Serial measurement of hFABP and high-sensitivity troponin I post-PCI in STEMI: how fast and accurate can myocardial infarct size and no-reflow be predicted?

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Uitterdijk A, Sneep S, van Duin RW, Krabbendam-Peters I, Gorsse-Bakker C, Duncker DJ, van der Giessen WJ, van Beusekom HM. Serial measurement of hFABP and high-sensitivity troponin I (hsTnI) via serial measurements to identify early time points to accurately quantify infarct size and no-reflow in a preclinical swine model of ST-elevated myocardial infarction (STEMI). Myocardial necrosis, usually confirmed by hsTnI or TnT, takes several hours of ischemia before plasma levels rise in the absence of reperfusion. We evaluated the fast marker hFABP compared with hsTnI to estimate infarct size and no-reflow upon reperfusion (2 h occlusion) and nonreperfused (8 h occlusion) STEMI in swine. In STEMI (n = 4) and STEMI + reperfusion (n = 8) induced in swine, serial blood samples were taken for hFABP and hsTnI and compared with triphenyl tetrazolium chloride and thioflavin-S staining for infarct size and no-reflow at the time of euthanasia. hFABP increased faster than hsTnI upon occlusion (82 ± 29 vs. 180 ± 73 min, P < 0.05) and immediately increased upon reperfusion while hsTnI release was delayed 16 ± 3 min (P < 0.05). Peak hFABP and hsTnI reperfusion values were reached at 30 ± 5 and 139 ± 21 min, respectively (P < 0.05). Infarct size (containing 84 ± 0.6% no-reflow) correlated well with area under the curve for hFABP (r² = 0.92) but less for hsTnI (r² = 0.53). At 50 and 60 min reperfusion, hFABP correlated best with infarct size (r² = 0.94 and 0.93) and no-reflow (r² = 0.96 and 0.94) and showed high sensitivity for myocardial necrosis (2.3 ± 0.6 and 0.4 ± 0.6 g). hFABP rises faster and correlates better with infarct size and no-reflow than hsTnI in STEMI + reperfusion when measured early after reperfusion. The highest sensitivity detecting myocardial necrosis, 0.4 ± 0.6 g at 60 min postreperfusion, provides an accurate and early measurement of infarct size and no-reflow.

heart-specific fatty acid binding protein; high-sensitivity troponin I; markers of myocardial necrosis; no reflow; STEMI model; acute myocardial infarction

FOR THE ASSESSMENT of novel therapies to treat acute ST-elevated myocardial infarction (STEMI), it is of great importance that at baseline, infarct size and no-reflow are determined precisely to be able to validate, tailor, or adjust experimental therapies. The efficacy of treatment for STEMI in (pre)clinical studies can only be accurately determined if the outcome can be compared with initial infarct size and subsequent area of no-reflow. Contemporary imaging modalities for determination of these parameters such as cardiac magnetic resonance imaging or echocardiography are often unsuitable for this purpose because of practical, temporal, resolutional, or economic considerations. These imaging modalities are also inadequate to assess relatively small infarct sizes (11). Furthermore, it is unclear whether the development of acute edema, often used to determine the area at risk, interferes with acute assessment of these parameters (2).

Standard, fast detectable markers such as creatine kinase and myoglobin may be regarded as considerably unspecific. Myoglobin, for example, the faster marker, with peak values at 12 h after onset of infarction and at 1–2 h after onset of reperfusion in patients (10), is not cardiac specific and is also prevalent in skeletal muscle tissue. Baseline values are especially high in a setting of soft tissue trauma or extreme exercise (8, 20). Creatine kinase MB (CKMB), specific for heart and brain, and the cardiосpecific troponins are of great predictive value for presence of myocardial necrosis. Troponins are bound to the contractile apparatus and therefore released relatively slowly. As a consequence of slow troponin clearance, late (72 h) assessment is very accurate but by its nature does not allow early detection of efficacy in acute interventions (5). Both CKMB and troponin T show peak values following reperfusion between 7 and 8 h (18). This disqualifies troponins and CKMB both as early and acute markers for infarct size determination. Consequently, a heart-specific, early-released, and accurately detectable marker for an early estimation of infarct size and no-reflow is needed (7, 22).

