

Assessment of a heart-type acid-binding protein test in the prehospital diagnosis of acute myocardial infarction

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Objective: To assess the usefulness of testing for heart-type fatty acid-binding protein (h-FABP) to obtain complementary information for the diagnosis of acute myocardial infarction (AMI) in the prehospital setting, particularly in patients whose symptoms began less than 3 hours earlier.

Methods: Prospective cross-sectional study in patients with probable AMI attended by 3 advanced life support units belonging to the health service of Castile-La Mancha, Spain. The recorded variables were clinical and epidemiologic data, electrocardiograms, and the results of a rapid h-FABP test. The patients were followed while in hospital, and the definitive diagnosis was considered to be the one eventually made in hospital. Indices reflecting the diagnostic yield of the test were calculated.

Results: Seventy-three patients were tested. The h-FABP test had to be repeated for 21 patients because of problems related to storage of the strips or interpreting the results, and in 7 cases (9.6%) test results were judged unusable. In 50 of the 66 patients for whom readable h-FABP results were obtained, the test was done within 3 hours of the onset of symptoms. In this group, the h-FABP test achieved a sensitivity of 91% (95% confidence interval [CI], 76%-98%), specificity of 37% (95% CI, 15%-65%), efficiency of 74% (95% CI, 64%-85%), positive predictive value of 76% (95% CI, 60%-88%), and negative predictive value of 67% (95% CI, 30%-93%). The specificity of the h-FABP test was higher when calculated for the entire patient sample regardless of time elapsed since the onset of symptoms, but the other indices were similar. The diagnostic yield of the test was lower in patients with atypical clinical pictures and/or electrocardiograms than in those with more characteristic signs and symptoms.

Conclusion: The h-FABP test result does not help make a prehospital diagnosis of AMI when symptoms have started less than 3 hours before the patient reaches the hospital, and the diagnostic yield of the test is particularly low in cases in which the clinical picture is nuclear. [Emergencias 2009;21:333-338]

Key words: Cardiac biomarkers. Myocardial infarction. Heart-type fatty acid-binding protein (h-FABP). Biological markers.

Introduction

Myocardial markers have assumed growing importance since about 5 decades ago when they were quantified and enzyme determinations were first correlated with myocardial lesions¹. Subsequent research in this field has been constant, in order to incorporate increasingly sensitive and specific biomarkers into clinical practice to help in the diagnosis of early acute myocardial infarction

(AMI). Since the early 90s, clinical trials have applied the Heart Fatty Acid Binding Protein (h-FABP) test, a myocardial marker that some researchers consider the earliest and most sensitive test for AMI compared to those in current use² and which may even be superior to myoglobin³ and the MB fraction of creatine kinase CKMB at very early stages⁴, although there is still some controversy over which of them should be considered the gold standard^{5,6}. This protein is abundant

in cardiac muscle fibers, and in healthy individual plasma its concentration is found between 1.6-19 ng/ml, increasing gradually with age. These basal concentrations largely depend on its presence and metabolism/catabolism in other tissues such as skeletal-muscle (in relation to exercise⁷), kidney, liver, intestine, brain, adipose tissue and nerve cells in the form of different isoenzymes.

In ischemia and/or myocardial injury, h-FABP is released early into the plasma, which allows detecting a progressive increase in levels above 19 ng/ml from 20 minutes (some authors refer 90 minutes), reaching a peak at 4 hours after onset and maintaining high values during 12-24 hours. For the detection of hFABP, easily applied technology has been developed, which allows us to determine its level using a procedure based on dry chemistry⁸ with monoclonal antibodies specific for cardiac h-FABP, and that has demonstrated good correlation with respect to the classical procedure by ELISA (Enzyme-linked Immunosorbent Assay)⁹. This technique has allowed inclusion of this marker in daily clinical practice in some hospitals and, in some countries, in out-of-hospital Advanced life support units (ALSU)¹⁰.

Currently, out-of-hospital diagnosis of AMI is based on two criteria: clinical symptoms and electrocardiogram (ECG). The existence, in addition, of medical history and risk factors for coronary heart disease supports the presumptive diagnosis. However, if one of the two criteria is not met, we can only suspect AMI and this prevents us from applying early treatment. It is therefore necessary to find another diagnostic criterion to complement the diagnosis. Currently, one of the possible lines of investigation is to perform a test to detect biochemical abnormalities when myocardial lesion occurs, particularly if there is a possibility of doing so early, by medical teams first on the scene. In this regard, h-FABP has been considered a good candidate as this third criterion. This test has been analyzed in hospitalized patients¹¹, but fewer studies have been performed in the pre-hospital setting.

