



Contents lists available at ScienceDirect

American Heart Journal Plus: Cardiology Research and Practice

journal homepage: www.sciencedirect.com/journal/american-heart-journal-plus-cardiology-research-and-practice



Research paper

Ultra-highly sensitive cardiac troponin I: Age and sex differences in healthy individuals

Mitra Mastali^{a,f,1}, Anum Asif^{a,1}, Qin Fu^{a,e}, Janet Wei^a, Frederick K. Korley^b, W. Frank Peacock^c, Kimia Sobhani^d, Galen Cook-Wiens^e, Marcio A. Diniz^e, C. Noel Bairey Merz^{a,*}, Jennifer E. Van Eyk^{a,f}

^a The Barbra Streisand Women's Heart Center, The Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

^b Department of Emergency Medicine, University of Michigan, Ann Arbor, MI, USA

^c Department of Emergency Medicine, Baylor College of Medicine, USA

^d Department of Pathology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

^e Biostatistics and Bioinformatics Research Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA

^f Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA



ARTICLE INFO

Keywords:

Cardiac troponin

Sex

Age

Simoa immunoassay

Quanterix

ABSTRACT

Background: Associations between elevated circulating cardiac troponin I (cTnI) levels and adverse cardiac outcomes were established prior to the ability to measure extremely low levels of cTnI. Immunoassays that achieve precise ultra-highly sensitive quantification of cTnI (u-hs-cTnI) will allow accurate measurement in healthy subjects. We aimed to evaluate the distribution of u-hs-cTnI values measured by (Simoa HD-1 Analyzer, Quanterix Corporation, Lexington, MA) in healthy subjects and characterize relations to sex and age.

Methods: Two independent, healthy cohorts (total of 200 women, 200 men) aged 18–86 years were analyzed in duplicate using the u-hs-cTnI Immunoassay. The u-hs-cTnI 99th percentiles were calculated as the upper limits considering a robust estimation against outliers with 90% confidence intervals. The Quanterix immunoassay analytical performance was established and compared to an existing clinical assay (ARCHITECT STAT High Sensitivity Troponin I, Abbott Laboratories, Wiesbaden, Germany).

Results: The lower limit of detection of the u-hs-cTnI assay was calculated to be 0.005 ng/L; we accurately quantified u-hs-cTnI in 95% of healthy individuals. The Quanterix immunoassay within overlapping concentrations correlated with the Abbott assay ($R^2 = 0.932$). The calculated combined 99th percentile was 7.94 ng/L (90% Confidence Interval [CI], 5.47–10.52). Women had lower mean u-hs-cTnI concentrations than men under the age of 40 years. The sex-specific 99th percentile for female vs. male individuals was 4.89 ng/L (90%CI, 3.71–6.25) and 10.49 ng/L (90%CI, 5.19–15.06), respectively.

Conclusion: The Quanterix immunoassay provides precise quantification in 95% of healthy individuals. Women under the age of 40 years have significantly lower levels of u-hs-cTnI than men.

1. Introduction

Cardiac troponin I (cTnI) and T (cTnT), are the internationally accepted biomarkers for detecting myocardial necrosis and the diagnosis of acute myocardial infarction (MI) [1,2]. There are ongoing efforts to improve the available assays' sensitivity to help early detection of acute coronary syndromes (ACS) and prognosis [3,4]. The forth universal definition of acute MI recommends myocardial injury should be used

when there is evidence of elevated cardiac troponin (cTn) with at least 1 value above the 99th percentile upper reference limit (URL). The myocardial is considered acute if there is a rise and/or fall of the cTn values [5]. The proposed definition for high-sensitivity assays is having the ability to detect cTnI concentrations precisely, with a coefficient of variation <10% at or the below the 99th percentile upper reference of normal, and measurable in >50% of normal healthy individuals [1,6]. The 99th percentile of various assays may vary according to age and sex

* Corresponding author at: 127 S San Vicente Blvd #A3600, Los Angeles, CA 90048, USA.

E-mail address: Noel.BaireyMerz@cshs.org (C.N.B. Merz).

¹ Both authors contributed to the paper equally.

