Q J Med 2004; **97**:187–198
doi:10.1093/qjmed/hch037

Review

QJM

Do we need additional markers of myocyte necrosis: the potential value of heart fatty-acid-binding protein

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Summary

Heart fatty-acid-binding protein (FABP) is a small cytosolic protein that is abundant in the heart and has low concentrations in the blood and in tissues outside the heart. It appears in the blood as early as 1.5 h after onset of symptoms of infarction, peaks around 6 h and returns to baseline values in 24 h. These features of H-FABP make it an excellent potential candidate for the detection of acute myocardial infarction (AMI). We review the strengths and weaknesses of H-FABP as a clinically applicable marker of myocyte necrosis in the context of acute coronary syndromes.

Introduction

The fatty-acid-binding proteins (FABP) are a family of cytosolic proteins that shows a large degree of structural homology. Discovered by Ockner in 1972 in studies on the intestinal absorption of fatty acids,[1] they are called FABP because they exhibit a high affinity for the non-covalent binding of fatty acids. These proteins are widely distributed and are present in the fatty-acid-metabolizing tissues of many mammals. Their presence has also been reported in various species, including birds, insects and fish.[2] There are several types, and all have low molecular mass (12–15 kDa), but they differ markedly in tissue distribution, concentration within tissue, isoelectric point (PI), binding capacity, and binding specificity.[3–10] The FABP are relatively tissue-specific, and are designated by a letter that refers to their tissue of origin, e.g. L-FABP, H-FABP, I-FABP, referring to liver, heart and intestine FABP, respectively;[11] tissue-specific FABP have also been reported in muscle, adipose tissue, kidney, brain and nerve cells. Tissue-specific FABP such as liver (L-FABP) and intestinal (I-FABP) have been used to detect pathologies in these tissues using specific antibodies raised against these proteins.[12,13] Different FABP share between 30–80% amino acid sequence homology. The heart and the liver contain the highest concentrations of these proteins.[9]

Function

Fatty acids are the major energy source of the heart.[14] They are also important molecules for the synthesis of membrane lipids and lipid mediators such as prostaglandins, leukotrienes and thromboxanes.[15] In general, 50–80% of the heart's energy is provided by lipid oxidation. The heart is a poor fatty acid synthesizer, and contributes only 0.1% of total body fatty-acid synthesis,[16] but accounts for 10% of the total body turnover of fatty acids.[17]

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Fatty acids are insoluble in the intravascular and extravascular space, and also in the intracellular space. In plasma they are transported bound to albumin, or as part of the lipoproteins complex.[14,18] Heart-FABP may constitute the intracellular equivalent of albumin for the intracellular transport of the insoluble fatty acids within the cells. These proteins are truly cytoplasmic, in the sense that they do not exist anywhere else (e.g. plasma or extracellular space) under normal conditions.[19,20] Recent work has suggested more complex regulatory functions for these proteins beyond lipid transport,[21–27] but the precise physiological functions of these abundant proteins are not fully understood.

Early diagnosis of acute coronary syndrome and its impact on patients' care

Early diagnosis of acute coronary syndrome based on multiple samples would contribute to patients' care in the following ways.

1. Triage of patients from accident and emergency department

Biochemical markers of early damage can help with the triage of patients from the emergency department. Those patients with positive results for ischaemia need to be admitted to the CCU or to a monitored bed. Those with 'true negative' results (i.e. after sufficient time for liberation of marker into the circulation) can be considered for early discharge if there is a low probability of ischaemia and of severe coronary artery disease, and the patient remains free of recurrence. These strategies will optimize the effective use of expensive resources in the CCU and other acute units for the appropriate groups of high and moderate risk patients.[28–30]

2. Acute myocardial infarction and non-diagnostic electrocardiogram

Early cardiac markers can be helpful in the diagnosis of AMI in the following situations when there is a high clinical suspicion of infarct. However the diagnostic value of the admission ECG may be limited: (i) when the ECG cannot be interpreted or has reduced diagnostic accuracy, e.g. the presence of conduction disorders including left bundle branch block (LBBB) or paced rhythm; (ii) if Q waves and ST-T changes are already present, e.g. old infarcts and digoxin effects, respectively; (iv) with ST-T changes of marked left ventricular hypertrophy (LVH); (iv) in posterior infarct or right ventricular infarct, which may produce no clear-cut diagnostic ECG changes on the standard 12-lead ECG; (v) when diagnostic changes of AMI are present in one lead only; and (vi) In the 30% of patients who have no diagnostic changes on their admission ECG.[28–32] In clinical practice today reperfusion therapy, thrombolysis or percutaneous coronary intervention (PCI), is only given to patients with clinical evidence of ischaemia and ST segment elevation.

