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Short- and Long-Term Biological Variation in Cardiac Troponin I Measured with a High-Sensitivity Assay: Implications for Clinical Practice

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BACKGROUND: The improved detection limit and precision in new-generation commercial assays for cardiac troponin I (cTnI) have lowered the 99th-percentile cutoff value, yielding higher frequencies of positive test results. Because serial testing is important in interpreting low concentrations, we evaluated the biological variation of cTnI in both the short (hours) and long (weeks) terms and determined reference change values (RCVs) and the index of individuality (II) for cTnI.

METHODS: To assess short- and long-term variation, we collected blood from 12 healthy volunteers hourly for 4 h and from 17 healthy individuals once every other week for 8 weeks, measured cTnI with a high-sensitivity assay (detection limit, 0.2 ng/L), and computed analytical, intraindividual, interindividual, and total CVs (CV_A, CV_I, CV_G, and CV_T, respectively; $CV_T = CV_A + CV_I + CV_G$) as well as the II. Because of the slight right-skewness of the data, RCVs were calculated with a lognormal approach.

RESULTS: Within-day CV_A, CV_I, and CV_G values were 8.3%, 9.7%, and 57%, respectively; the corresponding between-day values were 15%, 14%, and 63%. Within- and between-day IIs were 0.21 and 0.39, respectively. Lognormal within-day RCVs were 46% and −32%, respectively; the corresponding between-day values were 81% and −45%.

CONCLUSIONS: The low II indicates that population-based reference intervals are less useful for interpreting cTnI values than following serial changes in values in individual patients. This criterion is particularly important for interpreting results from patients who show cTnI increases at low concentrations measured with very high-sensitivity assays, from patients presenting with chest pain (short term), and for evaluating drugs for cardiotoxicity (long term).

Cardiac troponin assays have been the mainstay for diagnosing acute myocardial infarction (AMI)[5] and stratification of the risk for future adverse cardiac events. In the clinical context of myocardial ischemia, the European Society of Cardiology and the American College of Cardiology have produced a redefinition of AMI predicated on an increase in cardiac troponin concentration to greater than the 99th percentile of a healthy population, as measured with an assay with an imprecision of ≤10% *(1)*. Until recently, very few commercial assays were able to detect cardiac troponin concentrations in healthy individuals with the requisite precision. Singulex recently developed a new high-sensitivity troponin I (cTnI) assay that uses single-photon fluorescence detection *(2)*. In preliminary studies, this assay demonstrated a detection limit of 0.2 ng/L and a 10% CV at 1.8 ng/L. The concentration for the 99th-percentile cutoff was obtained from 2 different healthy populations: 7 ng/L in a study of 88 individuals *(3)* and 10 ng/L in another study of 150 individuals*(4)*. A similar cutoff of 10 ng/L has been proposed for the Nanosphere high-sensitivity cTnI assay *(5)*. Differences in the absolute values of 99th-percentile cutoffs or in the limits of detection for the various cTnI assays do not necessarily imply that one of the assays has a superior detection limit. In the absence of a cTnI reference method and universal adoption of the National Institute on Standards and Technology (NIST) cTnI stan-

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[5] Nonstandard abbreviations: AMI, acute myocardial infarction; cTnI, cardiac troponin I; CK-MB, creatine kinase isoenzyme MB; RCV, reference change value; ACS, acute coronary syndrome; BNP, B-type natriuretic peptide; CV_A, analytical CV; CV_I, intraindividual CV; CV_G, interindividual CV; CV_T, total CV; hsCRP, high-sensitivity C-reactive protein.

dard, it is difficult to compare absolute cTnI values. Differences in the 99th-percentile value can be seen on the same instrument when different populations are tested. For example, for the Abbott AxSYM cTnI assay, Abbott Laboratories reported a 99th-percentile value of 0.50 μg/L *(6)*, whereas Apple et al. reported a value of 1.1 μg/L *(7)*. Therefore, these limits are expected to vary from institution to institution and cohort to cohort.

The clinical need for a very high–sensitivity troponin assay has recently been reviewed *(8)*. There are 3 major areas where next-generation assays have the potential to improve current practice: earlier diagnosis than is currently possible with existing routine markers [troponin, creatine kinase isoenzyme MB (CK-MB), and myoglobin], improved stratification of the risk for future adverse cardiac events among patients being evaluated for AMI, and monitoring of therapeutic drugs that have the potential to cause cardiotoxicity.