<https://doi.org/10.1016/j.ahjo.2022.100110>

Received 5 August 2021; Received in revised form 17 February 2022; Accepted 21 February 2022

Available online 2 March 2022

2666-6022/© 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

distribution in the reference population. However, whether age and sex-specific reference ranges for cTnI or cTnT ought to be reported remains an unsettled debate. Prior studies suggest that women with ACS are often misdiagnosed and have worse outcomes than men [7,8]. Therefore, having a sex-specific reference range and criteria may allow clinicians to better diagnose and treat women with heart disease, especially women requiring acute care [7].

The purpose of our study is to evaluate the accuracy and precision of the ultra-high sensitivity assay (Simoa HD-1 Analyzer, Quanterix Corporation, Lexington, MA) and to determine how the distribution of uhsTnI measured using this assay varies according to sex and age.

2. Methods

2.1. Study populations

Sample set-1 was comprised of 20 normal/healthy male EDTA plasma samples purchased from BioIVT, with the rest of the samples collected at the emergency departments at Cedars-Sinai Medical Center, Los Angeles, CA (set 2), Baylor College of Medicine, Houston, TX (set 3) and Johns Hopkins Hospital, Baltimore, MD (set 4). Samples were processed in the clinical chemistry laboratory within 1–4 h of blood draw. Cohorts of healthy individuals (sample sets 1, 2, and 3) were selected based on a completed metabolic panel and from individuals who had no known cardiovascular disease, diabetes, hypertension, or renal dysfunction at the time of blood collection, based on circulating markers.

2.2. cTnI immunoassay

For the Quanterix immunoassays, the EDTA-plasma samples were thawed and centrifuged at 4 °C at 12,000 ×g for 8 min. The cTnI Immunoassay was carried out on the Simoa HD-1 Analyzer (Quanterix Corporation, Lexington, MA). Each cTnI kit (TnI kit, Cat#100133 Quanterix) contained eight calibrators, two controls, sample diluent, Bead, Detector, SBG (streptavidin beta-galactosidase), and RGP (fluorogenic β-galactosidase substrate resorufin) reagents. For all the samples: first plasma is centrifuged for 8 min at 12,000 ×g; 120uL of plasma, which is sufficient for duplicate analysis, was diluted four-fold with Quanterix cTnI kit dilution buffer, 400 uL of the diluted sample was loaded into each well, and assay run finished according to the manufacture's protocol. Each plate consisted of 36 individual samples, an eight-point standard curve, and four quality control samples. Each sample was run in duplicate, and concentration was calculated based on Average Enzyme per Bead (AEB). Any sample with the percent coefficient of variation (%CV) higher than 20% was repeated with appropriate calibrators and controls.

2.3. Statistical analysis

Variables are described by median (quantile 25% - quantile 75%) with box plots to examine the data's distribution. Scatter plots are presented to study the relationship between variables. Comparisons between groups are performed by the Mann-Whitney (1947) and Brunner-Munzel tests (2000), where appropriate. The Anderson-Darling test (1952) and the Levene test (1960) are utilized to verify the normality and homogeneity of variances assumptions [9,10]. Outliers were removed based on Cook distance. Multivariable Generalized Additive Model for Location, Scale and Shape (GAMLSS) [11] with the Box-Cox t distribution described the relationship between the median of Troponin Concentration and demographic characteristics with age modeled either as continuous covariate with cubic splines effect or as a categorical covariate. In addition, cohort was considered a random effect. Residual analysis was performed by worm-plots [12]. We calculated 90% bootstrap confidence intervals for estimated 99th percentiles. We reported contrasts for the median estimated on the regression models as (contrast

estimate ± standard error). Sexes were compared under and above age of 40 years old, and *p*-value were adjusted [13].

Furthermore, agreement between assays was evaluated using the Bland-Altman plot [14], paired *t*-test and Intraclass correlation based on ANOVA with mixed-effects for agreement.

3. Results

The performance characteristics of the Quanterix cTnI immunoassays were determined by comparing the performance of the eight-point standard curve (Fig. 1A) and a low 0.119 ng/L and high 302 ng/L cTnI standards (Fig. 1B) of 6 different kits. Fig. 1C shows the analysis of 20 healthy individuals' run-in duplicates on three consecutive days. The mean cTnI values were 0.071 ± 0.004 ng/L with both inter- and intra-assay CV of less than 5%, and all values measured for the healthy males fall within the standard curve (Online Supplement Table 1 and Online Supplement Fig. 1).

