**Highly Sensitive Cardiac Troponin T Values Remain Constant after Brief Exercise- or Pharmacologic-Induced Reversible Myocardial Ischemia**

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**BACKGROUND:** Using a new precommercial high-sensitivity cardiac troponin T (hsTnT) assay, we evaluated whether hsTnT increases after reversible myocardial ischemia.

**METHODS:** In 195 patients undergoing nuclear stress testing (ST) using single-photon emission computed tomography (SPECT) for suspected ischemic heart disease, we measured hsTnT before and 18 min, 4 h, and 24 h after the stress test. Thirty patients were excluded before ST because of cardiac troponin T (cTnT) >30 ng/L (0.03 μg/L) as measured by the fourth-generation commercial test. Another 65 patients were excluded because of a combination of fixed and reversible perfusion defects (PDs) after SPECT.

**RESULTS:** We studied 18 patients with reversible PDs, 41 patients with fixed PDs, and 41 patients without any PDs. Of these 100 patients, 61 received dynamic ST and 39 pharmacological ST. Median baseline hsTnT concentrations (25th, 75th percentile) were comparable in patients with reversible, fixed, and no PDs [5.57 (2.47, 12.60), 8.01 (4.55, 12.44), and 6.90 (4.63, 10.59) ng/L, respectively]. After ST, median hsTnT concentrations did not change in the reversible, fixed, or no PD groups from baseline to 18 min [−0.41 (−0.81, 0.01), 0.01 (−0.75, 0.79), and 0.36 (−0.42, 1.01) ng/L] or from baseline to 4 h [−0.56 (−1.82, 0.74), 0.24 (−0.60, 1.45), and 0.23 (−0.99, 1.15) ng/L]. Median baseline hsTnT concentrations tended to be higher in patients undergoing pharmacological vs dynamic ST; however, there were no significant increases in hsTnT concentrations after either type of ST.

**CONCLUSIONS:** Elevation of cTnT is rather a consequence of irreversible myocyte death than reversible myocardial ischemia after exercise or pharmacologic myocardial ischemia.

Cardiac troponin (cTn) is the preferred biomarker for detection of myocardial cell injury. Reasons for increased cTn include irreversible myocardial necrosis in patients with an acute coronary syndrome (ACS) and, in the absence of ischemia, any direct or indirect myocardial cell damage (1–3). Although it has been speculated that reversible membrane leakage may lead to an egress of non–structurally bound cTn (4–7), experimental data clearly demonstrate that cTn release is restricted to irreversible cell damage (8). To clarify this question, ultrasensitive cTn assays could provide useful information.

We studied patients with suspected significant or confirmed coronary artery disease (CAD) who were undergoing routine stress testing. We used single-photon emission computed tomography (SPECT) to objectively document transient reversible ischemia. For detecting cTn, we used a newly developed precommercial troponin T assay with improved sensitivity (hsTnT).

**Materials and Methods**

We screened 195 consecutive patients who underwent thallium SPECT. The protocol was approved by the ethics committee of the University of Heidelberg, and all patients gave informed consent. Patients either underwent bicycle exercise or received weight-adjusted dipyridamole. Two cardiologists unaware of biomarker results categorized the SPECT images. Differences in opinion were resolved by consensus. Based on differences between the images taken at rest and under stress in each individual, tracer uptake was categorized as none, only reversible, only fixed, or a combination of reversible and fixed perfusion defects. Regional tracer uptake of reversible defects was visually graded as 3, normal; 2, mildly reduced; 1, severely reduced; and 0, absent. A summed score was obtained by adding the scores of all 17 segments for a maximum score of 51 (9). We excluded patients with a combination of fixed and reversible defects to avoid difficulties in estimating the degree of reversibility at the edges of predominantly fixed defects.

**cTnT**

All laboratory measurements were performed in the research laboratory of Roche Diagnostics in Penzberg, Germany, using the latest precommercial version of the hsTnT assay. The lower detection limit of this assay was 2 ng/L (0.002 μg/L). Improvement of sensitivity and precision was achieved by a) increasing the sample vol-
ume to 50 μL, b) optimizing the degree of ruthenyla-
tion of the signal antibody, and c) optimizing the buffer
composition to reduce background signal.

As described previously (10), the interassay CV
was 8% at 10 ng/L and 2.5% at 100 ng/L, and the in-
traassay CV was 5% at 10 ng/L and 1% at 100 ng/L.

Preliminary data demonstrated detectable concentra-
tions in 2 normal reference populations with a 99th
percentile value of 12 ng/L (personal communication,
Hallermayer, Roche Diagnostics, Penzberg, Germany;
data on file).

cTnT was measured using the fourth-generation
commercial 1-step enzyme immunoassay based on
electrochemiluminescence technology. The lower de-
tection limit is 0.01 μg/L, with a recommended di-
gnostic threshold of 0.03 μg/L (11).

