Biological Variation and Reference Change Value of High-Sensitivity Troponin T in Healthy Individuals during Short and Intermediate Follow-up Periods

Lutz Frankenstein,1,7* Alan H.B. Wu,2 Klaus Hallermayer,3 Frank H. Wians, Jr.,4 Evangelos Giannitsis,1 and Hugo A. Katus1

1 Department of Cardiology, Angiology, Pulmonology, University of Heidelberg, Heidelberg, Germany; 2 University of California, San Francisco, San Francisco, CA; 3 Assay Development, Roche Diagnostics, Penzberg, Germany; 4 Department of Pathology, Baylor University Medical Center, Dallas, TX; * address correspondence to this author at: Department of Cardiology, Angiology, Pulmonology, University of Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany. Fax +0049-6221-56-6547; e-mail Lutz. Frankenstein@med.uni-heidelberg.de.

BACKGROUND: Acute myocardial infarction is defined by a troponin concentration > 99th percentile with an acute increase and/or decrease, the magnitude of which has not yet been well defined. To aid the interpretation of changes in cardiac troponin concentration, we sought to establish biological variation and reference change values (RCVs) by applying both the normal and lognormal approaches for cardiac troponin T (cTnT) sampled at hourly and weekly intervals in healthy individuals and measured on the Roche E 170 and Elecsys® 2010 automated platforms.

METHODS: High-sensitive cTnT (hsTnT) was measured at baseline, and after 1, 2, 3, and 4 h and after 1, 2, 3, and 4 weeks in 20 and 17 healthy individuals, respectively. A healthy status was established by physical examination, MRI analysis at rest and during dobutamine stress, lung function testing, and blood sample testing.

RESULTS: Hourly total and within-individual CVs were 18% and 15%, respectively, for the E 170 assay, and 24% and 21%, respectively, for the Elecsys 2010 assay. Weekly total and within-individual CVs for these assays were 32% and 30%, respectively, for the E 170 assay, and 32% and 30%, respectively, for the Elecsys 2010 assay. The RCVs for the E 170 and Elecsys 2010 assays were ≥ 46% and 62% (hourly), respectively, and ≥ 87% and 86% (weekly), respectively. The corresponding lognormal values were +64%/−39% and +90%/−47% (hourly), and +138%/−58% and +135%/−58% (weekly).

CONCLUSIONS: RCVs appear attractive for interpreting hsTnT results. The short-term biological variation of hsTnT is low but becomes somewhat more important at intermediate sampling intervals. Knowledge of this variation is important for interpreting results from patients in whom cTnT values increase from low concentrations.

The Joint Task Force for the Redefinition of Myocardial Infarction predicted its redefinition of acute myocardial infarction on detecting an increase or decrease in cardiac troponin [cardiac troponin T (cTnT)3 or cardiac troponin I (cTnI)], with at least 1 value greater than the 99th percentile reference value in patients with evidence of myocardial ischemia (1). The advent of high-sensitive cTnT (hsTnT) and/or cTnI (hsTnI) assays has enabled measurement of previously undetectable cardiac troponin concentrations in healthy individuals and in patients with an acute coronary syndrome (ACS) (2–4). Despite the introduction of changes in cardiac troponin concentrations into the definition, the magnitude of an increase or decrease during serial sampling that is indicative of acute myocardial infarction has not been fully determined. A change ≥ 20% has been suggested for patients with cardiac troponin increases from baseline (5).

It is in these situations that biological variation and derived measures become important. For both biological and methodologic reasons, however, biological variation needs to be established for each biomarker and assay. Whereas Wu et al. reported relatively low biological variation for a cTnI assay (6), Vasile et al. found higher variation for a cTnT assay (7).

We provide data on biological variation and use the normal and lognormal approaches to calculate hourly and weekly reference change values (RCVs) for the hsTnT assays on the Roche Elecsys 2010® and E 170® hsTnT instruments.

Materials and Methods

SELECTION OF STUDY PARTICIPANTS

After obtaining written informed consent, we recruited 2 cohorts of healthy volunteers by means of a standard protocol approved by the local ethics committee. A healthy status was verified (as described previously (2)) through a physical examination, MRI analysis includ-
ing adenosine perfusion or dobutamine stress, lung function testing, and blood sample testing.