3. Unstable angina and non-Q-wave myocardial infarction

Clinical trials have shown most benefit from treatment in the unstable angina (UA) and non-Q-wave MI groups with positive biochemical marker evidence of ischaemia. Those patients with no biochemical marker evidence of ischaemia show least benefit (or no benefit) from treatment compared to placebo.[33] Cardiac markers can help with risk stratification of patients early in the course of ischaemia.[33,34] In those patients with UA and non-Q-wave MI, early diagnosis results in the admission of these patients to the CCU or to a monitored bed in a higher dependency area. Administration of antithrombotic agents (aspirin, clopidogrel, LMWH, and GPIIb/IIIa receptor antagonists) is associated with a significant reduction of subsequent complications (AMI and death).[35,36] In addition to early identification and implementation of treatment, further risk stratification in these patients can guide the use of exercise tolerance testing, perfusion scans or angiography and, where appropriate, PCI or CABG (coronary artery bypass grafting).

4. Prevention of inappropriate discharge of patients

In the very early stages of AMI, some patients may present with atypical chest pain and non-diagnostic ECG changes. Without an appropriately timed biochemical marker to rule out AMI, these patients could be misdiagnosed and inappropriately discharged. Based on previous studies, between 2% and 10% of patients with AMI are discharged from A&E departments.[37–39] This is more likely to happen in high-volume medical institutions where the turnover of patients is high and there is limited availability of beds. Common features of cases of missed AMI include factors such as age (young patients), sex (females), ethnic factors, atypical history of chest pain, absence of previous cardiac history, and being reviewed by inexperienced physicians.[38,39]

5. Financial implications

Previous studies estimated that less than 30% of patients admitted to the CCU with suspected AMI were eventually diagnosed with AMI.[37] Conservative policies that opt for the safe admission of patients without clear-cut diagnosis of ischaemia, rather than risking inappropriate discharge, result in the admission of a large number of patients without ACS. The cost of caring for such patients is very substantial.[37] Decisions based on cardiac markers for the triage of patients result in a considerable reduction of this financial burden without compromising the safety of patients.[40]

Heart fatty-acid-binding protein

Heart-FABP is a small (15 kDa) soluble non-enzyme protein. It is composed of 132 amino acids.[41] It is one of the most abundant proteins in the heart, and comprises 5–15% of the total cytosolic protein pool in the aqueous cytoplasm. This is equal to 0.5 mg/g wet weight of tissue.[42–45] Minor concentrations of H-FABP specific to the mitochondrial function have also been reported.[46] The gene is located on chromosome 1.[47] Heart-FABP binds two molecules of fatty acids, and is involved with the delivery of fatty acyl coenzyme A for oxidation with the generation of energy in the mitochondria.[42] Myocardial ischaemia results in a significantly higher level of fatty acids in the plasma and the myocardial tissue, which can be harmful to the heart.[48–51] The presence of H-FABP may serve a protective function for the myocardial cells against the oxidation of these fatty acids while still having these substances readily available for the metabolic needs of the cell. During ischaemia (e.g. AMI), H-FABP leaks out of myocardial tissue and the concentration increases in plasma.[44] The leakage of H-FABP from the myocardium may make the myocardium more vulnerable to the harmful effects of fatty acids during reperfusion, and may account for some of the complications seen during reperfusion, e.g. arrhythmias. Some reports have suggested another protective role for H-FABP, as scavengers of free radicals that are present in the heart during ischaemia.[52,53] H-FABP exists in high concentrations in the heart only. However, this protein is not totally cardiac-specific and occurs in other tissues, although at much lower concentrations.[54,55] It occurs in skeletal muscles in concentrations varying between 0.05 and 0.2 mg/g wet weight of tissue, depending on muscle fibre type studied.[45] It has also been reported in very low concentrations in tissues such as the kidney, aorta, testes, mammary glands, placenta, brain, adrenal glands, adipose tissue, and stomach.[54–56] However, the detection of H-FABP in these tissues does not necessarily means its presence in all cells of that tissue. Also, the evidence was obtained in some of these studies by immunohistochemical methods using antibodies to H-FABP. The different FABP from heart, liver and intestine share between 20–35% amino acid sequences homology, and heart, nerve, and adipose tissue FABP share 60–80% amino acid sequence homology.[9] Antibodies raised against heart, liver, or intestine in the earlier studies may thus have up to 5% cross-reactivity with each other, and have a detection limit of around 1 ng/ml. It is therefore possible that cross-reactivity with other FABP, or other as yet unidentified proteins, in these tissues is an alternative explanation for the reported presence of H-FABP in these tissues.[57–61] The newer assays have a much improved sensitivity and can detect H-FABP in concentrations as low as 0.25 ng/ml; the cross-reactivity with other tissues FABP is $< 0.005\%$.[62,63] The use of these newer assays might show a more accurate picture of the true distribution of H-FABP in the various tissues outside the heart.

