Use of Observed Within-Person Variation of Cardiac Troponin in Emergency Department Patients for Determination of Biological Variation and Percentage and Absolute Reference Change Values

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BACKGROUND: Many patients presenting to the emergency department (ED) for assessment of possible acute coronary syndrome (ACS) have low cardiac troponin concentrations that change very little on repeat blood draw. It is unclear if a lack of change in cardiac troponin concentration can be used to identify acutely presenting patients at low risk of ACS.

METHODS: We used the hs-cTnI assay from Abbott Diagnostics, which can detect cTnI in the blood of nearly all people. We identified a population of ED patients being assessed for ACS with repeat cTnI measurement who ultimately were proven to have no acute cardiac disease at the time of presentation. We used data from the repeat sampling to calculate total within-person CV (CV_T) and, knowing the assay analytical CV (CV_A), we could calculate within-person biological variation (CV_i), reference change values (RCVs), and absolute RCV delta cTnI concentrations.

RESULTS: We had data sets on 283 patients. Men and women had similar CV_i values of approximately 14%, which was similar at all concentrations <40 ng/L. The biological variation was not dependent on the time interval between sample collections (t = 1.5–17 h). The absolute delta critical reference change value was similar no matter what the initial cTnI concentration was. More than 90% of subjects had a critical reference change value <5 ng/L, and 97% had values of <10 ng/L.

CONCLUSIONS: With this hs-cTnI assay, delta cTnI seems to be a useful tool for rapidly identifying ED patients at low risk for possible ACS.

Cardiac troponin is a core component in the diagnosis of acute myocardial infarction, with the need for a demonstrated rise or fall in concentration and at least 1 result above the 99th percentile with some clinical evidence of ischemia (1). Determining the 99th percentile can be problematic for a variety of reasons, but especially because subclinical disease may cause artifactual increases in apparent cardiac troponin 99th percentile cut points (2, 3, 4).

The majority of patients assessed in the emergency department (ED)9 for possible acute coronary syndrome (ACS) prove to have no cardiac cause for their presentation (5, 6). Only a small proportion of patients being assessed will have a cardiac troponin above the 99th percentile (5). According to international guidelines (7, 8), the assessment process for possible ACS includes 2 or more blood samples collected for measurement of cardiac troponin concentration. Evidence suggests that the relative or absolute change in cardiac troponin concentration may be of particular value in assessing patients at risk for ACS in the Emergency Department (9).

We identified patients discharged home from the ED who did not have the diagnosis of ACS and used the data from this population to calculate total CV (CV_T), biological variation (CV_i), percentage reference

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9 Nonstandard abbreviations: ED, emergency department; ACS, acute coronary syndrome; RCV, reference change value; cTnI, cardiac troponin I; LoD, limit of detection.

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change value (RCV), and absolute (delta) RCV for cTnI concentration changes.

Materials and Methods

This study was approved by the Australian Capital Territory Health Human Research Ethics Committee.

STUDY POPULATION
To identify an ED cohort that was investigated for possible ACS, but in which no underlying serious acute disease was detected, we assessed all patients presenting to the EDs of the 2 acute hospitals we serve. The following criteria were used. (a) Patients had two or more samples collected for cardiac troponin I (cTnI) analysis on the 1 presentation to the ED. No time limit was applied between collections, and these varied between 1.5 and 17 h. (b) Both samples had results between the limit of detection (LoD) and the upper limit of the reference interval (see below). (c) Patients were discharged from the ED without admission to the hospital. (d) There were no further presentations to the ED or the hospital within the next 14 days.

Patients who met these criteria were considered not to have a diagnosis of an ACS at the ED presentation and time of blood collection. Hence any cTnI concentration changes reflect a mixture of analytical imprecision and biological variation, and not cTnI release resulting from an acute cardiac event.

BIOCHEMICAL ANALYSES
We introduced the high-sensitivity assay for cTnI from Abbott Diagnostics into routine clinical use from August 23, 2013. This assay has an LoD of 1.0 ng/L and a concentration corresponding to the 10% CV of 3.8 ng/L (10). The 99th percentile depends on the rigor with which subclinical cardiac disease was excluded in the reference population. For apparently healthy men of all ages, the 99th percentile is 36.8 ng/L, and for women, 17.6 ng/L, but after careful exclusion of persons with subclinical cardiac disease, the 99th percentile falls to 26.6 ng/L for men and 12.5 ng/L for women (4). Abbott Diagnostics quotes 99th percentiles for cTnI of 34.2 ng/L for males and 15.6 ng/L for females (11). Because of this variation in the 99th percentile, we collected data on all men and women who met the enrollment criteria above with results <40 ng/L.