**SAMPLING AND MEASUREMENT**

Sampling was performed after 30 min of rest (baseline) and after 1, 2, 3, and 4 h (cohort 1) or after 1, 2, 3, and 4 weeks (cohort 2). Samples were collected into EDTA-containing Vacutainers (Becton Dickinson), centrifuged immediately after collection at 4 °C to separate the plasma, and frozen at −80 °C. To minimize imprecision, we analyzed all samples together in a single run.

hsTnT was measured by electrochemiluminescence immunoassays (on the Elecsys 2010/cobas e 411 and Modular Analytics E 170/cobas e 601 immunoanalyzers; Roche Diagnostics). Details of the test principle and assay characteristics have previously been described (2, 8).

**STATISTICAL ANALYSIS**

hsTnT values lower than the limit of the blank (3 ng/L) were excluded from analysis. RCVs were evaluated with both the normal (10, 11) and lognormal (12) approaches.

With the normal approach, within-individual biological imprecision (i.e., CV$_i$) was calculated from the median CV$_t$ for duplicate measurements of hsTnT at all time points, as follows:

$$CV_i = (CV_t^2 - CV_s^2)^{1/2}.$$

The symmetrical limits of the normal RCV were calculated as follows:

$$RCV = Z \times 2^{1/2} \times (CV_a^2/n_a + CV_s^2/n_s)^{1/2},$$

where $Z = 1.96$ (Z score for 95% confidence), $n_a$ is the number of replicate hsTnT measurements, and $n_s$ is number of simultaneous blood samples per individual).

With the lognormal approach, the median normal deviation of the lognormal distribution ($\sigma$) was calculated from the median CV$_t$ (as a decimal value), as described by Fokkema et al. (12):

$$\sigma = [\ln(CV_t^2 + 1)]^{1/2}.$$

The asymmetrical limits for the upward (positive) value for the lognormal RCV ($RCV_{pos}$) and for the downward (negative) value for the lognormal RCV ($RCV_{neg}$), were determined as follows:

$$RCV_{pos} = \exp(1.96 \times 2^{1/2} \times \sigma) - 1 \times 100,$n$$

and

$$RCV_{neg} = \exp(-1.96 \times 2^{1/2} \times \sigma) - 1 \times 100.$n$$

**Results**

We recruited 20 volunteers [7 males (35%); median age, 32 years (range, 22–59 years)] for the hourly follow-up study and 17 volunteers [7 males (41%); median age, 36 years (range, 26–59 years)] for the weekly follow-up study. For the hourly measurements, 48 measurements for 14 of the 20 individuals yielded hsTnT concentrations below the limit of the blank (<3 ng/L). hsTnT concentrations remained <3 ng/L throughout the study. For the weekly measurements, 42 hsTnT measurements for 13 of the 17 volunteers yielded concentrations <3 ng/L. The hsTnT concentration in 2 of these individuals remained <3 ng/L throughout the study. Table 1 shows the hourly and

<table>
<thead>
<tr>
<th>Variable</th>
<th>E 170 assay Hourly</th>
<th>E 170 assay Weekly</th>
<th>Elecsys 2010 assay Hourly</th>
<th>Elecsys 2010 assay Weekly</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of values</td>
<td>98</td>
<td>100</td>
<td>62</td>
<td>56</td>
</tr>
<tr>
<td>CV$_a$, %</td>
<td>7.8</td>
<td>7.8</td>
<td>9.7</td>
<td>9.7</td>
</tr>
<tr>
<td>CV$_s$, %</td>
<td>15 (0.0–66)</td>
<td>30 (6.0–130)</td>
<td>21 (7.4–47)</td>
<td>30 (0.0–97)</td>
</tr>
<tr>
<td>CV$_i$, %</td>
<td>18 (7.1–66)</td>
<td>32 (11–130)</td>
<td>24 (12–48)</td>
<td>32 (8.9–97)</td>
</tr>
<tr>
<td>RCV, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>±47</td>
<td>±87</td>
<td>±62</td>
<td>±86</td>
</tr>
<tr>
<td>Lognormal</td>
<td>64, −39 (51)</td>
<td>138, −58 (98)</td>
<td>90, −47 (68)</td>
<td>135, −58 (96)</td>
</tr>
</tbody>
</table>

* CV$_i$ and CV$_s$ data are given as the median (range). RCV values obtained with the lognormal approach are given as the upward change value, the downward change value, and the mean between these values (in parentheses).

Table 1. Analytical and biological variation of hsTnT.

* Total number of values after omitting any values with a less-than (<) sign, which represent values less than the limit of blank (<3 ng/L) or missing values due to an inadequate sample volume.
weekly RCVs for hsTnT, which were obtained with both the normal and lognormal approaches to the hsTnT data measured with the E 170 and Elecsys 2010 instruments. Fig. 1 is a scatter plot of the individual values obtained with the Elecsys 2010 instrument.

