ORIGINAL ARTICLE

Evaluation of postoperative myocardial injury by heart-type fatty acid-binding protein in off-pump coronary artery bypass grafting surgery

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Abstract

Background and goal of study: Postoperative myocardial infarction is a serious and frequent complication of cardiac surgery. Nonetheless, diagnosis in this context is occasionally challenging. We sought to evaluate the kinetics and diagnostic accuracy of the new biomarker “heart-type fatty acid-binding protein” (h-FABP) in the early detection of myocardial injury in patients undergoing off-pump coronary artery bypass grafting, compared with classical biomarkers.

Materials and methods: A prospective study was conducted on 17 consecutive patients who underwent off-pump coronary artery bypass grafting during a 2-month period. Blood samples were drawn for measurement of myocardial ischemic injury biomarkers (h-FABP, troponin, creatine kinase [CK] and CK-MB), at baseline (T1), immediate post-coronary artery bypass grafting (T2), on ICU admission (T3), and after 4 (T4), 8 (T5), 24 (T6) and 48 h (T7). Perioperative ischemic complications, defined according to electrocardiographic, echocardiographic and hemodynamic criteria, were recorded.

Results: Earlier peak biochemical marker plasma values occurred at T4 with troponin (2.9 ± 5.2 ng/mL), and at T5 with h-FABP (37.9 ± 55.5 ng/mL). Maximum values of CK and CK-MB occurred later, both in T6 (741 ± 779 and 37 ± 51 U/L, respectively). The optimized cut-off obtained for h-FABP was 19 ng/mL, providing a sensitivity and specificity of 77 and 75%, respectively, for diagnosis of perioperative ischemic injury, with an area under the ROC curve for
Introduction

Perioperative ischemic events have a major impact on both long- and short-term surgical morbidity and mortality. Incidence following bypass grafting surgery ranges from 7% to 15%, the difference in reported rates being due to the lack of specific criteria (hemodynamic, ECG, echocardiographic and biochemical) and the different definitions used to diagnose such events in the perioperative period. The most commonly used myocardial injury biomarkers are troponin (I and T) elevation, myoglobin, and the MB fraction of creatine kinase (CK-MB), although cut-off levels for perioperative ischemia are still unclear.

A cardiac biomarker called heart-type fatty acid-binding protein (h-FABP), a small protein that transports fatty acids from the cell membrane to mitochondria for oxidation, has recently become available. H-FABP is 20 times more cardiac specific than myoglobin and is released as early as 30 min after an ischemic episode, thereby allowing early detection and consequently better management of ischemic patients. Few studies in patients undergoing bypass grafting surgery have investigated this marker, and those published to date have focused on surgery performed with a cardiopulmonary bypass (CPB) pump. In these studies, h-FABP was found to have greater sensitivity and specificity than troponins, myoglobin and CK-MB. In a prospective study in 1298 patients undergoing heart surgery with CPB, h-FABP measurement was found to be an independent predictor of death and ventricular dysfunction in both the immediate and delayed postoperative periods.
In recent years, off-pump coronary artery bypass grafting (OPCABG) surgery has gained ground as a means of avoiding the complications associated with CPB (intense inflammatory response, coagulation disorders and multiple organ dysfunction). However, although the technique is, in theory, less invasive, it still involves manipulation of the heart and coronary arteries and is associated with ischemia–reperfusion phenomena that can cause to myocardial injury.

We hypothesize that h-FABP determination in patients undergoing OPCABG surgery will enable early diagnosis of ischemic events and allow them to be correctly treated at an early stage.

The main aim of this study is to describe the release pattern of different cardiac biomarkers (troponin I, CK-MB and h-FABP) in OPCABG surgery and to establish h-FABP cut-off values for the diagnosis of perioperative myocardial injury and compare this biomarker with traditional predictors.

Materials and methods

Prospective observational pilot study in patients undergoing OPCABG surgery. The research project has been carried out in accordance with the principles of the Declaration of Helsinki, and was approved by the hospital’s independent Ethics Committee.

A total of 17 consecutive patients were included, all or whom were over the age of 18 and had been scheduled for OPCABG surgery. All patients signed an informed consent form.

