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Short Communication

Analytical and clinical evaluation of a new heart-type fatty acid-binding protein automated assay

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Abstract

Background: The accurate and rapid recognition of myocardial injury in patients presenting in the emergency department (ED) with chest pain continues to be a clinical challenge. Heart-type fatty acid-binding protein (H-FABP) appears to be one of the best candidates among the new early cardiac markers studied.
Methods: We evaluated the analytical characteristics of a new quantitative and fully automated H-FABP assay (Randox Laboratories Ltd., Crumlin, UK) and compared its clinical performance with respect to the myoglobin (Myo) assay (Dade Behring, Milan, Italy). A precision study was carried out by testing three levels of quality control (QC) material and two in-house pool (P) samples. To test the accuracy of H-FABP determinations in plasma (lithium-heparin) samples, H-FABP concentrations measured in a set of matched sera and plasma samples were compared. A total of 77 non-consecutive patients (51 males and 26 females; 62±16 years) who presented to the ED with chest pain suggesting myocardial ischemia were enrolled. The patients were classified into two groups (acute myocardial infarction, n=22; non-acute myocardial infarction, n=55) on the basis of the discharge diagnosis.
Results: The between-day imprecision for three levels of control material and serum pool samples was 6.26%–8.04% (range 2.32–44.03 μg/L) and 9.03%–12.63% (range 11.85–65.13 μg/L), respectively. In the serum vs. plasma study, bias was +0.178 (95% CI −0.033 to +0.389). The best cut-off and the associated diagnostic efficacy were 95 μg/L and 89.47% for Myo and 5.09 μg/L and 98.70% for H-FABP, respectively.
Conclusions: H-FABP determination in patients with ischemic symptoms may be a more reliable early indication of cardiac damage than myoglobin.
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Since the recent redefinition of acute myocardial infarction (AMI) (1), biochemical markers now play a more important role in the assessment of acute coronary syndrome, particularly in emergency clinical settings. However, it is sometimes difficult to diagnose early-stage AMI because of the delayed appearance in blood of serum cardiac markers such as creatine kinase MB and cardiac troponins I or T; moreover, early electrocardiogram changes are not always accurately interpreted (2). In fact, myoglobin (Myo), the earliest biochemical marker used in this setting, is significantly elevated in AMI patients as early as 1–3 h after the onset of symptoms, peaking within 3–15 h. However, Myo is not cardiac-specific and elevations in its serum concentrations have been documented in several clinical conditions (3). Therefore, the accurate and rapid recognition of myocardial injury in patients presenting in the emergency department (ED) with chest pain continues to be a clinical challenge that strongly affects patient outcome. Many attempts have been made in this particular field of clinical research to discover the ideal biochemical marker with the best clinical sensitivity and specificity. Heart-type fatty acid-binding protein (H-FABP) has been suggested as an early marker of cardiac injury (4, 5). Currently, nine distinct types of FABP have been identified, each showing a characteristic pattern of tissue distribution (6). To the best of our knowledge, however, a fully automated and quantitative H-FABP assay has not yet been made commercially available. Contradictory findings regarding rapid qualitative assays have been reported, whereas quantitative methods require a long assay time and are therefore unsuitable for clinical application, especially in the ED (7).

The aim of the present study was therefore to ascertain the analytical and clinical performance of a new quantitative and fully automated H-FABP assay. In particular, we evaluated and compared its usefulness in diagnosing patients presenting to the ED with chest pain compared to the Myo assay, which is routinely used in our laboratory (Dade-Behring, Milan, Italy). The H-FABP assay evaluated is part of a biochemical panel, the Evidence® Cardiac Panel (Randox Laboratories Ltd., Crumlin, UK), which allows quantitative and fully automated simultaneous determination of six markers: creatine kinase MB, Myo, glycogen phosphorylase BB, H-FABP, carbonic anhydrase III and car-

