Biomarkers of Myocardial Cell Damage: Heart-Type Fatty Acid Binding Protein (H-FABP) for the Early Evaluation of Suspected Acute Coronary Syndrome

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Abstract

Suspected acute coronary syndrome (ACS) represents a substantial healthcare problem and is responsible for a large proportion of emergency department admissions. Better triaging of patients with suspected ACS is needed to facilitate early initiation of appropriate therapy in patients with acute myocardial infarction and to exclude low-risk patients who can safely be sent home thereby limiting healthcare costs. Heart-type fatty acid-binding protein (H-FABP) is established to be the earliest available plasma marker for myocardial injury. In this chapter, the clinical utility of H-FABP for suspected ACS is evaluated. H-FABP shows added value in addition to cardiac troponin, especially in the early hours after onset of symptoms. Moreover, H-FABP identifies patients at increased risk for future cardiac events. It is concluded that measuring H-FABP along with troponin shortly after onset of symptoms improves risk stratification of patients suspected of having ACS in a cost-effective manner.

Keywords

Fatty acid-binding protein • H-FABP • FABP3 • Acute coronary syndrome • Acute myocardial infarction • Plasma biomarker • Early diagnosis • Point-of-care test

Abbreviations

ACS	Acute coronary syndrome
AUC	Area under curve
CABG	Coronary artery bypass grafting
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase MB
cTn	Cardiac-specific troponin
cTnI	Cardiac-specific troponin I
cTnT	Cardiac-specific troponin T
CV	Coefficient of variance
ECG	Electrocardiogram
GP	General practitioner
h	Hour
H-FABP	Heart-type fatty acid-binding protein
hs-cTn	High-sensitive cardiac-specific troponin
hs-cTnI	High-sensitive cardiac-specific troponin I
hs-cTnT	High-sensitive cardiac-specific troponin T

MI	Myocardial infarction
NPV	Negative predictive value
NT-proBNP	N-terminal B-type natriuretic peptide
PoC	Point of care
PPV	Positive predictive value
ROC	Receiver operating characteristic
UAP	Unstable angina pectoris

Key Facts of H-FABP

- Several molecules play a role in the function of heart cells and are detectable exclusively inside the heart cells.
- In a healthy situation, these molecules are not detectable on a significant level in the blood. In cases of damage to heart tissue, usually in cases of heart infarction, several of these substances can be released into the peripheral blood.
- Therefore, they become detectable in peripheral blood, for example, obtained through venous blood sampling or a capillary finger prick.
- This way, a rise in these molecules as measured in a blood sample is highly indicative for cell damage and possibly heart infarction in a patient, especially when complaints fitting this diagnosis are present simultaneously.
- Such molecular markers of a certain disease are named *biomarkers*.
- Before clinical use in patients is possible, the value of these biomarkers must become undisputed in studies including a large number of real patients with and without the studied disease (e.g., heart infarction).
- Heart-type fatty acid-binding protein (H-FABP) is an example of such a biomarker for heart cell damage that is currently studied for its use in daily medical practice.

Definitions

Acute coronary syndrome Clinical description of complaints suspicious for a cardiac ischemic cause. Those are new or worsened complaints when compared to an earlier, stable phase. To distinguish between acute coronary syndrome and other cardiac or noncardiac causes, plasma troponin measurement is necessary unless ST elevations on ECG are seen. In case of a confirmed acute coronary syndrome, unstable angina or myocardial infarction is present.

Angina pectoris Chest pain due to diminished blood flow in one or more coronary arteries. Stable angina pectoris: "predictable" chest pain occurring at a certain degree of exercise for a long time, due to myocardial ischemia caused by stable stenosis in a coronary artery without local thrombotic activity. Collateral circulation often compensates for diminished perfusion through the affected coronary artery. Stable angina is usually treated with medication, aiming at reduction of complaints as well as

secondary prevention of cardiovascular disease. Invasive intervention is sometimes indicated when severe complaints or increased mortality risks are present. Unstable angina pectoris: chest pain of new onset or chest pain occurring at lower intensities of exercise than in the recent past, mostly due to acute plaque rupture in a coronary artery, activating local thrombotic activity. Unstable angina pectoris is a clinical diagnosis; myocardial ischemia is present, but plasma troponin levels remain normal. Condition is usually treated with invasive intervention that may be necessary on a short term.

Biomarkers of myocardial cell damage Any molecule that is released into the circulation following myocardial cell damage and becomes measurable in peripheral blood; troponin and heart-type fatty acid-binding protein are examples of such biomarkers.

Chest pain Pain in (ventral and/or lateral and/or dorsal) thoracic region of any cause. Among possible causes are gastroesophageal causes, thoracic wall pain, and angina pectoris.

Diagnostic accuracy Term used for the overall capacity of a test to play a role in the diagnostic process. Accuracy in this context is a collective term for specificity, sensitivity, and positive and negative predictive value.

Myocardial infarction (Near-)complete occlusion of a coronary artery, most often caused by plaque rupture and local thrombotic activity in consequence. Myocardial damage is present; troponin is released from the damaged myocardial cells. ST elevations on ECG can be present or absent. Usually treated with urgent percutaneous coronary intervention or bypass surgery. Prognosis: depends on magnitude of myocardial cell loss.

Point-of-care test Test device able to measure one or more laboratory parameters and to deliver a result within a limited period of time. Result is known within the time of consultation of the patient; transport of patient material to externally located laboratory facilities is unnecessary. In a broader perspective, any test that delivers immediate results can be regarded as point-of-care test, for example, devices to measure temperature of blood pressure.

Primary care, general practice, and family medicine Synonyms used to describe the field of medicine where no limitation on type of complaints and disease is maintained. No thresholds for care are established. Attention to a broad patient perspective (somatic, psychological, social context) is a main goal in primary goal.

Reliability Other than diagnostic accuracy, this term describes the test ability to measure accurately and in a reproductive manner, without regarding the diagnostic potency when used in a clinical perspective.

Introduction: Heart-Type Fatty Acid-Binding Protein (H-FABP) and Acute Coronary Syndrome

In an earlier publication, heart-type fatty acid-binding protein (H-FABP) has been extensively reviewed (Glatz and Renneberg 2014). Referral is made to this chapter for a basic, mainly biochemical approach with elaborate references. In this present chapter, H-FABP is reviewed in a rather clinical perspective, to underline the future perspectives of H-FABP in dealing with acute coronary syndrome (ACS) in primary and secondary care.

A Major Healthcare Problem: Suspected Acute Coronary Syndrome

Cardiovascular diseases remain the leading cause of death in industrialized countries with coronary artery disease being the most prevalent manifestation (Fact sheet no. 317 Geneva, 2013). The clinical presentations of this include stable angina pectoris, unstable angina pectoris (UAP), myocardial infarction (MI), heart failure, manifestations of silent ischemia, and sudden death. Marked improvements in clinical treatment during the previous decades have resulted in increased survival of patients with acute coronary syndrome (ACS, i.e., MI or UAP). In contrast, diagnostic means have remained poor, especially in an early stage of acute coronary artery disease when patients may need immediate clinical treatment. While chest pain is the main symptom of chronic coronary artery disease and ACS, early assessment is hampered by the large number of patients presenting with chest pain of another, less severe, cause.

Dilemma in Chest Pain: ACS or Alternative Cause?

ACS represents a life-threatening manifestation of atherosclerosis causing a sudden and critical reduction in coronary blood flow due to intraluminal thrombosis. The therapeutic objective is to achieve rapid, complete, and sustained reperfusion by primary angioplasty or fibrinolytic therapy. Therefore, rapid triaging of patients presenting with chest pain is needed to facilitate early initiation of appropriate treatment in patients with acute MI. At the same time, low-risk patients who can safely be sent home without further expensive diagnostic analysis should be identified as well. Because the latter group currently represents up to 80 % of patients with suspected ACS (Bruins Slot et al. 2011; Goodacre et al. 2013; McConaghy and Oza 2013), adequate ruling out of MI and of UAP is important in view of not only patient burden but also the large costs involved, which may include ambulance transfer, extensive diagnostic procedures, and hospital stay.