The next clinical studies included two independent sample sets composed of 400 healthy individuals (Set 2 and 3). Each cohort was composed of a 1:1 ratio of males to females and ranged in ages from 18 to 86 years old. Of the 400 healthy subjects studied, Quanterix cTnI values were quantifiable (having CV% of <20%) in 368 individuals (92%) and the concentrations ranged >0.1 ng/L to 10 ng/L. Specifically, for the Quanterix assay, the median cTnI concentrations for these 400 individuals was 0.619 (0.36; 1.11) ng/L (CV of 6%), respectively. The assay's limit of detection (LOD) and 99th percentile were 0.005 ng/L and 17.60 ng/L (90% CI 9.04; 28.36), respectively. The assay LOD was calculated by extrapolating the background cTnI concentration plus three standard deviations of the background using a 4-parameter logistic fit. The median was cTnI concentrations were 0.43 (0.28; 0.82) ng/L and 0.83 (0.54, 1.53) for women and men ($p < 0.001$), respectively. (Fig. 2A, Table 1). Furthermore, the median cTnI concentrations were lower ($p < 0.001$) for people below 40 years of age, 0.45 ng/L (0.31; 0.71), versus those above 40, 1.00 (0.54; 1.93), (Fig. 2B, Table 1).

Using a multivariable Box-Cox t model, the cTnI concentration was associated with by both age ($p < 0.001$) as continuous and categorical covariate, and sex (increment for males on the median: 0.303864 ± 0.0407 $p < 0.001$). The interaction between sex and age as a continuous covariate was not statistically significant ($p = 0.458$), but it was significant for age as a categorical covariate ($p < 0.001$). For age as a categorical covariate, the 99th percentile was lower in women, 4.50 ng/L (90% CI 3.53; 10.43), than men, 8.74 ng/L (90% CI 6.73; 19.34), under 40 years old (increment for males on the median: 0.350 ± 0.0750 , $p < 0.001$). Similar results were observed for women, 8.90 ng/L (90% CI 7.28; 19.62), and men, 15.29 (90% CI 12.25; 33.91) above 40 years old (increment for males on the median: 0.572 ± 0.132 , $p < 0.001$). Fig. 2C illustrates 99th percentiles for males and females as function of age as a continuous covariate.

Finally, we determined the comparability of the cTnI concentrations measured on the Quanterix platform to the same samples run on a gold standard clinical chemistry platform, Abbott's highly sensitive cTnI Immunoassay. The samples (sample set-4) were plasma obtained from individuals diagnosed with MI based on AHA and WHO criteria [15], and a noncardiac (non-ACS) group from the same emergency department. A subset of individuals diagnosed with MI had cTnI concentration (Quanterix) elevated at the time of presentation (early MI, Online Supplement Fig. 2A) or had concentrations that rose to diagnostic levels after 3 h. (Late MI, Online Supplement Fig. 2B). This compared to the non-ACS control group which had cTnI levels in the same range as subsets 1, 2, and 3 (Online Supplement Fig. 2C). As shown in Fig. 3, the left plot of the values of the two assays against each other shows that they are correlated ($r = 0.99$, $p < 0.0001$, 95% CI 0.988, 0.997), but there is also a bias. The solid line is the identity line, where $x = y$, and is where the two scales would agree perfectly. The ICC (from ANOVA model with repeated subject measurements) for this is 0.89 with 95% CI (0.81, 0.94). The differences between the Quanterix and Abbott ranged