The rationale for the use of H-FABP as a marker for the early diagnosis of myocardial injury

The rationale for the use of H-FABP as a marker for the early diagnosis of myocardial injury is based on the following features: (i) the presence of this soluble protein in the myocardium in high concentration; (ii) virtual confinement to the cytoplasmic space; (iii) small molecular size; (iv) relative tissue specificity, with a relative distribution of H-FABP outside the heart similar to that of creatine kinase muscle brain (CK-MB),[45] and (v) early release into plasma and urine (within 2 h) after onset of myocardial injury. Heart-FABP bears a considerable resemblance to myoglobin (a well-accepted early marker of myocardial injury within 6 h) in terms of size, location within the cell, release and clearance kinetics. However, when compared to myoglobin, H-FABP concentration in the heart muscle is greater than that in skeletal muscle, and its normal baseline concentration is several fold lower than myoglobin. These advantages make H-FABP potentially a more suitable cardiac marker than myoglobin.[64–66]

Measurement of H-FABP and normal range

The method of measurement is based on sandwich enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies specific for H-FABP.[22,55,63,67,68] The normal ranges reported for H-FABP in plasma and serum are assay- and method-dependent. Tanaka *et al.* (1991) has reported the normal range for H-FABP to be 0.0–2.8 μg/l;[69] Wodzig *et al.* (1997) reported 0.3–5 μg/l as the normal limit;[63] and Tsuji *et al.* (1993) used 3 μg/l (normal range 0.0–0.6 μg/l).[70] One study used a cut-off concentration of 19 μg/l (mean ± 2 SD of controls).[71] Heart-FABP is not likely to be found in the blood stream under normal conditions. The normal plasma H-FABP is likely to be due to the continuous release of this protein from damaged skeletal muscle cells.

Plasma H-FABP and acute myocardial infarction

Heart-FABP was introduced by Glatz in 1988 as a potential novel biochemical marker for the early diagnosis of AMI.[73] This assumption was soon confirmed in several studies.[44,45,66,69,71,74,75] Under normal conditions H-FABP is not present in plasma or interstitial fluid, but is released into the blood upon cellular injury. The cytoplasmic to vascular concentration of H-FABP is of the order of 200 000:1.[76] The plasma concentration of H-FABP under normal conditions is < 5 μg/l. This makes the plasma estimation of H-FABP suitable for the early detection and quantification of myocardial tissue injury. The H-FABP is released into plasma within 2 h after symptom onset and is reported to peak at about 4–6 h and return to normal base line value in 20 h.[75] Within the period of 30–210 min after symptom onset, H-FABP has > 80% sensitivity for the diagnosis of AMI.[71] Within the interval of 0–6 h after symptom onset, the other cardiac markers such as creatine kinase, CK-MB mass or activity, cardiac troponin I (cTnI) and cardiac troponin T (cTnT) will only be starting to accumulate in the plasma, and their sensitivity has been reported to be around 64%.[77]

