Short Communication

Defining the serum 99th percentile in a normal reference population measured by a high-sensitivity cardiac troponin I assay

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ABSTRACT

Objectives: This study determined the serum 99th percentile reference value for cTnI measured using the high-sensitivity Erenna cTnI assay.

Design and methods: Serum was obtained from healthy adults (n = 348); aged 18–76 years of which 147 were males and 201 were females. Nonparametric analysis was performed to determine the 99th percentiles.

Results: For all subjects, the 99th percentile was 10.19 ng/L; mean concentration = 1.45 ng/L, range = 0.2 to 34.56 ng/L. By gender, the male and female 99th percentile values were as follows: male = 16.58 ng/L, mean concentration = 1.72 ng/L, and female = 9.36 ng/L, mean concentration = 1.25 ng/L, respectively (p = 0.108).

Conclusion: cTnI measured by the high-sensitivity Erenna cTnI assay measured 100% of normal subjects, allowing prospective diagnostic and risk assessment studies to be performed, which are essential for the early detection of cardiac disease and for the management of patients presenting with symptoms suggestive of acute coronary syndrome.

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Introduction

The universal definition of acute myocardial infarction (AMI) published in 2007 by the Global Task Force is predicated on a rising or falling cardiac troponin (cTn) concentration, with at least one concentration above the 99th percentile reference value, together with one of the clinical markers of ischemia; i.e., appropriate symptoms, ECG changes, or imaging evidence of new loss of myocardial function [1]. In clinical circumstances where cTn is increased as a consequence of a pathophysiologic mechanism other than ischemia, the diagnostic term used is myocardial injury secondary to the specific pathology, i.e., heart failure [1,2]. In addition, the Task Force classifies five difference types of myocardial infarction, with the type 2 MI secondary to ischemia due to either increased oxygen demand or decreased supply [1]. Therefore, cTn testing has become the standard for MI detection as well as a challenge to clinicians to be able to discern an increased cTn between the diagnosis of MI and a pathological mechanism in the absence of overt ischemic heart disease, with clinical judgment often the call. Over the past several years, manufacturers of cTn assays have improved the quality specifications of assays to allow for more precise quantitation of low cTn concentrations [3]. The Global Task Force [1] and the National Academy of Clinical Biochemistry (NACB) [2] guidelines have endorsed optimal total imprecision (XCV, coefficient of variation) of cTn assays at their 99th percentile reference value at ≤10% CV. The majority of contemporary assays in the marketplace currently do not meet these suggested guidelines [4]. However, studies have provided evidence that shows that assays with an imprecision up to 20% CV do not significantly affect diagnostic accuracy [5] or risk stratification [6] through misclassification of patients. Assays with imprecision >20% should be used with caution. More recently, prototype high-sensitivity cTnI assays have been developed by Beckman [7], Nanosphere [8], and Singulex [9], as well as for cTnT by Roche [10]. These prototype assays have been shown to demonstrate excellent imprecision (<10% CV) at and below the 99th percentile concentration reference levels, as well as measure almost 100% of normals in designated healthy volunteers. The purposes of the current study were to (a) determine the 99th percentile concentrations in a group of healthy men and women and (b) identify what percentage of healthy subjects has a measurable cTn value, using the Singulex Erenna Immunoassay System for cTnI.

Methods

Serum was obtained following informed consent (IRB-approved) from healthy adults (n = 348), aged 18 to 76 years of which 147 were male and 201 were female. By health questionnaire, no subject
reported any known current or past history of/ or medication use for coronary artery disease or cardiac related medical condition, diabetes, hypertension, or renal disease. Measurement of N-terminal pro-B-type natriuretic peptide (NT-proBNP, Roche Diagnostics) was performed also as a surrogate biomarker of left ventricular dysfunction to further assist in defining normality of the subject. Cutoff concentrations used were according to the Roche FDA cleared package insert: 125 ng/L for ages<75 years and 450 ng/L for ages ≥75 years. No patient NT-proBNP abnormalities influenced the normal distributions. Samples were frozen at 70 °C until analysis.

cTnI was measured (in triplicate, %CV<7%) using the high-sensitivity (hs) cTnI Singulex Erenna System (Singulex, Inc., Alameda, CA, USA) that is based on capillary flow single-molecule counting combined with microparticle immunoassay technology as previously described [9]. The assay turnaround time was 2 hours. The assay utilizes antibodies that recognize the following epitopes: capture, amino acids 41 to 49; detection, amino acids 27 to 41. The assay was standardized to the National Institute of Standards and Technology (NIST) material (SRM 2921). Nonparametric analysis for determination of 99th percentiles, based on age and gender, were determined along CLSI guidelines using the Kolmogorov–Smirnov test for normal distribution.