**Table 1. Baseline characteristics.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No perfusion defect</th>
<th>Reversible perfusion defect</th>
<th>Fixed perfusion defect</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>41</td>
<td>18</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>25 (61.0)</td>
<td>12 (66.7)</td>
<td>35 (85.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Age, years</td>
<td>68 (10)</td>
<td>70 (7)</td>
<td>67 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.59 (3.6)</td>
<td>29.58 (4.6)</td>
<td>27.79 (5.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Known CAD</td>
<td>27 (65.9)</td>
<td>15 (83.3)</td>
<td>34 (82.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Known MI</td>
<td>3 (7.3)</td>
<td>4 (22.2)</td>
<td>17 (41.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Known CABG</td>
<td>5 (12.2)</td>
<td>4 (22.2)</td>
<td>8 (19.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Known PCI</td>
<td>12 (29.3)</td>
<td>7 (38.9)</td>
<td>18 (43.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Angiogram before/after stress testing</td>
<td>28 (68.3)</td>
<td>14 (77.8)</td>
<td>31 (75.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active smoking</td>
<td>12 (29.3)</td>
<td>6 (33.3)</td>
<td>9 (22.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>33 (80.5)</td>
<td>15 (83.3)</td>
<td>34 (82.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>29 (70.7)</td>
<td>13 (72.2)</td>
<td>28 (68.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12 (29.3)</td>
<td>5 (27.8)</td>
<td>9 (21.95)</td>
<td>NS</td>
</tr>
<tr>
<td>Stress test data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dynamic examination</td>
<td>26 (63.4)</td>
<td>11 (61.1)</td>
<td>24 (58.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Length of dynamic examination, min</td>
<td>7.38 (2.8)</td>
<td>8.95 (3.7)</td>
<td>9.0 (2.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Peak work load, W</td>
<td>106 (33)</td>
<td>127 (34)</td>
<td>123 (29)</td>
<td>NS</td>
</tr>
<tr>
<td>Length of pharmacological test, min</td>
<td>5 (5, 5)</td>
<td>5 (5, 5)</td>
<td>5 (5, 5)</td>
<td>NS</td>
</tr>
<tr>
<td>Dipyridamol dose, mg</td>
<td>41.4 (5.6)</td>
<td>50.7 (5.6)</td>
<td>44.1 (5.8)</td>
<td>0.004b</td>
</tr>
<tr>
<td>PCI after examination</td>
<td>2 (4.9)</td>
<td>2 (11.1)</td>
<td>6 (14.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Data are n (%), mean (SD), or median (25th, 75th percentiles). NS, not significant; MI, myocardial infarction; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention.

b Reversible vs no perfusion defect.

**STATISTICAL ANALYSIS**

We compared continuous variables using t test or Mann-Whitney U test. We tested changes of hsTnT from baseline using Wilcoxon test for paired samples and categorical variables using χ² or Fisher exact test. For all analyses, a P value <0.05 was regarded as statistically significant. All statistical analyses were performed using MedCalc Version 9.2 for Windows (MedCalc Software).

**Results**

**BASELINE CHARACTERISTICS**

Of 195 patients screened, 30 (15.4%) were excluded because of a positive baseline conventional cTnT related to recent myocardial infarction and 21 (10.8%) because the sample volume was too low to allow measurement of hsTnT. Of the remaining 144 patients, 18 were found to have reversible perfusion defects, 41 had fixed perfusion defects, and 41 showed no perfusion defects. Table 1 presents baseline characteristics and stress test data of the study group.

**hsTnT concentrations in patients with and without inducible ischemia**

Baseline hsTnT concentrations were comparable in patients with reversible perfusion defects, fixed perfusion...
defects, and without perfusion defects [median (25th, 75th percentile): 5.57 (2.47, 12.60), 8.01 (4.55, 12.44), and 6.90 (4.63, 10.59) ng/L]. After stress testing, hsTnT concentrations for the 3 patient groups did not change from baseline to 18 min \([-0.41 \ (-0.81, 0.01), 0.01 \ (-0.75, 0.79), \) and 0.36 \((-0.42, 1.01)\) ng/L, respectively] or from baseline to 4 h \([-0.56 \ (-1.82, 0.74), 0.24 \ (-0.60, 1.45), \) and 0.23 \((-0.99, 1.15)\) ng/L] (Fig. 1).

hsTnT AND SEVERITY OF REVERSIBLE ISCHEMIA
In patients with reversible perfusion defects, semi-quantitative analysis of SPECT yielded a median of 3 segments showing stress-induced perfusion defects. The mean (SD) summed score \((n/17\) segments) was calculated as 2.78 \((0.10)\) with individual scores ranging from 2.78 to 2.59, suggesting a mild to moderate degree of ischemia. There was no significant correlation between the summed score and hsTnT change at 18 min \(r = 0.167 \ (0.374–0.625), P = 0.535\) or 4 h \(r = -0.193 \ (-0.642–0.354), P = 0.47\).