This justifies research that evaluates not only whether h-FABP is positive or negative in early AMI, but also its potential as a reliable means, easy to use and handle in an inherently more hostile setting for the practice of medicine. Good performance provides us with a third diagnostic criterion in cases where one of the two criteria is absent (clinical symptoms and ECG). Thus, the objectives of this study were to assess the reliability and feasibility of performing the test, as well as to determine its indices of diagnostic performance.

Method

We performed a prospective cross-sectional study in patients with probable AMI attended by 3 advanced life support units (ALSU) of the health services of Castile-La Mancha, Tomelloso and Valdepeñas (Ciudad Real) and Almansa (Albacete), belonging to GUETS, Castilla-La Mancha Health Service (SESCAM). These three units serve approximately 180,000 inhabitants of the Community of Castilla-La Mancha, and their activity is regulated by the Emergency Coordination Centre 112, which is exclusive for the whole Community. The referral hospitals for these three units are located in Tomelloso Valdepeñas Almansa, Alcazar de San Juan, Albacete and Ciudad Real. The study was approved by the Ethics Committee of Clinical Research, Hospital Complex of Toledo. Following the indications of that Committee, we obtained verbal consent from all patients before inclusion in the study.

Field work for the study lasted from January 1, 2006 to 31 March 2008. The study subjects were all patients with suspected AMI that were attended by one of the three ALSU, preferably those attended within 3 hours of symptom onset.

For the detection of h-FABP, we used the Cardio-Detect[®] card. The procedure consists of applying 4 drops of capillary and/or complete blood to the reactive strip. First, a red line (control test) appears, indicating that the test strip is in good condition to detect h-FABP with concentrations equal to or greater than 7 ng/ml of blood. Then, a second red line appears, parallel to the first but at the bottom of the test strip (line detection), which means that a significant level of h-FABP (> 19 ng/ml) has been detected.

If this second line does not appear, the result of the test is negative. These results appear within 15-20 minutes. On the back of the reactive card there is a circle whose colour indicates suitability for use. White (control test) means that the card is suitable, but if the circle turns gray it cannot be used and should be discarded. According to the manufacturer's instructions, this may happen when the conservation recommendations have not been followed: the cards must be kept at a temperature of 2-8°C until completion of the test.

The following patient variables were recorded: sex, type of chest pain (distinguishing between typical AMI pain and all other possibilities), time elapsed from symptom onset to performance of the test, ECG changes (differentiating ST elevation from other situations). When the pre-hospital

diagnosis was doubtful or did not include the two criteria, the final diagnosis (AMI and no AMI) was that made by the referral hospital and accepted as the gold standard. The result of h-FABP test was recorded as positive, negative or zero. Other non-basic variables were collected such as age, and risk factors such as smoking, hypertension, hyperlipidemia, diabetes, obesity, alcoholism and previous heart disease, time from symptom onset to bedside attention by the ALSU team, and scene of the event (home, in public or health center).

After data collection, statistical analysis of the h-FABP test performance indices (sensitivity, specificity and efficiency, and positive or negative predictive values) was performed, both for patients whose symptom evolution was less than 3 hours and for all patients. For these analyses, we excluded cases where the h-FABP test result was zero. For patients with symptom evolution of less than 3 hours, the analyses were repeated in two subgroups of patients: those with typical clinical symptoms and ECG ST elevation (with no diagnostic doubt) and the remaining patients (with diagnostic doubts). Each of the indices of these two subgroups were compared using Fisher's exact test. The results were expressed with a confidence interval of 95%, and differences were considered significant when the *p* value was less than 0.05.

Results

The study included a total of 73 people, 19 (26%) women and 54 (74%) men. Most (74%) were initially treated in a health centre, 22% at home and 4% in a public place. One or more cardiovascular risk factors were present in 94% of the women and 90% of the men. Table 1 shows the distribution of the remaining variables according to time elapsed between initial symptom onset and performance of the test.

