Biologic Variation of a Novel Cardiac Troponin I Assay

To the Editor:

Cardiac troponin is the marker of choice for evaluating myocardial injury (1). High-sensitivity assays improve analytical detection limits, thereby allowing concentrations to be measured in the majority of healthy individuals. This capability allows an assessment of biologic variation (BV) to determine what constitutes an important change in the cardiac troponin concentration, a critical metric for identifying acute events. Such an event is often a myocardial infarction, but any acute cardiac injury can cause increasing and/or decreasing values (1). Accordingly, we evaluated BV for a recently developed high-sensitivity cardiac troponin I (hs-cTnI) assay (2) from Beckman Coulter. We performed this study with the same cohort used to define BV for the high-sensitivity cardiac troponin T (hs-cTnT) assay (3) and according to a protocol approved by our institutional review board.

For assessment of short-term BV, we collected blood into serum separator tubes (Becton Dickinson) at 0, 1, 2, 3, and 4 h, centrifuged the tubes, and stored the serum samples immediately at −70 °C. Samples are stable under these conditions (2). For the long-term study, samples were obtained biweekly for 8 weeks and processed similarly. After thawing the samples, we analyzed the samples without recentrifugation in duplicate on the Access analyzer (Beckman Coulter). The limit of blank for this assay is 1.03 ng/L, the limit of detection is 2.06 ng/L, the lowest concentration with an imprecision (CV) <10% is 8.66 ng/L, and the 99th percentile value for serum is 8.00 ng/L, as previously reported (2). Reference change values (RCVs) were calculated as previously described (4) according to the method of Fokkema et al. ANOVA (generalized linear model procedure in statistical analysis software; SAS Institute) was used to calculate the sums of squares for the analytical and biological components. Total (SDT), analytical (SDA), intraindividual (SDI), and interindividual (SDG) variances were determined by the maximum-likelihood approach. Values were averaged across participants; variances were homogeneously distributed. The Cochran test was used to identify outliers; no values were removed. The index of individuality was computed as (CV_A^2 + CV_I^2)/CV_G, where CV_A is the analytical CV, CV_I is the intraindividual CV, and CV_G is the interindividual CV. Because the data were skewed, RCVs were calculated after lognormal transformation. A 95% CI was used for short- and long-term RCVs.

The mean age of the study participants was 39 years (range, 25–56 years); the median age was 36 years (interquartile range [IQR], 31.3–46.3 years). Sixty percent were women. None of the participants had a history of cardiovascular disease or other conditions known to affect cardiac troponin, and none were taking cardiovascular medications. One individual had only 2 initial blood draws in the short-term study, and 4 individuals missed 1 time point during the long-term evaluation.

No participants had values below the limit of blank. Baseline values ranged from 1.04 ng/L to 11.01 ng/L. The median value for short-term BV was 2.20 ng/L (IQR, 1.51–2.80 ng/L), and the median values for the short-term and long-term studies were similar [2.20 ng/L (IQR, 1.52–2.80 ng/L) and 2.19 ng/L (IQR, 1.5–2.82 ng/L)], respectively.

### Table 1. Biologic and analytical variation metrics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Short term (0–4 h)</th>
<th>Long term (0–8 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical variationa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV_A, %</td>
<td>3.5</td>
<td>2.7</td>
</tr>
<tr>
<td>CV_I, %</td>
<td>3.4</td>
<td>2.6</td>
</tr>
<tr>
<td>CV_G, %</td>
<td>45.3</td>
<td>41.6</td>
</tr>
<tr>
<td>Index of individuality</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>RCV (lognormal increase), %</td>
<td>45.2</td>
<td>14.0</td>
</tr>
<tr>
<td>RCV (lognormal decrease), %</td>
<td>−15.8</td>
<td>−10.6</td>
</tr>
<tr>
<td>Mean δ increase, %d</td>
<td>58.0a</td>
<td>20.0a</td>
</tr>
<tr>
<td>Mean δ decrease, %d</td>
<td>21.0f</td>
<td>18.0f</td>
</tr>
</tbody>
</table>

* Based on duplicate results.

** CV_A, analytical CV; CV_I, intraindividual CV; CV_G, interindividual CV.

* A CI of 95% was used to calculate the RCV for short- and long-term biologic variation at a z value of 0.84 (1-sided test).

** Mean δ increase/decrease: mean change (increase/decrease) in an individual’s troponin values compared with baseline (first time point); data are expressed as a percentage.

* Median (IQR), 44.7% (22.6%–68.3%).

* Median (IQR), 20.0% (10.5%–26.1%).

* Median (IQR), 24.2% (15.7%–67.9%).

* Median (IQR), 21.9% (14.7%–25.9%).
tively]. The RCVs were $+45.2\% - 15.8\%$ and $14.0\% - 10.6\%$ for short-term and long-term BV, respectively (Table 1). There were no significant differences between the sexes according to the t-test.

These results provide index data for a research hs-cTnI assay. The results are similar to those obtained for another hs-cTnI assay (4) but lower than those observed with the hs-cTnT assay (3). The high degree of BV in our hs-cTnT data has been suggested to be due to underlying cardiovascular co-morbidities. The present data are more consistent with the hypothesis that the differences are related to differences in the precision of the assays at very low troponin concentrations.

These data add to the evidence-based information necessary to use BV as a metric to evaluate important changes and perhaps a more refined definition of acute cardiovascular events (1).

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