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

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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-sensitivity 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 31%, respectively, for the Elecsys 2010 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 predicated its redefinition of acute myocardial infarction on detecting an increase or decrease in cardiac troponin [cardiac troponin T (cTnT)5 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-sensitivity 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 already elevated at 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.

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 including adenosine perfusion or dobutamine stress, lung function testing, and blood sample testing.

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), and serum was collected at each time point. The hsTnT results were measured on the E 170 and Elecsys 2010® instruments.

NONSTANDARD ABBREVIATIONS: cTnT, cardiac troponin T; cTnI, cardiac troponin I; hsTnT, high-sensitivity cTnT; hsTnI, high-sensitivity cTnI; ACS, acute coronary syndrome; RCV, reference change value; CV a, within-run analytical imprecision; CV i, within-individual biological imprecision; CV t, total imprecision.
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). We determined within-run analytical imprecision (CVa) according to the CLSI EP5-A2 protocol (9). The CVa was 7.8% with the Roche E 170 hsTnT assay and 9.7% with the Elecsys 2010 assay.

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 (CVi) was calculated from the median coefficient of the total imprecision (CVt) for duplicate measurements of hsTnT at all time points, as follows:

\[ CV_i = \frac{1}{2}(CV_t^2 - CV_a^2) \]

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

\[ RCV_{\text{Normal}} = [\exp(1.96 \times 2^{1/2} \times \sigma) - 1] \times 100 \]

\[ RCV_{\text{pos}} = [\exp(1.96 \times 2^{1/2} \times \sigma) - 1] \times 100, \]

\[ RCV_{\text{neg}} = [\exp(-1.96 \times 2^{1/2} \times \sigma) - 1] \times 100. \]

With the lognormal approach, the median normal deviation of the lognormal distribution (σ) was calculated from the median CVt (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_{\text{pos}}) and for the downward (negative) value for the lognormal RCV (RCV_{\text{neg}}), were determined as follows:

\[ RCV_{\text{pos}} = [\exp(1.96 \times 2^{1/2} \times \sigma) - 1] \times 100, \]

\[ RCV_{\text{neg}} = [\exp(-1.96 \times 2^{1/2} \times \sigma) - 1] \times 100. \]

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 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.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>E 170 assay</th>
<th>Elecsys 2010 assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hourly</td>
<td>Weekly</td>
</tr>
<tr>
<td>No. of values</td>
<td>98</td>
<td>100</td>
</tr>
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<td>7.8</td>
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<tr>
<td>CVa, %</td>
<td>15 (0.0–66)</td>
<td>31 (6.0–127)</td>
</tr>
<tr>
<td>CVa, %</td>
<td>18 (7.1–66)</td>
<td>32 (11–128)</td>
</tr>
<tr>
<td>RCV, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>±47</td>
<td>±87</td>
</tr>
<tr>
<td>Lognormal</td>
<td>64, −39 (51)</td>
<td>138, −58 (98)</td>
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*CVa and CVi 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). 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.
The question remains 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:

\[
Z = \Delta / [2^{1/2} \times (\text{CVa}^2 + \text{CVi}^2)^{1/2}],
\]

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% might 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.

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 patho-

\[\text{Fig. 1. Scatter plot for hsTnT hourly-testing values for all volunteers tested with the Elecsys 2010 assay.}\]

The dots represent individual hsTnT values from each sampling point for a given individual. For this graph, the numbering of patients is arbitrary and is for plotting purposes only. Because of the nature of the graph, any identical values in the graph are superimposed.

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 the 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 still allows, by definition, 1% of healthy individuals to have concentrations greater than this threshold by chance.
logic 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).

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.

**References**


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