The troponin values produced by the first-generation assays were not considered early markers of AMI because these assays lacked the necessary detection limit and precision and because relatively high cutoff concentrations were used for diagnosis. Thus, increases in the troponin concentration were not seen before increases in myoglobin or CK-MB *(9)*. Consequently, many clinical laboratories felt a need to retain the latter 2 tests. With the development of more sensitive troponin assays and lowering the cutoff to the 99th percentile of a healthy population, AMI can be diagnosed with troponin measurements as soon as with myoglobin or CK-MB data, leading some to question the need for these 2 markers *(10)*. Sixty-four percent more AMI diagnoses were made by assaying the first emergency department blood with the most sensitive commercially available cTnI assay than with a less-sensitive troponin assay *(11)*.

A similar evolution in troponin testing has occurred with respect to risk stratification. In the TIMI-TACTICS II trial, use of high-sensitivity troponin assays and a 99th-percentile cutoff identified 12% more patients who were subsequently determined to be at high risk for future adverse cardiac events *(12)*. The most recent risk-stratification data suggest prognostic value for use of a cutoff concentration even lower than the 99th percentile *(13)*. With respect to cardiotoxicity, injury to the heart caused by the use of chemotherapeutic drugs such as the anthracyclines releases small amounts of troponin *(14)*. Use of a high-sensitivity troponin assay may enable detection of other cardiotoxic drugs, such as trastuzumab (Herceptin), which is used to treat breast cancer patients who are positive for human epidermal growth factor receptor 2 (HER2/neu) *(15)*.

The diagnosis of AMI has always been predicated on serial testing, and this criterion dates from the original definitions established by the World Health Organization (WHO) *(16)*. With the high analytical and clinical specificity for cardiac troponin assays and the requirement for an early decision, there has been a tendency of late to diagnose AMI on the basis of a single abnormal troponin value. To reduce the diagnostic confusion caused by high-sensitivity troponin assays, guidelines produced by emergency medicine, cardiology, and clinical laboratory groups have all advocated serial testing *(17–19)*. Troponin concentrations that are stable over an appropriate sampling interval are more likely to be caused by chronic diseases, such as renal failure, heart failure, sepsis, and myocarditis. The National Academy of Clinical Biochemistry has recommended a 20% change from the baseline value to be suggestive of an AMI that is either evolving (a troponin increase) or resolving (a troponin decrease) *(19)*. This limit was based on a calculation of 3 times the imprecision at the cutoff concentrations and not on the marker's biological variation. To date, there have been no recommendations on how serial troponin results should be interpreted regarding the stratification of risk for future adverse events or with respect to cardiac toxicity due to therapeutic drugs.

Measurement of the biological variation of an analyte allows determination of reference change values (RCVs) that can be used to interpret data produced by serial testing. Such studies are usually conducted with healthy individuals with no evidence of active cardiovascular disease; however, such studies were not possible for troponin until assays had become available that could reliably detect troponin in healthy individuals. Studies of the biological variation in troponin concentrations cannot be conducted on patients with acute coronary syndromes (ACSs) or with chronic diseases such as heart and renal failure, because troponin concentrations change over time in such patients. Therefore, we used the prototype Singulex cTnI assay to evaluate short- and long-term biological variation in cTnI concentrations in healthy individuals. This assay has a 10-fold lower detection limit and a 40-fold lower 99th-percentile cutoff limit than the Siemens ADVIA® Immunoassay Systems TnI-Ultra™ Assay.

Materials and Methods

PATIENTS AND SAMPLES

Two separate cohorts of volunteers were recruited for this study by means of a protocol that has been reviewed and approved by the University of California Committee for Human Research and the San Francisco General Hospital General Clinical Research Center. All study participants signed a consent form. To assess

short-term biological variation, we recruited 12 individuals (6 male and 6 female; age range, 23–54 years). General Clinical Research Center staff inserted a heparin lock into the antecubital vein and collected blood at 0, 1, 2, 3, and 4 h through the heparin lock into Vacutainer Tubes (BD Medical Systems) containing no anticoagulant. The line was flushed of heparin with a small amount of blood prior to blood collection, and a small amount of heparin was injected into the intravenous lines after collection to maintain line patency. We allowed the collected blood to clot, centrifuged the tubes within 30 min, aliquoted the serum samples, and stored the samples at −70 °C until analysis. To assess long-term biological variation, we recruited 17 volunteers (9 male and 8 female; age range, 19–58 years). Blood was collected by venipuncture every other week for 6–8 weeks (3 or 4 samples per individual). Samples were centrifuged, stored at −70 °C, and thawed (once) before analysis. The same phlebotomist was used for each individual to minimize preanalytical variation. For the long-term study, we collected blood on the same day of the week at approximately the same time of day. For both cohorts, the participants self-reported no symptoms or history of heart disease. To assess baseline renal and left ventricular cardiac function, we measured serum creatinine and B-type natriuretic peptide (BNP) concentrations by enzymatic assay in all individuals with the ADVIA 1800 chemistry analyzer and the ADVIA Centaur CP Immunoassay System (Siemens Medical Solutions), respectively. All individuals had results within the appropriate creatinine reference interval (5–11.0 mg/L for females, 7–13 mg/L for males), an estimated glomerular filtration rate exceeding 60 mL/min, and a BNP concentration well below 100 ng/L. Although such values for these variables do not rule out the presence of preexisting cardiac disease, the lack of overt renal and/or heart failure in these individuals suggested that they were appropriate for an assessment of biological variation.

