# Heart-type fatty acid binding protein (hFABP) in the diagnosis of myocardial damage in coronary artery bypass grafting ☆

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## Abstract

**Objectives**: Heart-type fatty acid binding protein (hFABP) is an intracellular molecule engaged in the transport of fatty acids through myocardial cytoplasm and has been used as a rapid marker of myocardial infarction. However, its value in the evaluation of perioperative myocardial injury has not yet been assessed. **Methods**: 32 consecutive patients undergoing coronary artery bypass grafting were included in a prospective, randomized study using standardized operative procedures and myocardial protection. Three patients with perioperative myocardial infarction were added. Serial blood samples were taken preoperatively, before ischemia, 5 and 60 min after declamping, 1 and 6 h postoperatively and on postoperative days 1, 2 and 10 and were tested for hFABP, creatin kinase isoenzyme MB (CKMB) and troponin I (TnI). **Results**: Hospital mortality was zero. The kinetics of the biochemical parameters revealed a typical pattern for each marker. In routine patients, hFABP levels peaked as early as 1 h after declamping, whereas CKMB and TnI peaked only 1 h after arrival in the intensive care unit. Patients with perioperative infarction displayed peak levels some hours later in all marker proteins. Peak serum levels of hFABP correlated significantly with peak levels of CKMB ($r = 0.436$, $P = 0.011$) and TnI ($r = 0.548$, $P = 0.001$), indicating the degree of myocardial damage. **Conclusions**: hFABP is a rapid marker of perioperative myocardial damage and peaks earlier than CKMB or TnI. The kinetics of marker proteins in serial samples immediately after reperfusion is more suitable for the detection of perioperative myocardial infarction than a fixed cut-off level. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords*: Heart-type fatty acid binding protein; Marker protein; Myocardial damage; Coronary artery bypass grafting

## 1. Introduction

Myocardial ischemia during cardiac surgery results in functional and structural changes and ultimately in protein release from injured cardiomyocytes. The release pattern of different proteins varies as well as their cardiospecificity [1–4]. Creatin kinase (CK) and its isoform CKMB have been the most frequently used tool to diagnose myocardial ischemia [5]. In recent years, structural myofibrillar proteins, such as Troponin T and Troponin I with their cardiospecific isoforms, have entered the clinical field and have been used in daily routine as well as in scientific clinical research [6–11]. However, the time delay between the operative damage and the detection of a measurable amount of protein in peripheral blood remains a major concern, rendering difficult the rapid evaluation of the degree of myocardial injury.

Heart-type fatty acid binding protein (hFABP) is a small intracellular protein consisting of 132 amino acid residues and weighing 14.5 kDa. It is water soluble and abundant in the cytoplasm. Its physiological role is the transport of hydrophobic long-chain fatty acids from the cell membrane to their intracellular sites of metabolism in the mitochondria, where they enter the citric acid cycle. After membrane damage, it is released to the extracellular space, and due to its small size and physical properties, immediately enters the blood compartment [12].

Since evidence of hFABP release from damaged myocardium was established in an ischemic working rat heart model, several groups have studied the release pattern in patients with myocardial infarction [13–16]. They demonstrated plasma concentration curves that were markedly different from those of other marker proteins, with a very early peak and a rapid decline thereafter. However, due to the lack of a test with appropriate availability and tissue specificity, hFABP measurement has so far not achieved widespread clinical use. Moreover, its value in cardiac

surgery remains to be elucidated with only very few data published in the recent medical literature [17,18].

Recently, a direct sandwich enzyme-linked immunosorbent assay (ELISA) test has been developed [19], and a commercially available kit has been introduced to the Japanese market using two distinct types of murine antihuman monoclonal antibodies specific for hFABP. Using this kit, we tested the release pattern of hFABP and its relevance to myocardial injury in patients undergoing coronary artery bypass grafting (CABG) with and without evidence of perioperative myocardial infarction, and compared it to other marker proteins of myocardial ischemia.

