergoing dipyridamole stress testing.

Methods: Thirty consecutive ED patients with chest pain but no myocardial ischaemia were challenged with dipyridamole--atropine and followed by echocardiography. Blood samples for assessing H-FABP and HS-TnI were collected before the dipyridamole challenge, immediately after, and 6 h afterwards.

Results: The concentration of HS-TnI in the whole cohort of patients did not vary significantly throughout the study period, whereas H-FABP significantly increased after the test (4.2 ng/mL, $p = 0.003$), but not 6 h afterwards (3.8 ng/mL, $p = 0.372$) compared to baseline (4.0 ng/mL). The kinetics was similar in patients with positive or negative results of stress testing. The frequency of biomarker increase after the test was greater for H-FABP than for HS-TnI immediately after the pharmacological challenge (77% vs. 53%), but was lower 6 h afterwards (30% vs. 63%). The number of patients with values exceeding the diagnostic threshold of both biomarkers remained constant throughout the study period.

Conclusions: These results suggest that dipyridamole stress echocardiography does not trigger substantial myocardial injury. We have also shown that release of H-FABP from stressed myocardium occurs without progression towards irreversible necrosis, and is more precious than that of TnI.

Key words: stress testing, provocative testing, dipyridamole, troponin, heart type fatty acid binding protein (H-FABP)

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INTRODUCTION

Cardiospecific troponins, either troponin I (TnI) or troponin T (TnT), are now considered the biochemical gold standards for diagnosing myocardial injury [1], and their measurement represents a cornerstone for the identification of patients with ST elevation myocardial infarction (MI) and — especially — of those with non persistent segment elevation MI [2, 3]. The recent introduction of novel and more analytically efficient

Address for correspondence:
Prof. Giuseppe Lippi, UO Diagnostica Ematochimica, Azienda-Ospedaliero Universitaria di Parma, Via Gramsci 14, 43126 – Parma, Italy, e-mail: glippi@ao.pr.it
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methods, conventionally defined as high-sensitivity (HS), has magnified the diagnostic potential of these biomarkers, wherein myocardial ischaemia can now be detected earlier and more efficiently than using the former conventional (i.e. contemporary-sensitive) methods [2, 3]. Recent evidence has also been provided, however, that the diagnostic efficiency of HS troponin immunoassays is still suboptimal at emergency department (ED) admission, in that a remarkable number of patients with MI, up to one quarter, present with non diagnostic values [4]. Noticeably, this aspect cannot be considered a specific drawback of HS immunoassays, but is instead attributable to the biology and peculiar kinetics of troponin release after acute myocardial stress and/or injury. The molecular weights of TnI and TnT are respectively 22.5 kDa and 37 kDa, and the free cytosolic pool of both proteins is very modest, typically comprised between 3% and 8%, since the largest amount of these proteins in myocardiocytes is structurally bound to myofilaments [5]. These two aspects justify the slow and delayed extracellular release and their typical kinetics in blood, with a significant increase of concentration that only occurs after 60 to 90 min after the onset of myocardial ischaemia [5]. It is hence predictable that further refinements of analytical techniques will not be thoughtfully efficient to overcome this inherent biological limit, thus paving the way for translational research to identify early and complementary biomarkers which may help improve the diagnostic sensitivity of HS troponin immunoassays at patient presentation to the ED.

The heart type fatty acid binding protein (H-FABP) is a low molecular weight protein (i.e. 15 kDa) that is almost exclusively localised in the cytoplasm of cardiomyocytes [6]. A recent meta-analysis of the literature, including eight studies and 2,735 patients, reported that a combination of H-FABP with a conventional troponin immunoassay seems indeed favourable for increasing the sensitivity of the former biomarker, but also concluded that additional studies should be planned to define the diagnostic effectiveness of combining H-FABP with novel HS troponin immunoassays [7].

