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Brief Communications

Analytic and Clinical Utility of a Next-Generation, Highly Sensitive Cardiac Troponin I Assay for Early Detection of Myocardial Injury

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BACKGROUND: Improvements in cardiac troponin (cTn) assays have increased the rapidity with which clinicians can identify patients with changing cTn concentrations (rise or fall) indicative of acute myocardial injury. The aim of the present study was to characterize a new, high-sensitivity cTnI (hs-cTnI) assay and examine whether increased sensitivity can result in still earlier detection of evolving injury.

METHODS: We determined the limit of detection, precision profiles, and preliminary estimates of the 99th percentile for the Beckman Coulter hs-cTnI assay in 125 healthy individuals (age <55 years, 54% male). We compared AccuTnI® and hs-cTnI to assess whether change criteria for early concentration changes (i.e., ≥3SD for low concentrations and 20% difference for concentrations >0.10 μg/L) were exceeded in the first 2 specimens (median time between specimens, 1 h; 25th–75th percentile, 1–3 h) from subjects with symptoms suggestive of cardiac ischemia (n = 290).

RESULTS: The limit of detection for the hs-cTnI assay was 2.06 ng/L, and the 20% CV and 10% CV concentrations were 2.95 and 8.66 ng/L, respectively. The preliminary 99th percentile estimates in lithium heparin, serum, and EDTA plasma were 9.20, 8.00, and 8.60 ng/L, respectively. In 108 patients with myocardial injury based on the peak AccuTnI concentration, applying the change criteria on the 2 earliest specimens identified 81% (95% CI 73%–88%) of patients using the hs-cTnI assay compared to 62% (53%–71%) using the AccuTnI assay ($P < 0.001$).

CONCLUSIONS: Although more extensive validation studies are required, this Beckman Coulter hs-cTnI assay appears to detect patients with evolving myocardial injury earlier.

Guidelines from laboratory and cardiology groups *(1, 2)* have adopted the 99th percentile as the cutoff for cardiac troponin (cTn)[5] for the diagnosis of myocardial infarction (MI). Recent data document that chronic heart disease may cause persistent elevations above the 99th percentile *(3)*. Thus, using a changing pattern of cTn values for the diagnosis of acute disease has been advocated *(4)*. More sensitive assays would facilitate these recommendations and reduce the time required to evaluate patients with acute coronary syndromes (ACS) *(5, 6)*. This study examines the analytical and clinical characteristics of a highly sensitive cTn assay for identifying evolving myocardial injury earlier.

Materials and Methods

The high-sensitivity cardiac troponin I (hs-cTnI) assay from Beckman Coulter employs the same antibodies (i.e., recognizes the same epitopes) as the current AccuTnI assay. Increased sensitivity and precision is achieved by the use of an increased sample volume (100 μL compared to 40 μL for the current assay), increased incubation time, and changes in the microparticle capture bead. Specifically, the capture antibody is biotinylated and coated to a streptavidin-bound paramagnetic particle. The concentration units for the hs-cTnI assay are ng/L (or pg/mL); the present AccuTnI assay uses μg/L (or ng/mL). The analytical range for the hs-cTnI assay is 0–9780 ng/L, with a time to first result of 30 min.

We determined limit of the blank (LoB) and limit of detection (LoD) as per Clinical and Laboratory Standards Institute (CLSI) document EP17-A. Thirty measurements of the zero calibrator were performed over 2 days, and the 95th percentile concentration (nonparametric) was determined. The LoD was determined from the precision profile from duplicate measurements on 8 samples of decreasing concentrations. We prepared samples using a high-concentration stock of cTnI derived from pooled clinical samples spiked into a negative serum pool. We tested samples in duplicate in 20 independent series on 3 Access 2 systems. Results were pooled and a model fit was created to estimate concentrations at 20%, 15%, and 10% CV. For the

[5] Nonstandard abbreviations: cTn, cardiac troponin; MI, myocardial infarction; ACS, acute coronary syndromes; hs-cTnI, high-sensitivity cardiac troponin I; LoB, limit of the blank; LoD, limit of detection; CLSI, Clinical and Laboratory Standards Institute; IQR, interquartile range; RCV, reference change value.

LoD, we used concentrations and their SDs corresponding to 15% and 20% CVs in the following calculation: LoD = LoB + c_β(SDs), where c_β is derived from the 95th percentile of the standard gaussian distribution (and the correction factor) and SDs is an estimate for the population standard deviation. We determined the within-run and total imprecision using 3 quality control materials (QC1, 10.2 ng/L; QC2, 124 ng/L; QC3, 622 ng/L; Bio-Rad) according to the CLSI EP15-A2 protocol under 1 calibration.