Thus, one of the main demands in the diagnostic process of coronary artery disease is to distinguish chest pain caused by coronary obstruction from chest pain

with a benign course (thoracic wall pain, gastroesophageal reflux disease, etc.) (Hamm et al. 2011). In the former situation, urgent specialist care is needed, which is rarely the case in the latter situation. Therefore, in a patient presenting with chest pain to primary or secondary care facilities, the presence or absence of ACS should be crystal clear as soon as possible in order to design the most cost-effective diagnostic strategy in patients presenting with chest pain suggestive of MI. Among the most promising tools to reach this goal are biomarker tests that deliver immediate results at the point of care.

High-Sensitive Troponin and Additional Biomarkers

As a sensitive biomarker of cardiac injury, high-sensitive troponin (hs-cTn) is of great importance in the current diagnostic strategy in suspected ACS. Using hs-cTn, acute MI can be ruled out based on a negative test result in an emergency setting as early as 3 h after onset of symptoms (Bandstein et al. 2014). However, extension of these diagnostic rule-out strategies could lead to further cost reduction and more convenience for patients. Besides, time of onset of complaints can be uncertain. Altogether, in this field of increasing rule-out capacity, additional biomarkers that appear in plasma at an earlier point in time after MI can be of interest. In the following paragraphs, the specific demands of ruling out ACS in primary care and at an early moment in secondary care are made explicit. Furthermore, it is described how available evidence indicates that heart-type fatty acid-binding protein (H-FABP, also designated FABP3) fulfills the criteria to be useful for triaging patients with acute chest pain particularly in the early hours after onset of symptoms. In this chapter, therefore, the clinical utility of H-FABP for the early evaluation of suspected ACS is depicted. Other biomarkers representing different aspects of an evolving acute MI such as markers for vascular stress (copeptin, N-terminal B-type natriuretic peptide or NT-proBNP), oxidative stress (myeloperoxidase), or plaque instability (placental growth factor) are not discussed in this chapter, since their diagnostic value is not well defined or considered not useful (Keller et al. 2011; Collinson et al. 2013).

ACS in Primary Care

Because a large number of patients with symptoms suggestive of MI will first be presented to primary care physicians, often during out-of-office hours, the general practitioner (GP) plays a crucial role in the early diagnosis and referral of these patients (Bruins Slot et al. 2011; Goodacre et al. 2013; McConaghy and Oza 2013). In case of suspected ACS, patients will be urgently referred to a secondary care facility, since early treatment of ACS markedly increases survival and quality of life (Reimer and Jennings 1979; Gersh et al. 2005). The majority of patients presenting with chest pain to a physician (either in primary or in secondary care), however, do not suffer from an acute cardiac condition at all. In specialized care facilities such as

coronary care units, only 50 % of patients presenting with chest pain are diagnosed with ACS, whereas in primary care, ACS is diagnosed in no more than 1.5–22 % of cases (Bruins Slot et al. 2011; McConaghy and Oza 2013; Willemsen et al. 2015). In the remainder of cases, chest pain is mostly caused by a condition with beneficial outcome (e.g., gastroesophageal reflux disease, thoracic wall pain, etc.). Given the high prevalence of noncardiac chest pain, expenses to exclude severe disease in such patients result in a significant societal burden (Mourad et al. 2013). Moreover, even after expensive diagnostic research, reassuring patients is challenging (Dumville et al. 2007). Referring every patient with chest complaints would overwhelm secondary care facilities; however the GP is faced with serious diagnostic dilemmas since milder diseases with beneficial outcome can mimic ACS and vice versa (Body et al. 2010). To distinguish chest pain caused by ACS from chest pain of another cause therefore remains challenging.

ACS in Primary Care: Diagnostic Means

Contemporary diagnostic means are insufficient to overcome the difficulties in distinction between chest pain due to ACS and thoracic complaints due to alternative causes. This is caused by several reasons with two common factors: all tools are of limited availability in general practice or lack acceptable negative predictive value (NPV) and sensitivity. A few points, partly based on current literature and partly based on experience in daily practice, can be made. First, literature confirms that symptoms and signs vary widely in chest pain possibly due to ACS, from none (in circa 25 %) to severe, and thus have limited diagnostic value in a significant amount of cases (Brieger et al. 2004; Bruyninckx et al. 2008; Body et al. 2010). Second, validated decision rules for general practice to rule out AMI or ACS have been developed, but evidence for superiority of using these decision rules above the GP's judgment without these rules is lacking (Bösner et al. 2010; Haasenritter et al. 2012). Third, the value of electrocardiography is limited, since only about 50–65 % of patients with ischemic cardiac disease have classic electrocardiogram (ECG) findings in the first time period after start of the complaints, while an ECG is sometimes not even available in general practice (Rutten et al. 2000). Fourth, since the definition of AMI is for an important part based on biomarker levels and AMI can in a significant amount of cases not be ruled out otherwise, blood analysis, especially measurement of the concentration of troponin, is a cornerstone in diagnosing as well as ruling out AMI. Venous blood samples, obtained in general practice, however cannot be analyzed on the spot, and adequate monitoring of the patient in expectation of the results is impossible. Moreover, serial measurement of plasma hs-cTn, the cornerstone in diagnosing as well as ruling out MI (Newby et al. 2012; Thygesen et al. 2012), is impossible to perform in primary care. This impairment could partly be overcome by usage of point-of-care (PoC) tests. Unfortunately, contemporary PoC troponin tests are less accurate due to detection limits

higher than the widely used cutoff values for a positive test, usually set at the 99th percentile of a healthy population (Nilsson et al. 2013).

Ruling Out ACS in Primary Care: Specific Demands

Since diagnostic means accessible for GPs lack potency to safely rule out ACS, a low threshold for referring patients with possible ACS is maintained. Although ACS is present in the minority of cases, a majority is referred to a cardiologist to rule out ACS. In a Dutch cohort of such patients, 27 % of patients were not referred, in 8 % of whom ACS was diagnosed in a later stage, leading to a false-negativity rate of 2 %. Seventy-three percent of patients were referred, 75 % of whom were not diagnosed with ACS (false-positivity rate or "unnecessary referral rate" 54.8 %) (Bruins Slot et al. 2011, 2013b). Patients that were referred and appeared to be ACS negative were diagnosed with alternative diseases with advantageous courses. Thus, over-referral of patients presenting with chest pain in primary care leads to a low number of missed cases of ACS but is an (expensive) burden to secondary care facilities (Graff et al. 1997). Since unnecessary referral considerably outnumbers missed cases in the triaging of patients with suspected ACS in primary care, focus is rather on ruling out ACS and other urgent medical conditions as early as possible. Thus improvement of diagnostic tools aims at making referral unnecessary, without missing more cases of ACS, enabling limitation of overall healthcare costs. Besides, anxiety in patients undergoing unnecessary diagnostic procedures is reduced.

A PoC test with a high negative predictive value for ACS that delivers a clear result within several minutes is needed to reach this goal. Notably, such a PoC test is among the most demanded future tests by GPs (Cals et al. 2014). An improved triage of patients with signs and symptoms suggestive of ACS would reduce unnecessary referral and associated cost and anxiety. Therefore, to enrich the diagnostic tools of a GP in thoracic symptoms and to reduce unnecessary referral in cases of clinical doubt, novel, immediately measurable biomarkers with strong potency to rule out myocardial infarction in single measurements are needed. Combined with signs and symptoms, such tools should be able to safely rule out ACS in a significant number of otherwise referred patients, without a rise in missed cases of ACS. Importantly, this would lead to a significant cost reduction. The number of referred patients would decrease, and in the remaining patients who are referred, ACS could be confirmed or ruled out as is common in secondary care.

In the field of pulmonary embolism and respiratory tract infections, diagnostic tools combining clinical signs and symptoms with the result of a PoC test have recently been introduced. Both increased efficiency by reducing unnecessary referral (in cases of suspected pulmonary embolism) or unnecessary treatment (in respiratory tract infections) (Cals et al. 2011; Geersing et al. 2012; Little et al. 2013). For ACS, a similar procedure has not yet been defined.