diac troponin I. The Evidence® assay combines protein biochip array technology with the sandwich principle. All the cardiac panel immunoassays are performed simultaneously on the surface of a biochip (a 9-mm^2 solid substrate) presenting an array of discrete test regions (deposited in exact pre-defined coordinates by nanodispensing technology) consisting of different (capture) antibodies specific for each analyte. Each biochip accommodates a single patient sample and is located in a multi-chip carrier (nine biochips per carrier) transported within the analyzer to the different operation stations required for sample processing. Horseradish peroxidase, used as a label for the detection antibodies, in the presence of luminol and peroxide, used as substrates, starts the reaction cascade, which generates light (enhanced chemiluminescence) measured using a charge coupled device (CCD) camera. Imaging technology is used to quantify light output from the reaction sites on the biochip surface, and the software system mathematically computes the corresponding concentration result. Sample pre-treatment is not necessary, and the time required to process sample and obtain all cardiac panel results, as well as the single biochemical marker value, is approximately 80 min. Of the new biomarkers measured by the Evidence® Cardiac Panel, we focused our study on the H-FABP assay owing to the interesting but contradictory results reported so far in the literature regarding its clinical usefulness as an early marker (7). Serum, representing the specimen of choice recommended by the manufacturer, was used. The measurement range was 0.3–79 μg/L. Values higher than the upper limit of this range were diluted with the dedicated sample diluent provided by the manufacturer. No hook effect was observed for values up to 740 μg/L. The precision study was carried out by testing three levels of quality control (QC) material daily nine times each for a total of 6 days (54 measurements) and two in-house pool (P) samples tested daily nine times each for 10 days (a total of 90 measurements for P1 and 86 measurements for P2, as four results were above the upper limit of the measurement range). To test the accuracy of H-FABP determinations in plasma (lithium-heparin) samples, H-FABP concentrations measured in 30 matched sera and plasma samples were compared. A total of 77 non-consecutive patients (51 males and 26 females; age 62 ± 16 years, mean ± SD) referred to the ED of the University Hospital of Padova were enrolled between October 3 and December 8, 2005. All patients enrolled had chest pain suggesting myocardial ischemia (1). The median time interval between the onset of pain and ED presentation was 3.2 h (range 2.5–12.0 h). After collection, blood samples were delivered to the laboratory and centrifuged (5 min at 3500 × *g*); the sera obtained were stored at −20°C until analysis. The protocol was approved by the Ethics Committee of our institution, and fully informed consent was obtained from the subjects studied. The Bland-Altman test was performed to compare H-FABP concentrations measured in serum and plasma samples. Student's t-test was used to compare Myo and H-FABP concentrations in the population studied (normal distributions were obtained after logarithmic transformation of data). $p < 0.05$ was considered statistically significant. Calculations of clinical sensitivity, specificity, diagnostic efficacy and accuracy were carried out using ROC curve analysis. Statistical analysis was performed using MedCalc software version 8.1.1.0 (Mariakerke, Belgium). The mean concentration and within-run and between-day imprecision were: QC1, 2.32 μg/L, 5.26% and 6.46%; QC2, 16.62 μg/L, 4.46% and 6.26%; QC3, 44.03 μg/L, 7.31% and 8.04%; P1, 11.85 μg/L, 4.59% and 9.03%; and P2, 65.13 μg/L, 5.93% and 12.63%, respectively. Bias between H-FABP levels measured in matched serum and those in plasma samples was not of statistical significance (+0.178, 95% CI −0.033 to +0.389).

Patients enrolled in the study were classified into two groups (AMI and non-AMI) on the basis of the discharge diagnosis. The diagnosis of AMI was based on clinical and biochemical findings as recommended by the ESC/ACC Consensus Conference (1). Non-AMI patients were evaluated by revision of their clinical records after hospital discharge. For patients discharged from the ED, a 2-month follow-up was carried out to rule out recurrent chest pain and/or hospital readmission for any cardiac cause, or any other major events. Group 1 was the AMI patients (n = 22). Group 2 was the non-AMI patients (n = 55), who had pericarditis (n = 2), unstable angina (n = 6; diagnostic electrocardiogram findings and/or critical coronary lesions at PTCA without troponin elevation) or non-cardiac chest pain (n = 47; cardiac cause of the symptoms ruled out on the basis of all available clinical reports and patient monitoring). None of the patients had acute or chronic renal failure (creatinine 85 ± 22 mmol/L, mean ± SD). The median value and ranges measured were as follows: AMI, Myo 267.00 μg/L (47.00–5821.00 μg/L), H-FABP 56.93 μg/L (6.11–290.20 μg/L); non-AMI, Myo 47.00 μg/L (20.00–118.00 μg/L), H-FABP 2.56 μg/L (1.04–7.14 μg/L). Significant differences were found between the groups for both Myo and H-FABP concentrations ($p < 0.0001$). The best cut-off and associated diagnostic efficacy were 95 μg/L and 89.47% for Myo, and 5.09 μg/L and 98.70% for H-FABP, respectively. The corresponding sensitivity and specificity (with 95% CI) are shown in Figure 1.