## 2. Material and methods

Thirty-two consecutive patients scheduled for CABG were enrolled in a prospective clinical trial. Three patients with the diagnosis of perioperative myocardial infarction were added to the analysis. The clinical data of these patients are outlined in Table 1. All patients had three vessel coronary heart disease and suffered from angina CCS II or III. Emergency, redo and combined procedures were excluded. Informed consent was obtained from all patients and the study was approved by the Ethics Committee of the Heinrich-Heine University, Düsseldorf.

### 2.1. Operative procedure

Any vessel showing an obstruction of at least 70% in the preoperative angiogram was grafted at the operating surgeon's convenience. Complete revascularisation was intended in all patients, using the internal mammarian artery (IMA) for revascularisation of the left anterior descending artery and the saphenous vein for other grafts. Other arterial conduits (radial artery, right internal mammarian artery) and complete arterial revascularization were excluded.

Standardized anesthesia procedure included induction with Thiopentane (1–3 mg/kg) and fentanyl (3–5 mg/kg)

Table 1
Preoperative clinical data[a]

| Parameter | $n = 32$ |
|---|---|
| Age (years) | $64.08 \pm 10.0$ |
| Male/female | 27/5 |
| CCS | |
|     Class II | 50% |
|     Class III | 50% |
| Ejection fraction | $62.5 \pm 13.2$ |
| Hypertension | 37% |
| Diabetes | 13% |
| Hyperlipoproteinemia | 63% |
| Nicotine abuse | 37% |
| Prior myocardial infarction | 58% |
| Height (cm) | $172 \pm 7$ |
| Weight (kg) | $78.0 \pm 9.6$ |

[a] CCS, Canadian coronary score.

and neuromuscular blockade using pancuronium bromide (0.1–0.15 mg/kg). Anaesthesia was maintained with Etrane via inhalation, or, during extracorporeal circulation (ECC), via continuous infusion.

After median sternotomy and preparation of the IMA, the ascending aorta and superior and inferior caval veins were cannulated and extracoporeal circulation (ECC) was established. A vent catheter was routinely inserted via the right superior pulmonary vein. Blood temperature was reduced to 30°C. At first, all distal anastomoses were performed using either hypothermic cardiac arrest with cold antegrade cristalloid (Bretschneider) cardioplegia (17 patients) or intermittent aortic crossclamping (15 patients). During the last peripheral anastomosis, rewarming was begun. After final release of the aortic crossclamp, if necessary, the heart was electrically defibrillated and allowed to beat during completion of the proximal anastomoses in partial aortic occlusion. After reaching a core temperature of 34.5°C, the patients were weaned from ECC. Positive inotropic support was given when systolic blood pressure fell below 90 mmHg and vasodilators were administered when preload, as measured by left atrial pressure, rose to more than 15 mmHg.

After meticulous hemostasis, chest tubes were inserted and placed retrosternally, intrapericardially and intrapleurally. The thorax was then closed as usual and the patient transferred to the intensive care unit.

### 2.2. Analysis

Serial venous blood samples were collected on hospital admission, before ECC, immediately before aortic crossclamping, 5 min after declamping, 60 min after declamping, 1 h after admission to the intensive care unit, 6 h after admission to the intensive care unit, and on the 1st, 2nd, and 10th postoperative day for biochemical analysis as indicated below. They were drawn into vacuum tubes containing dry heparin. The tubes were placed immediately into ice and plasma was separated by centrifugation and stored in plastic tubes at −75°C until further usage. Continuous basic clinical and hemodynamic monitoring included systemic blood pressure, right atrial pressure, heart rate, the administration and dosage of inotropic support, single lead ECG rhythm surveillance and peripheral oxygen saturation.

Electrocardiogram: a 12 lead ECG was recorded before surgery, 1, 6, and 12 h after admission to the intensive care unit, and on the 2nd and 10th postoperative day. ECG records were analyzed by an experienced cardiologist blinded to the actual clinical situation in collaboration with two medical students specially trained in ECG analysis. Diagnostic criteria for myocardial ischemia were defined as follows: new development of Q waves of equal to or more than 0.4 mm, major ST-segment elevation or depression, or R-wave reduction of more than 25% in at least two leads. Bundle branch blocks were not considered as indicating ischemia [20,21].