Urinary H-FABP and acute myocardial infarction

Urinary indicators of myocardial injury are almost unknown, and only myoglobin has been tested as a urinary indicator of myocardial injury.[78–80] Heart-FABP is eliminated from the circulation by the kidney, but the precise mode of renal handling of H-FABP is unknown. A rise in serum and urine H-FABP concentration above normal values is seen in patients who present with AMI as early as 1.5 h after symptom onset.[69] Studies in animals have also shown decreased myocardial tissue content and rising plasma and urine concentrations of H-FABP very early after coronary artery ligation.[44,81] Measurement of plasma or urine concentration of H-FABP was diagnostic of AMI as early as 30 min after ligation. Assays that measure H-FABP in urine samples were able to accurately diagnose patients with AMI and provide reliable estimation of infarct size.[82] However, the measurement of infarct size based upon urinary H-FABP may be influenced by several factors, such as renal blood flow, perfusion pressure, glomerular filtration rate, tubular absorption, and diseases of the kidney. Measurement of urinary and plasma H-FABP in the presence of kidney diseases may lead to underestimation and overestimation, respectively, of the size of infarct due to impairment of excretion of H-FABP.[83] Heart-FABP circulates for longer (> 25 h) after AMI in the presence of renal failure.[71] Several sensitive assays that can measure H-FABP in urine samples are available.[67,69,70,82]

Limitations of H-FABP assays

The human skeletal muscle FABP has been reported to be identical to that of H-FABP.[56] The H-FABP content of skeletal muscle is variable, and is reported to range between 0.05 and 0.2 mg/g wet weight of tissue, depending on muscle type.[72,84] Skeletal muscle damage during the course of AMI (e.g. intramuscular injections, electric cardioversion, traumatic cardiopulmonary resuscitation) may result in the leakage of H-FABP, and this could interfere with the results of the assays.[84] Diagnosis of AMI in these groups of patients using H-FABP alone can be difficult. H-FABP is increased in the plasma of healthy volunteers after strenuous exercise as a result of release from skeletal muscle, but in these patients the ratio of myoglobin to H-FABP is below the 6% cut-off value considered specific for skeletal muscle injury.[85] One study however did not report any increase of H-FABP in urine or serum in a patient with crush injury, whereas myoglobin was markedly elevated.[69]

Surgery (both cardiac and non-cardiac) causes elevation of H-FABP concentration. H-FABP is excreted by the kidney, and renal insufficiency results in decreased clearance of H-FABP, thereby elevating the concentration and prolonging the

circulation time.[86] In situations of AMI and renal failure, measurement of plasma H-FABP could lead to overestimation of myocardial infarct size, and could interfere with its use for the detection of re-infarction.[83] However, renal failure is readily detectable in standard biochemical analysis and should not confound the diagnostic specificity of H-FABP, (as distinct from infarct size measurements) for the vast majority of patients.

Isoforms of H-FABP

Heart-FABP could be an ideal early marker of myocyte injury in ACS, if there is an isoform of this protein that is 100% specific to the heart. Several investigators have addressed the possibility for the existence of possible isoforms of H-FABP.[87] Glatz *et al.* (1985) isolated FABP from the human heart. This protein had a molecular mass of 15 kDa and an isoelectric point of 7.5.[88] Unterberg *et al.* (1986) reported the isolation of H-FABP with a molecular mass of 15.5 kDa, pI of 5.3, and a an amino acid sequence that included two cysteine residues.[89] Offner e*t al.* (1988) also reported the isolation of H-FABP, with a molecular mass 14 768 Da; pI 5.25, and an amino acid sequence that contained no cysteine residues.[41] These results suggest that isoforms of H-FABP may exist in the human heart. Similarly, in some studies in rats, the nucleotide sequence of two rat H-FABP cDNAs differed in the 5′ and 3′ untranslated regions. The existence of H-FABP isoforms has also been reported in bovine species.[92–95] Further studies using more sensitive techniques are needed to resolve this matter.