Pharmacologic stress testing is a common diagnostic procedure for establishing the burden of coronary artery disease. Basically, cardiovascular stress is induced by pharmacologic agents and imaging modalities are then used for assessing the extent and adequacy of coronary circulation to increase flow (i.e. coronary flow reserve) [8]. It is hence a reliable model for establishing the early kinetics of cardiac biomarkers after cardiac stress, identifying potential relationships among them, and translating these basic findings into clinical practice [9]. Since there are no previous studies that have assessed the kinetics of H-FABP after pharmacologic stress echocardiography, we planned a specific investigation to verify whether H-FABP may provide distinctive and/or adjunctive information over HS-TnI in a series of ED patients undergoing dipyridamole stress testing.

METHODS

Experimental design

The study population consisted of 30 patients (mean age 66, range: 43–84 years; 20 males and 10 females) referred to the observational unit of the Academic Hospital of Parma (Italy) over two consecutive months for assessment of chest pain at moderate-to-high probability (defined according to family history, previous myocardial ischaemia, smoking and presence of hypercholesterolaemia, renal failure, hypertension and diabetes). All patients were challenged with dipyridamole–atropine. Double echocardiographic assessment was also performed both at baseline and at the end of the challenge. In all patients, the presence of an acute coronary syndrome was excluded in the ED according to the criteria of the third universal definition of MI [10].

The study protocol consisted in a dipyridamole–atropine echocardiography with myocardial perfusion assessment between the end of dipyridamole infusion (0.84 mg/kg/10 min) and the beginning of atropine administration. Exclusion criteria were based on a hospital protocol, including atrial fibrillation, glomerular filtration rate lower than < 30 mL/min and severe valvular disease. All patients were also cleared from pharmacologic treatments with beta-blockers and teophylline. Wall motion was investigated with an iE33 echocardiograph (Philips Ultrasound, Bothell, WA, USA), whereas myocardial perfusion was assessed with SonoVue (Bracco Imaging Italia, s.r.l., Milan, Italy). Reversible wall motion abnormality was defined as the onset of a new dyssynergy in segments with normal function at baseline or deterioration of hypokinesia at baseline in one or more segments. Normal perfusion following dipyridamole administration was defined as complete myocardium replenishment 1.5 to 2.0 s after the end of flash impulse. Abnormal perfusion was defined as lack of myocardium replenishment after the same period. A 4 s threshold was chosen for defining normal replenishment following aminophylline administration. Perfusion defects were classified as either stable or reversible according to their persistence throughout the recovery period. The result of contrast-enhanced stress echocardiography was defined as abnormal in the presence of one or more of the following criteria, in one or more myocardial segments: (i) reversible wall motion abnormality, (ii) reversible myocardial perfusion defect, and (iii) stable impairment of myocardial perfusion in a patient with no previous history of acute coronary syndrome. The results of the test were assessed off-line by two expert echocardiographers. The study was performed in accordance with the Declaration of Helsinki, under the terms of relevant local legislation and informed consent was obtained from all patients.

Laboratory procedures

Blood was drawn into evacuated blood tubes containing no additives (Becton-Dickinson, Oxford, UK) by direct veni-

puncture, immediately before stress echocardiography (i.e. 'pre'), within 1 h after conclusion of the test (i.e. 'post'), and 6 h after the end of the test (i.e. '6 h'). Blood samples were transported to the core laboratory within 30 min from collection, separated by centrifugation at 1.500 × g for 10 min at room temperature, divided in aliquots and stored at –70°C until measurement.

The concentration of HS-TnI was measured with the prototype Beckman Coulter HS-AccuTnI on Access 2 (Beckman Coulter Inc, Chaska, MN, USA). The assay is characterised by a limit of detection of 2.5 ng/L and a 99th percentile of the upper reference limit (URL) of 32 ng/L [11]. The concentration of H-FABP was assayed with a novel immunoturbidimetric assay (Randox Laboratories Ltd., Crumlin, UK). In a recent investigation, the 99th percentile URL for this assay was 9.1 ng/mL, with no significant differences between genders, and the imprecision corresponding to a 10% coefficient of variation was found at a concentration of 2.4 ng/mL [12].