Table 1 Characteristics of plasma biomarkers for acute myocardial infarction

Marker protein	Molecular mass (kD)	First elevation in plasma after AMI[a] (h)	Peak plasma concentration (h)	Normalization of plasma level[b] (days)
H-FABP	14.5	1–2	6–12	1–1.5
Myoglobin	17.8	2–3	6–12	1–2
Cardiac troponin I	22.5	3–8	12–24	7–10
Cardiac troponin T	37.0	3–8	12–24	7–10
Creatine kinase MB	86	2–6	12–24	2–3

Several characteristics (molecular mass, first elevation in plasma after acute myocardial infarction (AMI), peak plasma concentration, and normalization of plasma level) of several widespread used biomarkers of cardiac ischemia

Abbreviations: *AMI* acute myocardial infarction, *h* hours, *H-FABP* heart-type fatty acid-binding protein, *kD* kilodalton

[a]First elevation above the upper reference level of the marker protein

[b]Dependent on (time of) reperfusion of the occluded vessels

ACS in Secondary Care

Various marker proteins are known to be released into plasma after MI, each showing a distinct tissue specificity and unique release pattern (see Table 1). Of these, the cardiac troponins, i.e., troponin T (cTnT) and troponin I (cTnI), are more specific and, as measured by the latest generation of troponin tests, more sensitive than the traditional cardiac enzymes, such as creatine kinase (CK) and its isoenzyme creatine kinase MB (CK-MB), and therefore have become the standard in establishing a diagnosis and stratifying risk (Thygesen et al. 2012). In patients with MI, plasma troponins initially rise about 3–4 h after symptom onset and remain elevated for up to 2 weeks due to slow proteolysis of the contractile apparatus in damaged cardiac myocytes. There is no fundamental difference between cTnT and cTnI. The NPV of contemporary fifth-generation hs-cTn tests in an emergency care department has increased to 98–99 % (Mueller 2014). Thus, evidence is growing that cardiac ischemia can be ruled out within 3 h after onset (Bandstein et al. 2014). Moreover, UAP seems to be diagnosed less because of the increasing sensitivity of hs-cTn (Mueller 2014). In new onset or altered chest pain where hs-cTn is negative, (severe) stable coronary artery disease is becoming increasingly diagnosed instead of UAP. Conversely, in cases where hs-cTn is slightly positive, MI is diagnosed according to the third universal definition of MI (Thygesen et al. 2012). An elevated hs-cTn value, i.e., above the 99th percentile of a normal reference population, is a strong indicator of myocardial cellular damage and has a very low false positivity.

Ischemia is not the sole cause of myocardial injury, however. Several other diseases also lead to myocardial cellular damage, including pneumonia, pericarditis,

and left ventricular stress. Using older-generation troponin assays, 30 % of patients testing positive had no coronary occlusion (Reichlin et al. 2009). With the current high-sensitivity assays, this percentage is probably even higher. To solve this issue, in the third universal definition of MI, AMI is diagnosed when, besides an elevated plasma hs-cTn, a change over time is measured. When such a change is detected, a coronary cause of the cardiac injury is likely (Thygesen et al. 2012). The magnitude of the change that is indicative of acute coronary occlusion is still open to debate. In the lower range of troponin results, an absolute change of 7 ng/L between two measurements is probably indicative of acute coronary disease, whereas in the higher range, a relative change of 20 % is needed (Biener et al. 2013).

Ruling Out ACS in Secondary Care: Specific Demands

Further improvement of the care process could be realized if patients presenting with chest pain of acute onset in secondary care – either after referral by a GP or otherwise – would undergo rule-out as soon as possible. Ideally such rule-out would occur within 1 h after onset of complaints. Furthermore, rule-out would ideally be based on a solitary measurement, while positive results due to other causes of myocardial injury should be limited as far as technically possible.

Ruling Out ACS in Primary and Secondary Care

As expounded above, main demands for ruling out ACS in primary as well as secondary care are early rule-out using a highly sensitive test with a high NPV for ACS. Such test could be an algorithm combining signs, symptoms, and a biomarker result. Moreover, especially in primary care, results should be available for assessment within several minutes. However, time to assessment is of significance in secondary care too, when definitive rule-out is demanded as early as possible (within 1 h). Key words in the field of future diagnostic means in ACS therefore are *point-of-care devices* and *high negative predictive value*. In the next subheadings, both requirements for efficient triaging are depicted.

Point-of-Care Tests

Contrary to pharmaceuticals, the legislation for diagnostic tests in general, and point-of-care tests in particular, is very limited. PoC tests may enter the European market after receiving no more than a CE certificate, which includes several fundamental, mostly technical, and laboratory aspects of the test. Proven reliability and diagnostic accuracy in daily clinical care do not belong to CE certification requirements. Consequently, primary care professionals took the initiative to start listing criteria to which PoC tests in their view should apply, and they simultaneously performed

studies in daily clinical care, on the performance of relevant PoC tests (Howick et al. 2014; Schols et al. 2015).

In primary care, PoC tests can be divided over (1) tests for (home) monitoring of patients who are unable to visit their GP; (2) tests for screening purposes, mostly as a service toward (future) patients; and (3) tests for ruling out particular diseases in patient presenting with symptoms possibly representing an underlying serious disease, like ACS. Preferably, all tests belonging to one or more of the above indications are fast, meaning they produce a test result within the duration of one consultation (i.e., 10 min). Furthermore, a PoC test for primary care must be reliable in the hands of non-laboratory trained personnel, it must have a better diagnostic accuracy than existing alternatives, its diagnostic accuracy must be investigated with the new PoC test as part of existing and accepted diagnostic algorithms, test results must adequately steer treatment or referral decisions, the test should be cost effective, costs of PoC testing must be properly reimbursed, PoC tests must be appreciated by both medical professionals and patients, and, last but not the least, a PoC test must be easy to implement in both the daily work-up of a GP and existing (laboratory) facilities of a particular clinic. Very few of currently in primary care used PoC tests apply to all requirements (Cals et al. 2013).

Infectious diseases and ACS belong to the relatively small group of (potentially) life-threatening diseases presented in daily primary care, needing PoC tests, like H-FABP, for quickly reaching accurate diagnostic conclusions and referral decisions. But before deciding on a definite introduction of H-FABP in primary care, the above-listed criteria must be evaluated in daily general practice circumstances.

Early Diagnosis of ACS: Plasma Marker Requirements

The "ideal" plasma marker to be used for evaluation of myocardial injury in patients presenting with chest pain suggestive of acute coronary syndromes in primary or secondary care would need to meet three criteria, i.e., (i) show absolute myocardial specificity and (ii) be instantaneously released into the circulation upon myocardial injury, while (iii) a test should be available that allows the accurate and rapid (minutes) assessment of its elevated concentration in plasma so as to permit the use of the test result in triaging of the patient. Unfortunately, such "ideal" marker does not exist (Gravning and Kjekshus 2008):

i. *Cardiac specificity.* Although the troponins show virtually absolute cardiac specificity and therefore have been adopted as primary marker for ACS diagnostics to be included in both the US and European guidelines for the management of ACS (Hamm et al. 2011), they appear in plasma only 3–8 h after onset of myocardial injury which in a substantial number of cases is too late to influence the initial triaging process. Hence, in cases where MI is not revealed on ECG, echocardiogram, or other imaging techniques, patients will be monitored up to 9–12 h to rule in or rule out an MI based on an elevated plasma troponin. For

those patients that turn out not to have MI, the latter procedure involves a marked financial burden that should be avoided when possible.

ii. *Early release into plasma*. H-FABP is the earliest marker to be elevated in plasma following myocardial injury and yet does not show absolute cardiac specificity because an elevation of plasma H-FABP could also be due to skeletal muscle injury. However, in view of the presence of H-FABP only in red (oxidative) skeletal muscle fibers and only in minute amounts (i.e., <5 % of that in the myocardium), a significant release of H-FABP from skeletal muscle takes place merely in specific cases such as eccentric exercise (Sorichter et al. 1998). In addition, in reported studies on the use of H-FABP for MI diagnostics to our knowledge, no such cases have been described in which plasma H-FABP was falsely elevated due to skeletal muscle injury.

iii. *Rapid test result*. The availability of an appropriate test that allows the rapid assessment of elevated marker concentrations in plasma is crucial to aid in the diagnosis of MI. Because H-FABP nor the troponins show enzymatic activity, such test has to be based on immunochemical detection of the protein. For both markers tests are available for use in the hospital emergency room or chest pain unit. For instance, Randox has developed a turbidimetric H-FABP assay that provides a quantitative result with a range of 2.5–120 ng/ml in serum in 14 min (see Table 2). Tests for cTnT and cTnI have been developed and marketed by most diagnostic companies, yet not all provide a high-sensitive test that complies with the requirements of current standard definition (Thygesen et al. 2012). To meet these requirements, high-sensitivity or ultrasensitive troponin assays (hs-cTnT and hs-cTnI) are needed. The limit of detection of these assays is 10–100-fold lower than that of the conventional troponin assays. This suggests their application for diagnosing smaller MIs otherwise undetected or for identifying MI earlier when abnormal troponin levels are below detection by conventional assays. Recently, PoC tests have become available for both H-FABP and the troponins; these will be discussed in a separate paragraph.

