Biological and Analytical Variability of a Novel High-Sensitivity Cardiac Troponin T Assay

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BACKGROUND: High-sensitivity cardiac troponin assays will augment the frequency of increased results, making important the determination of reference change values to distinguish acute from chronic increases. We assessed short- and long-term biological variability of cardiac troponin T (cTnT) in healthy subjects with a novel high-sensitivity (hs) assay.

METHODS: We collected blood from 20 healthy volunteers at 5 time points for short-term study and biweekly at 4 times from the same volunteers for long-term study. We analyzed serum samples in duplicate with a hscTnT assay on the Roche Modular E170 and computed reference change values (RCVs) for analytical, intraindividual, interindividual, and total change values (CV_A, CV_I, CV_G, and CV_T, respectively) and the index of individuality (II). We calculated RCVs by using a log-normal approach, owing to the skewed results of the data.

RESULTS: Short- and long-term CV_A values were 53.5% and 98%. CV_I and CV_G were 48.2% and 85.9%, respectively, for short-term studies and 94% and 94% for long-term studies. Mean δ values for the within-day study were 58% and -57.5%, and between-day mean δ values were 103.4% and -87%. Within- and between-day IIs were 0.8 and 0.14, respectively.

CONCLUSIONS: The biological variation demonstrated with the hscTnT assay is higher than prior data for cardiac troponin I. This may be attributed to differences in biology or assay imprecision at low concentrations. A short-term change (RCV log normal) of 85% and a long-term change of 315% is necessary to define a changing pattern.

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The diagnosis of acute myocardial infarction (AMI)[3] relies on determinations of cardiac troponin (cTn) *(1)*. Although all of the clinical and analytical guidelines endorse a cTn cutoff value greater than the 99th percentile of a healthy population for the diagnosis of cardiac injury, few commercially available assays are able to quantify cTn in most if not all healthy subjects *(2)*. So-called high-sensitivity (hs) troponin assays (hscTnI or hscTnT) are able to quantify concentrations of troponin in the low ng/L range *(3–9)*.

cTn assays, including many contemporary assays, facilitate detection of AMI, predict future cardiovascular events, and allow for determination of prognostic risk *(4, 10–12)*. These assays should further aid in identifying troponin concentrations associated with the cardiotoxicity of certain drugs *(13)*, a concept already proven with contemporary assays *(14–16)*. Thus, newer hscTn assays may provide superior information over current assays *(11, 12)*.

Current guidelines advocate serial cTn testing for the diagnosis of AMI *(1, 17)* so that a rising or falling pattern can be observed. Both the National Academy of Clinical Biochemistry and the American College of Cardiology/American Heart Association/European Society of Cardiology (ACC/AHA/ESC) task force *(1, 17)* recommend a 20% change from an increased baseline value as indicative of injury but are silent in regard to these criteria if the initial value is not increased. The only data-driven information on how to operationalize the determination of the optimal change to use for either short- or long-term evaluation was provided by Wu et al. *(18)*. They measured biological variation with determination of reference change values (RCVs), which allow for the definition of criteria for statistically significant changes that may occur with serial testing. Such studies include both biological and analytical variation. These are needed for all assays with sufficient analytical sensitivity to determine normal concentrations. Accordingly, we performed

a study to define the RCVs for the high-sensitivity fifth-generation cTnT assay.

Materials and Methods

SUBJECTS AND SAMPLES

The protocol was reviewed and approved by the Mayo Clinic institutional review board. The medical records of the participants were reviewed before recruitment, and individuals with any condition that could potentially influence cTn values were excluded. All participants provided written informed consent. To assess short-term biological variation, we recruited 20 healthy individuals. We inserted a saline lock into the antecubital vein and collected blood at 0, 1, 2, 3, and 4 h into serum separator tubes (Becton Dickinson). The line was flushed with saline before blood collection. A small void volume was discharged before obtaining the sample, and a small amount of saline was injected into the intravenous lines after collection to maintain line patency. Blood was allowed to clot and centrifuged within 30 min of being drawn. We stored serum aliquots at −70 °C until analysis. We studied the same 20 healthy volunteers to assess long-term biological variation. We collected blood by venipuncture every other week for 8 weeks. Sera were processed and frozen in a manner identical to the short-term specimens.