To the best of our knowledge, this is the first study to demonstrate the accuracy of a new quantitative, fully automated and commercially available H-FABP assay (8), thus suggesting that H-FABP may be a promising biomarker for routine use, as it is measurable in patients referred to the ED with chest pain as the chief symptom. In fact, the precision performance appeared satisfactory, showing reproducible results across the whole measurement range. The possibility of using both serum and plasma (lithium heparin) specimens seems of value owing to the lack of clotting time and the corresponding reduced turnaround time. Furthermore, although no significant difference was found between the diagnostic accuracy of the Myo and H-FABP assays ($p = 0.079$) (Figure 1),

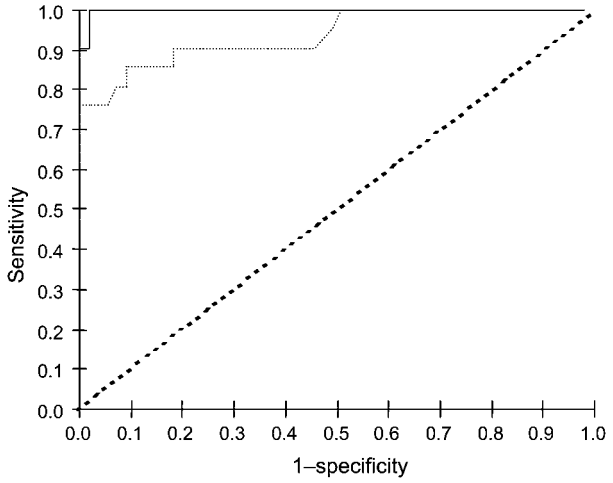

Figure 1 Evaluation of diagnostic accuracy: Myo (·····) and H-FABP (—) ROC curves. Areas under the ROC curve (95% CI): Myo, 0.938 (0.858–0.980); H-FABP, 0.998 (0.950–1.000). Difference between areas (95% CI): 0.061 (−0.007 to +0.128) (p=0.079). The best cut-off and the corresponding sensitivity (%) and specificity (%) with their 95% CI: Myo 95.00 μg/L, sensitivity 85.70% (63.60–96.80%), specificity 90.90% (80.00–96.90%); H-FABP 5.09 μg/L, sensitivity 100.00% (84.40–100.00%), specificity 98.20% (90.20–99.70%).

the diagnostic efficacy of H-FABP (98.70%) was greater than that of Myo (89.47%). Results obtained in this study are in agreement with previous findings by other authors, demonstrating the potential clinical usefulness of H-FABP measurement in acute coronary syndrome patients (9, 10). These data, although limited, confirm that H-FABP determination in patients with ischemic symptoms may be a more reliable early indication of cardiac damage than Myo. Although the assay time required might appear too long for use in an emergency situation, it is advantageous if compared to the usual 4–6 h required for the biochemical follow-up of patients complaining of chest pain and presenting an equivocal electrocardiogram or biochemical pattern on admission. Therefore, H-FABP determination might be of pivotal importance in the early hours of acute coronary syndrome, allowing the accurate identification of patients at higher risk of cardiac events and the correct decision on the best possible treatment strategy.

In conclusion, if used in combination with cardiac troponins, H-FABP may allow the biochemical detection of cardiac injury from the early stages of its onset until complete recovery in the ED. However, an automated and quantitative H-FABP assay with a reasonable turnaround time is needed before research evidence can be translated into clinical application.

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