Such a candidate could be heart-specific fatty acid binding protein (hFABP), which is a small (15 kDa) protein and is located in the cytoplasm (13). hFABP is a heart-specific isoform of a larger family of FABP members and 10 times more specific for cardiac tissue than myoglobin (28). In view of its small size and cytoplasmic localization, an early, diffusion-driven, and perfusion-facilitated release pattern is expected. However, the performance of hFABP as a biomarker of necrosis has not been explored in a large animal model of STEMI. Consequently, to determine whether this rediscovered marker is suitable for the determination of infarct size upon reperfusion, we compared timing and release of hFABP to the gold standard high-sensitivity troponin I (hsTnI) (24 kDa) following STEMI (sustained and reperfused) in swine using planimetrical infarct size determination by triphenyl tetrazolium chloride (TTC). In addition, we aimed to understand the release of these biomarkers in relation to no-reflow which was determined by thioflavin-S staining.

METHODS

Experiments were performed in 5- to 6-mo-old farm-bred swine (38 ± 1 kg, n = 14) of either sex as described before (9). Experiments...
were conducted in compliance with the “Guide for the Care and use of Laboratory Animals” and after written approval of the Animal Ethics Committee of the Erasmus MC. In short, animals were sedated with an intramuscular injection of midazolam (1 mg/kg, Actavis, Baarn, The Netherlands) and ketamine (20 mg/kg, Anisane, Raamsdonkveer, The Netherlands). Following an intravenous ear catheter placement, anesthesia was induced with an intravenous injection of 600 mg pentobarbital sodium. Animals were intubated and mechanically ventilated (O₂:N₂ = 1:3). Anesthesia was maintained with pentobarbital sodium (15 mg·kg⁻¹·h⁻¹). Body core temperature was continuously measured and when necessary adjusted with heating pads (9). Fluid loss was compensated for by an intravenous drip (100 ml/h saline). After the placement of an intra-arterial sheath (9F, Super Sheath, Boston Scientific, Nieuwegein, The Netherlands), 10,000 IU of heparin and 250 mg acetylsalicylic acid (Aspégic, Sanofi Aventis, Gouda, The Netherlands) were administered for anticoagulation followed by 5,000 IU of heparin every additional hour.

**STEMI model and blood sampling.** The left circumflex coronary artery (LCx) was catheterized with a standard clinical guiding catheter (IL3.5, Boston Scientific) and quantitative coronary angiography (CAAS II, PIE Medical, Maastricht, the Netherlands) was performed following 1 mg isosorbide dinitrate (Cedocar, Nycomed, Hoofddorp, The Netherlands) and using ioxilanol as a contrast agent (Visipaque, GE Healthcare BV, Eindhoven, The Netherlands). Then, an over the wire coronary angioplasty balloon (Axep PTCA Dilatation Catheter, Boston Scientific) on a standard guide wire (Luge, 0.37 mm × 128 cm, moderate support, Boston Scientific) was carefully positioned under fluoroscopic guidance to create a broad range of infarct sizes. Following balloon inflation, occlusion of the target vessel was confirmed by angiography at baseline and every subsequent hour. In the sustained occlusion group (n = 4), the occlusion was maintained for 8 h without reperfusion. In the STEMI + reperfusion group (n = 10), the occlusion was released after 2 h, followed by 6 h of reperfusion. A minimum of 2 h of occlusion was chosen to ascertain a transmural infarction that corresponds with the plateau phase of infarct development in swine (23).

Blood samples (BD Vacutainer, 10.8 mg K2E, Becton Dickinson, Breda, The Netherlands) were immediately put on ice prior to centrifugation and plasma was stored (−80°C) for later marker analyses. Blood samples were collected at baseline and every 30 min during occlusion, every 10 min upon the first hour of reperfusion followed by every 30 min during the remaining hours.