For the reference interval study, we analyzed serum, lithium heparin plasma, and EDTA plasma specimens from 131 individuals (age <55 years) who were deemed healthy (exclusion criteria included pregnancy, current cold or infection, chronic inflammatory disease, treated for cardiac disease or lipid management, diabetes, smoking, high blood pressure or treated for high blood pressure, immediate family history of cardiovascular disease, or increased interleukin-6 or C-reactive protein). Of the 131 individuals, 4 were excluded owing to the presence of heterophilic antibodies and 2 were excluded as true positives for myocardial injury (as demonstrated by inhibition upon addition of cTnI monoclonal antibody), leaving 125 subjects (54% male) for basis of the preliminary 99th percentile estimates for each sample type. The preliminary 99th percentile estimates were determined by the software Analyze-It, which uses the nonparametric calculation of 99th percentile reference limit. This reference limit is obtained by simple interpolation in Analyze-It. Because it is nonparametric, no outliers were excluded except those subjects who met the exclusion criteria (see above). The CI of the 99th percentile reference limit is not given because of the small number of subjects (see CLSI C28-A2 document).

For the emergency department chest pain population, subjects, study design, and methods have been reported *(6–9)*. All available heparin plasma specimens that were analyzed in 2003 with the AccuTnI assay and had sufficient sample quantity remaining were thawed and reanalyzed with the hs-cTnI assay (n = 1437 from 418 subjects). Subjects presenting to the emergency department with symptoms suggestive of ACS had blood collected based on the subjects' reported time from onset of symptoms and hourly until 6 h after onset, then at 9, 12, 24, and 48 h or until the patient was discharged, declined further participation, or was removed by their caregivers. We used Deming regression analysis to confirm the stability of cTnI in the stored specimens by repeating the AccuTnI measurement in a subset (n = 44) of the heparin samples after 5 years of further storage at −70 °C. Correlation and Bland–Altman bias plot analyses were performed between the AccuTnI and hs-cTnI assays (n = 1177 samples for which both measurements were available); see Supplemental Fig. 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue3.

In 290 subjects with 2 or more specimens available, we used peak AccuTnI concentration to determine if myocardial injury was present (peak AccuTnI >99th percentile; >0.04 μg/L). Concentration change criteria were applied (>3SD or >20%) consistent with the 2007 MI definition and our previous work *(2, 6, 7)*, using both the AccuTnI and hs-cTnI values in this cohort to assess if a change was present. For concentrations below 0.10 μg/L (or 100 ng/L), we used the performance of the QC material with the lowest concentration of cTnI to determine the SD for both assays [AccuTnI, Bio-Rad liquicheck LT (lot 31100) with mean 0.11 μg/L, SD 0.01 μg/L, n = 60; hs-cTnI, Bio-Rad cardiac markers control (lot 31201) with mean 10.2 ng/L, SD 1.11 ng/L, n = 42]. We used a 3SD change as recommended by the global task force—for AccuTnI this was ≥0.03 μg/L, and for hs-cTnI, ≥3.34 ng/L, to define a significant change. For high concentrations (>0.10 μg/L or >100 ng/L) a difference of at least 20% between values was considered significant *(2)*. These change criteria were applied to the 2 earliest specimens (i.e., at time of presentation and the next available), and in a separate assessment, to any 2 specimens measured in 1 patient: difference between the highest and the lowest concentrations observed [median 4 specimens/subject; interquartile range (IQR) 2–6]. The gold standard for documenting myocardial injury for this evaluation was the group of 108 patients that had an AccuTnI concentration that at any time exceeded the 99th percentile without consideration of changes over time. Additional analyses for determining sensitivity were performed for the 2 earliest specimens using ROC curves as well as using the short-term reference change value (RCV). A recent report for another hs-cTnI assay has documented that short-term changes (within 4 h) must exceed a 46% increase or a 32% decrease in serial samples *(10)*, which we used for the RCV in our current analysis, as the RCV for the Beckman Coulter hs-cTnI assay has not been determined. The 95% CIs, Deming regression, ROC curve analyses, and comparisons between groups were performed using Friedman, Kruskal–Wallis, Dunn's multiple, and McNemar tests using the Graphpad Prism and Analyze-it statistical software. The study was approved by the research ethics board.