Exclusion criteria were: urgent cardiac surgery, myocardial infarction in the 2 weeks prior to surgery or during surgery, fibrinolitics given 48 h prior to surgery, patients with previous cardiac surgery, impaired ventricular function (EF < 30%), concomitant valvular disease, kidney failure (creatinine > 2 mg/dl), corticoid therapy, liver disease (AST/ALT or bilirubina more than twice or 1.5 higher, respectively, than normal levels), and patients receiving intravenous inotropic agents. Patients in whom the indication for off-pump or on-pump surgery was changed intraoperatively were also excluded.

Patients were monitored by means of ST segments observed in leads II and V, BIS®-titrated depth of anesthesia (Covidien, Mansfield, MA, USA), invasive blood pressure, central venous pressure and transesophageal echocardiography. Anesthesia was induced with etomidate or propofol combined with fentanyl and rocuronium. It was maintained with sevoflurane or propofol, at the discretion of the anesthesiologist, and continuous perfusion of remifentanil (0.25–0.5 µg/kg/min), and muscle relaxation was maintained with rocuronium bolus to maintain suitable surgical and ventilation status.

Intraoperative anticoagulation was managed with 1.5–2 mg/kg sodium heparin to maintain ACT levels of between 250 and 300 s. After surgery, heparin was reversed with protamine sulphate.

Hemodynamic stability was maintained with crystalloids and colloids together with changes in certain operating table positions. Vasoactive agents to maintain hemodynamic conditions within normal parameters (heart rate 40–100 bpm and SAP between 120 and 140 mmHg) were administered at the discretion of the anesthesiologist.

After surgery, patients were taken to the critical care unit (CCU) under sedation with propofol (1–2 mg/kg/h) and morphine (1–2 mg/h), where they were reanimated and extubated according to established protocols.

All patients underwent OPCABG surgery. First, distal anastomoses were performed, followed by partial lateral aortic clamping to facilitate proximal anastomoses. During partial aortic clamping, SAP was maintained at around 80–90 mmHg to minimize the risk of injury to the aorta or thrombosis. Coronary arteries were immobilized and controlled by means of a suction stabilizer. Intracoronary shunts were used at the discretion of the surgeon in the event of visualization due to excessive bleeding at the site of the arterectomy (despite the use of suction devices), electrophysiological evidence of new areas of myocardial ischemia, or ventricular dysfunction that would prevent distal blood flow during anastomosis.

Study parameters and variables

Serum levels of myocardial ischemic injury and necrosis markers, troponin I, CK, CK-MB and h-FABP, were measured at: pre-anesthesia induction baseline determinations (T1), post-coronary bypass grafting (T2), completion of surgery and transfer to the CCU (T3), and at 4 h (T4), 8 h (T5), 24 h (T6) and 48 h (T7).

The need for inotropes and vasopressors was recorded together with baseline (pre-surgery) 12-lead electrocardiogram results at transfer to the recovery room, on suspicion of an ischemic event, and every 24 h for the first 3 days post-surgery.

Transsthoracic echocardiography was performed to evaluate left ventricular dysfunction and/or new segmental contractility disorders before discharge from the CCU. Left ventricular function was defined as mildly depressed (EF 30–50%) or good (>50%).

Cardiovascular complications were identified, and defined as follows:

- Myocardial injury due to the presence of one of the following criteria: (1) appearance of a new and persistent Q wave lasting longer than 40 ms on at least 2 continuous leads; (2) R wave disappears or is reduced by at least 25%, in 2 leads; (3) appearance of J point ST segment elevations in at least 2 or more continuous leads (greater than 0.2 mV in leads V1, V2 and V3 or greater than 0.1 mV in other leads); (4) new left bundle branch block; and (5) new and persistent myocardial contractility disorders on transesophageal on transthoracic echocardiogram.

- Low cardiac output syndrome following bypass grafting surgery, defined as the need for intermediate or high dose inotropes, the use of 2 or more inotropes, or placement of an intra aortic balloon pump once electrolyte disturbances and acid–base imbalance have been ruled out and after preloading in certain situations (cold extremities, absence of pedis pulse, cardiac output below 2.2 L/min/m², kidney failure and oliguria [diuresis < 0.5 mL/kg/h], metabolic acidosis, lactate levels ≥ 3 mmol/L for more than 2 h, mixed venous oxygen
saturation ≤ 60% and impaired neurologic function, after ruling out other causes).
- Malignant arrhythmias, defined as an episode of ventricular fibrillation or ventricular tachycardia.
- Length of stay in the CCU and the hospital.
- In-hospital mortality and 30-day mortality.