Heart-Type Fatty Acid-Binding Protein as Plasma Marker of Cardiac Injury

The cytoplasmic protein FABP has a relatively small size (14.5 kDa) and functions as an intracellular fatty acid carrier in parenchymal cells, thus supplying essential substrates for energy production in the myocytes (Glatz and Van der Vusse 1990). It comprises as much as 1–2 % of total cardiac cytosolic proteins, making it one of the most abundant cytosolic proteins. H-FABP is also found in small amounts in (slow-twitch oxidative) skeletal muscle, in distal tubule cells of the kidney, and in some parts of the brain (Schaap et al. 1998). The potential of H-FABP to be used as plasma marker of myocardial injury was suggested first in 1988 (Glatz et al. 1988). Its cytosolic occurrence, cardiac tissue abundance, and small size make that, upon myocardial cellular damage, H-FABP is released rapidly and in appreciable amounts to the interstitial space, from where it escapes through the endothelial clefts into the

Table 2 Overview of H-FABP assays in plasma

H-FABP assay	Test principle	Sample type	Detection limit (ng/ml)	Time to result (min)	Regulatory status (RUO/CE)	Reference
Laboratory immunoassays						
Randox Evidence Investigator Cardiac Array	Biochip	Serum/plasma	0.15	20	CE	www.randox.com
Randox immunoturbidimetric H-FABP	Turbidimetry	Serum/plasma	0.75	14	CE	www.randox.com
Roche Diagnostics H-FABP	Turbidimetry	Serum/plasma	1.1	8	NR	
Markit-M H-FABP (Dainippon Pharmaceutical)	ELISA	Serum/plasma	1.25	75	RUO	www.bmassay.com
Hycult Biotechnology H-FABP	ELISA	Serum/ plasma/urine	0.1	50–120	RUO	www.hycultbiotech.com
Oxis Research H-FABP	ELISA	Serum	NR	90	RUO	www.oxisresearch.com
Point-of-care (bedside) tests[a]						
Rapicheck (Dainippon Pharmaceutical)	Lateral flow	Whole blood	6.2	15	CE	www.bmassay.com
CardioDetect (8sens.biognostic)	Lateral flow	Whole blood	7	15–20	CE	www.biognostic.de
QuickSens H-FABP (8sens. biognostic)	Lateral flow	Plasma/whole blood	0.6	15–20	CE	www.biognostic.de
H-FABP True Rapid Test (FABPulous)	Lateral flow	Whole blood	4	5	CE	www.fabpulous.com

Several characteristics (test principle, sample type, detection limit, time to result, regulatory status, and reference) of currently available point-of-care and laboratory assays for testing heart-type fatty acid-binding protein

Abbreviations: *CE* European conformity quality mark, *H-FABP* heart-type fatty acid-binding protein, *NR* not reported, *RUO* research use only

[a]Rapicheck, CardioDetect, and H-FABP True Rapid Test are qualitative tests; QuickSens H-FABP uses a reader providing a quantitative test result

vascular space. While larger cytosolic proteins such as CK-MB (86 kDa, i.e., five times larger than H-FABP) appear in the interstitium simultaneously with H-FABP, these larger proteins are delayed in their plasma appearance because the speed of reaching the plasma compartment is governed by the permeability of the endothelial barrier (which is dependent on protein size) and by lymph drainage (Van Nieuwenhoven et al. 1996). The troponins also appear in plasma markedly later than H-FABP, despite their relative small size (troponin T, 37 kDa, i.e., three times larger than H-FABP; troponin I, 22 kDa, i.e., 1.5 times larger than H-FABP). This is due to the fact that following cellular damage the troponins first need to be proteolytically cleaved from the contractile matrix. As a result, H-FABP is the earliest available plasma marker of cardiac injury (Glatz 1998; Glatz et al. 2002). Release of H-FABP from injured myocardium is essentially complete, indicating that infarct size can be estimated from the cumulative release of H-FABP into plasma (Glatz et al. 1994; De Groot et al. 1999). Because the subsequent clearance of H-FABP from plasma occurs via the kidneys, renal insufficiency could hamper such estimation (Wodzig et al. 1997b). Renal clearance of small proteins such as H-FABP is rapid and thus contributes markedly to maintaining a relatively low plasma reference concentration. In apparently healthy subjects, the plasma H-FABP concentration is between 1 and 2 ng/ml (Pelsers et al. 1999; Pagani et al. 2002; Niizeki et al. 2007; Bathia et al. 2009; Glatz and Mohren 2013), which is only <0.0001 % of the tissue content (estimated at 170 μmol/L (Vork et al. 1993) which is equivalent to 2500 μg/ml). As a result, there is a steep gradient of H-FABP from myocardial cells to plasma which adds to the high sensitivity of this marker for tissue injury detection. Circulating levels of H-FABP are somewhat higher in males (ca. 1.9 ng/ml) than in females (ca. 1.5 ng/ml) and slightly increase with age, especially after 50 years, which most likely is explained by the decrease in renal function in elderly people (Wodzig et al. 1997a; Glatz and Mohren 2013).

H-FABP and Troponin are Sensitive Markers of Myocardial Tissue Injury

Representative mean plasma release curves of H-FABP and cTnT and, for comparison, myoglobin are shown in Fig. 1. These curves were recorded for 15 patients with MI, treated with reperfusion therapy, from whom blood samples were obtained frequently during the first 24 h of hospitalization (Glatz et al. 2002; Pelsers et al. 2005). Peak plasma concentrations of FABP and myoglobin are reached at about 4 h after onset of symptoms, whereas for cTnT this takes about 15 h (see Fig. 1) and for CK-MB about 12 h (data not shown). Plasma FABP and myoglobin return to their respective reference values already within 24 h after MI, indicating the usefulness of both markers particularly for the assessment of a recurrent infarction (Van Nieuwenhoven et al. 1995) which might be missed by CK-MB or the troponins as these markers return much slower to their normal plasma value. Importantly, for MI patients not treated with thrombolytics, H-FABP peaks after approximately 8 h and remains elevated up to 24–36 h after chest pain onset (Van Nieuwenhoven