Results

The LoB was 1.03 ng/L and the LoD was 2.06 ng/L for the hs-cTnI assay. The concentrations at which 20% and 10% CVs were achieved were 2.95 ng/L and

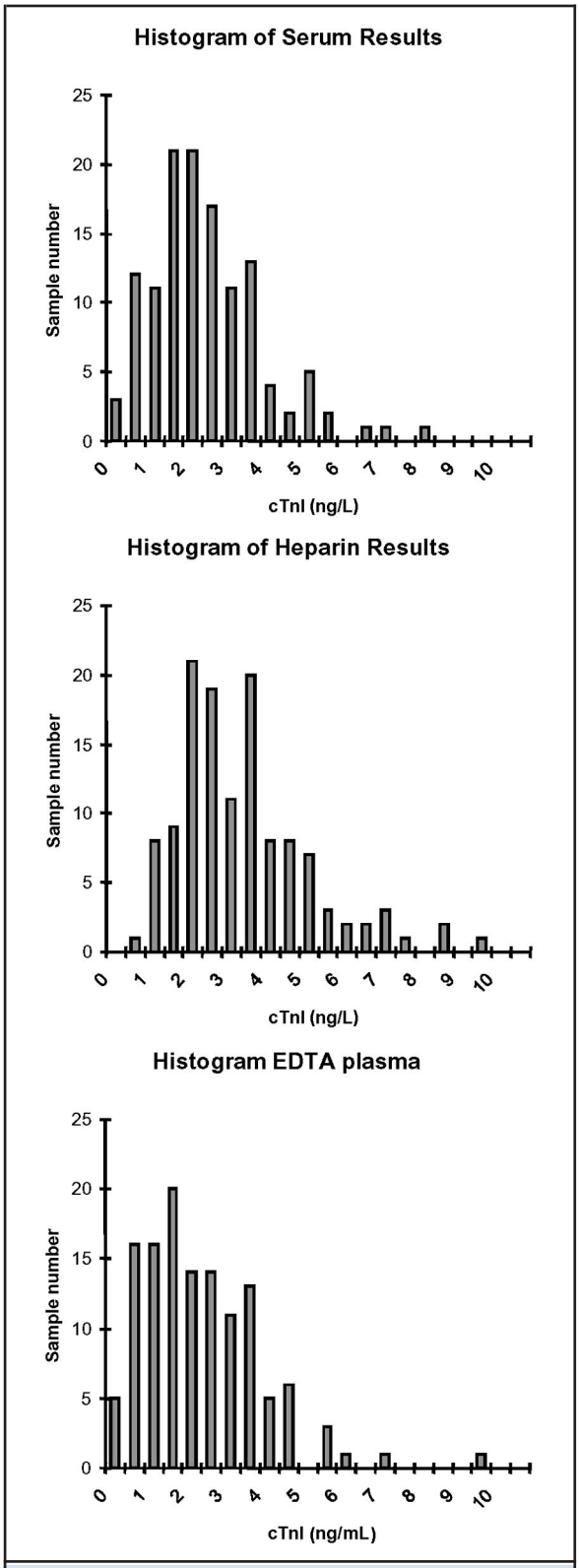

Fig. 1. Distribution of hs-cTnI concentrations in 125 healthy individuals in different matrices.

8.66 ng/L. Within-run precision and total precision for hs-cTnI was 6.0% and 7.1% for QC1 (10.2 ng/L); 1.3% and 4.5% for QC2 (124 ng/L); and 2.4% and 3.5% for QC3 (622 ng/L). There was good recovery of the 2003 AccuTnI concentrations in the 2007 AccuTnI reassays ($cTnI_{2007} = 0.977(cTnI_{2003}) + 0.005$; $r = 0.98$), and Deming regression analysis yielded the following: $hs\text{-}cTnI_{2007} = 0.862(AccuTnI_{2003}) - 0.001$; $r = 0.87$; $P < 0.001$ (n = 1177; see online Supplemental Fig. 1). The preliminary 99th percentile (median concentrations) for heparin plasma, serum, and EDTA plasma from the 125 controls were 9.20 ng/L (2.70 ng/L), 8.00 ng/L (1.93 ng/L), and 8.60 ng/L (1.68 ng/L), respectively, for the hs-cTnI assay ($P < 0.001$ for the 3 median concentrations, Fig. 1), with 70% of the heparin plasma specimens above the LoD and 92% above the LoB.