Regarding the feasibility and reliability of the h-FABP test, we found that many of the cards' control test indicated unsuitability for use, despite having been stored according to the manufacturer's instructions. This was reported to the laboratory manufacturer who informed us that, in recent tests, the detector cards had shown greater stability when stored in a dry place at a temperature not less than 15°C, and that the true suitability of the test was indicated by the appearance of the first red line at the top of the strip after applying the blood sample. However,

theoretically defective cards whose control test circle had turned gray were replaced by the manufacturer, and this new batch of cards no longer presented the control test circle on the back. Regarding in situ reading of the test, we found it difficult to determine the results, especially with viewing the 2nd red line of the h-FABP detector, which was sometimes almost imperceptible, but even so was considered positive. There were times when no lines appeared, and retesting was performed with a new card.

This difficulty of card conservation and the zero result made it necessary to repeat 21 of the tests performed. Therefore, a final 94 cards were used for 73 patients. However, seven of the 73 cases analyzed (9.6%) were excluded due to the irregular behaviour of the card used (no control line or h-FABP detector despite proper collection of the sample).

The indices of diagnostic performance for the h-FABP test were calculated for the 66 patients where a positive or negative result was obtained, as shown in Table 2. For patients with symptom duration of less than 3 hours (50 cases), the test showed lower specificity, while for the other patients, the indices showed similar values (Table 3). In general, we noted high sensitivity and low specificity; positive and negative predictive values for this sample of patients ranged from 66% to 78%.

Regarding patients where the h-FABP test was performed within 3 hours of symptom onset, in the subgroup of patients without diagnostic doubts we obtained good diagnostic performance indices, but these were poor for patients with diagnostic doubts (Table 4) and significantly lower, specifically for efficiency (43% versus 97%, respectively; *p* < 0.001) and positive predictive value (33% versus 100%, respectively, *P* < 0.001).

Table 1. Characteristics of the series

	Total (n = 73)	Less than 3 hours (n = 54)	More than 3 hours (n = 19)
Sex female [n (%)]	19 (26.0)	14 (19.1)	5 (6.9)
Age [mean (SD)]	66 (14.8)	65 (15.3)	69 (13.3)
Typical symptoms [n (%)]	45 (61.6)	36 (66.7)	9 (47.4)
ECG with ST elevation [n (%)]	48 (65.8)	40 (74.1)	8 (42.1)
Existence of risk factors [n (%)]	66 (90.4)	57 (78.0)	9 (12.32)
Final diagnosis of AMI [n (%)]	48 (65.8)	37 (68.5)	11 (57.9)
h-FABR test null [n (%)]	7 (9.6)	4 (7.4)	3 (15.8)
h-FABR test positive [n (%)]	50 (68.5)	41 (75.9)	9 (47.4)

ECC: Electrocardiogram; AMI: acute myocardial infarction.
SD: Standard deviation.

Table 2. Results of the heart-type fatty acid-binding protein test (h-FABP) in all patients where a result was obtained (n = 66)

Final Diagnosis	h-FABP test results		
	Positive	Negative	
No AMI	11	11	22
AMI	39	5	44
Total	50	16	66

Sensitivity: 82% (CI 95%: 75%-96%). Specificity: 50% (CI 95%: 28%-72%). Efficiency: 76% (CI 95%: 64%-85%). Positive predictive value: 78% (CI 95%: 64%-88%). Negative predictive value: 69% (CI 95%: 41%-89%). AMI: acute myocardial infarction.

Discussion

According to our results using the brand Cardio-Detect®, the h-FABP test had high sensitivity but insufficient specificity^{12,13}, so that a priori it does not appear to be a reasonably sure as an additional test to confirm the diagnosis of AMI¹⁴. It represented no improvement, in our experience, in diagnostic prediction when one of the two usual diagnostic criteria was not present. When the 2 criteria of clinical symptoms and abnormal ECG were met (ie, when the h-FABP test was theoretically unnecessary), it showed high sensitivity (and even specificity), but when theoretically needed, in the absence of both criteria, the test had almost no sensitivity or specificity in our experience. However, other studies have yielded data indicating its utility^{15,16}.

An added difficulty was the question of card conservation, due to discrepancies about optimal temperature and the confusing information provided by the laboratory manufacturer. In certain cases, we experienced great difficulty in reading the cards and occasionally required a magnifying lens to determine the presence of the line indicating a positive h-FABP¹⁷. This possibly helps explain the non-negligible percent-

Table 3. Results of the heart-type fatty acid-binding protein test (h-FABP) in patients with symptom evolution time of less than three hours where a result was obtained (n = 50)

Final Diagnosis	h-FABP test results		
	Positive	Negative	
No AMI	10	6	16
AMI	31	3	34
Total	41	9	50

Sensitivity: 91% (CI 95%: 76%-98%). Specificity: 37% (CI 95%: 15%-65%). Efficiency: 74% (CI 95%: 60%-85%). Positive predictive value: 76% (CI 95%: 60%-88%). Negative predictive value: 67% (CI 95%: 30%-93%). AMI: acute myocardial infarction.