Biochemical analysis of blood samples

Ultrasensitive troponin I, CK, CK-MB and h-FABP were analyzed using immunohistochemistry. The results are shown in Annex 1.

Statistical analysis was performed on SPSS® version 18.0.1 (SPSS Inc., Chicago, IL, USA). Values were expressed as mean and standard deviation for normal distribution, and as median and interquartile range for non-normally distributed data. The Student’s T or Mann-Whitney U-test was used for quantitative variables, and the chi-squared or Fisher’s test for qualitative variables. The Student’s T or Mann-Whitney U-test was used for comparative quantification of inter-group ischemic injury parameters, and evolution of intra- and inter-group ischemic injury markers was analyzed using repeated measures ANOVA, varying according to the type of distribution of each marker. The chi-squared test was used to study inter-group clinical complications. Different ischemic injury parameters were correlated using Spearman’s rho correlation test and multiple linear regression.

The usefulness of different markers as predictors of cardiac morbidity and mortality associated with each anesthesia technique was quantified by calculating the area under the ROC curve for these parameters.

A p value of <0.05 was considered statistically significant.

Results

A total of 17 patients were included in the study from 1 June to 30 August 2012.

Demographic variables, pre-operative characteristics, comorbidity and medication, together with intra-operative events, are shown in Table 1.

Post-operative characteristics are shown in Table 2 (ECG and echocardiography changes) and Table 3 (post-operative complications). Six patients (35.3%) presented clinical signs of ischemia as defined in the “Materials and methods” section.

With regard to biochemical parameters, average T1–T7 values for the entire series are shown in Table 4 and Fig. 1. The average peak plasma value of troponins occurred at T4 (4 h following admission to the CCU), in contrast to that of h-FABP, which peaked at T5 (8 h following admission). The average peak plasma value of the CK and CK-MB markers occurred at T6 (24 h post-surgery).

The highest biochemical marker levels observed in the group of patients diagnosed with perioperative ischemia vs. the no ischemia group are shown in Table 5. A trend toward higher h-FABP levels was observed in the group diagnosed with perioperative ischemia, although this was not statistically significant (Table 5).

The highest correlation (Spearman’s rho) between markers was found between h-FABP and CK (correlation coefficient 0.75, p < 0.0001). Correlation with troponins was 0.55 (p = 0.02).

The relationship between ischemic events (defined as the presence of any of the changes described in the “Materials and methods” section) and h-FABP levels was analyzed by means of ROC curves. The area under curve...
Table 3  Postoperative complications.

<table>
<thead>
<tr>
<th>CCU complications</th>
<th>Numbers (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No complications</td>
<td>13 (76.4)</td>
</tr>
<tr>
<td>Needed NIV</td>
<td>2 (11.7)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>2 (11.7)</td>
</tr>
<tr>
<td>Reintervention</td>
<td>2 (11.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CCU inotropes</th>
<th>Numbers (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3 (17.64)</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>6 (35.2)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>5 (29.4)</td>
</tr>
<tr>
<td>Dobutamine and phenylephrine</td>
<td>2 (11.7)</td>
</tr>
<tr>
<td>Solinitrine</td>
<td>2 (11.7)</td>
</tr>
<tr>
<td>Extubation (min)</td>
<td>474.7 ± 340</td>
</tr>
<tr>
<td>Hospital stay (d)</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td>Complications 30 days</td>
<td>0</td>
</tr>
</tbody>
</table>

CCU: critical care unit; NIV: non-invasive ventilation.
Data expressed as numbers (percentage of patients) or mean ± standard deviation.

for defined ischemic events was 0.83 (95% CI 0.6–1.0) for h-FABP, 0.63 (95% CI 0.33–0.83) for troponin I, 0.8 (95% CI 0.6–1.1) for CK, and 0.61 (95% CI 0.3–0.9) for CK-MB.

ROC curves were used to obtain a cut-off value of 19 ng/mL for h-FABP and 1.9 ng/mL for troponin I, giving a sensitivity and specificity of 77% and 75%, respectively, for the diagnosis of perioperative ischemic injury. In the case of CK, sensitivity and specificity were 66% and 75%, respectively, with a cut-off value of 625 U/L, and for CK-MB they were 88% and 75%, respectively, with a cut-off value of 17 U/L.

Figure 1  Evolutionary trends in troponin I and heart-type fatty acid binding protein. Study times: 1 (T1): baseline, 2 (T2): anastomosis grafting completed, 3 (T3): transfer to CCU, 4 (T4): 4h post-surgery, 5 (T5): 8h post-surgery, 6 (T6): 24h post-surgery, 7 (T7): 48h post-surgery.