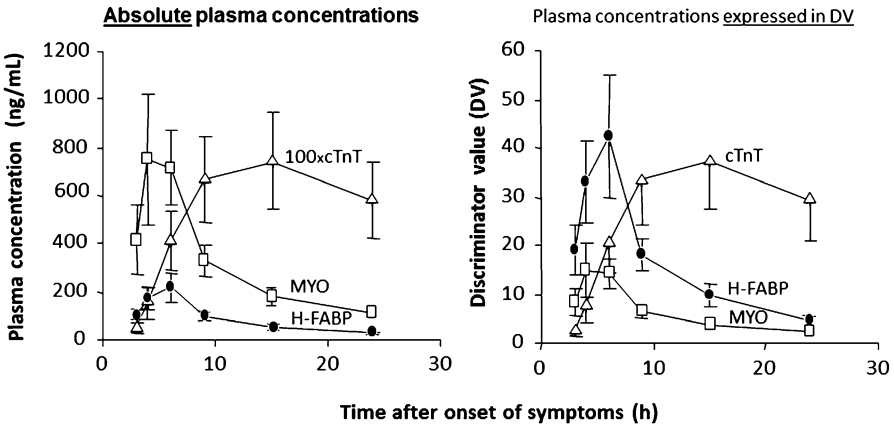

Fig. 1 Plasma release curves for three cardiac marker proteins. Mean plasma concentrations of heart-type fatty acid-binding protein (H-FABP) (●), myoglobin (MYO) (□) and cardiac troponin T (cTnT) (Δ) as a function of time after acute myocardial infarction for 15 patients who were treated successfully with reperfusion therapy and from whom serial blood samples were obtained up to 24 h after onset of symptoms. The data are presented as plasma concentrations in ng/mL (*left panel*) or relative to the upper reference limit for H-FABP (6 ng/mL), MYO (60 ng/mL) and cTnT (0.1 ng/mL) (*right panel*). Data refer to mean ± S.E.M (Adapted from Glatz et al. (2002), with permission). Abbreviations: *cTnT* cardiac troponin T, *DV* discriminator value, *h* hours, *H-FABP* heart-type fatty acid-binding protein, *MYO* myoglobin

et al. 1995). This latter finding indicates that the so-called diagnostic window of H-FABP for detection of myocardial injury in patients presenting with chest pain stretches to 24–36 h after onset of symptoms.

When expressed relative to the upper reference limit (or discriminator value) of each marker protein, it is clear that the rise in plasma concentrations is highest for H-FABP, closely followed by cTnT, and is much lower for myoglobin (see Fig. 1, right panel). This difference is explained mainly by the markedly lower relative plasma reference concentrations of H-FABP and cTnT when compared to myoglobin. Taken together, the sensitivity of H-FABP and cTnT for cardiac injury detection markedly outperforms that of myoglobin (see Fig. 1), as well as that of CK-MB (data not shown).

In more recent years, the performance of H-FABP for acute MI diagnosis has been centered on its comparison with cTnT/cTnI and/or hs-cTnT/hs-cTnI, thereby focusing on early exclusion of MI. Table 3 lists the larger and more recent clinical studies that have directly compared H-FABP and troponin applying quantitative assays that are currently in use. Quantitative tests are independent of the cutoff level that is chosen and thus allow a proper evaluation of the markers. In contrast, the performance of a qualitative test (such as a PoC test) depends on the assigned cutoff (see discussion below).

The emerging overall picture is that the area under the receiver operating characteristic (ROC) curve (AUC) for H-FABP is similar to that for the conventionally analyzed troponins (see Table 3, upper part). However, when analyzed with high-

Table 3 Diagnostic performance of heart-type fatty acid-binding protein (H-FABP) and cardiac troponin (cTn) for acute myocardial infarction

Reference	Admission time[a] (h)	H-FABP				cTn (cTnT or cTnI)			
		Cutoff (ng/ml)	AUC	Sensitivity	Specificity	Cutoff (ng/ml)	AUC	Sensitivity	Specificity
Conventional cTn tests									
Mion et al. 2007 ($n = 132$)	3.8 (FR 1.5–13)	5.8	0.92	0.83	0.93	0.032 cTnI	0.75	0.55	0.98
McCann et al. 2008 ($n = 415$)	0–4	5.0	0.77	0.73	0.71	0.03 cTnT	0.78	0.55	0.95
	5.3 (IQR 2.7–8.9)		0.74	0.76	0.61		0.88	0.75	0.94
Haltern et al. 2010 ($n = 94$)	0–4	7.3	0.76	0.86	0.66	0.03 cTnT	0.71	0.42	1.00
	4 (CI 3–6)		0.71	0.71	0.65		0.87	0.74	1.00
Alhadi and Fox 2010 ($n = 100$)	<6	5.0	NR	0.80	0.92	0.032 cTnI	NR	0.56	0.81
Gururajan et al. 2010 ($n = 485$)	<6	17.7	0.97	0.87	0.93	0.032 cTnI	0.77	0.54	0.95
Kurz et al. 2011 ($n = 94$)	6.0 (IQR 2.5–15)	6	0.81	0.89	0.62	0.03 cTnT	0.72	NR	NR
Body et al. 2011	3.5 (IQR 1.8–7)	58	0.86	0.75	0.89	0.055 cTnI	0.70	0.42	0.96
Keller et al. 2011 ($n = 1{,}818$)	4.3 (IQR 2.0–13)	5.8	0.89	NR	NR	0.032 cTnI	0.92	0.79	0.95
McMahon et al. 2012 ($n = 1{,}128$)	0–3	5.2	0.84	0.64	0.84	0.037 cTnT	0.76	0.50	0.93
	3–6		0.89	0.85	0.89		0.85	0.68	0.94
	6–12		0.94	0.90	0.94		0.90	0.81	0.94
	12–24		0.97	0.90	0.91		0.98	0.96	0.94
	24–48		0.91	0.63	0.91		0.98	0.97	0.95
	>48		0.87	0.66	0.91		0.94	0.88	0.94
Ruff et al. 2013 ($n = 343$)	8.7 (IQR 5–14)	5	0.78	0.63	0.79	0.10 cTnI	0.91	0.77	0.97
High-sensitive (hs-)cTn tests									
Kurz et al. 2011 ($n = 94$)	6.0 (IQR 2.5–15)	6	0.81	0.89	0.62	0.014 hs-cTnT	0.82	0.82	0.76

Keller et al. 2011 ($n = 1{,}818$)	4.3 (IQR 2.0–13)	5.8	0.89	NR	NR	0.030 hs-cTnI	0.99	0.82	0.92
Eggers et al. 2012 ($n = 360$)[b]	<8	5.8	0.71	0.39	0.95	0.014 hs-cTnT	0.74	0.79	0.75
Kagawa et al. 2013 ($n = 114$)	NR	6.2	0.59	0.78	0.22	0.028 hs-cTnI	0.89	0.81	0.79
Reiter et al. 2013 ($n = 1{,}074$)[b]	<3	4.2	0.85	NR	NR	0.014 hs-cTnT	0.92	NR	NR
	<12		0.84	0.72	0.80		0.94	0.93	0.77
Ruff et al. 2013 ($n = 343$)	8.7 (IQR 5–14)	5	0.78	0.63	0.79	0.040 hs-cTnI	0.96	0.92	0.92
Cappellini et al. 2013 ($n = 67$)	<8	3.5	0.84	1.00	0.39	0.014 hs-cTnT	0.81	0.81	0.56
Collinson et al. 2014 (Heart) ($n = 838$)	3.7 (IQR 2.6–5.8)	3	0.84	0.65	0.94	0.040 hs-cTnT	0.92	0.78	0.96
Bank et al. 2015 (ACB) ($n = 453$)	3.0 (IQR 1.8–6.8)	7	0.73	0.54	0.81	0.014 hs-cTnT	0.88	0.71	0.90
Jacobs et al. 2015 (ACB) ($n = 584$)	3.0 (IQR 1.8–5.1)	4	0.81	0.60	0.86	0.045 hs-cTnI	0.88	0.68	0.96

Comparison of recent clinical trials using quantitative tests of H-FABP and cTn and acute myocardial infarction as an outcome. Cutoff level (being the 99th percentile upper reference level used), area under the curve, sensitivity, and specificity are given for both H-FABP and cTn

Abbreviations: *AUC* area under receiver operating characteristic (ROC) curve, *CI* 95 % confidence interval, *cTn* cardiac-specific troponin, *cTnI* cardiac-specific troponin I, *cTnT* cardiac-specific troponin T, *FR* full range, *h* hours, *H-FABP* heart-type fatty acid-binding protein, *hs-cTnI* high-sensitive cardiac-specific troponin I, *hs-cTnT* high-sensitive cardiac-specific troponin T, *IQR* interquartile range, *non-STEMI* non-ST-elevated myocardial infarction, *NR* not reported

[a]Median time from symptom onset to admission, with full range (FR), 95 % confidence interval (CI), or interquartile range (IQR)