Of the 1216 heparin specimens analyzed from the 290 chest pain subjects with the hs-cTnI assay, only 53 (4%) had concentrations ≤ LoD compared to 534 (44%) that had AccuTnI concentrations ≤ LoD (0.01 μg/L) *(6)*. Applying change criteria to the 2 earliest specimens from each subject (median time interval 1 h; IQR 1–3 h), the hs-cTnI assay identified 81% (95% CI 73%–88%) of the 108 patients with myocardial injury as having a changing pattern, compared to only 62% (53%–71%) identified using the AccuTnI assay ($P < 0.001$), a 19% absolute difference or a 30% increase in detection. When change criteria were applied to any 2 specimens—not necessarily consecutive specimens—the hs-cTnI assay identified 107 of the 108 subjects with myocardial injury (e.g., sensitivity 99%; 95%CI 94%–99%), whereas the AccuTnI assay had a sensitivity of 88% (95%CI 80%–93%) ($P < 0.001$) (Table 1).

In the subjects with peak AccuTnI concentrations <99th percentile, there were significantly more individuals who met hs-cTnI change criteria than those who met the AccuTnI change criteria (104 vs 13). These 104 patients had significantly higher peak hs-cTnI concentrations than the 78 subjects negative for both the hs-cTnI criteria and peak AccuTnI [median (IQR) 31.9 ng/L (15.7–101) vs 6.2 ng/L (4.8–9.4), $P < 0.05$] (Table 1). When we applied the RCV to our 2 earliest samples with the hs-cTnI assay, only 65% (95%CI 55%–73%) of the 108 patients were identified compared to 81% using the >3SD or >20% change ($P < 0.001$). However, there was overall good agreement in classifying evolving injury between the RCV and change criteria (89% agreement; $\kappa = 0.78$). ROC curve analysis (area under the curve 0.67, 95% CI 0.61–0.73; $P < 0.001$) for the absolute percent change between the 2nd and 1st specimen yielded the following estimates: >10% change yielded a sensitivity of 0.90 (0.83–0.95) and specificity of 0.27 (0.21–0.35); >20% change yielded a sensitivity of 0.79 (0.70–0.86) and specificity

Table 1. Changing concentration patterns classified by AccuTnI and hs-cTnI and corresponding cTn concentrations in the different group classifications.

Change criteria	Myocardial injury present[a]	Myocardial injury absent[a]
Earliest pair (median interval 1 h; IQR 1–3 h)[b]		
hs-cTnI change positive	88 [44.0 ng/L (19.0–153)]	75 [15.4 ng/L (6.3–34.7)]
hs-cTnI change negative	20 [13.4 ng/L (5.9–54.5)]	107 [5.4 ng/L (4.0–9.7)]
AccuTnI change positive	67 [0.06 μg/L (0.02–0.21)]	7 [0.00 μg/L (0.00–0.01)]
AccuTnI change negative	41 [0.05 μg/L (0.02–0.16)]	175 [0.00 μg/L (0.00–0.01)]
Any specimen pair (median 4 specimens/subject; IQR 2–6)[c]		
hs-cTnI change positive	107 [323 ng/L (77.0–4099)]	104 [31.9 ng/L (15.7–101)]
hs-cTnI change negative	1	78 [6.2 ng/L (4.8–9.4)]
AccuTnI change positive	95 [0.55 μg/L (0.11–5.5)]	13 [0.03 μg/L (0.03–0.04)]
AccuTnI change negative	13 [0.09 μg/L (0.05–0.18)]	169 [0.01 μg/L (0.01–0.02)]

[a] Peak AccuTnI concentration was used to define if myocardial injury was present (>99th percentile) or absent (≤99th percentile).
[b] Data are n [median cTnI concentration at presentation (IQR)].
[c] Data are n [median cTnI concentration at peak (IQR)].

of 0.45 (0.38–0.53); >30% change yielded a sensitivity of 0.71 (0.62–0.80) and specificity of 0.53 (0.46–0.61); and >44.5% change maximized conjoint sensitivity and specificity [sensitivity 0.62 (0.52–0.71) and specificity 0.63 (0.55–0.70)].

Discussion

This work extends reports of highly sensitive cTn assays that measure in the ng/L range and give detectable values for most normal individuals *(10–13)*. These assays show increased clinical sensitivity, with the ability to identify more individuals with evolving myocardial injury earlier. This increased analytical sensitivity and precision of the hs-cTnI assay also appears to allow earlier identification of changing patterns. We acknowledge that our study only addressed this issue in patients with ACS, in whom one could argue larger deltas might be present. We have not calculated the biological variation for this hs-cTnI assay, which could be useful for the identification of changing patterns *(10, 14, 15)*. However, the RCV obtained with another hs-cTnI assay displays good agreement with our operationalized criteria (>3SD or 20%), indicating that RCV may be helpful in patients presenting with ACS.