Table 1. Clinical and demographical data of the study population

	Dipyridamole stress echocardiography		P
	Negative	Positive	
N	20	10	
Age [years]	66 (61–73)	62 (57–73)	0.42
Gender (male/female)	14/6	6/4	0.58
Hypertension	5 (25%)	4 (40%)	0.40
Diabetes	7 (35%)	5 (50%)	0.43
GFR [mL/min/1.73 m^2]	83 (70–95)	85 (72–105)	0.14

Numerical variables are described as median and interquartile range; GFR — glomerular filtration rate (calculated with the Modification of Diet in Renal Disease equation)

Statistical analysis

The results of testing were finally presented as median and interquartile range (IQR). The significance of differences was assessed by Wilcoxon-Mann-Whitney test (for continuous variables) and Pearson's χ^2 test (for categorical variables). The relative increase of HS-TnI and H-FABP over baseline was calculated as the ratio between the concentration of each biomarker obtained within 1 h (i.e. post/pre) or at 6 h (i.e. 6 h/pre) and the relative values before dipyridamole stress testing. The statistics was performed using Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK).

RESULTS

The main characteristics of the study population are shown in Table 1. The dipyridamole–atropine echocardiography was positive in 10/30 patients (33%). Compared to baseline values (3.6 ng/L, IQR 2.9–9.0 ng/L), the concentration of HS-TnI in the whole cohort of patients did not differ significantly after the end of the test (4.6 ng/L, IQR 2.5–9.4 ng/L, p = 0.167) and 6 h afterwards (4.7 ng/L, IQR 3.0–8.9 ng/L, p = 0.170) (Fig. 1). At variance with these findings, the concentration of H-FABP was found to be significantly increased immediately after the test (4.2 ng/mL, IQR 3.5–5.5 ng/mL, p = 0.003), but not 6 h afterwards (3.8 ng/mL, IQR 3.1–4.9 ng/mL, p = 0.372) compared to the baseline (4.0 ng/mL, IQR 3.4–5.0 ng/mL) in the whole cohort of patients (Fig. 1). When patients were stratified according to the outcome of dipyridamole–atropine echocardiography, the concentration of HS-TnI did not dif-

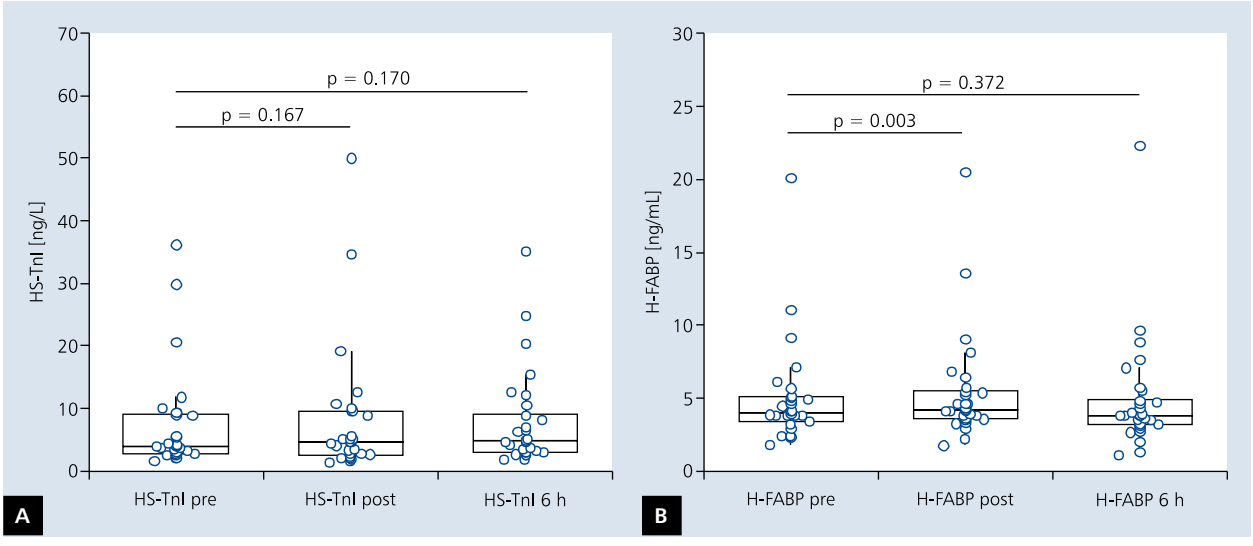

Figure 1. Early kinetics of high-sensitivity troponin I (HS-TnI) (**A**) and heart-type fatty acid binding protein (H-FABP) (**B**) in 30 patients undergoing dipyridamole stress echocardiography