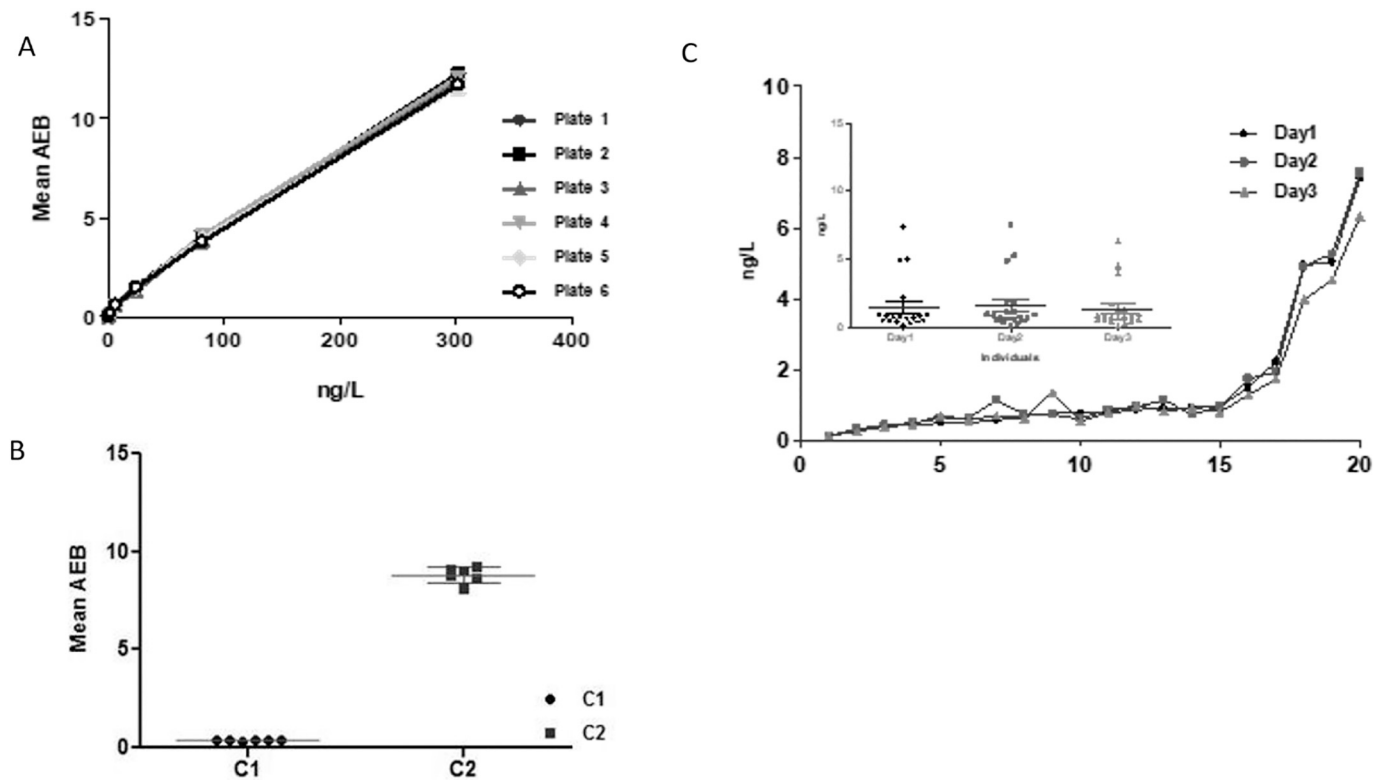


Fig. 1. Cardiac TnI immunoassay inter and intra-day performance. Panel A shows the standard curves (mean AEB of cTnI vs. concentration) obtained from the standard curve samples from six different cTnI kits ran on six different days. Panel B shows the plot of the mean AEB for the low (Control 1, filled circle) and high (Control 2, filled square) concentration cTnI standard from the same six cTnI kits. Panel C is the plot of the concentration of cTnI measured for 20 healthy male plasma samples measured in 3 different kits on three different days (day 1, filled circle, day two filled square, and day 3, filled triangle). The insert is the cTnI values obtained on each day.

from -3.5 to 1936.5 . The average difference (bias) was 240.4 , taking the Quantex - Abbott. This is significantly different from 0 (t -test $p = 0.0061$, 95% CI 74.1 , 406.6). Quantex is, on average, higher than Abbott. Fig. 3 right shows the difference between Quantex and Abbott plotted against the average of the two assays. The solid line shows where the difference is zero, perfect agreement. The middle-dashed line shows where the average difference (bias) is. The outer two dashed lines show the limits of agreement based on the standard deviation of the differences. The region between these two lines is where we would expect 95% of the differences to lie if the differences had a normal distribution about their mean. This range is -632.2 to 1113 . The correlation between the differences and their average is significantly different from 0 ($r = 0.98$, $p < 0.0001$), so this is evidence of a linear relationship between the errors and their mean. As the mean increases the difference increases suggesting a difference of scale in the two assays.