hsTnT AFTER REVERSIBLE ISCHEMIA RELATED TO TYPE OF STRESS
Of the 100 patients, 61 (61%) received dynamic stress (DS) tests and 39 (39%) pharmacological stress (PS) tests. Compared to patients receiving DS tests, patients receiving PS tests were older \((71.51 \ (9.13)\) years vs 65.90 \((10.40), P = 0.01\) and had a lower glomerular filtration rate (GFR) \((64.72 \ (29.03)\) vs 80.95 \((31.90), P = 0.01\). Median baseline hsTnT levels (25th, 75th percentile) tended to be higher in those receiving PS vs DS tests: 8.09 \((4.76, 15.13)\) vs 6.12 \((3.80, 10.30)\) ng/L, \(P = 0.07\). There was no significant increase of hsTnT concentrations after any type of stress.

Discussion
In this study, we evaluated the effect of reversible myocardial ischemia on concentrations of cTnT measured with a new precommercial hsTnT assay. Our key finding is that reversible ischemia does not induce a significant change of hsTnT. Baseline concentrations of hsTnT were not found to be different in patients without perfusion defects compared to those with fixed and reversible perfusion defects and did not change after 18 min and 4 h in either group.

In this study, SPECT was performed for objective identification of reversible myocardial perfusion defects. To test the effects of reversible myocardial ischemia, we used a precommercial hsTnT assay with a 5-fold lower detection limit than the standard assay. The finding that hsTnT does not increase after reversible myocardial ischemia supports the experimental results of Fishbein et al. (8) demonstrating that troponin release was restricted to irreversible myocyte necrosis.
Our results are also supported by the findings of Schulz et al. (12), who tested a sensitive cTnI assay on 47 outpatients undergoing stress testing for stable CAD. Detectable cTnI concentrations below the 99th percentile remained unchanged 3 and 5 h after exercise in patients with and without detectable ischemia. Baseline values, however, were higher in patients who later developed stress-induced myocardial ischemia. In our study, there was a trend toward higher baseline values, but hsTnT did not change significantly after either stress type. Previously, Venge et al. (13) reported an age-dependent increase in cTnI in patients without CAD, raising the question as to the reason for higher cTnI. Later, Zethelius et al. (14) found that low cTnI predicted death and first coronary event in seemingly healthy persons. Similar findings were reported for patients presenting with possible ACS (15) and in patients with stable CAD who had stress-inducible myocardial ischemia (12). Conversely, Wu et al. (16) found extraordinarily low concentrations of cTnI in patients without evidence of heart disease. Consistently in our study, patients deemed unable to perform or complete a DS test and were therefore referred to PS testing by their physicians demonstrated a trend to higher baseline hsTnT values. These patients were older and had more impaired renal function, suggesting more cardiovascular morbidity. These findings are preliminary, however, and should prompt further investigations into whether hsTnT may be useful for identifying different levels of subclinical cardiovascular disease. Interestingly, several studies, mainly on professional and recreational athletes, found short reversible increases of cTnT or cTnI and speculated on a release of non-structurally bound cTn through reversible membrane leakage (17). The reasons for these increases of cTn in apparently healthy athletes remain controversial.

In this study, the presence of reversible ischemia was documented objectively using SPECT, and semiquantitative classification of the perfusion defects demonstrated only a mild to moderate degree of ischemia. We used a standard stress protocol applying strict criteria for discontinuation of stress after the first evidence of myocardial ischemia. Therefore, we cannot exclude the possibility that longer or more severe myocardial ischemia could have caused troponin release. In addition, it remains unclear whether our findings apply to cTnI, which is smaller and thus potentially better membrane-permeable, or whether our results may be extrapolated to other advanced-generation cTnI assays, particularly when measured more sensitively using advanced technology such as the single-molecule fluorescent detector assay (Singulex) (18). At the moment, recent data on a sensitive cTnI assay (12) and our results on hsTnT are consistent in suggesting that ischemia alone is not capable of causing troponin release, or that more severe or more prolonged ischemia is required. Moreover, the degree of modification of the troponin molecule may be different in naturally occurring ischemia than in brief induced ischemia (19).

**STUDY LIMITATIONS**

The duration and severity of reversible myocardial ischemia induced by our protocol stress tests may have been too small to identify cTnT release from membrane leakage. Semiquantitative assessment of the severity of myocardial ischemia demonstrated mild to moderate reversible perfusion defects. Second, blood was collected before and at 3 time points after stress. Although we may have missed an increase of cTnI by measuring as early as 4 h after ischemia, recent data have demonstrated that lowering the decision cutoff from ROC cutoff to the 10% CV or even to the 99th percentile will shorten the time to appearance in blood significantly to <4 h (20). Last, patients were not randomized to receive DS or PS.

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**References**


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