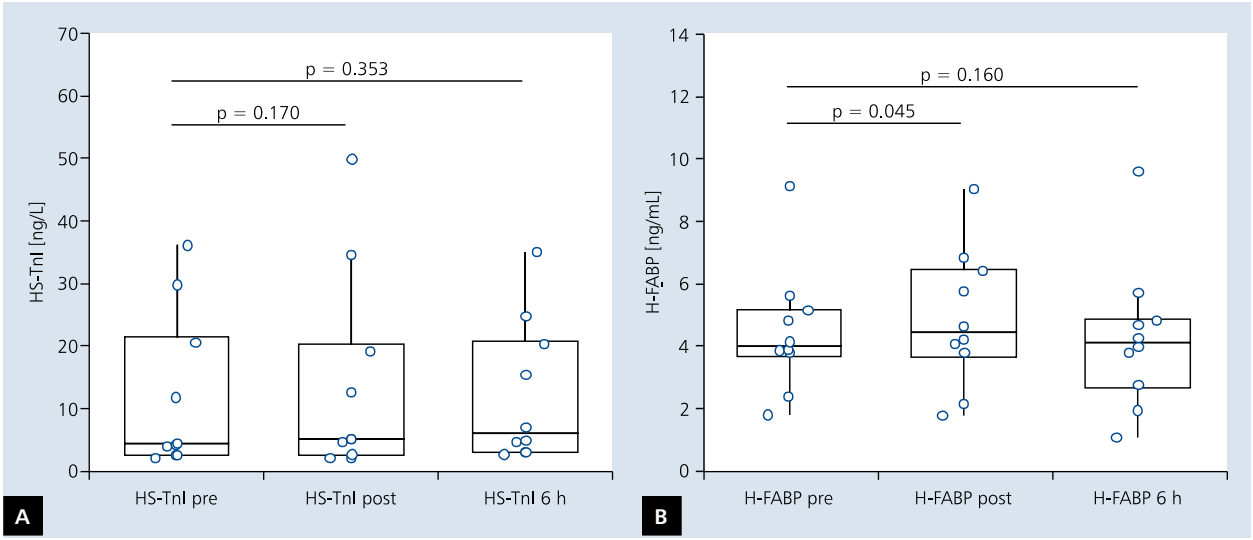

Figure 2. Early kinetics of high-sensitivity troponin I (HS-TnI) (**A**) and heart-type fatty acid binding protein (H-FABP) (**B**) in 10 patients with positive dipyridamole stress echocardiography