Table 4. Results of the heart-type fatty acid-binding protein test (h-FABP) in patients with more than three hours of chest pain, separated according to the subgroups "Without diagnostic doubt" (n = 29, top) and "With diagnostic doubts" (n = 21, below)

Final Diagnosis	h-FABP test results		
	Positive	Negative	
No AMI	0	2	2
AMI	26	1	27
Total	26	3	29

Sensitivity: 96% (CI 95%: 81%-100%). Specificity: 100% (CI 95%: 16%-100%). Efficiency: 97% (CI 95%: 87%-100%). Positive predictive value: 100% (CI 95%: 87%-100%). Negative predictive value: 67% (CI 95%: 9%-99%).

Final Diagnosis	h-FABP test results		
	Positive	Negative	
No AMI	10	4	14
AMI	5	2	7
Total	15	6	21

Sensitivity: 71% (CI 95%: 29%-96%). Specificity: 29% (CI 95%: 8%-58%). Efficiency: 43% (CI 95%: 22%-66%). Positive predictive value: 33% (CI 95%: 12%-62%). Negative predictive value: 67% (CI 95%: 22%-96%). AMI: acute myocardial infarction.

age (almost 10%) of invalid tests. Therefore, the test showed poor reliability and feasibility for application in a pre-hospital setting.

Other published studies on this detector in both hospitals and pre-hospital units conclude that it may improve¹⁸ or even be valid *per se*¹⁹ as an element for the early diagnosis of AMI¹⁹. Therefore, we believe future clinical trials should be performed, and that tests be developed to better quantify the detection of h-FABP in blood, with easy and accurate reading of the results.

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Valoración de la utilidad del test detector de la proteína transportadora de ácidos grasos específica del miocardio (h-FABP) en los infartos agudos de miocardio (IAM) diagnosticados en el medio extrahospitalario

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Objetivo: Valorar el test de la proteína cardiaca citoplasmática transportadora de ácidos grasos (h-FABP) como prueba complementaria para el diagnóstico del infarto agudo de miocardio (IAM) en el medio prehospitalario, y especialmente para aquellos pacientes con una clínica de menos de 3 horas de evolución.

Método: Estudio prospectivo transversal en pacientes con sospecha de IAM atendidos por 3 unidades de soporte vital avanzado del Servicio de Salud de Castilla-La Mancha (SESCAM). Se recogieron datos clínico-epidemiológicos, un registro electrocardiográfico y se les realizó un test rápido de determinación de h-FABP. Se realizó un seguimiento hospitalario del caso y se consideró su diagnóstico final en el hospital como el patrón oro. Se calcularon los índices de rendimiento diagnóstico de la prueba.

Resultados: Se incluyeron 73 pacientes. En 21 casos se repitió la determinación de h-FABP, ya que se detectó cierta dificultad en la lectura y conservación del test, y en 7 casos (9,6%) fue considerado finalmente nulo. En 50 de los 66 casos restantes con resultado legible de h-FABP dicha prueba se realizó durante las primeras 3 horas del inicio de los síntomas. En estos casos, la h-FABP obtuvo una sensibilidad del 91% (intervalo de confianza del 95%: 76%-98%), una especificidad del 37% (15%-65%), una eficiencia del 74% (64%-85%), un valor predictivo positivo del 76% (60%-88%) y un valor predictivo negativo del 67% (30%-93%). Cuando se consideró a la totalidad de pacientes sin tener en cuenta el tiempo transcurrido desde el inicio de la clínica, la h-FABP incrementó la especificidad, pero el resto de índices fueron parecidos. Para los casos con clínica y/o electrocardiogramas (ECG) no típicos, el rendimiento diagnóstico de la prueba fue bajo y significativamente inferior que para los casos con clínica y ECG típicos.

Conclusión: En el IAM de menos de tres horas de evolución, la h-FABP no ayuda al diagnóstico prehospitalario, y su rendimiento es especialmente bajo en aquellos pacientes en los que existen dudas diagnósticas. [Emergencias 2009;21:333-338]

Palabras clave: Marcadores cardiacos. Lesión miocárdica. h-FABP. Biomarcadores.