TROPONIN MEASUREMENTS

Frozen serum aliquots were allowed to thaw at room temperature. We measured hscTnT by use of the modified fourth-generation Roche Diagnostics troponin T assay on the Modular E170 (Roche Diagnostics) according to recommended procedures. The intraassay imprecision was 3.7% using a serum pool at 8.4 ng/L. Interassay imprecision of the serum pool was 13.4% at 6.9 ng/L. The limit of blank (LoB) was 2 ng/L, and the limit of detection (LoD) was 4.5 ng/L, representing the 95th percentile of 60 replicate results from zero calibrator. The 99th percentile value for a normal reference population was 13.5 ng/L measured with a CV <10% (2). The level of significance for a reference change was set at $\alpha = 0.05$.

STATISTICAL ANALYSIS

We evaluated RCVs according to the method of Fraser and Harris (19). We used ANOVA testing (generalized linear model procedure in SAS) for each individual to calculate the sums of squares for the analytical and biological components of variation. In addition, we determined total (SD_T^2), analytical (SD_A^2), intraindividual (SD_I^2), and interindividual (SD_G^2) variances by means of a maximum-likelihood approach. We then averaged these values across study participants. Variances were homogeneously distributed. To exclude outlying values from the individual subjects, we applied the Cochran test before performing calculations. The index of individuality (II) was computed as $II = (CV_A^2 + CV_I^2)^{1/2}/CV_G$, where CV_A is the analytical CV, CV_I is the intraindividual CV, and CV_G is the interindividual CV. Because the distribution of our hscTnT data was slightly skewed, we also evaluated RCVs after performing a log-normal transformation. A CI of 80% was used to calculate short-term RCV and an interval of 95% was used for long-term RCV.

Delta values were calculated as the difference between 2 different values representing the percentage change associated with a statistically significant increase (plus sign) or decrease (minus sign) in analyte concentration between serial values obtained over the short or long term.

Results

The mean age of the 20 subjects was 39 years (range 25–56 years); 40% (8 of 20) were male. None of the subjects had a history of cardiovascular disease or any other condition known to effect troponin values. During the short-term arm of the study, 1 subject had only the initial 2 blood draws (0 and 1 h). Three other subjects missed the second time point (week 4), and 1 subject missed the third time point (week 6) in the long-term study. The distribution of hscTnT for short- and long-term biological variability is shown in Fig. 1. The median value for short-term variation was 1.53 ng/L [interquartile range (IQR) 0–3.54 ng/L], whereas for the long-term arm of the study the median value was 1.67 ng/L (IQR 0.3–6.3 ng/L). For the overall study the median hscTnT was 1.67 ng/L (IQR 0–3.61 ng/L). For short-term variability, 6 individuals had duplicate values less than the limit of detection at all time points. Of the subjects followed up on the long-term study, 4 had duplicate undetectable values at all time points. Table 1 shows the RCVs for both short- and long-term biological variation, imprecision, and II. As with previous studies with hscTnI (18), both CV_I and CV_G values were lower for short-term variability compared with long-term. The long-term cohort had a higher II compared with the short-term component of the study.

Discussion

Biological variability data in healthy individuals is critical for interpretation and analysis of the clinical utility of hscTn assays. Current cardiac cTn assays fail to yield a detectable concentration in most healthy subjects, therefore precluding studies of biological variability. Consistent with the study of Wu et al. (18), we report a
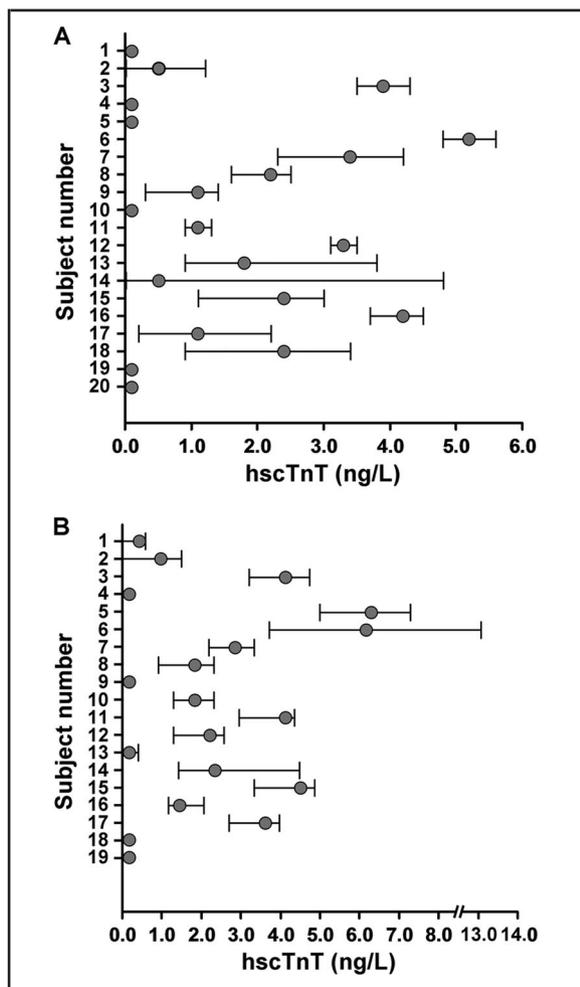
Fig. 1. hscTnT concentration ranges.