DATA ANALYSIS

Frozen samples were allowed to thaw at room temperature. All analytical assays for cTnI were performed with the Erenna Immunoassay System at Singulex, Hayward, California, and were tested in duplicate by the same technologist on the same day (different days for each of the short- and long-term studies). All calculations and statistical analyses were carried out with Microsoft Excel and SAS software. We evaluated standard RCVs according to the method of Fraser and Harris *(20)*. We then used ANOVA testing (GLM 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. With this approach, we averaged these values across study participants. 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 [the equation was not simplified to the more commonly used formula ($II = CV_I/CV_G$) because CV_A was equal to CV_I *(20)*]. Because the distribution of our cTnI data was slightly right-skewed, we also evaluated RCVs after performing a lognormal (natural logarithm) transformation of the data. Although one individual in the between-day dataset appeared to be an outlier, this observation disappeared when the data were log-transformed. Determination of lognormal RCVs was first described in equations formulated by Fokkema et al. *(21)*, who used the total CV (CV_T) of non–log-transformed data to evaluate the σ^2 parameter of the lognormal distribution. Use of log-transformed data is statistically preferable, however, when calculating all logarithmically transformed components of biological variation for our within-day and between-day TnI data. The data are then back-transformed (i.e., by exponentiation of log-transformed data) to obtain corresponding raw values. This approach provides nonsymmetric log CV values such that the corresponding log RCV values obtained from them do not represent a single percentage change, but rather 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 a specified time period (e.g., within or between days).

Results

The distributions of cTnI results for short- and long-term biological variation are shown in Fig. 1, A and B, respectively. Data are presented as the mean and range. None of the data were below the detection limit of the assay (0.2 ng/L), and the data for all but one of the individuals were below the 99th-percentile limit of 10 ng/L, which was previously established for 100 apparently healthy individuals *(4)*. Table 1 shows the short- and long-term biological variation in the cTnI data. CV_I and CV_G values were slightly lower for the short-term results compared with the long-term results. This finding was expected, given that changes to the heart are not expected to change in a healthy individual from hour to hour but may change slightly from week to week. Also shown are imprecision, inaccuracy, and index of individuality, with hour-to-hour values being slightly higher but not significantly different from week-to-week results.

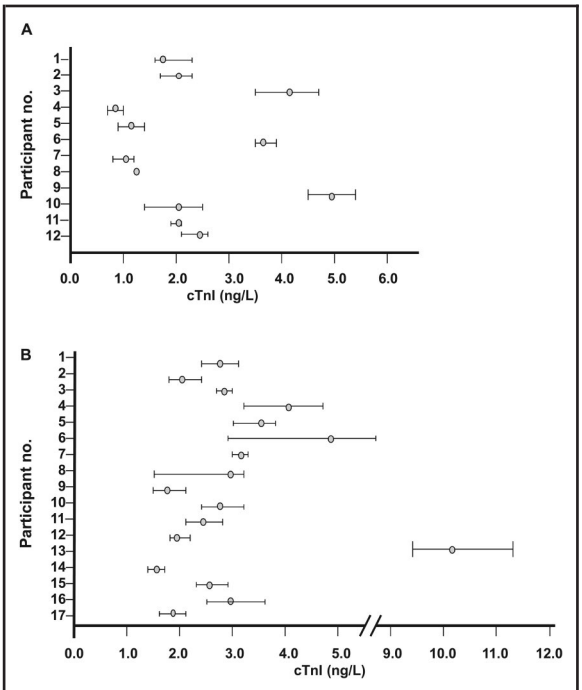

Fig. 1. cTnI concentration ranges.

Data are presented as the mean and range. (A), Short-term variation in cTnI concentration for 12 study participants over a 4-h period. (B), Long-term cTnI variation for 17 participants over an 8-week period. Statistically significant within-subject outliers were removed.

