Release kinetics of early ischaemic biomarkers in a clinical model of acute myocardial infarction

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INTRODUCTION

Acute myocardial infarction (AMI) is associated with high morbidity and mortality.1 Well-established biomarkers, such as the cardiac troponins, aid in the diagnosis of patients with acute coronary syndrome (ACS) and help determine risk stratification for individual patients.2–5 However, the majority of patients with chest pain and suspected ACS are diagnosed with other cardiac or extra-cardiac conditions.6 The main issue with cardiac troponins is that there is a diagnostic gap in the first hours after onset of myocardial ischaemia and a need for a second blood draw to detect changes in their concentration. Accordingly, many patients are unnecessarily hospitalised or incorrectly discharged.7

Recent studies have suggested that using biomarkers such as soluble fms-like tyrosine kinase (sFlt-1), ischaemia modified albumin (IMA), and heart-type fatty acid binding protein (hFABP) in combination with cardiac troponins might help diagnose or rule out AMI.8–14 Several studies have shown that animal models of AMI can be used to provide information about the release kinetics of biomarkers.15–17 However, such findings cannot be extrapolated to humans due to differences in physiological parameters (e.g., differences in metabolism and release).18 19

We recently reported the early release kinetics of cardiac troponin T (cTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), copeptin, and several microRNAs.20–23 There are currently no data regarding the exact release kinetics of sFlt-1, IMA, and hFABP in patients with AMI. Because the exact time point when myocardial ischaemia begins is often unclear or is reported incorrectly, and because of patient related delays before presentation at the hospital, the early release kinetics of these early myocardial ischaemic biomarkers are not well described.

The aim of the present study was to characterise the time course of changes in sFlt-1, IMA, and hFABP concentrations in patients undergoing transcoronary ablation of septal hypertrophy (TASH). Notably, this procedure creates conditions similar to those seen in patients with AMI.

METHODS

Study design

From January 2010 until June 2011, 21 consecutive patients with hypertrophic obstructive cardiomyopathy (HOCM) undergoing TASH were included in the study. Details of the pre- and postprocedural management of the patients has been previously published.20–23 Briefly, the clinical history and results of physical examination, 12-lead ECG, laboratory tests, echocardiography, and coronary angiography were determined for all patients. A final diagnosis of HOCM was made according to the current guidelines based on severe symptoms during physical activity, asymmetrical septal hypertrophy >15 mm, systolic movement of the anterior mitral valve leaflet, and an intraventricular pressure gradient ≥30 mm Hg at rest and/or...
>50 mm Hg after the Valsalva manoeuvre. TASH was performed according to standard clinical practice with temporary septal branch occlusion for selective therapeutic injection of 96% ethanol after heparin administration (bolus, 5000 IU heparin). Post-procedural management included monitoring at the intensive care unit for 48 h.

All patients provided written informed consent before participation in the study, and approval was obtained from the ethical board of the state of Hessen, Germany (FF 31/2010). The investigation adhered to the principles outlined in the Declaration of Helsinki.

**Laboratory assessment**

Venous blood samples for determination of sFlt-1, IMA, and hFABP were collected before and at 15, 30, 45, 60, 75, 90, and 105 min and, 2, 4, 8, and 24 h after induction of myocardial infarction. Serum and EDTA-plasma were processed immediately and frozen at −80°C until assayed.

sFlt-1 was measured in serum using an electrochemiluminescence immunoassay (sFlt-1 assay, Elecsys 2010 Chemical Analyzer, Roche Diagnostics, Mannheim, Germany). The lower detection limit for the sFlt-1 assay is 10.0 ng/L, and the highest concentration measurable is 85 000.0 ng/L.

IMA was measured in plasma using a quantitative sandwich immunoassay (Human Ischemia Modified Albumin ELISA kit, CUSABIO Biotech Co, Ltd, Wuhan, PR China). The package insert indicates that the minimum detectable concentration of IMA is 3.12 U/mL, and the highest measurable concentration is 200.0 U/mL.

The hFABP concentration was measured in serum using a latex enhanced immunoturbidimetric assay (Randox H-FABP assay, Randox Laboratories Limited, Crumlin, Co Antrim, UK) adapted to the Architect c8000 analyzer from Abbott Diagnostics. The minimum detectable concentration of hFABP is 0.75 ng/mL.

