Letters to the Editor

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)\(^1\) to determine what constitutes a clinically 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 \(-70{\text{°C}}\). 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 refugation 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 \((SDT^2)\), analytical \((SDA^2)\), intraindividual \((SDI^2)\), and interindividual \((SDG^2)\) 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 \((CV_A^2 + CV_I^2)^{1/2}/CV_G\), 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]. 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 comorbidities (5). 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.

---

\(^1\) Nonstandard abbreviations: BV, biologic variation; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; RCV, reference change value; IQR, interquartile range.

---


Paola Concolino*
Enrica Mello
Angelo Minucci
Cecilia Zuppi
Ettore Capoluongo

Laboratory of Molecular Biology
Institute of Biochemistry and
Clinical Biochemistry
Catholic University
Rome, Italy

* Address correspondence to this author at:
Catholic University
Largo A. Gemelli 8
00168 Rome, Italy
Fax +39-0630156706
E-mail paolaconcolino78@libero.it

Previously published online at DOI: 10.1373/clinchem.2011.162230

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)\(^1\) to determine what constitutes a clinically 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 \(-70{\text{°C}}\). 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 refugation 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 \((SD_T^2)\), analytical \((SD_A^2)\), intraindividual \((SD_I^2)\), and interindividual \((SD_G^2)\) 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 \((CV_A^2 + CV_I^2)^{1/2}/CV_G\), 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]. 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 comorbidities (5). 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).

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: None declared.
Consultant or Advisory Role: A.S. Jaffe, Beckman Coulter, Critical Diagnostics, Inverness Medical, Radiometer, Pfizer, and Amano.
Stock Ownership: None declared.
Honoraria: A.S. Jaffe, Roche and Abbott Laboratories.

References

Research Funding: A.K. Saenger, Roche Diagnostics; G.G. Klee, Beckman Coulter; A.S. Jaffe, Beckman Coulter.
Expert Testimony: None declared.
Other Remuneration: G.G. Klee, Beckman Coulter.

Acknowledgments: Reagents were supplied by Beckman Coulter.

To the Editor:

Miller and colleagues (1) compared the results obtained by direct measurements of HDL cholesterol (HDL-C) and LDL-C with those obtained with reference methods requiring ultracentrifugation. They used frozen pooled serum samples and fresh serum samples from 37 healthy controls and 138 individuals with dyslipidemia or heart disease that they had obtained from the NIH. Assay kits, calibrators, and controls were provided by 7 manufacturers. The authors reported CVs of 5.4% to 4.4% for LDL-C for the frozen samples, biases for fresh serum from the healthy controls of −5.4% to 4.4% for HDL-C and −6.8% to 1.1% for LDL-C. The bias for the patients with disease were −8.6% to 8.8% for HDL-C.

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 variation&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5</td>
<td>2.7</td>
</tr>
<tr>
<td>BV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV&lt;sub&gt;IV&lt;/sub&gt; %</td>
<td>3.4</td>
<td>2.6</td>
</tr>
<tr>
<td>CV&lt;sub&gt;IR&lt;/sub&gt; %</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), %&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.2</td>
<td>14.0</td>
</tr>
<tr>
<td>RCV (lognormal decrease), %&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−15.8</td>
<td>−10.6</td>
</tr>
<tr>
<td>Mean δ increase, %&lt;sup&gt;d&lt;/sup&gt;</td>
<td>58.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Mean δ decrease, %&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.0</td>
<td>18.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on replicate results.
<sup>b</sup> CV<sub>IV</sub>, analytical CV; CV<sub>IR</sub>, intrasubject CV; CV<sub>IR</sub>, intersubject CV.
<sup>c</sup> 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).
<sup>d</sup> 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.

Previously published online at DOI: 10.1373/clinchem.2011.162545

Letters to the Editor

Vlad C. Vasile
Amy K. Saenger
Jean M. Kroning
George G. Klee
Allan S. Jaffe<sup>*</sup>

CCLS Division
Department of Laboratory Medicine and Pathology
Mayo Clinic
Rochester, MN

* Address correspondence to this author at: Cardiovascular Division Gonda 5
Mayo Clinic and Medical School
200 First St. S.W.
Rochester, MN 55905
Fax 507-266-0228
E-mail Jaffe>Allan@Mayo.edu

Limitations of Direct Methods and the Reference Method for Measuring HDL and LDL Cholesterol

Miller and colleagues (1) compared the results obtained by direct measurements of HDL cholesterol (HDL-C) and LDL-C with those obtained with reference methods requiring ultracentrifugation. They used frozen pooled serum samples and fresh serum samples from 37 healthy controls and 138 individuals with dyslipidemia or heart disease that they had obtained from the NIH. Assay kits, calibrators, and controls were provided by 7 manufacturers. The authors reported CVs of <3.7% for HDL-C and <4.4% for LDL-C for the frozen serum pools, biases for fresh serum from the healthy controls of −5.4% to 4.4% for HDL-C and −6.8% to 1.1% for LDL-C. The biases for the patients with disease were −8.6% to 8.8% for HDL-C.