The distributions of the within-day and between-day cTnI results were not normally distributed but were right-skewed. A lognormal transformation was performed before the calculation of RCV. This transformation produced nonsymmetric RCV results, with higher limits signifying a statistical change for increasing cTnI results and lower limits for decreasing results. The higher RCV limit for an increasing cTnI concentration reflects the right-skewed distribution of results for healthy individuals and greater statistical uncertainty when values are increasing. For the short-term, the calculated RCV results are about 1.5–2 times higher than the single limit of a 20% change in serial results that has been recommended by the National Academy of Clinical Biochemistry *(19)*. This result is partly due to the higher imprecision observed in healthy individuals at lower cTnI concentrations. Criteria for long-term RCV for use with troponin had not previously been determined or suggested. Results from the present study suggest that the RCVs should be 81% and −45% for increasing and decreasing concentrations, respectively.

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

Variable	Short term (0–4 h)	Long term (0–8 weeks)
Analytical variation		
CV_A, %[a]	8.3	15
Biological variation		
CV_I, %	9.7	14
CV_G, %	57	63
Index of individuality	0.21	0.39
RCV: log-normal increase, %	+46	+81
RCV: log-normal decrease, %	−32	−45

[a] Based on duplicate results.

Discussion

Measurement of the biological variation in cardiac troponin was not previously possible because the assays lacked lower limits of detection adequate to reliably measure troponin in the blood of healthy individuals. The recent availability of high-sensitivity troponin assays now enables reliable detection of troponin (with the requisite imprecision of ≤10%) at concentrations that reflect the typical turnover of cardiac myocytes in healthy individuals. Two different research assays have now shown that the distribution of cardiac troponin in healthy people is nonparametric *(4, 5)*. The results of this study are consistent with these previous findings.

Table 2 compares the short-term biological variation in cardiac troponin with that of other commonly used cardiac biomarkers of ACS. Total CK, CK-MB activity, CK-MB mass, and myoglobin *(22)* have been described as "event markers," such as for plaque rupture or erosion in cases of AMI. The CV_I values for troponin and CK-MB mass are low relative to the CV_G, thereby producing a low II. A low II (i.e., <0.6) indicates that reference intervals will be of less value for interpreting individual results than with a high II (i.e., >1.4) *(23)*. Table 2 shows that the traditional ACS markers have low IIs. In this study, the short-term cTnI II of 0.21 was also much lower than the IIs of the other markers, an observation that reinforces the notion that serial testing is an important criterion for assessing the diagnostic implications of cTnI increases that are just above the 99th-percentile cutoff concentration.

These biological-variation data do not influence the interpretation of troponin measurements in patients who have very high concentrations, e.g., a 10-fold increase relative to the 99th-percentile cutoff. These patients should be admitted immediately and

Table 2. Summary of biological variation for cardiac markers.

Marker	CV_A, %	CV_I, %	CV_G, %	II	RCV, %	Monitoring duration	Reference
Acute-disease markers							
Creatine kinase	14	22	42	0.52	72.2	Daily	Ross et al. *(22)*
CK-MB, activity	29	4.9	14	0.35	81.8	Daily	Ross et al. *(22)*
CK-MB, mass	6.8	18	61	0.30	54.4	Daily	Ross et al. *(22)*
Myoglobin	13	18	47	0.38	61.2	Daily	Ross et al. *(22)*
cTnI	8.3	9.7	57	0.21	+46, −32	Hourly	This study
Chronic-disease markers							
Myoglobin	6.0	11	14	0.80	35.0	Weekly	Panteghini et al. *(24)*
C-reactive protein	5.2	42	92	0.46	118	Weekly	Macy et al. *(25)*
C-reactive protein	1.0	37	62	0.59	102	4 Days	Cho et al. *(26)*
Serum amyloid A	4.0	25	61	0.40	70.1	Weekly	d'Eril et al. *(27)*
Myeloperoxidase	4.0	36	30	1.20	100	Weekly	Dednam et al. *(28)*
BNP	8.4	40	41	0.98	113	Weekly	Wu et al. *(29)*[a,b]
BNP					+198, −66	Weekly	Fokkema et al. *(21)*[b]
NT-proBNP[c]	3.0	35	35	1.00	98	Weekly	Bruins et al. *(30)*[d]
NT-proBNP	1.6	33	36	0.90	92	Weekly	Wu et al. *(29)*
NT-BNP					+157, −61	Weekly	Fokkema et al. *(21)*
cTnI	15	14	63	0.39	+81, −45	Weekly	This study