**Statistical analysis**

All data for continuous variables are expressed as mean±SD or as median and IQR, as appropriate. Categorical variables are reported as number and percentage. Continuous variables were compared using the Wilcoxon signed-rank test. Within-subject comparisons are made across repeated observations without correction for multiple comparisons. The data were distributed non-parametrically as tested by the Kolmogorov-Smirnov test. All statistical tests were performed with SPSS software, V15.0. A two-tailed p value <0.05 was considered to be statistically significant.

**RESULTS**

The baseline characteristics of the patients enrolled in this study (13 men, eight women, mean (SD) age 59.0 (13.29) years) are shown in table 1 and were described in detail previously. All TASH procedures were performed in a single session using single septal branch occlusion. The mean (SD) ethanol administered during the procedure was 1.77 (0.59) mL. The median occlusion time was 20.0 min (IQR 14.5–31.0).

The median sFlt-1 concentration was significantly increased 15 min after myocardial infarction was initiated (3657.5 ng/L, IQR 2302.3–4475.0 ng/L) compared with the median baseline concentration (76.0 ng/L, IQR 71.2–88.8 ng/L; p<0.001). sFlt-1 concentrations returned to baseline value after 480 min (85.9 ng/L, IQR 70.8–92.6 ng/L), compared with the median baseline concentration (reported above; p=0.77) (figure 1A). We observed an increase in the sFlt-1 concentration in every patient at the first post-induction time point. All patients showed significant increases in sFlt-1 compared to the baseline value at the 15 min time point (range of the percentage increase (min–max): 3811.8–4186.8%; range of absolute increase (min–max): 1996.62–4773.0 ng/L).

Measurement of serum IMA concentrations revealed a significant increase 30 min after induction of myocardial infarction (26.0 U/mL, IQR 21.8–38.6 U/mL) compared with the median baseline concentration (15.6 U/mL, IQR 10.1–24.7 U/mL; p=0.016). IMA concentrations showed a continuous increase until the 75 min time point and then decreased until 24 h after initiation of myocardial infarction (figure 1B). Every patient had a significant increase in his/her IMA concentration compared to his/her baseline value after 45 min (range of the percentage increase (min–max): 45.6–67.7%; range of the absolute increase (min–max): 3.35–18.16 U/mL).

The median hFABP concentration was significantly increased at the 15 min time point (9.0 ng/mL, IQR 7.0–15.4 ng/mL) compared with the median baseline concentration (4.6 ng/mL, IQR 3.4–7.1 ng/mL; p<0.001). The median hFABP concentration showed a continuous increase until the 8 h time point, with a plateau phase between 1–2 h after induction of AMI (figure 1C). All patients had a significant increase of >40% compared to the baseline value after the first post-induction time point (range of the percentage increase (min–max): 44.2–377.9%; range of the absolute increase (min–max): 1.19–182.3 ng/mL).

The sFlt-1, IMA, and hFABP concentrations at each of the time points are shown in table 2. The biomarker values with percentage change of all patients during the first 240 min after induction of AMI are shown in figure 2. We observed a highly significant difference between the diverse kinetics presented in percentage change (p<0.0001; Friedmann’s test for multiple comparisons). In addition, we performed a correlation analysis between the maximum hs-cTnT concentration, representing the extent of myocardial injury, and the different biomarker concentrations for the first 240 min after induction of AMI. The concentrations of sFlt-1 (r=0.304, p=0.22), hFABP (r=0.377, p=0.10), and IMA (r=0.049, p=0.86) did not show any significant correlation with hs-cTnT. Furthermore, the maximum concentrations of sFlt-1, hFABP, IMA, and hs-cTnT during the first 240 min after induction of AMI did not show any significant correlation with the length of the balloon occlusion time or the amount of the administered ethanol. There were no sex specific differences observed among the rates of increase for the different biomarkers.