H-FABP and myoglobin

Myoglobin has been introduced as a marker for early diagnosis of AMI (within 3 h after symptom onset).[96–101] In a 1994 study, myoglobin was superior to CK-MB mass and cTnT for ruling out AMI within the period of 3–6 h after symptom onset.[102] Myoglobinuria has long been known to be useful in the diagnosis of AMI.[78,103] Myoglobin and H-FABP share many key features:[104] (i) low molecular mass (17 and 15 kDa, respectively); (ii) found in abundant concentrations in the cytosol of myocardial cells; (iii) provide substrates for mitochondrial oxidation (oxygen and fatty acids, respectively); and (iv) released within 2 h after symptom onset, peak early (6 h) and return to normal baseline concentration within 24 h. Both are present in the heart and skeletal muscle. However, concentration of myoglobin is approximately two-fold lower in cardiac than skeletal muscle (2.5 and 4.0 mg/g wet weight of tissue, respectively). In contrast, H-FABP concentrations are 2–10-fold higher in heart than in skeletal muscle (0.5 vs. 0.05–0.2 mg/g wet weight).[84,104] In addition, the normal plasma concentration of H-FABP (< 5 μg/l) is 10–15-fold lower than that of myoglobin (20–80 μg/l). H-FABP is therefore more cardiospecific than myoglobin and because of this superior specificity, the use of H-FABP as a marker may be preferable for the early diagnosis of AMI.[65,66,104]

The main disadvantage of myoglobin or H-FABP as early markers of myocardial injury is lack of complete specificity, due to the presence of both in skeletal muscle. Severe skeletal muscle injury may result in the release of both proteins in sufficient quantity to interfere with the specificity of the assay. Both proteins are released into plasma after injury at about the same time and in a ratio similar to the concentration of the proteins in the tissue of origin, therefore the measurement of the myoglobin: H-FABP ratio could be useful for discriminating between cardiac and skeletal muscle damage. A myoglobin:H-FABP ratio ∼5 is considered to be specific for the heart; a ratio ∼21–70 is more indicative of skeletal muscle damage.[84] The combination of the two markers in a ratio has been reported by some investigators to increase the diagnostic specificity for the diagnosis of AMI more than relying on either marker alone. However, the use of this ratio should not be a rigid criterion, as overlaps do occur. Some investigators did not find any additional value in myoglobin:H-FABP ratio over the measurement of H-FABP alone.[66,84,105]

H-FABP and unstable angina

H-FABP may be useful for the identification of patients with UA based upon detection of myocyte injury. However, there have been no detailed studies evaluating its usefulness for the diagnosis, or risk stratification in patients with UA. In a study by Tsuji (1993) using H-FABP with a normal range of 0.0–0.6 μg/l and an upper limit of normal of 3 μg/l, in patients suspected with a diagnosis of UA, the concentration of H-FABP was 3.5 ± 1.7 μg/l. In patients with AMI, the range was 12.3 ± 9.6 μg/l.[70] Other investigators have also observed an increase in H-FABP serum concentration in UA patients.[66] One study reported that H-FABP was normal in a patient diagnosed with UA.[71] In this study, a relatively high upper limit of normal concentration was used (19 μg/l), and this high cut-off concentration may have affected the sensitivity, or could be due to UA without myocardial necrosis. At present we have limited information on the ischaemic

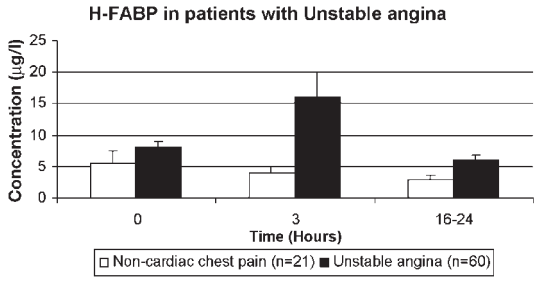

Figure 1. Concentrations of H-FABP in patients with unstable angina and non-cardiac chest pain. Data from our pilot study in patients with acute chest pain.

threshold for leakage of H-FABP from myocytes. Preliminary results from our pilot study have suggested a possible role for H-FABP in the diagnosis of UA (Figure 1). There is a need for larger-scale studies designed to look specifically at the role of H-FABP for the diagnosis of patients with UA.