## 2.3. Biochemical analysis

### 2.3.1. Heart-type fatty acid binding protein

As previously mentioned, a sandwich enzyme linked immunosorbent assay for the determination of hFABP levels has been developed (Markit-M H-FABP®, Dainippon Pharmaceutical Co. Ltd.) using two distinct murine anti-human FABP specific monoclonal antibodies. Briefly, h-FABP within the test sample bound to a monoclonal anti h-FABP antibody coated on microplate wells. Enzyme labeled anti h-FABP antibody was added to the wells to form a sandwich immune complex. After addition of substrate to start the enzymatic reaction, absorbance was measured at 492 nm and at 620 nm in a microplate reader. (The kit was a generous gift from Dainippon Pharmaceutical Co. Ltd.).

### 2.3.2. CK and CKMB enzyme activity

For the determination of CK- and CKMB-enzyme activity, a plate analysis test was used (Vitros CK or CKMB Analysenplättchen, Vitros, Johnson & Johnson Clinical Diagnostics). Briefly, serum samples were added to a plate prepared in multilayer technology. The containing enzyme catalyzed the generation of creatin and ATP by phosphorylation of creatinphosphate and ADP. A subsequent colouring reaction was read at a wavelength of 670 nm reflecting the enzyme activity.

### 2.3.3. Cardiac Troponin I

Cardiac troponin I was measured using a microparticle immunoassay (Troponin I, B3C291, Axsym Systems, Abbot GmbH Wiesbaden Germany). In summary, TnI, contained in serum samples, bound to microparticles, coated with anti-Troponin I. The complexes were bound to a glass fibre matrix again using an antibody reaction and were subsequently visualized and measured by a colouring reaction, as already described.

## 2.4. Statistical analysis

For each patient, 300 variables were registered and stored on the hard disc of a microcomputer using the commercially available software Excel® (Microsoft). The data were divided into discrete and continuous variables. For the continuous variables mean, median and standard deviation were calculated. For the discrete variables the number of values in each category were calculated and given as a percentage.

The Student's $t$-test for non-paired values was used for comparison of the continuous variables, and the $\chi^2$ test for the discrete variables. In addition, an $\alpha$-adjustment was carried out, using the HOLM-procedure and comparing three groups of different markers. Probability values of $P < 0.05$ were defined as statistically significant and are presented, when appropriate. All statistical calculations including the correlation test, were carried out using the statistic tools of Microsoft Excel®.

## 3. Results

### 3.1. Clinical course

The intraoperative data of the study group are outlined in Table 2. There was no perioperative or in-hospital mortality. Postoperative course was undisturbed in all patients.

Transient ECG changes, mostly minor ST-segment depressions or bundle branch blocks, were observed in 15 patients and did not correlate to marker protein abnormalities. A new Q-wave, indicating perioperative myocardial infarction, was assessed in one patient in the absence of any laboratory abnormalities. Two other myocardial infarctions were diagnosed, due to a typical laboratory pattern (CK-CKMB ratio of 610/82 6 h postoperatively in one of them, and 417/41 on the first postoperative day in the other). In these latter two patients, ECG changes could not be observed. In the three patients with perioperative myocardial infarction, clinical course was also uneventful, with early extubation, mobilization and hospital discharge between the 7th and 10th postoperative day.

### 3.2. Time course of mean concentration

Each marker protein displayed a typical laboratory pattern. Mean values rose from a baseline concentration preoperatively to a postoperative peak indicating myocardial damage of a certain degree in all cases. Thereafter, concentrations declined and reached baseline values on the 10th day.

One hour after declamping, peak concentration of the h-FABP was assessed, whereas significant changes between baseline and the perioperative course of all ischemic markers were already earlier observed. Patients with perioperative myocardial infarction showed higher values from the beginning and throughout the whole perioperative period with a peak concentration after arrival at the intensive care unit. See Fig. 1.