**Table 1 Baseline characteristics of 21 patients undergoing transcoronary ablation of septal hypertrophy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>(n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>59.0 (13.3)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>13 (61.9)</td>
</tr>
<tr>
<td>Body mass index, kg/m², mean (SD)</td>
<td>30.19 (6.94)</td>
</tr>
<tr>
<td>Cardiovascular risk factors, n (%)</td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>10 (47.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (61.9)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>6 (28.6)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (28.6)</td>
</tr>
<tr>
<td>Family history</td>
<td>6 (28.6)</td>
</tr>
<tr>
<td>Laboratory measurements</td>
<td></td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>68.6 (IQR 66.9–81.8)</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate</td>
<td>90.53 (IQR 79.04–113.73)</td>
</tr>
</tbody>
</table>

DISCUSSION

Given the large number of patients hospitalised with chest pain, identifying patients with an ACS represents a daily clinical challenge, especially in the absence of clear symptoms and electrocardiographic changes. In such cases, evaluation of cardiac troponin values plays a central role in providing both diagnostic and prognostic information. However, when using cardiac troponin values in clinical practice, additional diagnostic validation is required, and routine clinical chemistry testing is necessary (ie, troponin concentrations after 3–6 h) following initial presentation, even in this ‘high sensitivity era’. Published data demonstrate that a multi-marker strategy could improve diagnostic accuracy in terms of diagnosing and/or ruling out AMI. However, the detailed release kinetics of the promising biomarkers sFlt-1, IMA, and hFABP had not been determined before the present study. Our study elucidates in detail the early release kinetics of these biomarkers after induction of myocardial infarction in patients undergoing TASH as a surrogate for AMI. We used the TASH procedure as a model of AMI because it closely mimics the pathophysiology of AMI and allows well-defined chronological assignment of biomarker release relative to the initiation of myocardial infarction.

sFlt-1 is elevated in patients with ACS, especially in patients presenting early (<60 min) after symptom onset. Our data clearly show that sFlt-1 is released rapidly within the first 15 min after induction of myocardial infarction; subsequently, values decrease and return to baseline concentrations after 4–8 h. This excessive release of sFlt-1 might be partly explained by the heparin administration, which displaces the sFlt-1 heparin binding site from heparin sulfate proteoglycans. Nevertheless, a considerably early release of sFlt-1 could also be demonstrated in a mice model of coronary occlusion with left coronary ligation. Despite the heparin induced sFlt-1 release there is evidence for diagnostic and/or prognostic use in patients with ACS. Previously published data demonstrated that the combination of sFlt-1 and high sensitivity cardiac troponin I (hs-cTnI) on admission provided a negative predictive value (NPV) of 98.4%, which was comparable to the highest NPV of 99.6% provided by hs-cTnI 3 h after admission; however, the combination of sFlt-1 and hs-cTnI on admission did not achieve the high positive predictive value of the relative change within a rule-in of AMI.

hFABP has been shown to be superior to other early ischaemic biomarkers for diagnosing AMI. In a post hoc-defined cohort, hFABP measurement on admission yielded an area under the curve of 0.89 (95% CI 0.87 to 0.91) for identification of AMI that was improved to 0.97 (95% CI 0.96 to 0.98; p=0.02) when combined with hs-cTnI on admission. Furthermore, hFABP determination can also rule out early AMI. Elevated hFABP is a predictor of death or recurrent AMI for up to 1 year and provides additional prognostic information independent of cTnT. In our study, we observed...
considerable early release of hFABP (within the first 15 min following TASH). In this study, measurement of hFABP was better than measuring IMA in terms of earlier detection after induction of AMI. We demonstrated recently that copeptin is released quickly (within the first 30 min) after the induction of myocardial infarction, reaches a maximum after 90 min, and returns to baseline concentrations after 24 h.\(^\text{21}\) IMA shows release kinetics comparable to those of copeptin, with the first significant difference detected at the 30 min time point. Like copeptin, IMA is an independent predictor of cardiac outcome after AMI.\(^\text{28}\) In contrast to the diagnostic value of copeptin in terms of early rule-out of AMI, IMA does not seem to be better than other established biomarkers.\(^\text{29} \text{ 30}\)

The characterisation of the kinetics of the appearance of detectable changes in early ischaemic biomarker concentrations in patients with AMI is of major clinical and socioeconomic importance, because chest pain is one of the most frequent causes of emergency department admissions. We previously demonstrated the early release of cTnT, as measured using a high sensitivity assay, compared to the release of other myocardial biomarkers.\(^\text{20}\) In brief, as assessed by the hs-cTnT assay, all patients had a significant increase of >50% compared to the baseline value at the 15 min time point (table 2). At the 30 min time point, all patients had hs-cTnT concentrations above the 99th centile value with continuous increases at all of the pre-specified time points.