Discussion

As expected, the means of the absolute values of the upward and downward RCVs obtained with the lognormal approach were in good agreement with the RCVs obtained with the normal approach (Table 1). This agreement served as an accuracy check on the mathematical calculations for determining RCVs by each of these approaches.

Our findings are in line with those for cTnI obtained by Wu et al. (6) for both hourly and weekly intervals. In contrast, a more recent study by Vasile et al. (7) for cTnT that used an assay similar to one used in our study obtained values that were substantially higher than ours. There are several potential reasons for this difference. First, as the editorial accompanying their article pointed out (13), there may have been differences in study design. The steps taken in our study to rule out occult myocardial injury (MRI, stress testing) and our treatment of outlier values differed. Second, we used the NCCLS precision protocol for within-run imprecision in our study. The resulting differences in assay imprecision will alter the results obtained with the normal formula (see Materials and Methods). Third, individual cTnT concentrations in the study of Vasile et al. were lower than ours. This difference raises the possibility that, irrespective of comorbidities, the results of Vasile et al. reflected increased analytical variation owing to the increase in imprecision at the lower end of the measurement spectrum.

Likewise, the higher cTnT concentrations in our study raise potential questions about the “normality”—or health status—of our cohort; however, given our extensive testing to assess health status, it is unlikely that our study participants had cardiac damage. Furthermore, use of the 99th percentile as a discriminator allows, by definition, only 1% of healthy individuals to have concentrations greater than this threshold by chance.

The question remains as to how to properly apply a given RCV or a percentage-change threshold derived by some other approaches to cardiac troponin. The CVi imprecision data reported thus far vary between 5% (6) and 24% (7), thereby restricting the application of RCVs, especially when concentrations are below the 99th percentile (where imprecision increases further), because analytical imprecision must be less than one-half of the within-person biological variation (14). The low index of individuality reported by Wu et al. (6), however, suggests that cardiac troponin results are person specific and best suited to the RCV model.

Given this statement, all RCVs published so far refer to an arbitrary P value of <0.05, meaning that there is a 5% chance that a change in cardiac troponin (Δ) greater than the RCV can occur in a healthy person. The relationship between the change in any individual and the probability that this change is statistically significant can be assessed according to the following equation:

\[
Z = \Delta/[\sqrt{\frac{(CV_{a}^{2} + CV_{i}^{2})}{2}}]
\]

and the resulting Z score can be retranslated into probabilities via tables.

Outside the statistical considerations of the RCV, percentage-change values have been suggested for identifying true ischemic events. The National Academy of Clinical Biochemistry guidelines (5) recommended a change ≥20% at 6–9 h after presentation; however, this recommendation applied only to patients with end-stage renal disease. For other subsets of patients, the extent of change needed is currently under debate. For ACS patients, Apple et al. suggested that a change in concentration of ≥30% be considered in addition to either baseline or 6-h follow-up values (15). More recently, after performing an ROC curve analysis, we suggested an RCV of ≥112% in addition to the 99th percentile cutoff value for cTnT in ACS patients with an initially negative cTnT value (16). In summarizing these studies (6, 7, 15, 16), we find it tempting to speculate that a change in cardiac troponin in ACS of ≥50% is no longer explained by...
biological variation and thus represents acute rather than chronic cardiovascular disease. Higher Δ change values may improve specificity but will likely cause lower sensitivities.

LIMITATIONS
We evaluated the biological variation in hstnT in healthy individuals only. Because baseline concentrations of this marker can be expected to be higher in such conditions as chronic heart failure, the resulting CVs and RCVs might be different from those in a healthy cohort. In the absence of data on such patients, we urge caution regarding extrapolating our results to these patients. On the other hand, in nonacute pathologic processes in which new homeostatic steady states are reached, biological variation around the new set points might be expected to be of the same order as those found in healthy individuals (17).

CONCLUSION
The use of RCVs appears attractive for interpreting hstnT results. The hourly biological variation of hstnT is low, but hstnT variation becomes slightly more important at weekly sampling intervals. Knowledge of this variation is important for interpreting results from patients in whom cTnT increases from low concentrations.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: K. Hallermayer, Roche Diagnostics.
Consultant or Advisory Role: E. Giannitsis, Roche Diagnostics, Bayer Vital, and Daiichi Sankyo.
Stock Ownership: None declared.
Honoraria: E. Giannitsis, Roche Diagnostics, Astra Zeneca, and Bayer Vital.
Research Funding: L. Frankenstein, other biomarker-related research; E. Giannitsis, Roche Diagnostics and Daiichi Sankyo.
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References


Previously published online at DOI: 10.1373/clinchem.2010.158964