**Detection of risk area, infarct size, and no-reflow.** After finalizing blood sampling, a sternotomy was performed and the balloon was reinflated to reocclude the LCx artery. In the STEMI + reperfusion group, 10 ml 4% (wt/vol) thioflavin-S solution (Sigma, Zwijndrecht, The Netherlands) was injected into the left atrium for negative staining of the area at risk. Following balloon inflation, occlusion of the target vessel was confirmed by angiography at baseline and every subsequent hour. In the sustained occlusion group (n = 4), the occlusion was maintained for 8 h without reperfusion. In the STEMI + reperfusion group (n = 10), the occlusion was released after 2 h, followed by 6 h of reperfusion. A minimum of 2 h of occlusion was chosen to ascertain a transmural infarction that corresponds with the plateau phase of infarct development in swine (23).

**Risk area, infarct, size and no-reflow by Evans blue, TTC, and thioflavin S.** The different infarct sizes, created by inflating the balloon at different locations along the coronary tree, resulted in a risk area of 8.3 ± 1.3 g [7.6–15.5% of left ventricle (LV)] and 19.4 ± 3.4 g (12.2–43.3% of LV) in the chronic occlusion and reperfusion group, respectively. Weight of the infarcted tissue varied from 7.9 ± 3.9 g (7.3–15.5% of LV) to 14.9 ± 3.6 g (4.6–39.4% of LV) in the chronic occlusion and reperfusion group, respectively. Linear regression showed that infarct size versus area at risk was similar for the two groups with a slope of 0.94 ± 0.09 (r² = 0.98) and 1.06 ± 0.12 (r² = 0.94), respectively.

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**Table 1. Peri-procedural hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>CAO</th>
<th>Δ from 2 h CAO ± R</th>
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<tr>
<td>HR, beats/min</td>
<td></td>
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<tr>
<td>8 h CAO</td>
<td>67 ± 6</td>
<td>68 ± 3</td>
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<tr>
<td>2 h CAO + R</td>
<td>67 ± 6</td>
<td>68 ± 3</td>
<td>79 ± 3*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>8 h CAO</td>
<td>78 ± 7</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>2 h CAO + R</td>
<td>78 ± 7</td>
<td>68 ± 4</td>
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Data are expressed as means ± SE. Hemodynamic monitoring during sustained occlusion (8 h CAO, n = 4) and during 2 h occlusion and 6 h reperfusion (2 h CAO + R, n = 8). HR, heart rate; MAP, mean arterial pressure; CAO, coronary artery occlusion; R, reperfusion; BL, baseline. *P < 0.05 vs. corresponding BL time point; †P < 0.05 vs. corresponding 2 h CAO + R time point.
Correlation between no-reflow and infarct size. A very tight correlation was found between no-reflow and infarct size (Fig. 1, \( r^2 = 0.97 \)), suggesting that no-reflow is dictated by infarct size. This duration of ischemia, chosen to reflect the maximum development of no-reflow as determined in rabbits (14), showed that \( ~84 \pm 0.6\% \) of the infarct size contained no-reflow, regardless of infarct size when larger than \( ~1.55 \) g. Only with small infarcts will no-reflow be negligible or fall below detectable levels with the demonstrated techniques.

Marker release during sustained occlusion. hFABP increased significantly faster than hsTnI upon occlusion in all animals with sustained 8 h occlusions (82 ± 29 vs. 180 ± 73 min, \( P < 0.05 \)), but peak values were not reached within the course of the experiment (Fig. 2A). Area under curve (AUC) for hFABP and hsTnI (76.2 ± 14.2 and 10 ± 3.1 ng·h·ml⁻¹, \( P < 0.004 \), corrected for baseline values of 6 ± 1 and 0.3 ± 0.1 ng·h·ml⁻¹, respectively) showed a moderate correlation (\( r^2 = 0.57 \) vs. \( r^2 = 0.34 \)) with infarct size (11 ± 2% of LV) produced by the 8 h sustained occlusions.