Results

Analytical studies demonstrated a limit of blank (LoB) of <0.088 ng/L, limit of detection (LoD) of 0.051 ng/L, and limit of quantitation (20% CV) of 0.253 ng/L. The total imprecision at 10% (10% CV) was found to be between 0.78 and 1.6 ng/L. All normal subjects (100%) had a measurable hs-cTnI concentration above both the LoB and LoD. The range of measured concentrations was from 0.2 to 34.56 ng/L. For all subjects, the 99th percentile value was 10.19 ng/L, mean 1.45 ng/L (95% CI 1.16–1.73 ng/L), as shown in Fig. 1A. The male and female 99th percentiles were 16.58 ng/L, mean 1.72 ng/L (95% CI 1.18–2.27 ng/L), and 9.36 ng/L, mean 1.25 ng/L (95% CI 0.94–1.55 ng/L), respectively (Figs. 1B and C). While the male 99th percentile trended higher than the female, it was not statistically significant (p = 0.108). Both the male and female hs-cTnI distributions were near Gaussian. hs-cTnI concentrations were not influenced by age (p = 0.932); aged 18 to 30 years, n = 105, 9.49 ng/L; aged 31 to 50 years, n = 134, 16.58 ng/L; aged >50 years, n = 109, 9.36 ng/L.

Discussion

Our findings extend preliminary, but underpowered, observations demonstrating that the Singulex hs-cTnI assay measures 100% of concentrations in all healthy subjects. These findings define a true normal reference range, following a near-Gaussian distribution using a hs-cTnI assay. While hs-cTnI levels for 99th percentile men and women were not statistically different, men trended higher, and thus a larger, more diffuse normal population may eventually show gender-defined cutoff values. The 99th percentile concentration by the Singulex hs-cTnI assay was found to be guideline acceptable as previously proposed based on a scorecard criteria [4] predicated on imprecision and the ability to measure all concentrations in normal subjects; with a <10% CV at the 10.19 ng/L 99th percentile concentration.

The clinical implications of implementation of a hs-cTnI assay in practice are potentially very positive in patient diagnosis and risk stratification. First, clinical sensitivity for detection of myocardial injury and AMI will approach 100% in patients presenting very early with symptoms suggestive of acute coronary syndrome. This has already been demonstrated using the same Singulex assay by Sabatine [9] who demonstrated stress test-induced myocardial ischemia was associated with an increased hs-cTnI within 2 hours. Further, Wilson showed that in patients with AMI, 98% had an increased hs-cTnI measured by the Nanosphere hs-assay at 2 hours after presentation.

[8]. In comparison, using a contemporary cTnI assay <20% of subjects were detected at 2 hours. Further, utilizing the Beckman prototype hs-cTnI, Venge et al. [7] found an 85% sensitivity and 90% specificity for death or MI in a GUSTO-IV subset cohort. Therefore, as appropriately powered normal reference populations are used to establish statistically reliable 99th percentile cutoff concentrations, as we have performed in the current study, a clear line between normal and myocardial injury, accepted as representing irreversible cell death, is being drawn. Better analytical assays appear to add to clinical judgment.

However, lowering the analytic threshold to study what is normal is not without a clinical challenge or limitations. The new hs-cTn...
assays are now measuring concentrations in > 80% of normal subjects that are currently below the LoD of contemporary assays used in clinical practice [4,11]. This will result in a trade-off for clinical specificity for AMI, resulting in values in the 60% to 70% range. Increased hs-cTnI or hs-cTnT concentrations are not false positive values, but mechanistically result from other pathological etiologies that cause myocardial cell injury, cell death, and release of cTn into the blood [12]. To assist in differentiating the etiology of increases found for hs-cTn assays, investigators [9,13], including the NACB [2], have proposed following the change (delta) of two samples over a short time period, 2–6 hours, to assist in improving the clinical specificity. Using the Singulex hs-cTn assay, Wu et al. [13] have demonstrated that within-day and between-day reference interval change values (biological variation) ranged for an increasing and decreasing cTnI value between +46% and −32%, and +81% and −45%, respectively. In a recent study from the author’s laboratory using a contemporary cTn assay (Ortho-Clinical Diagnostics cTnI), a delta (change) over 6 hours of >30% enabled an improved clinical specificity to 91% from 75% using just the admission cTn value [14]. However, caution needs to be adhered to, as to what delta change value is used, as each cTn assay, whether contemporary or high sensitivity, needs to have its optimal individual delta value determined on its own. The recent study by Keller et al. [15] unjustifiably applied delta values of 20% recommended by the NACB (expert opinion) and 30% by Apple (derived for an individual assay) to evaluate diagnostic accuracy and risk stratification based on contemporary cTn assays (Roche cTnI, Siemens Ultra cTnI) that did not have their appropriate delta value established independently. This could result in misleading information for clinical interpretations. For an assay like the Singulex hs-cTn assay to be used in everyday clinical practice, its turnaround time will need to be shortened to accommodate guidelines that require < 60 minutes from the blood draw to result to provide for emergency medicine use. With specimen processing limitations, actual assay time, unless in a point-of-care format, will need to approach ≤ 40 minutes because this time has been demonstrated to influence patient flow through an emergency department [16]. Expert opinion leaders have predominantly taken the stance of describing assays such as the Singulex and other cTn assays of similar analytical performance as high-sensitivity assays. Readers should not be mislead by reading the literature as to the nature of assays that are named ‘Ultra’ or ‘sensitive’ per their manufacturer because these are really contemporary assays not high-sensitivity assays. Finally, expressing hs-cTn results in nanograms per liter instead of micrograms per liter will yield less chance for clinical interpretation error, thus eliminating many zeros after a decimal place. Future research defining the diagnostic accuracy (clinical sensitivity, clinical specificity, delta change) and risk stratification odds ratios for outcomes assessment will be necessary to translate our reference limit determination into real clinical practice.

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References

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<th>ID</th>
<th>Title</th>
<th>Pages</th>
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