There were statistical differences between values obtained with different sample types with the hs-cTnI assay, but the magnitude of these differences were so small that they are unlikely to have biological importance. The preliminary 99th percentile for the hs-cTnI assay was different from that of the AccuTnI assay *(16)*. This could in part be due to the younger reference population we analyzed (<55 years), since in some studies age can have an effect on the 99th percentile *(17)*. Further studies validating the clinical use of shorter-interval ordering protocols using hs-cTn assays are needed to confirm our study findings of the earlier detection of myocardial injury.

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References

1. Morrow DA, Cannon CP, Jesse RL, Newby LK, Ravkilde J, Storrow AB, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines: clinical characteristics and utilization of biochemical markers in acute coronary syndromes. Clin Chem 2007;53:552–74.
2. Thygesen K, Alpert JS, White HD, Joint ESC/AACF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. J Am Coll Cardiol 2007;50:2173–95.
3. Wallace TW, Abdullah SM, Drazner MH, Das SR, Khera A, McGuire, et al. Prevalence and determinants of troponin T elevation in the general population. Circulation 2006;113:1958–65.
4. Jaffe AS. Chasing troponin: how low can you go if you can see the rise? J Am Coll Cardiol 2006; 48:1763–4.
5. Melanson SE, Morrow DA, Jarolim P. Earlier detection of myocardial injury in a preliminary evaluation using a new troponin I assay with improved sensitivity. Am J Clin Pathol 2007;128: 282–6.
6. MacRae AR, Kavsak PA, Lustig V, Bhargava R, Vandersluis R, Palomaki GE, et al. Assessing the requirement for the 6-hour interval between specimens in the American Heart Association classification of myocardial infarction in epidemiology and clinical research studies. Clin Chem 2006;52:812–8.
7. Kavsak PA, MacRae AR, Lustig V, Bhargava R, Vandersluis R, Palomaki GE, et al. The impact of the ESC/ACC redefinition of myocardial infarction and new sensitive troponin assays on the frequency of acute myocardial infarction. Am Heart J 2006;152:118–25.
8. Kavsak PA, MacRae AR, Newman AM, Lustig V, Palomaki GE, Ko DT, et al. Effects of contemporary troponin assay sensitivity on the utility of the early markers myoglobin and CKMB isoforms in evaluating patients with possible acute myocardial infarction. Clin Chim Acta 2007;380:213–6.
9. Kavsak PA, Newman AM, Lustig V, MacRae AR, Palomaki GE, Ko DT, et al. Long-term health outcomes associated with detectable troponin I concentrations. Clin Chem 2007;53:220–7.
10. Wu AH, Lu QA, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. Clin Chem 2009; 55:52–8.
11. Wu AH, Fukushima N, Puskas R, Todd J, Goix P. Development and preliminary clinical validation of a high sensitivity assay for cardiac troponin using a capillary flow (single molecule) fluorescence detector. Clin Chem 2006;52:2157–9.
12. Latini R, Masson S, Anand IS, Missov E, Carlson M, Vago T, et al. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. Circulation 2007;116:1242–9.
13. Kurz K, Giannitsis E, Zehelein J, Katus HA. Highly sensitive cardiac troponin T values remain constant after brief exercise- or pharmacologic-induced reversible myocardial ischemia. Clin Chem 2008;54:1234–8.
14. Wu AH, Jaffe AS. The clinical need for high-sensitivity cardiac troponin assays for acute coronary syndromes and the role for serial testing. Am Heart J 2008;155:208–14.
15. Melanson SE, Conrad MJ, Mosammaparast N, Jarolim P. Implementation of a highly sensitive cardiac troponin I assay: test volumes, positivity rates and interpretation of results. Clin Chim Acta 2008;395:57–61.
16. Venge P, Lindahl B, Johnston N, James S. Evaluation of the ultra-sensitive AccuTnI assay and establishment of normal levels of cardiac troponin I [Abstract]. Clin Chem 2008;54:A91.
17. Clerico A, Fortunato A, Ripoli A, Prontera C, Zucchelli GC, Emdin M. Distribution of plasma cardiac troponin I values in healthy subjects; pathophysiological considerations. Clin Chem Lab Med 2008;46:804–8.

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