H-FABP and acute myocardial infarction after surgery

H-FABP peaks early, and may be useful for the early detection of myocardial injury after surgery. The plasma concentration of H-FABP is increased relatively early, compared to CK-MB and cTnT, after aortic declamping in CABG surgery. The time to peak concentration was significantly shorter for plasma H-FABP (1.4 ± 0.5 h) than for CK-MB (2.5 ± 0.5 h) or cTnT (6.6 ± 1.3 h).[106] Similar findings were reported in other studies.[107] H-FABP was not increased in low-risk patients after CABG surgery without cardiopulmonary bypass.[108] The myoglobin to H-FABP ratio (see 'H-FABP and myoglobin' above) was useful in the diagnosis of AMI after non-cardiac surgery. However, the sensitivity of this ratio for the diagnosis of AMI in patients after cardiac surgery was less clear, and ranged from 11.3 ± 4.7 to 32.1 ± 13.6.[84]

H-FABP and detection of reperfusion

Establishment of reperfusion in the infarct-related artery is associated with significant reduction in morbidity and mortality. However, thrombolytic treatment is associated with successful reperfusion in only 50–80%.[109,110] New or alternative treatment options are being examined to try to see the best way to deal with patients who do not reperfuse after the first course of thrombolytic treatment.[111–113] Clinical trials are now underway randomizing patients who do not reperfuse to either another course of thrombolytic treatment, PCI, or conservative treatment. In clinical practice, reperfusion is ascertained indirectly by the reliance on clinical features such as disappearance of chest pain, resolution of ST segment elevation, and occurrence of reperfusion arrhythmias (e.g. accelerated idioventricular rhythm).[114] Reliance on clinical features alone is not sensitive for the detection of reperfusion, but H-FABP has been reported to be a sensitive marker for the detection of reperfusion after thrombolytic treatment. Abe *et al.* (1996) demonstrated that a rise of H-FABP ratio of ≥ 1.5 (compared to pre-treatment concentration), 30 min after thrombolytic treatment, was associated with 100% accuracy for the detection of reperfusion. This accuracy dropped to 94% at 60 min after thrombolytic treatment.[74] The advantage of using H-FABP is that reperfusion is ascertained very quickly, in some studies as early as 15 min. In a study by Ishii *et al.* (1995), the predictive accuracy of H-FABP ratio > 1.8 for the detection of reperfusion within 60 min of initiation of treatment was 93% at 15 min, 98% at 30 min, and 100% at 60 min after reperfusion.[115] The few additional studies that have examined the role of H-FABP for the detection of reperfusion also support this view.[64]

H-FABP and detection of re-infarction

Re-infarction is a well-recognized complication following AMI, but one which may be difficult to detect clinically. This may be attributable to re-occlusion of the infarct-related artery after an initial successful reperfusion or to a vessel occlusion at another site. Re-infarction can manifest as a recurrence of chest pain, or haemodynamic deterioration such as hypotension, acute pulmonary oedema, and arrhythmia with or without new ST segment changes. In the presence of AMI, recurrence of chest pain with or without ST segment changes could be misinterpreted and without a confirmatory test, the diagnosis of re-infarction could be missed. Re-infarction carries a worse prognosis, and may necessitate further pharmacological, supportive (e.g. balloon pump) or intervention treatment with PCI, or (rarely) urgent CABG. It is vital that this complication is recognized and appropriate interventions implemented. The most definitive method for the confirmation of re-infarction is coronary angiography, but the diagnosis of re-infarction may be possible using cardiac markers. The high sensitivity, simplicity, cost and safety

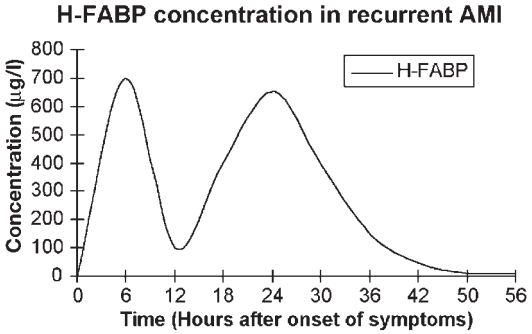

Figure 2. Release pattern in a patient with recurrent AMI.[62]

profile make cardiac markers a practical option for the detection of re-infarction.

The features of an ideal marker for early re-infarction are early release and clearance from the circulation, thus permitting a prompt return to pre-infarction concentrations. H-FABP fulfils these features, appearing within 3 h after infarction, peaking early at about 5 h, and returning to baseline concentrations about 20 h after symptom onset.[71] Re-infarction is shown by a rapid rise in H-FABP concentration in serum compared to the previous value. Heart-FABP can detect re-infarction when it occurs 10 h after symptom onset (Figure 2).[84] Other cardiac markers such as CK-MB, cTnI, cTnT, and LDH take several days to return to the pre-infarction levels, and thus are not sufficiently sensitive for the detection of re-infarction.