Marker release upon reperfusion: infarct size. Reperfusion after 2 h of ischemia resulted in an immediate increase in hFABP while hsTnI release was only apparent after 16 ± 3 min (Fig. 2B, \( P < 0.05 \)). hFABP and hsTnI peak values were reached in 30 ± 5 and 139 ± 21 min (\( P < 0.05 \), respectively. Infarct size by TTC correlated very well with 8 h AUC for hFABP (Fig. 3A, \( r^2 = 0.91 \)) but only moderately for hsTnI (Fig. 3, \( r^2 = 0.51 \)). Reperfusion peak values showed an equally good correlation with infarct size for hFABP (Fig. 3C, \( r^2 = 0.92 \)) and a similarly moderate correlation for hsTnI (\( r^2 = 0.56 \)). The highest correlation was found at 50 min postreperfusion (\( r^2 = 0.94 \)) with hFABP.

Marker release upon reperfusion: no-reflow. No-reflow correlated well with hFABP AUC (Fig. 3B, \( r^2 = 0.94 \)) but again only moderately with hsTnI (Fig. 3B, \( r^2 = 0.48 \)). Reperfusion peak values of hFABP also correlated well (Fig. 3D, \( r^2 = 0.91 \)) and again hsTnI performed less well (\( r^2 = 0.48 \)). The highest correlation with no-reflow was found at 50 min postreperfusion (\( r^2 = 0.96 \)) with hFABP.

Optimal time points and lower levels of detection for myocardial necrosis and no-reflow. We examined which time points correlated best with infarct size and no-reflow by plotting the coefficients of determination of all time points analyzed before reperfusion and of those time points in which an average discernible marker elevation was apparent (Fig. 4). hsTnI did not show any strong correlations with a maximum of \( r^2 = 0.59 \) at 120 min postreperfusion and continued fluctuating with a lower limit of \( r^2 = 0.40 \) at 210 min within the chosen time frame.

Correlation for hFABP remained high and was best at 50 and 60 min postreperfusion for both infarct size as well as no-reflow (\( r^2 = 0.94 \) and 0.93; \( r^2 = 0.96 \) and 0.94 (Fig. 3, E and F)). In addition, the first 90 min postreperfusion continuously produced useful correlations with regard to hFABP levels (infarct size: \( r^2 = 0.87–0.94 \); no-reflow: \( r^2 = 0.87–0.96 \)). The most considerable correlation for choosing the 50 or 60 min time point is the sensitivity to detect small myocardial infarctions [\( 2.3 \pm 0.6 \) and 0.4 ± 0.6 g (2.7 and 0.5% of LV)] and no-reflow [0.6 g (4% of infarct size)] by determining the x-intercept at \( y = 0 \).

DISCUSSION

The aim of the present study was to determine the value of hFABP for in vivo assessment of infarct size and no-reflow in a large animal model of reperfused STEMI. We found that hFABP rose within 60 min after onset of occlusion and, upon reperfusion, showed an immediate and steep incline. We observed a strong correlation between infarct size and hFABP...
reperfusion peak values \( r^2 = 0.92 \) at 30 ± 5 min of reperfusion. While a good correlation can be found as early as 10 min postreperfusion \( r^2 = 0.88 \) the sensitivity to detect small amounts of myocardial necrosis is low \( (4.6 \pm 0.8 \text{ g}). \) An optimal correlation with infarct size was found at 50–60 min postreperfusion \( (r^2 = 0.94–0.96) \) allowing detection of \( 2.3 \pm 0.6 \) and \( 0.4 \pm 0.6 \text{ g} \) of necrotic myocardium.

