Release Kinetics of Cardiac Biomarkers in Patients Undergoing Transcoronary Ablation of Septal Hypertrophy

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BACKGROUND: The release kinetics of cardiac troponin T measured with conventional vs high-sensitivity cardiac troponin T (hs-cTnT) assays in patients with acute myocardial infarction (AMI) is difficult to establish.

METHODS: We analyzed the release kinetics of cTnT measured by fourth generation and high-sensitivity assays, creatine kinase-MB (CK-MB), and myoglobin in patients with hypertrophic obstructive cardiomyopathy undergoing transcoronary ablation of septal hypertrophy (TASH), a model of AMI. Consecutive patients (n = 21) undergoing TASH were included. Serum and EDTA-plasma samples were collected before and at 15, 30, 45, 60, 90, and 105 min, and 2, 4, 8, and 24 h after TASH.

RESULTS: cTnT concentrations measured by the hs assay were significantly increased at 15 min [21.4 ng/L, interquartile range (IQR) 13.3–39.7 ng/L vs 11.3 ng/L, IQR 6.0–18.8 ng/L at baseline; P = 0.031]. In comparison, cTnT concentrations measured by the conventional fourth generation assay increased significantly at 60 min (30.0 ng/L, IQR 20.0–30.0 ng/L vs <10.0 ng/L, IQR <10.0–10.0 ng/L; P < 0.01), CK-MB at 90 min (8.4 μg/L, IQR 6.9–14.4 μg/L vs 0.9 μg/L, IQR 0.4–1.1 μg/L; P < 0.01), and myoglobin at 30 min (188.0 μg/L, IQR 154.0–233.0 μg/L vs 38.0 μg/L, IQR 28.0–56.0; P < 0.01).

CONCLUSIONS: cTnT concentrations measured by the hs assay were significantly increased after TASH at all of the time points, with a doubling at 15 min after induction of AMI, confirming earlier evidence of myocardial injury compared to the fourth generation cTnT assay and CK-MB and myoglobin.

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Coronary artery disease is associated with high morbidity and mortality (1). The identification of patients with an acute myocardial infarction (AMI) among the large proportion of patients admitted with chest pain is a diagnostic challenge, especially in the absence of clear symptoms and electrocardiographic changes. In these cases, cardiac troponins play a central role because they allow distinction between non–ST-elevation myocardial infarction and unstable angina, thus providing both diagnostic and prognostic information (2, 3). Consequently, the introduction of cardiac troponins (troponins T and I) and their implementation into the guidelines has dramatically influenced risk stratification and care of patients with acute coronary syndromes (4–6). The central problem of conventional troponin assays is the low diagnostic sensitivity within the first hours after symptom onset in AMI patients (7–9). For earlier detection of myocardial necrosis, high-sensitivity (hs) assays for cardiac troponin T (cTnT) have been successfully evaluated. Studies have shown that hs assays can detect cardiac troponin concentrations in serum of healthy individuals with the required imprecision (10, 11). Guidelines recommend the use of the 99th percentile of troponin concentrations from a healthy reference population as a cutoff with a guideline-equitable imprecision of <10% (CV <10%) (4, 12)

In addition, controversial data exist with regard to the combined use of myoglobin in the early rule out of AMI in patients with suspected acute coronary syndrome (13–15). Because of the inaccurate definition of the exact time point of the beginning of myocardial ischemia and patient-related delay before presentation to the hospital, the early release kinetics of cTnT and myoglobin are not well described. Thus, the objective of the present study was to characterize the time course of cTnT increase measured by an hs assay in patients work, and all should be considered as first authors.

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2 Nonstandard abbreviations: AMI, acute myocardial infarction; hs, high sensitivity; cTnT, cardiac troponin T; TASH, transcoronary ablation of septal hypertrophy; CK-MB, creatine kinase-MB; IQR, interquartile range.
undergoing transcoronary ablation of septal hypertrophy (TASH) as a correlate for patients with AMI.