In contrast, the release kinetics of sFlt-1 and hFABP are completely different. sFlt-1 was highly elevated 15 min after induction of myocardial infarction and decreased continuously afterwards, reaching baseline values 4 h after induction of myocardial infarction. Recently published data demonstrated only limited diagnostic information resulting from sFlt-1 when used together with hs-cTnT for the early diagnosis of AMI.\(^\text{8}\) We can speculate that Hochholzer et al did not look for the right patients—the early presenters only. Regarding the release kinetics of sFlt-1, the impact of this biomarker in terms AMI diagnostics should be validated once more.

Whereas hs-cTnT concentrations increased continuously during all time points, hFABP concentrations decreased 24 h after TASH. Regarding these different release kinetics, hFABP could be helpful in order to diagnose the possible phenomenon of myocardial re-infarction.

Understanding the time course of the release of cardiac troponins and other biomarkers and correlating the values with patient symptoms and the results of ECG and imaging studies is important for early diagnosis, individual risk stratification, and individualised therapy, especially in the hours early after symptom onset. If it was unnecessary to additionally validate cardiac troponin values in patients with suspected ACS, a shorter observation period would be needed, which would have major resource implications.\(^\text{31}\) However, in the real world, this validation needs to be performed to establish fast-track work-up protocols in the chest pain unit.

### Study limitations

This is the first study to measure early ischaemic biomarkers in patients with HOCM undergoing TASH. However, the study has some limitations. The studied patients did not have coronary artery disease and therefore did not have the possible phenomenon of ischaemic preconditioning; this may have influenced

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**Table 2** Concentrations of the indicated biomarkers in 21 patients undergoing transcoronary ablation of septal hypertrophy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>sFlt-1* (ng/L) Median (IQR)</th>
<th>IMA (U/L) Median (IQR)</th>
<th>hFABP (ng/mL) Median (IQR)</th>
<th>hs-cTnT (ng/L) Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>76.01 (71.18–88.81)</td>
<td>15.6 (10.1–24.7)</td>
<td>4.6 (3.4–7.1)</td>
<td>11.3 (6.3–18.8)</td>
</tr>
<tr>
<td>15 min</td>
<td>3657.5 (2302.3–4475.0)</td>
<td>21.8 (15.3–32.5)</td>
<td>9.0 (7.0–15.4)</td>
<td>21.4 (13.3–39.7)</td>
</tr>
<tr>
<td>30 min</td>
<td>2948.0 (2146.5–3608.5)</td>
<td>26.0 (21.8–38.6)</td>
<td>21.4 (15.8–28.2)</td>
<td>51.3 (36.7–146.9)</td>
</tr>
<tr>
<td>45 min</td>
<td>2062.9 (1761.5–2846.5)</td>
<td>33.8 (17.8–40.5)</td>
<td>32.9 (25.2–44.1)</td>
<td>103.5 (78.4–201.6)</td>
</tr>
<tr>
<td>60 min</td>
<td>1620.0 (1346.0–2062.0)</td>
<td>30.6 (18.4–47.9)</td>
<td>34.9 (25.2–47.6)</td>
<td>194.3 (115.3–294.2)</td>
</tr>
<tr>
<td>75 min</td>
<td>1345.0 (1055.0–1424.5)</td>
<td>36.6 (22.4–44.4)</td>
<td>45.4 (30.2–59.9)</td>
<td>218.2 (143.3–316.3)</td>
</tr>
<tr>
<td>90 min</td>
<td>970.4 (834.1–1106.0)</td>
<td>35.3 (26.0–46.9)</td>
<td>42.9 (34.2–60.2)</td>
<td>321.2 (215.1–517.3)</td>
</tr>
<tr>
<td>105 min</td>
<td>692.7 (579.9–845.6)</td>
<td>21.2 (12.9–40.3)</td>
<td>44.3 (35.9–60.2)</td>
<td>351.9 (202.8–467.3)</td>
</tr>
<tr>
<td>2 h</td>
<td>536.8 (395.3–680.2)</td>
<td>25.9 (19.7–54.8)</td>
<td>46.7 (38.6–62.5)</td>
<td>429.4 (234.4–547.6)</td>
</tr>
<tr>
<td>4 h</td>
<td>102.8 (78.2–140.0)</td>
<td>21.1 (10.6–31.8)</td>
<td>59.2 (48.7–79.7)</td>
<td>687.7 (472.8–1040.0)</td>
</tr>
<tr>
<td>8 h</td>
<td>85.9 (70.8–92.6)</td>
<td>19.3 (12.7–25.1)</td>
<td>71.9 (44.7–91.2)</td>
<td>1314.0 (1033.2–1953.5)</td>
</tr>
<tr>
<td>24 h</td>
<td>75.9 (69.1–79.2)</td>
<td>20.6 (12.2–29.4)</td>
<td>20.9 (16.5–27.1)</td>
<td>2239.0 (1831.5–2832.0)</td>
</tr>
</tbody>
</table>