No-reflow, as quantified by absence of thioflavin-S, correlated excellently with infarct size \( (r^2 = 0.97) \). Consequently, the correlation of hFABP with no-reflow was equally good with AUC \( (r^2 = 0.94) \) and reperfusion peak values \( (r^2 = 0.91) \), but best at 50–60 min reperfusion \( (r^2 = 0.96–0.94) \). Lower limit detection of no-reflow was calculated to be \( < 0.6 \text{ g}. \)

hsTnI performed consistently worse than hFABP, and peak values were obtained significantly later \( (P < 0.005). \)

Release patterns of cardiac markers following injury. The delayed release of biomarkers in sustained occlusion is due to the fact that release is diffusion driven and perfusion facilitated. The cytosolic localization of hFABP and subsequent fast release is reflected in the significantly faster release compared with hsTnI \( (P < 0.05). \)

hFABP, a small cytosolic protein, is easily released from injured and permeabilized cardiomyocytes. In contrast, cardiac

![Fig. 3. Correlation of the area under curve (AUC) and reperfusion values to infarct size and no-reflow in reperfused STEMI. The relation between release of hFABP (■) and hsTnI (●) with infarct size (A, C, and E) and no-reflow (B, D, and F) as determined by the AUC (A and B), reperfusion peak values (C and D), and values at 60 min postreperfusion (E and F) in reperfused STEMI \( (n = 8) \). No-reflow correlated well with hFABP AUC \( (B, r^2 = 0.94) \) but only moderately with hsTnI \( (B, r^2 = 0.48) \). Reperfusion peak values of hFABP again correlated well with no-reflow \( (D, r^2 = 0.91) \) and again hsTnI performed poorly \( (D, r^2 = 0.48) \). At 60 min postreperfusion, the correlation was optimal for detecting myocardial necrosis \( (< 0.4 \pm 0.6 \text{ g}) \) \( (E, r^2 = 0.93) \) and no-reflow \( (F, r^2 = 0.94). \)
troponins are bound to the contractile apparatus, which significantly delays release (34). Indeed, our results show significant differences in release of hFABP and hsTnI, both in terms of onset of release and time to peak.

No-reflow upon reperfusion is characterized by microvascular obstruction, microvascular damage, and mechanical compression by myocyte edema (36) and could theoretically affect the release of markers due to decreased perfusion. However, the fast release by myocyte edema (36) and could theoretically affect the release of hFABP, which takes place predominantly before the major obstruction, microvascular damage, and mechanical compression on washout kinetics of hFABP.

Our study showed a tight correlation between infarct size and the extent of no-reflow with 84% of the infarction showing no-reflow. Hence the relation between biomarker release was equally strong for infarct size and no-reflow. Interestingly, no-reflow is increasingly being appreciated as an important prognosticator for long-term outcome and was recently suggested to be, at least in part, independent of myocardial necrosis, thus questioning the well-established concept that no-reflow is dictated by infarct size (15, 27). Future studies using interventions to affect no-reflow independently of effects on necrosis can shed light on the influence of no-reflow on washout kinetics of hFABP.

Since hFABP plasma levels decrease fast by renal clearance, this is responsible for the differences in the plasma elimination rates as cardiac troponins are known to remain elevated for longer periods of time (5, 17). It indicates the need for proper timing when sampling hFABP and indicates why hsTnI is more sensitive as a late marker for ischemia when hFABP is no longer detectable.

Sensitivity of hFABP for infarct size and no-reflow. Our data are the first to describe the precise “rise and fall” of circulating plasma levels of hFABP upon reperfusion of STEMI in a large animal model. Previously, only a single time point was measured in swine at 2 h postreperfusion (33). We performed the same analysis and found a good correlation with infarct size ($r^2 = 0.78$), but this resulted in a lower sensitivity to detect small infarctions (lower level of detection 5.6 g). In comparison, peak and 50–60 min postreperfusion values show a calculated lower level of detection of 3.1, 2.3, and 0.4 g necrotic myocardial tissue, which on average corresponds with 3.6, 2.7, and 0.5% of the LV. Therefore, these values are not only obtained faster but result in a higher sensitivity to detect necrosis and no-reflow.