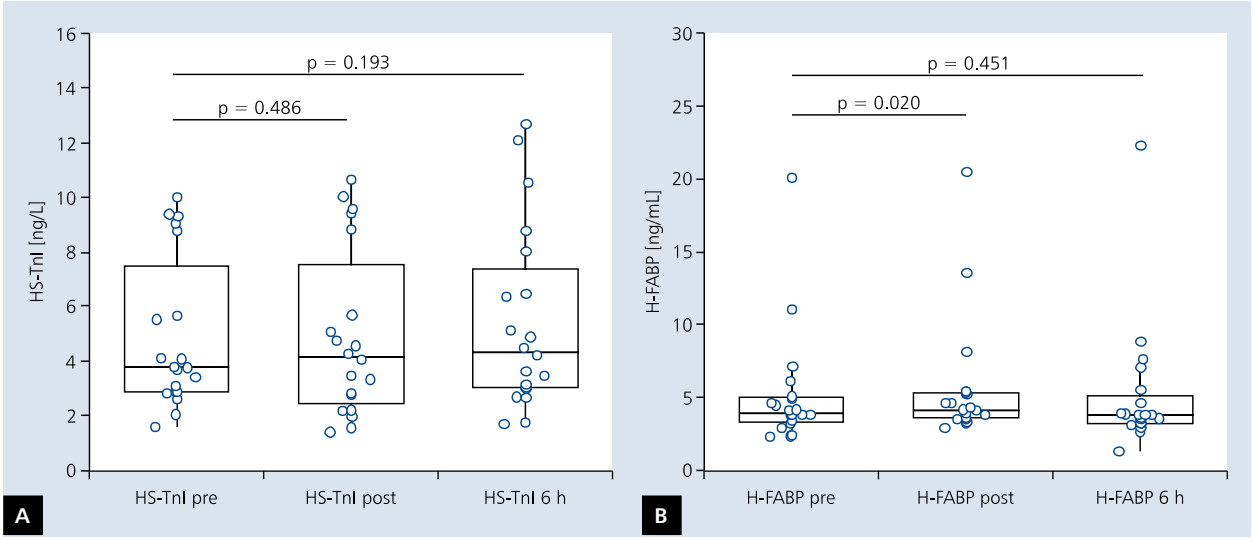

Figure 3. Early kinetics of high-sensitivity troponin I (HS-TnI) (**A**) and heart-type fatty acid binding protein (H-FABP) (**B**) in 20 patients with negative dipyridamole stress echocardiography

fer from baseline at both time points in either patients with positive or negative echocardiographic findings (Figs. 2, 3), whereas that of H-FABP was found to be significantly increased after the test, but returned to values non significantly different from baseline 6 h afterwards, irrespective of positive or negative echocardiographic findings (Figs. 2, 3). The frequency of patients with biomarker increase (i.e. ratio > 1) did not differ between H-FABP and HS-TnI immediately after the test (i.e. 77% vs. 53%, Pearson's χ^2 statistic 3.59, p = 0.058), but was instead significantly lower for H-FABP than HS-TnI 6 h afterwards (i.e. 30% vs. 63%, Pearson's χ^2 statistic 6.70, p = 0.001). Nevertheless, the number of patients with values exceeding the 99th percentile URL did not differ throughout the study period for either HS-TnI (pre 3%, post 7%, 6 h 3%, Pearson's χ^2 statistic 0.52, p = 0.770) or H-FABP (2% at all time points). Interestingly, the ratios of HS-TnI and H-FABP were not significantly correlated immediately after stress testing (r = –0.24, p = 0.211), but the correlation become significant after 6 h (r = 0.46, p = 0.011) (Fig. 4).

DISCUSSION

The first important conclusion that can be drawn from results of this study is that dipyridamole stress echocardiography does not appear to elicit a significant and irreversible myocardial