Data are mean and range. (A), Short-term variation (20 subjects) of hscTnT concentration over a 4-h period. (B), Long-term hscTnT variation (19 subjects) over an 8-week period. Statistically significant within-subject outliers were not removed.

Table 1. Short- and long-term biological variation in hscTnT.

Variable	Short-term (0–4 h)	Long-term (0–8 weeks)
Analytical variation		
CV$_A$, %[a]	53.5	98
Biological variation		
CV$_I$, %	48.2	94
CV$_G$, %	85.9	94
II	0.84	1.4
RCV, log-normal increase, %[b]	84.6	315
Mean δ increase, %	58	103.4
Mean δ decrease, %	57.5	87

[a] Based on duplicate results.
[b] CIs of 80% and 95% were used to calculate the RCV for short- and long-term biological variation, respectively.

nonparametric distribution of troponin concentrations in healthy individuals.

Data regarding biologic variation is integral to defining the appropriate δ (change over time) necessary to diagnose AMI. In addition, it is now well appreciated that some patients with stable heart disease *(20)* have chronic cTn increases. These chronic increases will no doubt be far more widespread with the use of the new hscTn assays. Thus, the definition of acute cardiovascular events is likely to require a distinct, defined changing pattern of serial values, referred to in the guidelines as a pattern of rising or falling *(1, 21)*. Previous attempts to precisely define a changing pattern could rely only on assay analytic variability because of

the inability to quantify biological variability. hscTn assays allow for further refinement of the δ criteria, taking both analytical and biological variability into account. It is conceivable that the biological data differ for cTnT and cTnI. However, because the RCV includes both analytical and biological variation, a higher value could reflect differences in low-end imprecision rather than biological change. This is especially likely when many of these low values are in a zone that is considered "unstable" analytically.

A CV$_A$ value equaling a 0.5 CV$_I$ value is usually considered adequate for a decision point. Our study demonstrates relatively high RCVs of 84.4% for a short-term change (0–4 h, at a *z* value of 0.84, 1-sided test) and 315% for a longer-term change (0–8 weeks). This is in contrast to the data with the Singulex hscTnI assay observed by Wu et al. *(18)*. Our data suggest that it will be impossible to distinguish between a rising pattern indicative of acute cardiac injury from biological and analytical variation if only minor to modest changes in serial results with the hscTnT assay occur. Acute changes as high as 84% from the baseline will be needed to make any clinical judgments; for the long term, a >3-fold increase in troponin will be required. The magnitude of these differences in RCVs between this hscTnT assay and the Singulex hscTnI assay may explain why individuals with ischemia by stress testing could be detected with the latter *(22)* but not the former *(23)*.

Some researchers have disputed the utility of biological variation for determining when analytically, and thus likely clinically, significant serial changes occur for other biomarkers *(24, 25)*. The results from our study should be verified in various patient populations.

If there were sufficiently large clinical studies, one could attempt to determine a minimum clinical change that would indeed be diagnostically useful. Until this other approach is tested, we would advocate for the serial determination of hscTnT using a minimum change of 85% over a 6-h period to detect a δ value. Biological variation using hscTn could also be used in patients with stable heart disease such as stable coronary artery disease or congestive heart failure, as advocated by other groups (25, 26). However, such studies must be interpreted cautiously due to the high likelihood for a variety of interceding events such as angina or heart failure that could induce the release of cTn. The II for the short-term arm of our study was 0.8, exceeding the 0.6 value that is the cutoff used by Fraser and Harris (19) to identify unusual values. This is perhaps because our population consisted of healthy individuals with little hscTnT variation over the 5-h duration of the short-term study.

Many have suggested that eventually hscTn concentrations will be monitored repeatedly in patients with no overt heart disease to detect the early onset of cardiovascular comorbidities (11). To use hscTnT in this manner, a >300% change is needed to be confident that the RCV value has been exceeded in conjunction with the clinical presentation. Our data for the hscTnT assay, along with prior data for hscTnI, begins to develop the conceptual constructs necessary to use a changing pattern of results with hscTn assays.

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