[b]Non-STEMI patients only

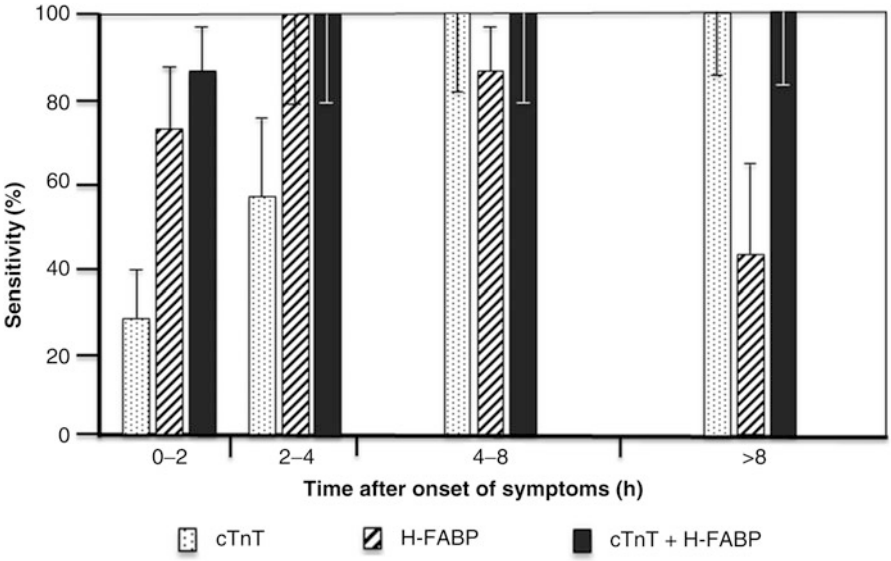

Fig. 2 Diagnostic performance of plasma heart-type fatty acid-binding protein (H-FABP) and cardiac troponin T (cTnT) as a function of time after onset of symptoms suggestive of acute coronary syndrome. Sensitivities for myocardial infarction of the markers separately and a combined approach (either one positive) are presented with varying symptom durations (Reprinted from Haltern et al. (2010), Copyright (2010), with permission). Abbreviations: *cTnT* cardiac troponin T, *h* hours, *H-FABP* heart-type fatty acid-binding protein

sensitivity assays, troponin exhibits a significantly greater AUC than H-FABP (see Table 3, lower part). Irrespective of the assay format used, the overall specificity is higher for troponin than for H-FABP; however the overall sensitivity is lower for troponin than for H-FABP. When distinction is made for patients seen early after onset of symptoms (e.g., within 3–4 h) versus patients admitted to the emergency room at a later point in time, the performance of H-FABP is significantly better in the first hours after MI (McCann et al. 2008; Haltern et al. 2010; McMahon et al. 2012; Reiter et al. 2013). This finding is illustrated in the report by Haltern et al. (2010), who evaluated patient groups according to symptom duration (see Fig. 2). Sensitivity of H-FABP at presentation was > twofold higher than that of conventional cTnT when symptom duration was <2 h and increased to 100 % in the group with symptom duration of 2–4 h. In this latter group, the sensitivity of cTnT was only 55 %. For patients admitted >4 h, the sensitivity of the two markers switched: the sensitivity of cTnT reached 100 %, while that of H-FABP decreased significantly (see Fig. 2). The data reported by McMahon et al. (2012) (see Table 3) reveal a similar bell-shaped curve for the sensitivity of H-FABP as a function of the admission delay. The corollary is that combining H-FABP and cTnT (i.e., either marker elevated) provides a significant improvement in sensitivity for patients presenting <4 h after symptom onset while being maintained at 100 % for patients presenting >4 h (see Fig. 2). These data indicate the usefulness of combining H-FABP and

troponin for improved early diagnosis of ACS. In conclusion, each of the biomarkers has its own characteristics, with H-FABP being the preferred marker to diagnose AMI in the early hours after onset of symptoms and (hs-)cTnT or (hs-)cTnI the preferred marker from 3 to 4 h onward after presentation.

Combining H-FABP and Troponin for Ruling Out ACS

As discussed above, especially in the early hours after onset of symptoms, H-FABP shows a superior sensitivity to troponin, even when high-sensitivity troponin tests are used. However, published data indicate that measurement of H-FABP alone cannot enable a safe rule-out of AMI at presentation, i.e., NPV >97–98 % (see Table 3 and references therein). This is illustrated by the results of a meta-analysis of 16 studies including 3,709 patients with suspected AMI, which reported for H-FABP a pooled sensitivity of 84 % (95 % confidence interval (CI) 76–90 %) and a pooled specificity of 84 % (95 % CI 76–89 %) (Bruins Slot et al. 2010). As mentioned above, combining H-FABP and cardiac-specific troponin (cTn) significantly improves the diagnostic sensitivity (see Fig. 2), especially when using hs-cTnT or hs-cTnI assays. A systematic review by Carroll et al. (2013) on four clinical studies (total of 1,598 patients) on combinations of quantitatively assessed H-FABP and cTn versus cTn alone at presentation (Mion et al. 2007; McCann et al. 2008; Haltern et al. 2010; Body et al. 2011) revealed that the addition of H-FABP to cTn increased sensitivity from 42–75 % to 76–97 % but decreased specificity from 95–100 % to 65–93 %. In a subsequent review, Lippi et al. (2013) analyzed eight studies (totaling 2,735 patients), including four studies applying qualitative H-FABP tests, to observe that the addition of H-FABP to cTn increased pooled sensitivity from 73 % to 91 % which however was counterbalanced by a decreased pooled specificity from 94 % to 82 %. These reviews did not include the earlier extensive study (1,818 patients) described by Keller et al. (2011), combining quantitative H-FABP and hs-cTnI. These investigators reported that the addition of H-FABP to hs-cTnI increased sensitivity from 73 % to 85 %, decreased specificity from 95 % to 91 %, and decreased positive predictive value (PPV) from 66 % to 60 % but increased the NPV from 95.9 % to 97.6 % (Keller et al. 2011). The latter indicates that the NPV of the combined test fulfills the diagnostic requirements for application as a rule-out parameter. In this study, the time between chest pain onset and admission to the emergency room (first blood sample) was 4.3 h (range 2.0–13 h). It was not examined whether the performance of the combined markers is dependent on the time of presentation of the patient.

In a study by Ruff et al. (2013), similar data were found. The addition of H-FABP to a conventional cTnI assay significantly enhanced both the sensitivity (from 77 % to 92 %) and NPV (from 92 % to 97 %) of MI diagnosis. When H-FABP was added to hs-cTnI, the overall diagnostic accuracy was not improved when compared to the performance of hs-cTnI alone, but in early presenters (<6 h after onset of symptoms) the combination did improve both sensitivity and NPV (each to 100 %) (Ruff et al. 2013). In contrast, in the recent study by Reiter et al. (2013), no synergistic

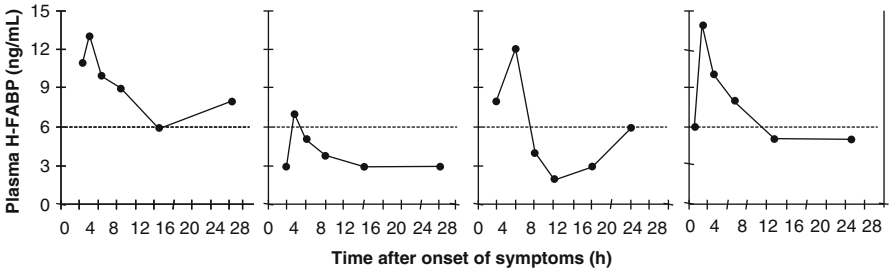

Fig. 3 Plasma release curves of heart-type fatty acid-binding protein (H-FABP) for patients with unstable angina pectoris. Examples of individual patients clinically diagnosed as having unstable angina pectoris. In each case plasma H-FABP was elevated above its discriminator value (of 6 ng/mL; *dashed line*) and shows a typical "rise and fall" pattern suggesting the occurrence of minor myocardial injury. Data obtained from the *EuroCardi* multicenter trial (Adapted from Glatz et al. (2002), with permission). Abbreviations: *h* hours, *H-FABP* heart-type fatty acid-binding protein

performance of H-FABP and hs-cTnT was reported, but in this study patients with ST-segment elevation in the initial ECG were excluded.