* hFABP, heart-type fatty acid binding protein; hs-cTnT, high sensitivity cardiac troponin T; IMA, ischaemia modified albumin; sFlt-1, soluble fms-like tyrosine kinase.

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**Figure 2** Release kinetics of soluble fms-like tyrosine kinase (sFlt-1), ischaemia modified albumin (IMA), heart-type fatty acid binding protein (hFABP), and high sensitivity cardiac troponin T (hs-cTnT). Biomarker values with percentage change of all patients during the first 240 min after induction of myocardial infarction.
the release of ischaemia related biomarkers. Furthermore, the release kinetics of the studied biomarkers after alcohol ablation might be different from the release from the stuttering thrombotic occlusion of an epicardial coronary artery where the vessel dynamically opens and closes during the early period of AMI. The baseline blood samples in our study were taken before heparin administration. The TASH procedure itself was performed after heparin administration (bolus, 5000 IU heparin). Despite the induction of myocardial infarction we cannot exclude the further influence of heparin on sFlt-1 concentrations. Nevertheless, our data clearly demonstrated a significant increase in sFlt-1 and hFABP at the 15 min time point showing different release kinetics compared to hs-CTnT. Even with a 50% difference in sFlt-1 concentrations due to heparin, the difference compared to sFlt-1 concentrations at 15 min would still be significant. Although methodological issues may have slightly interfered with the final sFlt-1 measurements, our results add important information to this field. sFlt-1 might be helpful in ACS diagnostics, whereas the increase of IMA concentrations at the 30 min time point provides no additional data.

CONCLUSION

sFlt-1 and hFABP concentrations increased very early after induction of myocardial ischaemia, showing different release kinetics compared to hs-CTnT in patients undergoing TASH, while IMA provided no additional data. These findings provide additional information that is helpful for developing the potential combined use of these early ischaemic biomarkers with cardiac troponins in patients with suspected myocardial infarction.

References


Key questions

What is already known about this subject?
Early ischaemic biomarkers like soluble fms-like tyrosine kinase (sFlt-1), ischaemia modified albumin (IMA), and heart-type fatty acid binding protein (hFABP) might be useful as diagnostic biomarkers in patients with acute myocardial infarction (AMI).

What does this study add?
The study adds important information about the exact release kinetics of sFlt-1, IMA, and hFABP in a human model of AMI.

How might this impact on clinical practice?
Understanding the time course of the release of these biomarkers and correlating the values with patient symptoms and the results of ECG and imaging studies is important for early diagnosis, individual risk stratification, and individualised therapy, especially in the hours early after symptom onset. If it is unnecessary to additionally validate cardiac troponin values in patients with suspected acute coronary syndrome, a shorter observation period would be needed, which would have major resource implications.

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Contributors

CL: concept, treatment of the patients, draft of the manuscript. HN: concept and proofreading of the manuscript. OD: treatment of patients and blood sample collection. LG: treatment of patients and proofreading the manuscript. JH: treatment of patients and proofreading the manuscript. AH: treatment of patients and blood sample collection. AR: treatment of patients and proofreading the manuscript. CT: blood processing and sFlt-1 and IMA measurement. KJL: blood processing and hFABP measurement. TK: treatment of patients and proofreading the manuscript. CWJ: proofreading of the manuscript. HM: concept and first draft of the manuscript.

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Competing interests

None.

Patient consent

Obtained.

Ethics approval

Ethical board of the state of Hessen, Germany (FF 31/2010).

Provenance and peer review

Not commissioned; externally peer reviewed.

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