4. Discussion

There is an increasing clinical interest in the implementation of u-hs-cTnI assays for early diagnosis of ACS partly based on exceeding the 99th percentile of a normal reference population. The 99th percentile is assay-specific and must be defined for each cTn assay. For some cTn assays, the 99th percentile varies according to the reference population's distribution age and sex [16]. The Quantex assay can detect femtomolar concentrations of analytes by utilizing single molecule counting technology. Using this method, the single-molecule arrays, which are femtoliter-sized reaction chambers, can isolate and detect a single enzyme molecule, resulting in increased analytical sensitivity [17]. We have shown the assay's limit of detection (LOD), and 99th percentile were 0.005 ng/L and 7.948 ng/L (90% CI 5.478 , 10.516), respectively, with reproducibly over time ($CV < 5\%$ over six days). Our study further

revealed that it is possible to accurately measure baseline levels of circulating cTnI in 95% of healthy individuals using the Quantex immunoassay platform. As well, the assay can measure diagnostic concentrations and matches other clinical chemistry assays.

Our study demonstrates that cTnI measurements are significantly lower in healthy younger females, than males overall. Previous studies indicated that women commonly have lower cTnI concentration and are less frequently diagnosed with acute MI. This may be the consequence of using non-sex specific higher cutpoints in women, the results of which is that MI's may go undetected, and thus untreated, when using current general 99th percentile cutoff points [18]. The lack of diagnosis and treatment may explain why women often have worse outcomes than men [7,8]. Lower cTnI levels are likely the result of lower left ventricular mass, which is correlated with cardiac troponin concentration, and is smaller in women [19,20]. A sex-specific reference range and criteria may allow clinicians to better diagnose and treat women with heart disease, especially those requiring acute care [7]. It is also worth considering that the possible benefits of having sex-specific cutoff are not just in terms of the increased rate of acute MI diagnosis and can potentially be expanded to a biomarker for recognizing women with increased risk of future cardiovascular events [21].

Few studies have determined the 99th percentile using highly sensitive assays for different age groups and showed that thresholds are greater for the older age group [22,23], while our data illustrate differences in cTnI concentration among different age groups. Specifically, we demonstrate cTnI values that are significantly higher for people above 40 years of age, and this was a general phenomenon in both males and females. An age-specific cutoff could potentially provide a more accurate diagnosis of acute MI, as other studies also described that mild elevation is common in older non-acute MI patients. Therefore, the optimal cutoff levels may be higher in the elderly in comparison to