CKMB levels were also elevated immediately after declamping of the aorta. However, values increased more

Table 2
Intraoperative data[a]

| Parameter | $n = 32$ |
| --- | --- |
| No. of grafts | |
| 3... | 50% |
| 4... | 47% |
| 5... | 3% |
| IMA | 92% |
| ECC time (min) | $109.3 \pm 25.1$ |
| Aortic occlusion time (min) | $43.4 \pm 10.9$ |
| Operation time (min) | $233.2 \pm 38.9$ |

[a] IMA, internal mammarian artery; ECC, extracorporeal circulation.
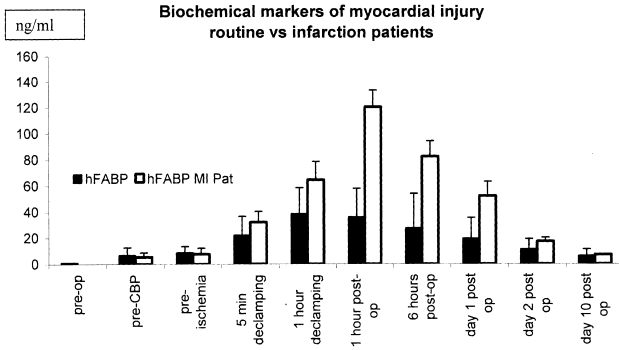
Fig. 1. Mean concentrations of hFABP after CABP in routine patients and in patients with perioperative myocardial infarction.

slowly and reached a peak at 1 h postoperatively. In the infarction patients, the curves showed a biphasic pattern with a second peak between postoperative day 1 and 2. For further details, see Fig. 2.

Cardiac troponin I, usually not present in the plasma, could also be detected early after reperfusion. As outlined in Fig. 3, peak concentrations were reached at 1 h postoperatively. Values of the patients with perioperative infarction were excessively high.

A significant difference between the h-FABP values 1 h after declamping and 1 h postoperatively was not observed, as it was not in the CKMB or in the TpI analysis.

### 3.3. Time to peak level

54.8% of the patients reached hFABP peak concentration at 60 min after declamping, whereas only 16.1% showed peak concentrations of CKMB or TnI at this time point. In contrast, serum concentrations of CKMB or TnI reached their maximum level at 1 h after arrival at the intensive care unit in 80.6% or 61.3% of the patients, respectively. For details see Fig. 4. The appearance of hFABP values is statistically earlier than that of TnI- ($P = 0.001$) or CKMB- ($P = 0.002$) values ($\chi^2$ test and HOLM-procedure).
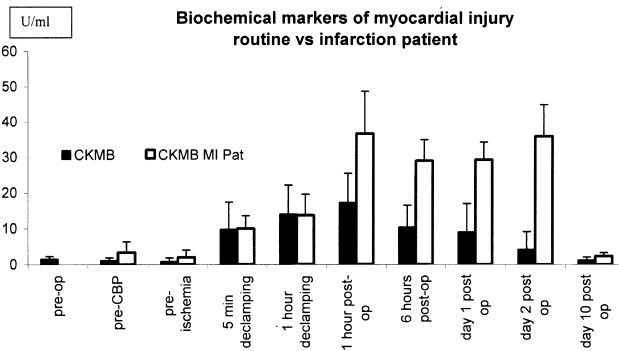


Fig. 2. Mean concentrations of CKMB after CABP in routine patients and in patients with perioperative myocardial infarction.
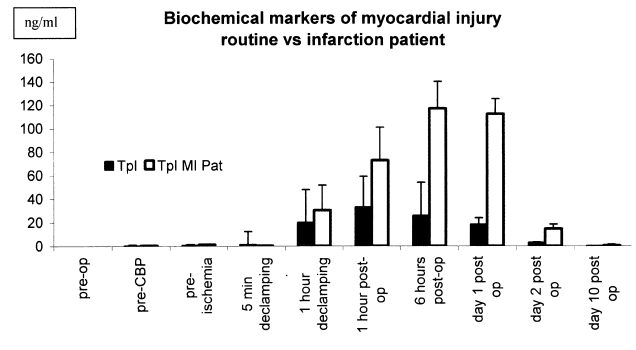


Fig. 3. Mean concentrations of Troponin I after CABP in routine patients and in patients with perioperative myocardial infarction.