STATISTICAL ANALYSIS
With these selection criteria and follow-up for 14 days (as for the TIMI trials (12)), we had a population without acute cardiac disease at the time of presentation and in whom the only contributions to the total variation were analytical variation and biological variation. As described previously (13), we calculated the total variation for each patient as the SD of the 2 or occasionally 3 measurements taken during their ED admission and expressed this as SD and total CV (CV_T) for each individual. We fitted a power function to our precision profile for this hs-cTnI assay \( y = 26.457x^{-0.6466}; r^2 = 0.92 \) (10) and calculated the assay CV (CV_A) at the mean cTnI concentration for each patient. This in turn allowed us to calculate the biological variation for each individual (CV_i) as the difference between the CV_T and the analytical variation:

\[
CV_i = \sqrt{(CV_T^2 - CV_A^2)}
\]

We independently verified these data by performing ANOVA.

We used our data from these measurements to calculate the index of individuality (CV_i/CV_T) and the RCV as described by Fokkema et al. (14). For 95% confidence that a change in a result is real and not simply a reflection of biological variation plus analytical variation, a change of 2.77 × SD is required (15–17). In the small number of cases where >2 samples were collected, the samples with highest and lowest concentrations were used.

Because changes may be in either a positive or negative direction, we looked to see if there was any systematic difference between rises and falls and found none. We identified outliers using the criteria of Dixon as recommended by the Clinical and Laboratory Standards Institute for nongaussian populations (18). We carefully reviewed all patients who met our enrollment criteria to see whether there could have been a missed diagnosis of ACS.

Results

During the course of our study, we performed 3182 hs-cTnI analyses on 2182 ED patients. From these data we identified 283 patients (164 men and 119 women) who met our study inclusion criteria. Two patients were outliers according to the criteria of Dixon and had absolute RCVs for cTnI >15 ng/L. These persons were reviewed at 30 days and were well.

A scattergram of the CV_i and the mean cTnI concentration for each individual patient is shown in Fig. 1. At very low concentrations, small changes resulted in larger increases in CV_i. Over the range of cTnI concentrations up to 40 ng/L, the median CV_i was 13.9% for women and 12.9% for men.

The effect on CV_i of the time differences between sampling in each individual are shown in Fig. 2. From these data showing the median and interquartile ranges for the biological variation at different times, only small changes in CV_i with time differences between sampling were noted.
Data on the reference change values found in this study and in other comparable studies using the Abbott hs-cTnI assay are shown in Table 1, demonstrating a low index of individuality in nearly all studies that covered time frames from a few hours to several years.

We show in Fig. 3 the absolute reference change values as related to the median cTnI concentration. There was little difference in the absolute RCV regardless of the starting cTnI concentration. In Fig. 4, we show the frequency distribution of the absolute RCV values. Of our 283 patients, 92.2% (261) had absolute
RCVs <5 ng/L and only 2.8% (8) had an RCV >10 ng/L.

The observed mean cTnI concentrations and the ranges are shown in Fig. 5 with the patients ranked from lowest mean concentration to highest.

Because there is uncertainty about 99th percentile values, and because there is a growing agreement that women should have a lower 99th percentile than men, we reviewed our data looking at the effect of using 20 ng/L as the upper limit for inclusion of women into our

<table>
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<tr>
<th>Criterion</th>
<th>This study (short)</th>
<th>LOOK (long)</th>
<th>Nordenskjold et al. (19) (short)</th>
<th>Nordenskjold et al. (19) (long)</th>
<th>Apple and Collinson (20)</th>
<th>Goldberg et al. (21) (short)</th>
<th>Goldberg et al. (21) (long)</th>
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<td>2–17 h</td>
<td>2–4 years</td>
<td>24 h</td>
<td>4–58 days</td>
<td>4 h</td>
<td>4–6 h</td>
<td>9 days</td>
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<td>LiHep plasma</td>
<td>EDTA plasma</td>
<td>EDTA plasma</td>
<td>Serum</td>
<td>Serum</td>
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**Table 1. Reference change values for cTnI for patients in the ED, compared to other studies using the same hs-cTnI assay.**

**Fig. 3. Absolute RCVs in relation to mean cTnI in ED patients without acute cardiac disease.**
study. The number of women included was 113 (119 if a cutoff of 40 ng/L was used), the biological variation for women was 14.2% (previously 13.9%), and for men and women combined, the biological variation was 13.9% (previously 13.8%). The index of individuality for men and women together was 0.16 (previously 0.17), 93.1% (258 of 277) of both men and women had an absolute RCV <5 ng/L, and only 2.5% (7 of 277) had an absolute RCV >10 ng/L.