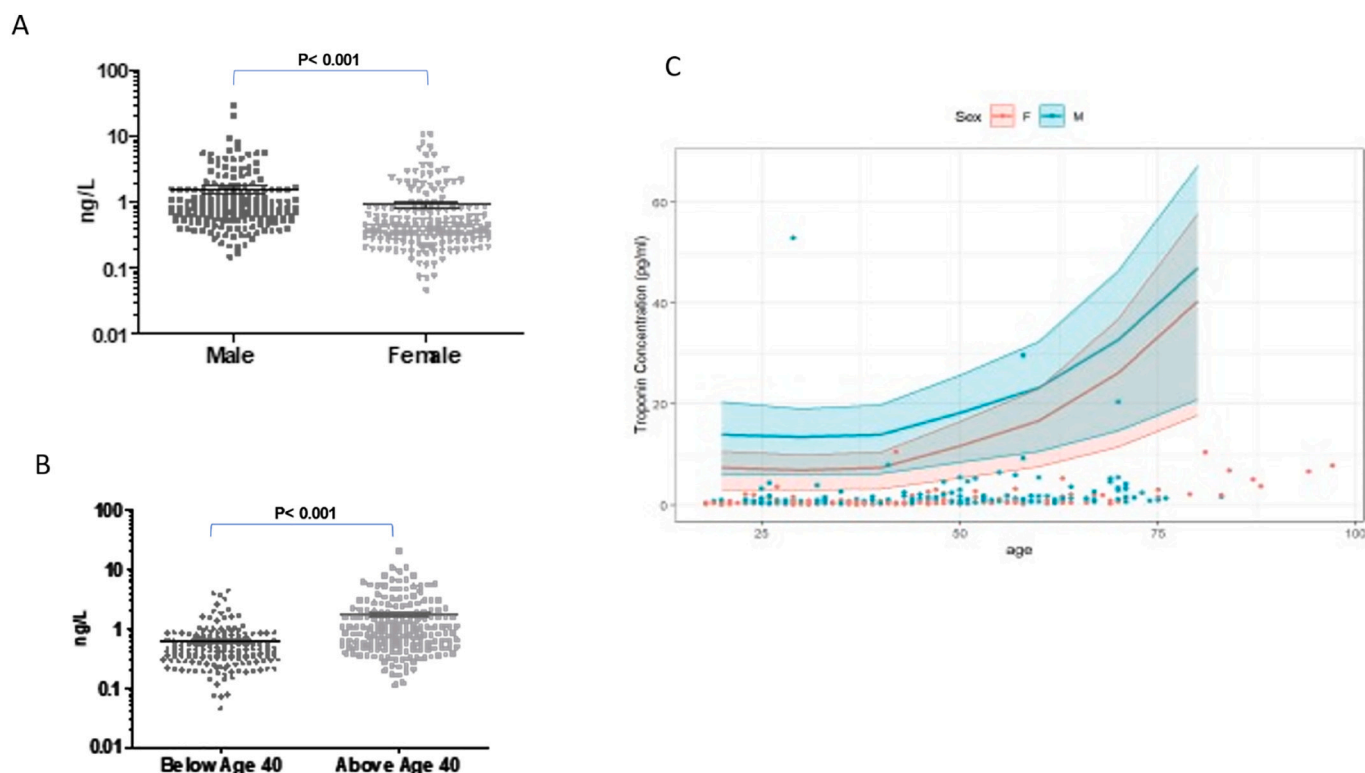


Fig. 2. cTnI concentrations differ in sex and age. The Quantex u-hs-cTnI values of 400 healthy individuals from two cohorts were plotted based on sex (panel A) or below or above 40 years old (panel B). Multivariable Generalized Gamma distribution model considering the log (TnI Concentration) as response: Age 0.025 ± 0.00398 , $p < 0.001$, and Sex 1.019 ± 0.24589 , $p < 0.001$ (Panel C).

Table 1

Sex and age stratification of u-hs-cTnI values in 400 healthy individuals measured on the Quantex immunoassay platform.

Factor	Group	Median ng/L (Q25% - Q75%)	p value
Sex	Female	0.43 (0.29; 0.82)	<0.001
	Male	0.83 (0.54; 1.53)	
Age (Overall Cohort)	≤40 yrs.	0.53 (0.34; 0.84)	<0.001
	>40 yrs.	0.79 (0.43; 1.58)	
Age (Female)	≤40 yrs.	0.34 (0.24; 0.46)	<0.001
	>40 yrs.	0.69 (0.42; 1.85)	
Age (Male)	≤40 yrs.	0.59 (0.46; 0.85)	<0.001
	>40 yrs.	1.09 (0.71; 2.07)	

younger patients [24].

Chest pain is a common presenting symptom; however, the majority of emergency chest pain admissions are not due to acute MI. As myocardial infarction is life-threatening, early diagnosis or rule out of acute MI might potentially improve morbidity and mortality. Further, early diagnosis can reduce the time to decision, thereby reducing emergency department overcrowding and overall treatment costs. Immunoassays measuring cardiac troponins define the diagnosis of myocardial infarction and are critical tools for diagnosing acute myocardial infarction. While most contemporary assays provide adequate diagnostic performance, the increased sensitivity and precision of the new, high sensitivity assays that have already been introduced into clinical practice provide the potential to further shorten intervals between blood draws or the time needed to detect the first significant troponin elevation. The new ultra-sensitive cTnI assays may allow the detection of the first low-level elevation of troponin within 90 to 180 min of symptom onset [25,26]. Our study shows that ultra-highly sensitive assays such as Quantex may enable clinicians to diagnose acute MI patients, particularly women, earlier and faster, thus allowing appropriate treatment, as well as safely discharging emergency

department patients earlier.