Methods

PATIENTS AND TREATMENT
From January 2010 until June 2011, 21 consecutive patients with hypertrophic obstructive cardiomyopathy undergoing TASH were included in the study. Clinical history, physical examination, 12-lead electrocardiogram, laboratory tests, echocardiography, and coronary angiography were assessed for all patients. The final diagnosis of hypertrophic obstructive cardiomyopathy was made according to the current guidelines and was based on severe symptoms during daily activity, asymmetrical septal hypertrophy >15 mm, systolic anterior movement of the mitral valve, and an intraventricular pressure gradient of ≥30 mmHg at rest and/or >60 mmHg after provocation by the Valsalva maneuver (16). TASH was performed according to standard clinical practice with temporary septal branch occlusion for selective therapeutic injection of 96% ethanol. Postprocedural management included monitoring at the intensive care unit for 48 h. All patients enrolled in the study provided signed informed consent, which included consent for biomarker analyses. The ethics board of the state of Hessen, Germany, approved the study (FF 31/2010).

LABORATORY ASSESSMENT
Venous blood samples were collected in plain and EDTA-containing tubes for determination of cTnT, creatine kinase-MB (CK-MB) mass, and myoglobin at 15, 30, 45, 60, 75, 90, and 105 min and 2, 4, 8, and 24 h after induction of myocardial infarction. The serum and EDTA plasma were processed immediately and frozen at −80 °C until assayed.

cTnT was measured in serum with the hs-electrochemiluminescence immunoassay (hs-cTnT assay, Elecsys Analyzer 2010, Roche Diagnostics). The lower detection limit for the hs-cTnT assay is 3.0 ng/L, with the 99th percentile at a concentration of 14.0 ng/L. The lowest concentration measurable with a CV <10% for this assay is 13.5 ng/L (11). We also measured cTnT using a commercial 1-step electrochemiluminescence immunoassay (conventional fourth generation cTnT, Elecsys 2010, Roche Diagnostics). The lower detection limit of this assay is 10.0 ng/L, the 99th percentile is <10.0 ng/L, and the lowest concentration measurable with a CV <10% is 30.0 ng/L (17, 18).

We measured CK-MB mass in plasma using a chemiluminescent microparticle immunoassay (ARCHITECT® STAT CK-MB, Abbott Laboratories). The 99th percentile of this assay is 7.27 μg/L for men and 3.4 μg/L for women. As shown by the package insert, a reference range study was conducted and was based on guidance from CLSI Protocol C28-A2. Plasma samples from apparently healthy individuals (n = 310) were evaluated in replicate with the ARCHITECT STAT CK-MB assay. The 99th percentile for the total cohort is 6.6 μg/L, and the imprecision is ≤10% total CV for samples ≥3.0 μg/L.

Myoglobin was measured in plasma by use of a chemiluminescent microparticle immunoassay (ARCHITECT® STAT Myoglobin, Abbott Laboratories). The 99th percentile of this assay is 154.9 μg/L for men and 106.0 μg/L for women. As shown by the package insert, plasma samples from apparently healthy individuals (n = 319) were evaluated in replicates of one by use of the ARCHITECT STAT Myoglobin assay. The 99th percentile for the total cohort was 140.1 μg/L, and the imprecision was ≤10% total CV for samples ≥40.0 μg/L.

STATISTICAL ANALYSIS
All data for continuous variables are expressed as mean (SD) or as median and interquartile range (IQR), as appropriate. Categorical variables are reported as number and percentage. We compared continuous variables using the Wilcoxon signed-rank test. The data were distributed parametrically as tested by the Kolmogorov–Smirnov test. The interaction term was calculated with ANOVA repeated measures. All statistical tests were performed with SPSS software, version 15.0. A 2-tailed P value <0.05 was considered to be statistically significant.

Results

Table 1 shows the clinical characteristics of all patients [13 men, 8 women, mean (SD) age 59.0 (13.29) years] enrolled in the study. Mean (SD) left ventricular ejection fraction was 61.5% (6.38%). All TASH procedures were performed in a single-session procedure by use of a single septal branch occlusion. During the procedure the mean (SD) administered ethanol was 1.77 (0.59) mL. The median occlusion time was 20.0 min (IQR 14.5–31.0). Plasma CK-MB concentrations showed a continuous increase during all prespecified time points, exceeding the 99th percentile at 90 min after induction of myocardial infarction [CK-MB 8.4 μg/L (IQR 6.9–14.4) vs baseline CK-MB 0.9 μg/L (IQR 0.4–1.1); P < 0.01; Fig. 1A]. Plasma myoglobin concentration exceeded the 99th percentile at 30 min after induction of myocardial infarction [188.0 μg/L (IQR 154.0–233.0) vs baseline CK-MB 0.9 μg/L (IQR 28.0–56.0); P < 0.01], peaked between 1 and 8 h, and returned to baseline values after 24 h (Fig. 1B). Measurement of serum cTnT concentrations by the fourth generation cTnT assay showed a first significant increase at 60 min after
interaction term with increased baseline cTnT and age was not significant (P for interaction = 0.106), nor was the interaction term with sex (P for interaction = 0.36). Additionally, no sex-specific differences between the rates of increase were observed for the different biomarkers.