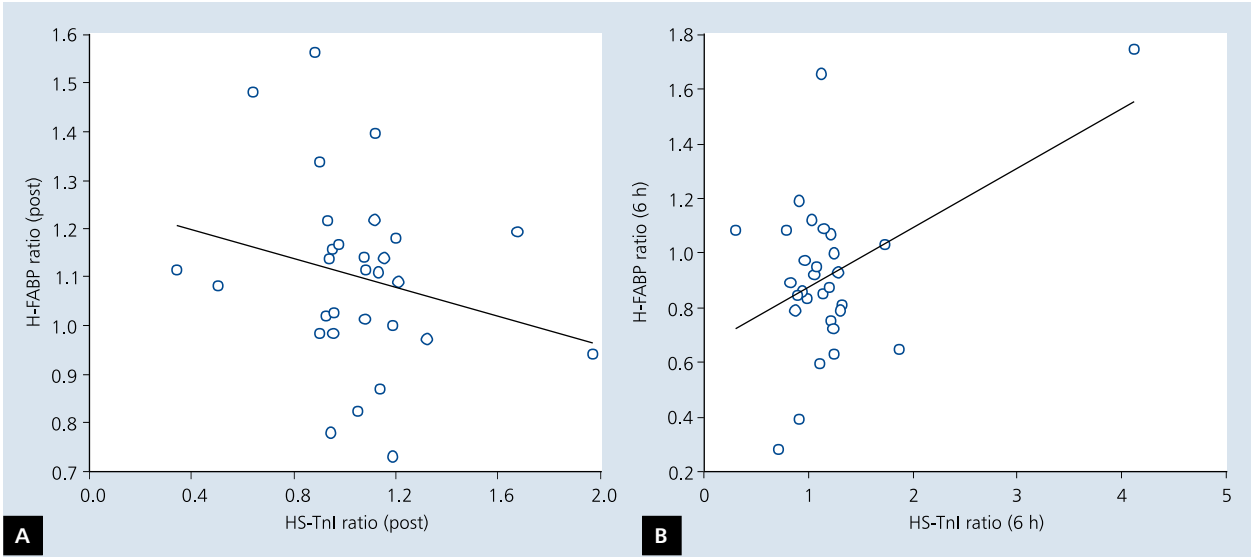

Figure 4. Correlation of ratio of increase of high-sensitivity troponin I (HS-TnI) (**A**) and heart-type fatty acid binding protein (H-FABP) (**B**) in 30 patients undergoing dipyridamole stress echocardiography immediately after stress testing and 6 h afterwards

injury, as attested by the lack of significant variation of HS-TnI concentration (Fig. 1), as well as by the constancy of subjects with values exceeding the 99th URL throughout the study period. The concentration of H-FABP, which appeared modestly increased at the end of the test as a result of the typical biochemistry and biology of this biomarker (small molecule with prevalent cytoplasmic localisation and rapid clearance from circulation) [13], returned to values not significantly different from baseline 6 h afterwards (Fig. 1), and the frequency of patients with concentration above the 99th URL did not differ throughout the study period. This is important evidence, since it has been previously shown that other types of stress testing, such as the dobutamine challenge, may trigger minor myocardial injury as reflected by a significant increase of HS-TnT in patients with coronary artery disease and healthy volunteers [14].

The second innovative aspect that we have addressed in this study is the direct comparison of early kinetics of HS-TnI and H-FABP in subjects undergoing pharmacological myocardial stress. This information does not replicate previous data gathered on patients with MI, since we could exactly establish the onset of myocardial stress (i.e. the beginning of the provocative test) and then reliably monitor cardiac release of both HS-TnI and H-FABP afterwards, up to 6 h. This is noteworthy since the drawback of most previous clinical studies lies in the virtual impossibility of identifying the exact time elapsed between onset of symptoms (or ED admission) and occurrence of myocardial stress and/or ischaemia (this typically varies between a few minutes to up to 10–12 h) [15]. Interestingly, the lack of correlation between the ratios of HS-TnI and H-FABP within 1 h from the end of the pharmacological challenge provides a firm biological support to previous clinical evidence that these biomarkers exhibit a different kinetics of immediate release after myocardial stress (Fig. 4), as also attested by the fact that values of H-FABP, but not those of HS-TnI, were significantly increased over baseline immediately after the test (Fig. 1). On the other hand, however, the significant correlation observed 6 h after the pharmacological challenge (Fig. 4) underlines that the kinetics of H-FABP and HS-TnI become virtually similar in the following period.

This evidence would hence suggest that a release of H-FABP from stressed myocardium occurs even without progression towards irreversible necrosis, and is also more precious than that of TnI, despite cut-off points for this marker have not been established to separate ischaemia from necrosis. This is not surprising if one considers the biochemistry and biology of H-FABP, wherein this protein is smaller than both TnI and TnT, and is prevalently localised in the cytoplasm of myocardiocytes rather than structurally bound to myofilaments [13].

Limitations of the study

The major limitation of this study is represented by the limited number of patients included (30 overall, 20 with positive and 10 with negative dipyridamole stress echocardiography). As such, larger studies are indeed needed to confirm our preliminary findings about the early kinetics of H-FABP and HS troponin(s) after dipyridamole stress echocardiography.

CONCLUSIONS

From a diagnostic perspective, the different early kinetics of H-FABP and TnI may have important implications for precocious diagnosis of acute coronary syndrome. It is in fact plausible that the combination of H-FABP with a HS troponin

immunoassay may be effective to fill the gap of suboptimal sensitivity of the latter test at early patient presentation to the ED (i.e. within 2 to 3 h after onset of myocardial ischaemia). However, the kinetics of H-FABP and its correlation with the release of cardiospecific troponin, coupled with the established evidence that the sensitivity of HS troponin immunoassays approximates to, or even reaches, 100% at 3 to 6 h [2, 3], would make the assessment of H-FABP virtually meaningless at later time points during sequential testing, since the diagnostic sensitivity of H-FABP may virtually replicate that of TnI, at the expense of a much lower specificity [7]. Indeed, this hypothesis represents a valid premise for planning further studies specifically focused on assessing the diagnostic performance of the combination of H-FABP with HS troponin immunoassays in patients with suspected myocardial ischaemia.