Discussion

Most studies reporting biological variation of cardiac troponin have assessed small numbers of people. In this article, we have been able to determine short-term CV$_{i}$ of cTnI in a large cohort of patients apparently without active acute cardiac disease at the time of assessment. We are aware of only 4 other studies that have examined biological variation using the hs-cTnI assay from Abbott as reported in this paper. Norden-skjold et al. looked at 24 patients admitted for elective coronary angiography and found that the short-term CV$_{i}$ was 14% and longer-term CV$_{i}$ (4–58 days) was 24% (19). This result for short-term biological variation is essentially identical to the results obtained in this paper: 13.9% for men and 12.9% for women. Apple and Collinson found that the short-term CV$_{i}$ was 15% (20), whereas Goldberg et al. found that the short-term CV$_{i}$ was 24% but the longer-term CV$_{i}$ (over 9 days) was 80% (21). A study looking at biological variation over several years in 453 healthy children found that the median CV$_{i}$ was 33% (13). Other than the study from Goldberg et al., these results are remarkably consistent in establishing that cTnI is an analyte with a small index of individuality. This, coupled with the excellent precision of the Abbott hs-cTnI assay at low concentrations of cTnI, results in small absolute delta cardiac troponin concentrations indicating real—i.e., pathological—changes.

The most important population for cTnI analysis is the ED population that requires assessment for ACS. International guidelines recommend analysis of serial blood samples at least 3 h apart to identify significant changes in cardiac troponin concentration when using a high-sensitivity cardiac troponin assay (7, 22). We have demonstrated that sampling only 2 h apart will identify those patients who can be safely discharged (5). However, for a variety of reasons emergency physicians may collect samples at varying time intervals, as occurred with the current pragmatic study. It is noteworthy that the biological variation and CV$_{T}$ in our population were little influenced by the time interval between blood collections.

The definition of a significant change in troponin concentration remains elusive at present. Our study is of particular relevance in this context. Of interest and importance is that the absolute delta changes for cTnI are consistent over the entire concentration range examined. More than 90% of our patients had a critical delta change in cTnI concentration <5 ng/L, and fewer
than 3% had a critical delta change >10 ng/L. It must be noted that we performed this study only on patients with cTnI concentrations <40 ng/L, the approximate upper reference limit for cTnI according to Abbott Diagnostics, the manufacturer of the kit used in this study. ED physicians are unlikely to discharge a patient with a higher cardiac troponin concentration without further assessment even if ACS is considered unlikely. These data suggest that a delta change >5 ng/L should be viewed with caution in assessing patients for exclusion of ACS. Interestingly, Mueller et al. (23), investigating patients with ACS with a high sensitivity assay for cTnT, found that an absolute change of 9.2 ng/L was the optimal cut point.

Currently it is the 99th percentile that forms the basis for decision making with regard to ACS, including acute myocardial infarction. However, it is apparent that many of the studies determining the 99th percentile are contaminated by people with subclinical cardiac disease (2–4). Although exact determination of the 99th percentile is problematic, for this study we used values up to 40 ng/L, a pragmatic value that is relevant in clinical care. With the index of individuality for cardiac troponin being low (in this study 0.17 and for other short-term studies with the same assay, 0.08 (19), 0.22 (20), and 0.24 (21)), it is quite feasible for a clinically important change to occur within the reference interval.

We propose that the 99th percentile should not be the only important metric for determining risk in the majority of ED patients assessed for possible ACS. Although a minority of ED patients (approximately 20%) assessed for ACS have cardiac troponin above the reported 99th percentile (5, 24), the greater challenge is the identification of those patients who are not at risk of a serious medical event and who can be safely discharged with cardiac troponin concentrations near or below the 99th percentile. Our study helps in this goal by demonstrating that with the Abbott Diagnostics high-sensitivity cTnI assay, nearly all ED patients who were assumed to have no serious underlying acute cardiac condition by our criteria, deemed safe for discharge and not re-presenting within 14 days, had absolute delta changes of <10 ng/L, with the great majority having changes <5 ng/L.

A potential limitation of our study is that we have assumed that persons who did not re-present had no serious underlying acute disease. It is possible that some patients re-presented to other health services in the 2-week period. However, this is unlikely, as we serve all the major acute hospitals in the Australian Capital Territory and there are no other hospitals with major facilities within 200 miles. It should be noted that if occult disease were contributing to the measured variation in cTnI, then in persons who truly had no
active cardiac disease, the delta changes would have been even smaller than we state. In conclusion, we have found that for patients presenting to the ED with symptoms of possible ACS, a delta cTnI of <10 ng/L (with the Abbott Diagnostics high-sensitivity cardiac troponin I assay) identified nearly all patients in whom there was no serious underlying acute cardiac disease. A larger prospective study is warranted to assess this finding, as a lack of change in serial troponin concentrations may assist in rapid assessment of a significant proportion of ED patients.

**Author Contributions:** All authors have 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.

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**References**