Discussion

Recently published data have illustrated the diagnostic and prognostic effect of cTnT after AMI (19–23). However, the diagnostic accuracy of cTnT assays in the diagnosis of AMI is limited within the first hours after onset of symptoms (7–9, 24, 25). hs-cTnT assays have improved diagnostic accuracy at the lower limit of detection and provide incremental diagnostic information, especially in the early phase of AMI (19–21, 26).

Our study reports the precise early release kinetics of cTnT measured with a conventional (Roche fourth-generation) and an hs assay after induction of myocardial infarction as a surrogate for AMI in a cohort of patients undergoing TASH. In clinical routine practice, it is difficult to estimate accurately the precise time point of the onset of myocardial ischemia in patients with AMI. Therefore, an exact temporal allocation of the release of biomarkers in the context of AMI onset is not feasible. Although animal models of myocardial infarction can provide information about the release kinetics of cardiac troponins (27–30), such findings cannot be directly extrapolated to patients because of the different physiological parameters (e.g., metabolism, release) (28).

Despite the lack of a stuttering thrombotic occlusion of an epicardial coronary artery during an AMI induced by plaque rupture, our investigation of biomarkers after TASH is a valuable model because it closely mimics the pathophysiology of AMI and allows a chronological assignment of biomarker release in relation to the initiation of myocardial infarction. The release kinetics of troponins depends on the circulation as well as on the clearance from the interstitium, from which troponins are released after loss of the integrity of cardiac myocytes. This process might be different after alcohol ablation in which the total occlusion of a septal branch inhibits troponin drainage. The main part of the clearance mechanism could be accomplished by lymphatic transport.

Our study shows that cTnT release measured by hs assay occurs quickly, within the first minutes after the induction of myocardial infarction, and increases during all prespecified time points. We detected the first significant difference in cTnT values compared to baseline values at the first postinduction time point of 15 min. Troponins measured by a conventional assay usually begin to increase within 3–4 h after myocardial

Table 1. Baseline characteristics of patients.

<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>Hypercholesterolemia</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, mg/dL*</td>
<td>0.78 (IQR 0.76–0.93)</td>
</tr>
<tr>
<td>Glomerular filtration rate, mL·min⁻¹·(1.73 m²)⁻¹</td>
<td>90.53 (IQR 79.04–113.73)</td>
</tr>
<tr>
<td>CK-MB, µg/L</td>
<td>0.9 (IQR 0.4–1.1)</td>
</tr>
<tr>
<td>C-reactive protein hs assay, ng/L</td>
<td>11.3 (IQR 6.0–18.8)</td>
</tr>
<tr>
<td>C-reactive protein fourth generation assay, ng/L</td>
<td>&lt;10.0 (IQR &lt;10.0)</td>
</tr>
<tr>
<td>Myoglobin, µg/L</td>
<td>38.0 (IQR 28.0–56.0)</td>
</tr>
</tbody>
</table>

*To convert milligrams per deciliter to millimoles per liter divide by 11.3.