Conflict of interest: *none declared*

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Wczesna kinetyka stężenia sercowego białka wiążącego kwasy tłuszczowe u chorych poddanych echokardiografii obciążeniowej z dipirydamolem oraz zależności ze stężeniem troponin oznaczonych metodą wysokoczułą

Giuseppe Lippi[1], Mariella Dipalo[1], Rosalia Aloe[1], Fiorenza Robuschi[2], Francesco Scioscioli[2], Livia Ruffini[3], Gianfranco Cervellin[2]

[1]Laboratory of Clinical Chemistry and Haematology, Academic Hospital of Parma, Parma, Włochy
[2]Emergency Department, Academic Hospital of Parma, Parma, Włochy
[3]Department of Radiology, Academic Hospital of Parma, Parma, Włochy

Streszczenie

Wstęp: Podstawową metodą w diagnostyce uszkodzenia mięśnia sercowego jest oznaczenie swoistych troponin sercowych, jednak czułość tego badania nie jest wystarczająca w przypadku pacjentów zgłaszających się na oddział ratunkowy. Dlatego też poszukuje się wczesnych biomarkerów zapewniających dodatkowe informacje, które umożliwiłyby zwiększenie czułości diagnostycznej w stosunku do oznaczenia troponin metodą wysokoczułą u pacjentów zgłaszających się na oddział ratunkowy.

Cel: Celem pracy było zbadanie, czy sercowe białko wiążące kwasy tłuszczowe (H-FABP) dostarcza odmiennych i/lub dodatkowych informacji w stosunku do oznaczenia stężenia troponiny I metodą wysokoczułą (HS-TnI) u chorych, u których przeprowadzono próbę dipirydamolową na oddziale ratunkowym.

Metody: U 30 kolejnych pacjentów, którzy zgłosili się na oddział ratunkowy z bólem w klatce piersiowej, jednak bez cech niedokrwienia mięśnia sercowego, przeprowadzono echokardiograficzną próbę obciążenia dipirydamolem–atropiną. Próbki krwi w celu oznaczenia H-FABP i HS-TnI pobrano przed przeprowadzeniem próby dipirydamolowej, bezpośrednio po niej oraz po 6 godzinach.

Wyniki: Stężenia HS-TnI w całej grupie chorych nie różniły się znamiennie w okresie badania, natomiast H-FABP zwiększyło się istotnie w stosunku do wartości wyjściowych (4,0 ng/ml) bezpośrednio po próbie dipirydamolowej (4,2 ng/ml; p = 0,003), ale nie 6 godzin później (3,8 ng/ml; p = 0,372). Parametry kinetyczne były podobne u pacjentów z dodatnim i ujemnym wynikiem próby wysiłkowej. Bezpośrednio po próbie obciążeniowej częściej obserwowano zwiększenie stężenia H-FABP niż HS-Tn-1 (77% *vs.* 53%), natomiast po 6 godzinach te proporcje się odwróciły (30% *vs.* 63%). Liczba chorych, u których wartości obu markerów przekraczały próg diagnostyczny, pozostawała niezmienna przez cały okres badania.

Wnioski: Wyniki sugerują, że echokardiograficzna próba obciążeniowa z dipirydamolem nie powoduje istotnego uszkodzenia mięśnia sercowego. Ponadto wykazano, że uwalnianie H-FABP z mięśnia sercowego w warunkach obciążenia nie wiąże się z progresją do nieodwracalnej martwicy i że ma większą wartość diagnostyczną niż oznaczenie TnI.

Słowa kluczowe: próba obciążeniowa, próba prowokacyjna, dipirydamol, troponina, sercowe białko wiążące kwasy tłuszczowe (H-FABP)

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Adres do korespondencji:
Prof. Giuseppe Lippi, UO Diagnostica Ematochimica, Azienda-Ospedaliero Universitaria di Parma, Via Gramsci 14, 43126 – Parma, Italy, e-mail: glippi@ao.pr.it
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