Discussion

Myocardial injury can be hard to diagnose in the perioperative cardiac surgery period because cardiac biomarkers can increase above pathological infarction levels in the non-surgical population, electrocardiographic changes are common, and symptoms can vague. According to some authors, measuring markers following cardiac surgery is of little use, since treatment is already fully optimized. Early diagnosis of possible ischemic complications due to occlusion of graft or existing vessels, however, can be resolved with rescue angioplasty or reintervention. Since comorbidity rates are steadily increasing, hybrid treatment can be a useful alternative.

H-FABP is a small cytosolic protein that is not found in the myofibrils of cardiomyocytes. Large quantities of the protein are rapidly released when the integrity of the membrane is threatened by ischemia before irreversible necrosis occurs. Many studies in non-surgical populations have shown the sensitivity of this protein as a predictor of mortality.

Table 4  Biochemical marker levels and sampling times.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Troponin I (ng/mL)</th>
<th>CK (U/L)</th>
<th>CK-MB (U/L)</th>
<th>h-FABP (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.02 (0.02)</td>
<td>83 (81)</td>
<td>15 (5)</td>
<td>4.8 (1.6)</td>
</tr>
<tr>
<td>T2</td>
<td>0.4 (0.6)</td>
<td>101 (76)</td>
<td>24 (11)</td>
<td>18.9 (21.5)</td>
</tr>
<tr>
<td>T3</td>
<td>1.9 (5.2)</td>
<td>181 (140)</td>
<td>26 (18)</td>
<td>22.5 (12.5)</td>
</tr>
<tr>
<td>T4</td>
<td>2.9 (5.2)</td>
<td>336 (247)</td>
<td>23 (13)</td>
<td>36.3 (49.9)</td>
</tr>
<tr>
<td>T5</td>
<td>2.6 (2.6)</td>
<td>553 (473)</td>
<td>31 (33)</td>
<td>37.9 (55.5)</td>
</tr>
<tr>
<td>T6</td>
<td>2.5 (4.3)</td>
<td>741 (779)</td>
<td>37 (51)</td>
<td>18.4 (15.3)</td>
</tr>
<tr>
<td>T7</td>
<td>1.2 (2.3)</td>
<td>542 (598)</td>
<td>17 (13)</td>
<td>9.4 (5.8)</td>
</tr>
</tbody>
</table>

Time intervals: T1: baseline; T2: anastomosis grafts completed; T3: transfer to critical care unit; T4: 4h post-surgery, T5: 8h post-surgery, T6: 24h post-surgery, T7: 48h post-surgery.
Data expressed as mean (standard deviation).

Table 5  Peak biochemical marker plasma values in patients with and without perioperative ischemia.

<table>
<thead>
<tr>
<th></th>
<th>Troponin I (ng/mL)</th>
<th>CK (U/L)</th>
<th>CK-MB (U/L)</th>
<th>h-FABP (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perioperative ischemia (n = 6)</td>
<td>2.1 ± 0.7</td>
<td>1031.7 ± 1149.7</td>
<td>25.4 ± 13.5</td>
<td>59.9 ± 75.8</td>
</tr>
<tr>
<td>No perioperative ischemia (n = 11)</td>
<td>5.1 ± 7.1</td>
<td>577.9 ± 358.7</td>
<td>43.7 ± 58.7</td>
<td>35.1 ± 36.7</td>
</tr>
<tr>
<td>p</td>
<td>0.32</td>
<td>0.23</td>
<td>0.51</td>
<td>0.37</td>
</tr>
</tbody>
</table>
and major cardiac events in patients with acute coronary syndrome; however, few have focused on its value and validity in the post-operative period. Because of its intrinsic properties, h-FABP is an interesting perioperative cardiac surgery marker due to its potential to help identify patients at high risk post-surgery, in whom symptoms and other markers of ischemia are unspecific.

Few studies have been conducted in surgical patients undergoing bypass grafting surgery, and those published to date have focused on patients undergoing performed with CPB pump. Chowdhury et al. compared biochemical markers in off-pump and on-pump patients, and their results for troponin I are similar to ours in the off-pump group. However, h-FABP levels in our series were significantly higher than those obtained by Chowdhury et al., and were nearer those obtained in on-pump patients. This could be due to the different measurement system used in both studies, or to variations in the surgical technique (different stabilizers, or the use or absence of intracoronary shunts). It is interesting to note that in these studies the h-FABP release kinetics in the off-pump group were largely consistent with those obtained in our study.