### 3.4. Correlation between maximum level of FABP and other marker proteins

A significant correlation was observed between the peak values of HFABP and CKMB ($r = 0.436$, $P = 0.011$) as well as between HFABP and Troponin I ($r = 0.548$, $P = 0.001$).

### 4. Discussion

Adequate management of patients after cardiac surgery depends on a rapid evaluation of perioperative myocardial damage [21,22]. ECG criteria remain uncertain and lack diagnostic accuracy in the early as well as in the late postoperative period [20,22]. Therefore, diagnosis depends mainly on the assessment of plasma levels of cardiospecific marker proteins [1,3,4,6,8,9,11,23]. These are intracellular molecules, soluble in the cytoplasm or bound to subcellular structures, that are released to the extracellular space after damage of the myocardial cell membrane.

Plasma levels of specific marker proteins follow a typical pattern. Small, water soluble molecules are more rapidly washed out and detectable in the serum than proteins with a larger molecular mass, mainly due to the permeability of the endothelial layer [1,12]. Ongoing occlusion or reperfusion are other factors with impact on the kinetics and the
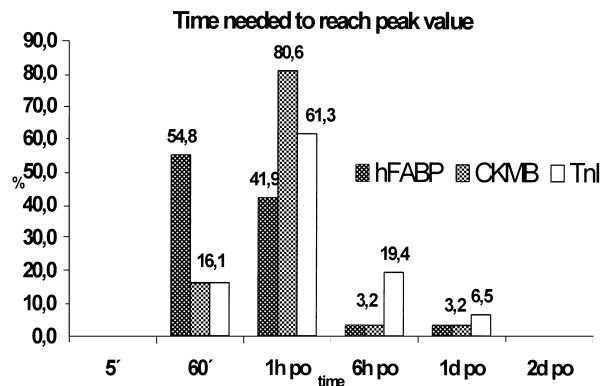


Fig. 4. Time needed to reach its individual peak concentration for each patient.

total amount of protein release from an injured myocardial area [1,3].

HFABP has been shown to be highly sensitive in the diagnosis of myocardial infarction and the degree of myocardial damage [12–16,24]. It appears very early after the onset of symptoms in myocardial infarction patients. However, there is only little information about hFABP in cardiac surgery patients. HFABP and myoglobin were shown to peak 0.5 h after reperfusion in 57 patients with various cardiac diagnoses and operations, as published by Fransen et al. [17]. Suzuki et al. [18] demonstrated in a group of 20 patients with coronary heart disease, that HFABP peaked $47.3 \pm 2$ min after release of the aortic cross-clamp [18].

In our study group, blood samples were taken at time points applicable in clinical routine. At 60 min after declamping, the operation usually approaches its end, and 1 h after arrival at the intensive care unit, routine patient's condition has normally stabilized, so that the ICU staff has time for sophisticated diagnostic activity. We found the mean peak level as early as 1 h after the onset of reperfusion. 55% of the patients reached their individual peak level at this time point. Thus, hFABP peaked significantly earlier than CKMB or TnI.

The diagnostic test that we applied takes about 1 h and requires the presence of laboratory personnel [19]. A rapid assay, using dry laboratory technology and allowing for quantification after only 20 min, is currently being developed [25].

Interestingly, myocardial infarction patients displayed their peak level only at 1 h after arrival at the intensive care unit and all of the three patients had their individual peak level at this time point. This was also noted by Fransen et al. [17], who observed a time delay up to 12 h for patients with myocardial infarction to reach their individual peak level. Suzuki et al. [18] reported on a pattern of hFABP levels in patients with perioperative myocardial infarction equal to routine cases. However, the myocardial infarction patients they mentioned had valvular or aneurysma surgery, hence, pathogenesis of myocardial damage may have been different from patients with coronary artery surgery alone. In a recent paper, Adams [1] points out that each marker protein has a typical 'rising and falling pattern' in the case of myocardial injury, and that high serum levels in the absence of this pattern may lead to false positive results.