In summary, our results demonstrate the robustness of the ultra-highly sensitive cTnI assay by utilizing the Quantex assay. This allows baseline levels of circulating cTnI to be measured in the majority of healthy individuals. We also found significant sex-based difference in reference ranges until after age 40, at which cutpoint could be similar. These differences could impact the diagnostic value of cTnI, particularly in younger women; applying this knowledge could improve the recognition of young women with acute MI and at increased risk of morbidity and mortality.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. W. Frank Peacock discloses research grants Abbott, Becton Dickinson, Brainbox, Calcimedica, CSL Behring, Cue, Ortho Clinical Diagnostics, Relypsa, Roche, Salix, Siemens, participation on Advisory board Abbott, Astra-Zeneca, Beckman, Bosch, Fast Biomedical, Forrest Devices, Ischemia Care, Dx, Instrument Labs, Janssen, Nabriva, Ortho Clinical Diagnostics, Osler, Relypsa, Roche, Quidel, Salix, Siemens, Upstream, and has stocks/ownership interests in AseptiScope Inc., Brainbox Inc., Braincheck Inc., Coagulo Inc., Comprehensive Research Associates LLC, Comprehensive Research Management Inc., Emergencies in Medicine LLC, Fast Inc., Forrest Devices, Ischemia DX LLC, Lucia Inc., Prevencio Inc., ScPharma, Trivirum Inc., Upstream Inc.; Dr. Kimia Sobhani discloses grants NINDS 1U01NS115658-01 Post-translational modification and protein qualification of plasma and CSF, NIDDK 5U01DK124019-02 Design and Validation of Easy-to-Adopt Mass Spectrometry Assays of Importance to Obesity, Speaker for Abbott on six sigma assays; Dr. C. Noel Bairey Merz, serves as Board of Director for iRhythm, fees paid through CSMC from Abbott Diagnostics and Sanofi, served on Bayer Advisory Board, Med Intelligence (Caladrius lecture),

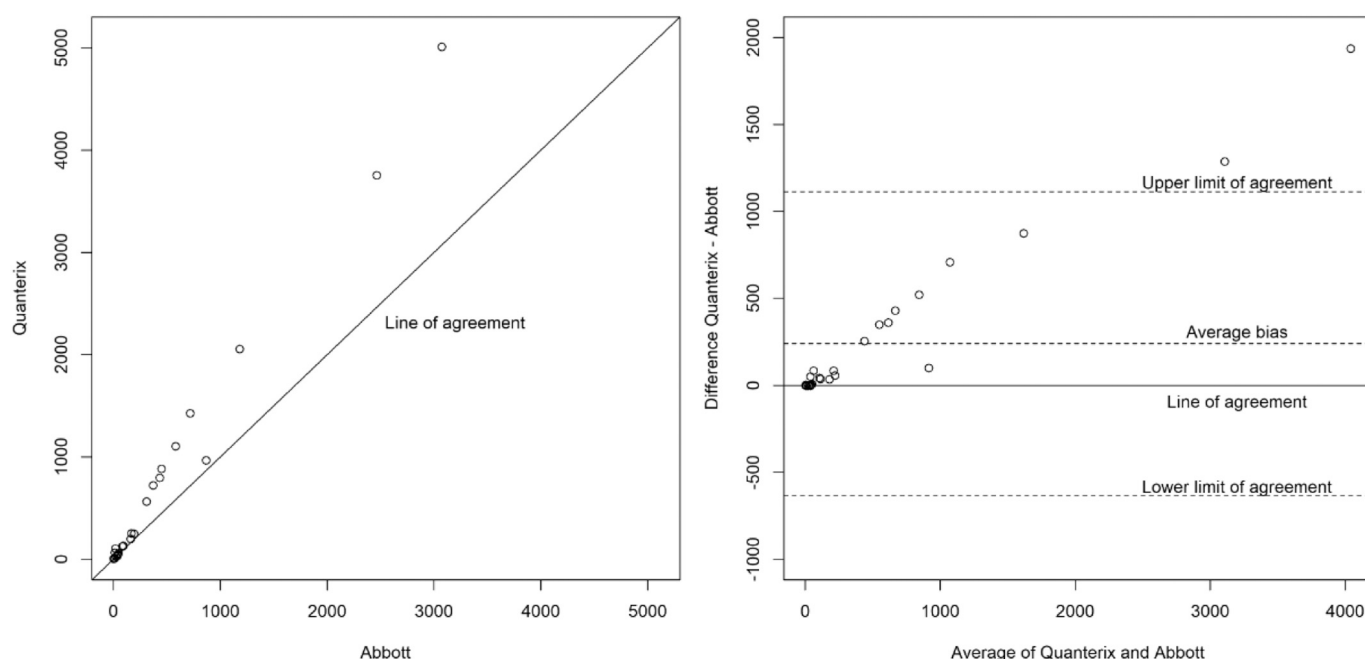


Fig. 3. Agreement between assays. Plot of Quantarix versus Abbott in late MI subjects (left) and Bland-Altman plot for the two assays (right).

grant contracts from California Institute for Precision Medicine, CDMRP Department of Defense, Normal Control, Microvascular Registry, NHLBI subcontract to Research Triangle Institute (RTI) International, NHLBI R01, Sanofi ACT14656, WISE HFpEF. All other authors have no disclosures to disclose.