myocardial infarction was initiated [30.0 ng/L (IQR 18.0–30.0) vs <10.0 ng/L (IQR <10.0–10.0); P < 0.01; Fig. 1C]. Measurement of serum cTnT concentrations by the hs assay revealed a significant increase at 15 min compared to baseline concentrations [21.4 ng/L (IQR 13.3–39.7) vs 11.3 ng/L (IQR 6.0–18.8); P = 0.031] after induction of myocardial infarction, with a continuous rise at all prespecified time points (Fig. 1D). All patients had a significant increase with the hs-cTnT assay of more than 50% compared to the baseline value after 15 min [range of percentage increase (minimum–maximum): 171.4%–257.5%; range of absolute increase (minimum–maximum): 3.71–38.7 ng/L]. For all patients, cTnT concentrations measured by hs assay at 30 min postprocedure were above the 99th percentile, and cTnT concentrations measured by the fourth generation assay at 75 min were above the 30.0 ng/L cutoff. There were 7 patients (33.3%) with cTnT concentrations detected by hs assay above the 99th percentile at baseline. However, the interaction term for the time point of blood draw and increased baseline cTnT above 14 ng/L was not significant (P for interaction = 0.31). Therefore, the cTnT change in patients with increased baseline cTnT was not more significant compared to that for patients without increased baseline cTnT (<14.0 ng/L). Furthermore, the
ischemia (7–9). Our data show that the increase in cTnT measured by a conventional assay begins at 45 min, with the first significant difference compared to baseline concentration occurring after 60 min.

Understanding the time frame of the cTnT increase measured by hs assay and the correlation with patient symptoms and electrocardiogram and imaging studies is important for early diagnosis, individual risk stratification, and individualized therapy. Many patients with acute coronary syndrome are needlessly hospitalized, and chest pain units must unnecessarily allocate beds because of the additional diagnostic validation required with routine clinical chemistry testing (troponin after 6–9 h) following the initial presentation (3, 4, 7, 31). Inconsistent data exist concerning the release kinetics of myoglobin after AMI. Our model provides the unique opportunity to compare the release kinetics with other biomarkers. We were able to demonstrate a considerable early release of myoglobin in comparison to cTnT detected by a conventional assay and CK-MB. However, the hs-cTnT assay used in our approach outperformed myoglobin in terms of earlier detection after induction of AMI.

There are different opinions on the amount of percentage change of cTnT compared to baseline value that must be detected before the diagnosis of myocardial infarction can be made. The Universal Definition of Myocardial Infarction group and the National Academy of Clinical Biochemists recommended a 20% change from the baseline value to indicate (re)infarction with the current troponin assays (4, 32). However, these recommendations are partly related to the “pre-high-sensitivity era.”

After implementation of hs-cTnT assays in clinical practice, the degree of serial change and biological variation has been a matter of ongoing discussion (33–37).

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**Fig. 1.** Biomarker concentrations [median (IQR)] of all patients at baseline and throughout the study for (A) CK-MB, (B) myoglobin, (C) fourth generation (4th gen.) cTnT assay, and (D) hs-cTnT assay.
Release Kinetics of Cardiac Troponin T

The recommendation for the use of a 20% increase from baseline value as indicative for myocardial infarction neglects the impact of biological variation in hs-cTnT. On the basis of other study results, investigators have suggested that the greater increase of troponin concentrations must be used, especially in the lower cTnT range (17, 38).

With the higher analytical sensitivity of the new generation of troponin assays and their ability to enable much earlier detection of the release of cardiac troponin, it seems that diagnostic validation can be performed in a shorter time frame. The characterization of the kinetics of the appearance of detectable changes in cardiac troponin concentrations in patients with suspected AMI is of major clinical and health system importance because chest pain is one of the most frequent causes of emergency department admissions. Thus, if a reduction in the interval between hs-cTnT assays could be validated, a resulting shorter required observation period for such patients would have major resource implications. However, this validation must be performed in a large-scale real-world scenario to establish fast-track workup protocols in the chest pain unit.

This study is the first to investigate serial cTnT measurement by hs assay in patients with hypertrophic obstructive cardiomyopathy undergoing TASH. However, the small number of enrolled consecutive patients from a single center is a major limitation of our study that must be considered. Additionally, the kinetics of biomarker release after alcohol ablation might be different from the release from the stuttering thrombotic occlusion of an epicardial coronary artery. The data were nevertheless sufficient to demonstrate a significant increase in serum cTnT concentrations at the prespecified time points.

In conclusion, in our study cTnT measured by the hs assay showed a continuous rise at all prespecified time points after induction of myocardial infarction. The first significant difference compared to baseline cTnT values occurred at 15 min after induction. Our results provide additional evidence that the use of hs troponin assays allows AMI to be detected sooner.

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