One limitation of our study is the relatively small number of patients with myocardial infarction. With only three patients, it seems not possible to define a time point, when patients with perioperative myocardial infarction can be clearly discriminated from those with uneventful perioperative course. Further studies including more infarction patients are intended to better characterize the course of protein markers in this important patient cohort.

There is general agreement that the amount of released marker protein reflects the degree of myocardial injury. Using human heart tissue samples it could be demonstrated that after myocardial infarction, hFABP is quantitatively released into the plasma allowing estimating the infarction size from hFABP levels [13]. That the release of troponin I from myofibrillar ultrastructures correlates with the amount of structural myocellular injury is not controversial [7,22]. The size of the infarction as assessed by CKMB release correlates closely with the volume of the infarction, the ejection fraction, the frequency of rhythm disturbances and the prognosis [5,21,22]. We found a significant correlation between hFABP and CKMB or TnI peak levels, so we can assume that there is a correlation between the level of hFABP and the degree of myocardial damage. Further studies, as already mentioned, are necessary to prove this observation in cardiologically as well as in surgically treated myocardial infarction patients.

## 5. Conclusion

The determination of HFABP levels represents a useful tool in the rapid evaluation of perioperative myocardial damage. HFABP peaks earlier than CKMB or TnI, and with the introduction of a rapid assay, perioperative myocardial damage can already be evaluated in the operating theatre.

The diagnosis of perioperative myocardial infarction depends on the kinetics of marker proteins in serial blood samples during the first 2 h after the onset of reperfusion, rather than on clear cut-off levels.

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## Appendix A. Conference discussion

**Dr W. Flameng** (*Leuven, Belgium*): Is there a correlation between the washout of let's say all three markers and the duration of cross-clamping?

**Dr Petzold**: We did not check this. I think we had too few patients to really find a correlation therefore. From literature it is known that there is a correlation. Fatty acid binding protein increases with the cross-clamp time.

**Dr Flameng**: I was a little bit surprised that you did not find a difference in time to peak from the troponin I and the CK-MB.

**Dr Petzold**: We were astonished too, but we found it.

**Dr F. Beyersdorf** (*Freiburg, Germany*): Was this marker also tested in patients who underwent acute myocardial infarction?

**Dr Petzold**: We did not do this in Dusseldorf, but in literature it has been tested, and it is known to be a very rapid marker. This was the reason why we undertook this study.

**Mr Ashraf** (*Swansea, UK*): Did you have postoperative any ECG protocol in this group of patients? Did you correlate it with any ECG changes with troponin I, CK-MB and free fatty acid?

**Dr Petzold**: It is very difficult to analyze the postoperative ECG. It is very insecure and it is hardly possible to correlate the ECG with a perioperative infarction. Actually we did our infarction diagnosis of these three patients, in one patient due to ECG changes which were a new Q-wave, and in the two others because of significant changes in the other marker proteins that we knew. But in the other patients we had no ECG changes, which could be significant for perioperative myocardial damage. Of course, we looked for it.

**Mr Ashraf**: A second question, was there any correlation of your level of ischemic damage with postoperative inotropic support?

**Dr Petzold**: No, it was not.

**Mr Ashraf**: Did you look into that?

**Dr Petzold**: We did that, but there was no correlation.

**Dr G. Lutter** (*Freiburg, Germany*): In case the patient has got a high hFABP value, i.e. after myocardial infarction, would you change your procedure out of this information?

**Dr Petzold**: Up to now it takes about one hour to one hour and a half to obtain the results of fatty acid binding protein, so this is too late to draw a conclusion. But we are working on a rapid test, a rapid assay that could give us the results within 20 minutes, and with this, I think we will have to draw consequences in the operation theatre.