Minimal Myocardial Injury

Patients with a clinical diagnosis of UAP often show an elevated plasma concentration of H-FABP (Katrukha et al. 1999; Valle et al. 2008). Analysis of serial plasma samples after onset of symptoms has revealed that, also in these patients, there is a characteristic rise and fall of plasma markers reminiscent of their release from injured myocardium (see Fig. 3; Glatz et al. 2002). This reflects the sensitivity of the marker H-FABP for myocardial cell injury and should not be labeled as false positive. Patients with such minimal (or minor) myocardial injury – also referred to as subclinical myocardial injury – may have a prognosis as serious as do patients with definite MI (Hamm et al. 1992) and therefore may benefit from similar medical treatment. Interestingly, H-FABP has also been applied as plasma marker to identify minimal myocardial injury in nonalcoholic fatty liver disease (Basar et al. 2013).

H-FABP and Kidney Function

H-FABP is cleared by glomerular filtration in the kidneys, and thus, H-FABP values can be elevated in case of severe kidney damage (eGFR <30 ml/min). After myocardial injury, H-FABP is eliminated by renal clearance and values return to normal after 24–36 h. Therefore, it can be used up to 24 h after onset of complaints.

Primary Care: Extension of Diagnostic Strategies in ACS and Potential Role of H-FABP

Because in primary care the median period between onset of symptoms of MI and diagnostic assessment by the GP in most countries is 2–3 h (Hooghoudt et al. 1998) and only in rural areas will be longer, the troponins cannot be used as the lead parameter for stratification of patients. Furthermore, as stated above, although troponin levels are of high importance in ruling out AMI in a secondary care setting, unacceptable practical limitations are faced in using troponin in a primary care setting. Therefore, attention is drawn to alternative biomarkers. Of the biomarkers studied to date, H-FABP is placed among the earliest of plasma markers (Dekker et al. 2010). In case of AMI, elevation of plasma H-FABP can be detected within the first 1–2 h after onset of complaints (Glatz 1998; Mad et al. 2007). Venous levels are increased to concentrations up to 40-fold the normal concentration (Pelsers et al. 2005). Especially in cases of AMI, H-FABP levels correspond impressively to hs-cTnT levels (Willemsen et al. 2015). Therefore, H-FABP may have meaningful potential in improving the triage of patients suspected of AMI.

The main requirement for any biomarker test in primary care is the possibility to measure and obtain a result within several minutes at the point of care, combined with a potency to rule out AMI with a high NPV that should be >97–98 %. The NPV largely depends on sensitivity of the test and prevalence of the disease and less on specificity. At this moment, studies reviewing early PoC markers are characterized by methodological imperfections (Bruins Slot et al. 2013a). The function of H-FABP and other early markers combined with signs and symptoms in risk classification in a low-prevalence setting such as primary care is still to be determined (Than et al. 2011; Tomonaga et al. 2011; Reiter et al. 2013). When H-FABP testing is combined with signs and symptoms in a *diagnostic algorithm*, NPV hypothetically improves, and thus the number of patients that are referred by a GP, but turn out to have no ACS, could be reduced. Even with a moderate amount of false-positive results, such an algorithm could improve daily practice since currently the majority of patients without underlying ACS are referred to secondary care facilities.

A primary care study evaluating a PoC test on H-FABP did not lead to implementation of PoC testing in daily practice. Limiting test characteristics in this study were insufficient sensitivity (using a test cutoff point for H-FABP of 7 ng/ml), robustness (11 % invalid results), and a time to result of 15–20 min that is considered too long for acute situations in general practice. However, the PoC device for H-FABP used in this study used a cutoff value of 7 ng/ml, which is above the 99th percentile of 5.7 ng/ml as found in a normal reference population (Glatz and Mohren 2013). Retrospective measurement of plasma H-FABP values revealed added value of H-FABP, although insufficient to reach a NPV of 98 % or more.

Reported 99th percentile values for H-FABP range from 5.2 to 7.3 ng/ml (Pelsers et al. 1999; Pagani et al. 2002; Niizeki et al. 2007; Bathia et al. 2009; Glatz and Mohren 2013; Haltern et al. 2010). However, when derived from ROC curves, optimal cutoff levels for H-FABP at presentation to discriminate AMI from other

Table 4 Diagnostic values of H-FABP and hs-cTnT at different cutoff points

Biomarker	Cutoff value	Sensitivity	Specificity	Expected NPV in primary care, with a prevalence of AMI of 17 %
H-FABP	4 ng/ml	0–3 h 56,1 %	0–3 h 67,5 %	0–3 h 88,3 %
		3–24 h 91,5 %	3–24 h 80,7 %	3–24 h 97,9 %
hs-cTnT	14 ng/ml	0–3 h 56,3 %	0–3 h 70 %	0–3 h 88,7 %
		3–24 h 91,5 %	3–24 h 68,4 %	3–24 h 97,5 %
H-FABP	7 ng/ml	0–3 h 20,8 %	0–3 h 95 %	0–3 h 85,4 %
		3–24 h 76,3 %	3–24 h 91,2 %	3–24 h 94,9 %
hs-cTnT	50 ng/ml	0–3 h 14,6 %	0–3 h 92,5 %	0–3 h 84,1 %
		3–24 h 78 %	3–24 h 94,7 %	3–24 h 95,5 %
hs-cTnT	100 ng/ml	0–3 h 8,3 %	0–3 h 100 %	0–3 h 84,2 %
		3–24 h 72,9 %	3–24 h 98,2 %	3–24 h 94,6 %

Sensitivity, specificity, and negative predictive value (NPV) for acute myocardial infarction of H-FABP and hs-cTnT at different cutoff points are given. Patients with an estimated glomerular filtration rate below 30 ml/min were excluded. NPV is calculated using a prevalence of AMI of 17 %, as has been reported among patients presenting with chest pain in primary care (Willemsen et al. 2015 extended data)

AMI acute myocardial infarction, *h* hours, *H-FABP* heart-type fatty acid-binding protein, *hs-cTnT* high-sensitive cardiac-specific troponin T, *NPV* negative predictive value

causes of chest pain generally are lower, ranging from 3.3 ng/ml (Freund et al. 2012) to 4.4 ng/ml (Reiter et al. 2013). Similarly, in a recent study of 218 consecutive patients with new-onset chest pain seen by a GP, the ROC-derived optimal cutoff for H-FABP was 4.0 ng/ml (Willemsen et al. 2015). Improved diagnostic performance of H-FABP using such lower values has been documented and advocated (Ruff et al. 2013; Cappellini et al. 2013; Carroll et al. 2013).

As a consequence, recently, a new PoC H-FABP test was designed to overcome the earlier mentioned limitations, by lowering the cutoff value to 4 ng/ml in a secondary care population, where 50 % of patients were diagnosed with AMI (Willemsen et al. 2015). This is below the 99th percentile of H-FABP – in a normal reference population determined by the manufacturer in a healthy reference population of blood donors between 40 and 70 years of age (Glatz and Mohren 2013). Setting the cutoff value at 4 ng/ml leads to a diagnostic performance equaling that of hs-cTn. Thus, H-FABP has the same properties as hs-cTn with the generally used cutoff value of 14 ng/ml for high-sensitive troponin T, used as gold standard for AMI, whereas the earlier used PoC H-FABP test with a cutoff point of 7 ng/ml correlates to high-sensitive troponin T with a cutoff point of 50 ng/ml (see Table 4).

Calculated NPV in a primary care population (with an incidence of ACS of 20 % or less) would reach 88.3 % in patients with a duration of complaints of less than 3 h and 97.9 % in patients with a duration of complaints of 3–24 h. Currently this PoC H-FABP test is studied in primary care (Willemsen et al. 2014). At the cutoff point of 4 ng/ml, this PoC H-FABP test is regarded as positive by its users in 95 % of cases, and coefficient of variance (CV) is <10 %. Furthermore, decrease of invalid results

to an amount of less than 2 % has improved robustness, and a time to result of 5 min increases usability in an acute setting.