H-FABP and estimation of infarct size

The measurement of infarct size after AMI can have important prognostic implications.[116–118] It may also have therapeutic applications in the selection of patients for ACE inhibitor or anticoagulation treatment. Those patients with large infarcts who are deemed at higher risk for complications such as congestive cardiac failure, adverse remodelling of the ventricles or intramural thrombosis may be selected for higher intensity treatment options. However, the measurement of infarct size is not performed routinely. This may be partly due to the complicated blood sampling protocol, which is both prolonged (several days) and time-consuming, but is necessary to establish a complete time-concentration curve profile necessary for this type of measurement. In clinical practice, infarct size is estimated indirectly (or qualitatively) by methods such as nuclear perfusion imaging, echocardiography (wall motion abnormalities, measurement of ejection fraction), ECG changes (e.g. number of leads involved; conduction abnormalities in anterior infarction), the presence of heart failure, and by reference to the maximum rise of cardiac marker concentrations after infarction. Accurate measurement of infarct size is possible using nuclear studies, but is not practical for routine use because it is expensive, requires high technology, and exposes patients to additional radiation.

Cardiac markers offer an alternative for the estimation of infarct size. The rapid and quantitatively robust release of H-FABP into plasma after symptom onset and its rapid clearance from the circulation within 24 h, make it potentially suitable for the early estimation of infarct size, provided that blood is sampled sufficiently frequently.[83] Sohmiya *et al*. (1993) showed good correlation between myocardial infarct size measured from plasma H-FABP and infarcted myocardium estimated from triphenyl tetrazolium chloride (TTC) staining.[82] A study by Glatz *et al*. (1994) using H-FABP for the early estimation of infarct size, showed a good correlation between H-FABP, CK-MB and α-hydroxybutyrate dehydrogenase (α-HBDH) for the estimation of infarct size. The advantage of H-FABP is that this measurement is completed much earlier than with the other two markers: 24 h, 48 h, and 72 h for H-FABP, CK-MB, and α-HBDH, respectively.[76]

Excretion of heart fatty acid binding protein

It is not clear at present whether H-FABP reaches the circulation trans-endothelially, or via the lymphatic system, or both, after its release from the cell into the intercellular space. The rapid appearance from blood may suggest the first route. The route of elimination from the circulation is assumed to be the kidney, based on direct and indirect evidence.

The indirect evidence comes from observations in clinical studies. Patients presenting with AMI demonstrate rising levels of plasma and urine H-FABP within 1.5 h after symptom onset.[70] Patients with renal insufficiency have raised levels of H-FABP, and circulation time is prolonged compared to those with normal renal function.[71,83]

The direct evidence comes from radioactive iodine-H-FABP excretion studies in animals. The compound is concentrated within the kidney and appears in the bladder within very short period after intravenous injection.[82] However, the reported amount of radioactive H-FABP excreted in the urine is variable. One study reported that only 14–29% of the total intravenous dose injected was excreted in the urine. The total clearance was 0.33 ml/min and the half-life value of total elimination was estimated

to be 270 min.[81] A study by Sohmiya (1993) reported only 6.5 ± 1.0% recovery of the radioactive H-FABP in the urine, and its disappearance half-time was 27.5 ± 8.4 min. They suggested that the administered H-FABP might be degraded elsewhere in the body and the undegraded H-FABP is excreted in the urine. The authors concluded that their results were comparable to the excretion studies of myoglobin (known to be excreted by the kidney).[119]

Pathological confirmation of acute myocardial infarction using anti-H-FABP antibodies on autopsy materials