Infarct size and, with increasing insight, no-reflow are the main determinants of LV remodeling. When therapy is started within 24 h a reliable assessment of infarct size and no-reflow is mandatory for assessment of therapeutic efficacy, especially in small treatment groups. Both contemporary imaging modalities as well as enzyme or biomarker release studies remain inadequate, noneconomical, or impractical for acute assessment of these end points. Here, we demonstrate that hFABP AUC, hFABP peak values, and primarily 50–60 min postreperfusion biomarker levels correlate very well with acute infarct size and no-reflow in reperfused STEMI. Although high-sensitivity troponin assays continue to improve (6), hFABP is of great value for settings where onset of reperfusion is closely controlled. Especially for longitudinal studies aiming to employ long-term follow-up, this relatively easy and fast sampling method allows both an accurate in vivo infarct size and no-reflow quantification method without the need for additional imaging modalities early after infarction.

Clinical relevance. hFABP can be of added clinical value, especially in a multimarker approach (3, 4, 24, 26, 31), but contradictory evidence exists (1, 19, 30). This may stem from difficulties in estimating the time from onset of ischemia to presentation, as well as variations in onset of spontaneous reperfusion and presence of collateral flow. In combination with rapid renal clearance, hFABP in this setting is difficult to interpret without this awareness (1). It does not disqualify hFABP as a useful marker for infarct size or no-reflow determination, especially in the setting of post-percutaneous coronary intervention (post-PCI) reperfusion in STEMI. In absence of spontaneous reperfusion for example, hFABP will yield an accurate and early determination of infarct size. Data can be obtained in the lab prior to opportunities such as MRI for determination of these prognostic parameters, especially with the recent development of fast (15–20 min) qualitative point-of-care tests (21). Moreover, no-reflow is increasingly being appreciated for its additional prognostic value and hFABP was shown efficacious in long-term prediction of survival (25, 32). In the clinical setting, the timing is of crucial importance and must be taken into account when studying the

Fig. 4. Temporal changes in coefficients of determination (i.e., $r^2$) for the circulating plasma concentrations of hFABP (●) and hsTnI (○) for infarct size (A) and no-reflow (B). Data show a window of opportunity between 20 and 90 min of reperfusion. The major consideration for choosing an optimal time point is the sensitivity to detect small myocardial infarctions. R = onset of reperfusion.
relevance of this marker. It must be noted that while hFABP remains of interest for clinical application, the present study principally demonstrates the suitability of serial hFABP measurement for preclinical purposes.

Study limitations. The range of infarct sizes tested in this study was limited to 4.6–39.4% of the LV. The predictive value of hFABP for infarct size or no-reflow determination in smaller or larger infarcts therefore remains to be determined, but the lower limit of detection is expected to be <1 g of myocardial tissue in animals of 38 ± 1 kg. An important limitation of the work is the limited follow-up time of 6 h postreperfusion as it is apparent that troponin continues to rise for 72 h (55). The data presented here should therefore be interpreted in light of the very early phase postreperfusion, taking into account that the study was designed for hFABP validation for baseline infarct size determination in an acute, preclinical setting.

Conclusions. hFABP release shows an early and distinct release pattern, correlates strongly to infarct size and no-reflow, and is detectable significantly earlier than hsTN.

hFABP plasma levels at 50–60 min postreperfusion provide an excellent, sensitive, accurate, and early biomarker to assess infarct size and no-reflow for longitudinal preclinical infarct-reperfusion studies and holds promise for clinical applications such as the controlled post-PCI setting.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES
1. Aldous S, Pemberton C, Troughton R, Than M, Mark Richards A. Heart fatty acid binding protein and myoglobin do not improve early rule out of acute myocardial infarction when highly sensitive troponin assays are used. Resuscitation 83: e27–e28; author reply e29–e30, 2012.