Muehlschlegel et al. studied 1298 patients undergoing bypass with CPB, finding similar mean h-FABP values to those obtained in our study. In their series, however, peak values occurred mainly after CPB disconnection, while in our series the h-FABP peak occurred at T5 (8 h after transfusion to CUC), roughly 9 h after completion of anastomosis.

Despite differences in the biochemical profile of troponin I and h-FABP demonstrated in many in vivo and in vitro studies, we found kinetic release to be similar, with overlaid curves. One possible explanation for this could be the presence of ischemia-reperfusion phenomena and the cytotoxic effect of circulating neurohormones. Troponin release is not exclusive to acute myocardial infarction, and high sensitivity troponin I can become elevated during perioperative myocardial stress due to cardiomyocyte apoptosis, increased myocardial wall stretch and proteolysis of the contractile apparatus, without the presence of myocardial necrosis. These questions can only be answered by further research into the behavior of this marker in our clinical setting.

The foregoing group established a first post-operative day cut-off value for h-FABP that was found to be an independent predictor of ventricular dysfunction, longer hospital stay and mortality at levels of over 26 ng/mL (18.2–51.5 ng/mL), with a positive predictive value of 13% and a negative value of 95%. In our series, the average first post-operative day value was found to be 18.4 (15.3) ng/mL. Chowdhury et al. established a cut-off value of 6.8 ng/mL to discriminate myocardial injury in patients undergoing on-pump bypass grafting. Our ROC curve-based cut-off value of 19.7 ng/mL (sensitivity 77%, specificity 75%) was significantly higher.

It is also interesting to note in the former study the correlation between long-term increased morbidity/mortality in patients whose second post-operative day values remained higher than post-CPB levels. In our series, 4 patients presented second post-operative day (T7) values above post-anastomosis (T2) levels, but we were unable to establish the clinical significance of this finding.

The correlation between the different markers studied in our series differs slightly from that of the foregoing studies, in which the greatest correlation was found between h-FABP and CK-MB, while our results showed a greater correlation between h-FABP and CK. The correlation between troponin I and h-FABP was lower. We have not been able to ascertain the significance of this finding.

The study is limited by its design, the small sample size, and by its preliminary nature. Other limiting factors are the different surgical techniques employed, since the procedures were performed by different surgeons, and the lack of standardized h-FABP measurement protocols. Possible bias in measurement could be due to the laboratory techniques used. Attempts were made to minimize this by calibrating laboratory instruments at regular intervals, in accordance with the criteria established by specialists and the device manufacturers.

In this preliminary study we have explored the behavior of ischemia and necrosis markers, h-FABP, comparing them with those currently in use in clinical practice in the OPCABG perioperative period. Further studies in larger series would allow us to establish cut-off points for ischemia with a reasonable degree of sensitivity and specificity, and to study the clinical impact and optimize treatment strategies in OPCABG.

Conflict of interests

The authors have no conflicts of interest.

Acknowledgements

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Annex 1.

High sensitivity troponin I (TnI) values were determined by a two-site (sandwich) chemiluminescence immunoenzymatic assay using the Beckman Coulter UniCel® Dxi 800 system. The Access AccuTnI assay is a two-site sandwich enzyme-linked immunosorbent assay. A sample is added to a reaction vessel along with monoclonal anti-cTnI antibody conjugated to alkaline phosphatase and paramagnetic particles coated with monoclonal anti-cTnI antibody. The human cTnI binds to the anti-cTnI antibody on the solid phase, while the anti-cTnI antibody-alkaline phosphatase conjugate reacts with different antigenic sites on the cTnI molecules.

h-FABP values were determined by immunoturbidimetry using the Olympus AU5400™ system. The sample reacts with the buffer and anti-h-FABP antibody-coated latex particles. The formation of immunocomplexes increases the turbidity of the solution, which is measured by light absorption at 700 nm. h-FABP levels are determined by plotting a curve using absorbance values from standard samples.

CK values were determined by means of UV kinetic assays using the Olympus AU5400™ system. CK-MB values were only determined in samples from patients whose CK levels were above high end of normal values. The method used by enzymatic immuno inhibition using the Olympus AU5400™ system.
Myocardial injury by heart-type fatty acid-binding protein

References