Pathological confirmation of AMI is possible using autopsy material from the heart. The diagnosis can be established microscopically or macroscopically using immunohistochemical methods. Haematoxylin and eosin (H&E) staining of the tissue sections can establish the microscopic diagnosis, whereas the macroscopic diagnosis is based on nitro blue tetrazolium (NBT) staining methods. This macroscopic technique reflects the intracellular activity of the enzyme. In viable tissue, this enzyme converts the NBT into dark blue insoluble pigment (formazan), while infarcted tissue remains unstained.[120] These two methods are only positive after at least 4–6 h after AMI. Anti-H-FABP has been used for the confirmation of AMI on autopsy materials. Using anti-H-FABP, it was possible to diagnose infarcts < 4 h old. In all biopsies that were positive by either H&E or NBT staining, the anti-H-FABP staining showed an absent or noticeably decreased staining of H-FABP in these tissues. Some biopsy material from patients with AMI who died within 4 h were positive using anti-H-FABP, but the H&E and NBT staining were negative. The authors of the study concluded that anti-H-FABP antibody is more sensitive than either H&E or NBT staining methods for the detection of subtle changes of AMI or very small or very recent (< 4 h) AMI on autopsy materials.[121,122]

Discussion

Heart-FABP is a novel cytosolic protein with the potential for accurate early diagnosis of AMI, early detection of re-infarction, detection of reperfusion, and estimation of infarct size. However, there is uncertainty in clinical practice about its additional value compared to the currently available markers such as myoglobin, CK-MB and troponins. Many studies have convincingly shown that the latter markers (with the exception of myoglobin) are relatively insensitive for the early detection of AMI in the first 6 h after symptom onset.[77] Reperfusion in this interval is associated with the greatest reductions in morbidity and mortality.[110,116,117,123–126]

Consistently negative serial samples within 6 h after symptom onset can be used to rule out AMI with high predictive accuracy. Measurement of H-FABP before and at 30 or 60 min after the administration of thrombolytic treatment can detect reperfusion of the infarct-related artery with high sensitivity, and permit further reperfusion therapy to be planned for those patients who do not reperfuse successfully. Early re-infarction is a well-recognized complication after initial infarct. Given the release kinetics of H-FABP, it is more suited to the detection of re-infarction than other markers (with the exception of myoglobin, which lacks specificity). The accurate estimation of myocardial infarct size has important prognostic and therapeutic applications. Heart-FABP can provide a reliable estimate of infarct size. The advantage of H-FABP over other markers is that this measurement can be provided within 24 h of admission.

It is premature to attach a specific clinical value for the detection of AMI from measurement of H-FABP in the urine. Further studies are needed to characterize the renal handling and metabolism of H-FABP under normal and disease states. Until such essential details are available, it can only be assumed that, if H-FABP is excreted mainly by the kidney, urinary H-FABP might offer an alternative method for the detection of AMI. Urinary H-FABP testing might be useful as a qualitative test for general practitioners to rule in or rule out AMI in the community.

Heart-FABP distribution outside the heart has been equated with that of CK-MB, which is currently regarded as the gold standard marker for the diagnosis of AMI.[45] Creatine kinase-MB lacks the required sensitivity to be of value for the very early diagnosis of AMI in the first 3 h after symptom onset. During this interval, the sensitivities of CK-MB and H-FABP for the diagnosis of AMI were 20% and 91.4%, respectively.[70] Heart-FABP shares many key features with myoglobin, but is more cardiospecific, because its concentration in the skeletal muscle is only a fraction of that of myoglobin. The normal concentration of H-FABP in the blood is 10–15-fold lower than that of myoglobin. Compared to the troponins, H-FABP is less cardiac specific. The value of cTnT and cTnI for the late diagnosis of AMI and for the diagnosis and prognosis and risk stratification of patients with UA is well-established. However, these markers have little role in the very early

diagnosis of AMI, i.e. within the first 4 h after symptom onset. They achieve their greatest sensitivity and specificity in the interval 12–16 h after symptom onset.

Conclusions

Heart-FABP is a sensitive marker for the detection of AMI, but is not 100% cardiac-specific, because of its presence in tissues outside the heart. In renal failure and skeletal muscle disease, it has limited diagnostic value for AMI, as it tends to overestimate infarct size. However, the features of H-FABP, which combine very early release after onset of symptoms and relative cardiac specificity suggest that it has great potential. Serial measurement of H-FABP within 24 h after symptom onset can: (i) define patients with AMI who need CCU admission and thrombolysis within 6 h after infarction; (ii) distinguish patients who reperfuse their infarct-related artery from those who do not and who need further intervention, as early as 30 min after starting thrombolytic treatment; (iii) detect re-infarction if it occurs 10 h after symptom onset; (iv) permit accurate estimation of myocardial infarct size and thus provide information concerning prognosis.

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