[a] Conducted with healthy individuals.
[b] Log-normal transformation of data of Bruins et al. *(30)*.
[c] NT-proBNP, N-terminal proBNP.
[d] Conducted in stable heart failure patients.

treated without undergoing serial troponin testing. In contrast, proper use of data on biological variation will be important for interpreting minor increases in troponin concentration (i.e., values at or just above the 99th-percentile limit) when assays of very high sensitivity are used. Serial cardiac troponin values that exceed the upper RCV in the clinical context of chest pain increase the likelihood that the patient is experiencing an evolving AMI. This interpretation may be valid even if all serial troponin results are below the population-based reference limit (because of the low II); however, other acute disease processes, such as sepsis, can increase troponin concentrations over the short term. Patients with serial troponin values that are mildly increased but stable are more likely to have a chronic cardiac condition known to cause cardiac damage (e.g., myocarditis, heart failure, kidney failure). Declining serial troponin values could be indicative of a resolving AMI, particularly if the patient has a history of chest pain over the preceding days or week. Of note is that patients with a positive troponin result due to a chronic disease can present with an acute exacerbation and increasing troponin values that mimic an AMI release pattern. Therefore, clinical judgment remains of paramount importance in interpreting the results of troponin testing.

Table 2 also compares long-term biological variation in cTnI with that of other biomarkers used for detecting chronic disease. These markers include myoglobin *(24)*, high-sensitivity C-reactive protein (hsCRP) *(25, 26)*, serum amyloid A *(27)*, and myeloperoxidase *(28)* for atherosclerosis and include BNP and N-terminal proBNP for heart failure *(29, 30)*. Both CV_I and CV_G values are higher in general for long-term "disease" markers than for short-term event markers. This finding is not unexpected, given that the interval between blood collections is weeks instead of hours. The low II values for inflammatory markers (0.40–0.59, Table 2) suggest that reference intervals will not be as useful as serial testing. In cases in which serial testing is not available, use of the reference interval may be the only alternative. In contrast, the higher II values for the natriuretic peptides (0.9–1.1, Table 2) suggest that reference intervals are more appropriate. For both the inflammatory markers and natriuretic peptides, the high biological variation produces relatively high RCV values (70%–198%). Therefore, minor changes in serial results will not be meaningful, as one

of us recently suggested for hsCRP *(31)*. Other investigators have challenged the utility of biological variation for measuring significant serial changes for hsCRP and BNP *(32, 33)*.

The cTnI results for monitoring cardiac disease appear to be appreciably different from those for the other markers of chronic cardiac disease. Intraindividual biological variation, IIs, and RCVs are much lower (Table 2). Although hsCRP, myeloperoxidase, and BNP are useful markers for risk stratification, the troponin results suggest that high-sensitivity assays could also be used to detect minor myocardial damage due to cardiotoxic drugs. Patients on these drugs are currently not routinely tested for cardiac troponin because existing commercial assays lack limits of detection adequate for detecting very minor and chronic increases. With the development of highly sensitive assays, the utility of cardiac troponin should be reexamined as a marker for this application.

There is continuing debate regarding the applicability of biological-variation data from healthy individuals to the interpretation of data from patients with ischemic heart disease. Some investigators have advocated calculating biological variation for patients with stable heart disease. Although such calculations have been done for patients with stable heart failure *(30)*, it cannot be done in ACS patients because ACS is a dynamic disease with changing biomarker concentrations. Troponin studies would have little relevance because this marker has no physiological function in blood and serves only as an indicator of tissue damage. Therefore, the validation of measures of biological variation derived in this work can be accomplished only through clinical studies in which results of serial changes in troponin concentration are compared against diagnoses at final discharge. Such studies also have drawbacks, however, because troponin results cannot be withheld from attending physicians for ethical reasons. For this reason, such results may influence and bias the clinical conclusion rendered. Evidence also exists that troponin concentrations below the 99th-percentile limit also have risk-stratification value *(13)*. Therefore, changes in cTnI with respect to the group reference interval will also need further evaluation in clinical studies.

If proved useful, the long-term biological variation and calculation of the RCV will be relevant for all new generations of cTnI assays. Roche Diagnostics recently developed a high-sensitivity troponin T assay, and similar biological-variation studies are necessary to determine how serial changes in cTnT can be interpreted in the context of acute and chronic cardiovascular disease. Although cTnT is used clinically in the same manner as cTnI, it has different kinetics with regard to release and clearance, and the cTnI biological-variation data likely will not apply.

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