Secondary Care: Extension of Diagnostic Strategies in ACS and Potential Role of H-FABP

In secondary care, where safe rule-out based on one measurement would be preferable above several measurements with a given time interval, ongoing studies focus on the potency of hs-cTn as well as the potency of other biomarkers to be combined with a single hs-cTn measurement. cTnT or cTnI measurement has become the cornerstone of diagnosing MI in secondary care (Hamm et al. 2011; Thygesen et al. 2012). Adding copeptin or H-FABP to troponin in an early phase in emergency room settings increases sensitivity for ACS, but so far the combination has failed to safely rule out ACS in an early stage (Body et al. 2011; Charpentier et al. 2011). Until recently, troponin assays have gained sensitivity due to usage of highly sensitive techniques (resulting in hs-cTn measurements). The additional value of H-FABP testing besides hs-cTn in some studies is small or unclear (Carroll et al. 2013; Lippi et al. 2013; Vaidya et al. 2014; Bank et al. 2015). Several studies however have described an added value of H-FABP when measured besides troponin in an emergency room setting in an early phase (McMahon et al. 2012; Carroll et al. 2013; Gami et al. 2015; Jacobs et al. 2015). As a solitary rule-out test at admission, hs-cTnT outperforms H-FABP slightly, but H-FABP tested in addition to hs-cTnT leads to an increase of sensitivity compared to hs-cTnT alone (Collinson et al. 2014). Recently, promising results were published of hs-cTn measurement combined with H-FABP measurement, ECG findings, and several clinical findings in early rule-out of severe underlying disease in patients presenting with chest pain (Body et al. 2014a, b).

Concluding Remarks

Twenty-five years after the first report on the potential use of H-FABP as a plasma biomarker for myocardial injury (Glatz et al. 1988), a large number of clinical studies performed by a variety of researchers applying a multitude of assay formats have now documented that H-FABP (i) is rapidly released from injured myocardium to be the earliest available plasma marker after an ischemic insult; (ii) shows a sensitivity for cardiac injury detection that is similar to that of cTn and markedly better than that of all other known cardiac marker proteins; (iii) for AMI diagnosis or exclusion shows added value on top of the recommended markers cTnT or cTnI, even when these are determined by high-sensitivity assays, with the added value being larger for patients presenting early (<4 h) after onset of symptoms; and (iv) in early (<4 h) presenters may be suited as stand-alone diagnostic test for safely ruling out AMI. The latter is relevant especially for primary healthcare and would markedly increase cost-effectiveness of AMI diagnosis but awaits appropriate prospective trials.

H-FABP also is established to be a robust early predictor of future cardiovascular events or mortality independent of other cardiac risk factors including plasma troponin. As a result, the clinical utility of H-FABP both as early marker for the evaluation of suspected ACS and as prognostic marker is undisputed, especially when it is part of a diagnostic assessment combining several early findings (Pelsers et al. 2005; Viswanathan et al. 2012; Body 2012; Lackner 2013; Renneberg et al. 2013; Body et al. 2014b).

Future Perspective

Despite the strong data available for H-FABP on its performance as biomarker for early triaging of patients with chest pain, FABP has not (yet) gained widespread use. Likely this will change in the near future, when more PoC tests (that employ optimal cutoff levels) and tests for clinical chemistry analyzers will become available. This would hold especially for tests that would give results within the time of a typical primary care consultation of 7–10 min. H-FABP then may be adopted as "early" plasma marker to be applied alone or beside a "late" marker such as cTnT or cTnI. In emergency care diagnostics, the future focus will be on very early exclusion of AMI. H-FABP could be excellently suited for this purpose especially when measured in combination with several other early findings in patients presenting with chest pain. In this way, a major reduction of costs otherwise spent on hospitalization and extensive diagnostic follow-up of non-AMI patients is enabled (Body et al. 2014a,b). Given the large numbers of patients who present with chest pain (for instance, in Germany >750,000 annually) (Nilsson et al. 2003), due to its diagnostic accuracy and due to the reliability of contemporary PoC tests, H-FABP may well become part of a new golden standard for improving quality yet reducing cost of care.

Potential Applications to Prognosis and Other Diseases or Conditions

Besides its (future) use as a biomarker for acute ischemic heart disease, H-FABP has been reported to be valuable as a prognostic marker to assess future risks on major cardiac events in patients.

H-FABP is an early and independent predictor of future cardiovascular events and thus may help to improve long-term risk stratification of patients with acute chest pain (O'Donoghue et al. 2006; Kilcullen et al. 2007; McCann et al. 2009; Garcia-Valdecasas et al. 2011; Viswanathan et al. 2010). Increased plasma H-FABP is a robust predictor of major cardiac events (such as death or MI) within 2 years in patients with chest pain and remained significant in a multivariate analysis that included both various plasma biomarkers and echocardiographic assessment of cardiac morphology and function (O'Donoghue et al. 2006; Kilcullen et al. 2007; McCann et al. 2009; Reiter et al. 2013). The

NPV regarding 1-year and 2-year mortality was 99 % (CI 98–100) and 98 % (CI 96–99), respectively, for plasma H-FABP <2.7 ng/ml (Ruff et al. 2013). H-FABP plasma concentration identifies patients at risk for death and major cardiac events even when troponin and/or NT-proBNP are not elevated (O'Donoghue et al. 2006; Kilcullen et al. 2007; Reiter et al. 2013). These findings are consistent when H-FABP is compared to troponin values obtained with hs-cTnT assays (Reiter et al. 2013; Viswanathan et al. 2010). These findings confirm H-FABP to be a rapidly released and sensitive biomarker of minor myocardial injury as caused by ongoing and recurrent myocardial ischemia.

Similarly, in patients with congestive heart failure, plasma H-FABP identifies those at high risk for future cardiac events, independent of cTnT (Niizeki et al. 2008; Kutsuzawa et al. 2012). In patients undergoing coronary artery bypass grafting (CABG), H-FABP is a superior independent predictor of postoperative mortality and ventricular dysfunction (Muehlschlegel et al. 2010). H-FABP appears a promising early biomarker also for risk stratification of normotensive patients with acute pulmonary embolism and was found to perform markedly better than either plasma cTnT or right ventricular dysfunction (Kaczynska et al. 2006; Puls et al. 2007; Dellas et al. 2010; Boscheri et al. 2010). In case of a negative H-FABP test, these patients had an excellent prognosis regardless of echocardiographic findings, while patients with an elevated plasma H-FABP had a complication rate of 23 %. In sepsis, H-FABP appears to be an independent prognostic factor for 28-day mortality (Jo et al. 2012; Zhang et al. 2012). Finally, an elevated H-FABP concentration measured during the follow-up of MI (median of 20 days post-MI) predicted long-term all-cause mortality and readmission for heart failure significantly better than did plasma cTnT, for a time interval up to 5 years post-MI (Matsumoto et al. 2013).

In conclusion, when plasma H-FABP is elevated, a decreased clinical outcome can be expected in patients with chest pain, congestive heart failure, and pulmonary embolism, in patients after CABG, and in post-MI patients.

Summary Points

- An increased number of patients present with chest pain of unknown cause. There is a need for additional diagnostic tools to facilitate a cost-effective strategy for these patients.
- Plasma marker proteins of myocardial injury have become the most reliable parameter for diagnosis of patients with chest pain.
- The small but abundant myocardial heart-type fatty acid-binding protein (H-FABP) appears a promising plasma marker for myocardial injury detection.
- Among plasma marker proteins, H-FABP and troponin display the highest sensitivity for detection of myocardial injury.
- Subclinical myocardial injury was found to result in elevated plasma H-FABP concentrations.
- No plasma marker exists that is ideally suited for myocardial injury detection.

- Because of its more rapid release from injured myocardium when compared to troponin, H-FABP is applicable in particular for early monitoring of myocardial injury.
- A multimarker approach, i.e., combining H-FABP and troponin, markedly improves diagnostic performance of the markers when compared to each of the markers alone.
- Because point-of-care tests with low cutoff values between positive and negative are available for H-FABP, H-FABP is a promising biomarker for ruling out myocardial infarction in this setting.
- Several point-of-care tests for H-FABP have been described and are expected to facilitate diagnosing patients with chest pain especially in primary care.
- Further research in primary care and early after presentation in secondary care is needed to define the future role of H-FABP in ruling out acute coronary syndrome in patients with chest pain of unknown cause.

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