Acknowledgments

We would like to recognize funding from The Barbra Streisand Women's Cardiovascular Research and Education Program (NBM, JVE), Erika Glazer Endowed Chair in Women's Heart Health (JVE), Advanced Clinical Biosystems Research Institute (JVE), The Smidt Heart Institute (NBM, JVE), The Linda Joy Pollin Women's Heart Health Program, Edythe L. Broad and the Constance Austin Women's Heart Research Fellowships at Cedars-Sinai Medical Center, Los Angeles, California.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ahjo.2022.100110>.

References

- [1] E.A. Bohula May, M.P. Bonaca, P. Jarolim, E.M. Antman, E. Braunwald, R. P. Giugliano, et al., Prognostic performance of a high-sensitivity cardiac troponin I assay in patients with non-ST-elevation acute coronary syndrome, *Clin. Chem.* 60 (1) (2014) 158–164.
- [2] K. Reddy, A. Khaliq, R.J. Henning, Recent advances in the diagnosis and treatment of acute myocardial infarction, *World J. Cardiol.* 7 (5) (2015) 243–276.
- [3] G. Wang, J. Wang, S. Wu, W. Zheng, H. Zhang, J. Ma, et al., Clinical impact of using a more sensitive troponin assay in patients with acute chest pain, *Clin. Cardiol.* 42 (5) (2019) 561–567.
- [4] J. McCord, Will high-sensitivity troponin assays lead to improved outcomes in patients with acute coronary syndrome? *Coron. Artery Dis.* 24 (8) (2013) 713–715.
- [5] T. Keller, F. Ojeda, T. Zeller, P.S. Wild, S. Tzikas, C.R. Sinning, et al., Defining a reference population to determine the 99th percentile of a contemporary sensitive cardiac troponin I assay, *Int. J. Cardiol.* 167 (4) (2013) 1423–1429.
- [6] F.S. Apple, A new season for cardiac troponin assays: it's time to keep a scorecard, *Clin. Chem.* 55 (7) (2009) 1303–1306.
- [7] A.S. Jaffe, F.S. Apple, High-sensitivity cardiac troponin assays: isn't it time for equality? *Clin. Chem.* 60 (1) (2014) 7–9.
- [8] D. Radovanovic, P. Erne, P. Urban, O. Bertel, H. Rickli, J.M. Gaspoz, et al., Gender differences in management and outcomes in patients with acute coronary syndromes: results on 20,290 patients from the AMIS plus registry, *Heart* 93 (11) (2007) 1369–1375.
- [9] H.B.W.D. Mann, On a test of whether one of two random variables is stochastically larger than the other, *Ann. Math. Stat.* 18 (1947) 50–60, <https://doi.org/10.1214/aoms/1177730491>.
- [10] T.W.D.D. Anderson, Asymptotic theory of certain "goodness of fit" criteria based on stochastic processes, *Ann. Math. Stat.* 23 (2) (1952) 193–212.
- [11] R.A.S.D. Rigby, Generalized additive models for location, scale and shape, *J. R. Stat. Soc. Ser. C Appl. Stat.* 54 (3) (2005) 507–554.
- [12] S. van Buuren, M. Fredriks, Worm plot: a simple diagnostic device for modelling growth reference curves, *Stat. Med.* 20 (8) (2001) 1259–1277.
- [13] T. Hothorn, F. Bretz, P. Westfall, Simultaneous inference in general parametric models, *Biom. J.* 50 (3) (2008) 346–363.
- [14] J.M. Bland, D.G. Altman, Statistical methods for assessing agreement between two methods of clinical measurement, *Lancet* 1 (8476) (1986) 307–310.
- [15] K. Thygesen, J.S. Alpert, A.S. Jaffe, M.L. Simoons, B.R. Chaitman, H.D. White, et al., Third universal definition of myocardial infarction, *Circulation* 126 (16) (2012) 2020–2035.
- [16] J.Y. Liu, Q.W. Jia, X.L. Zang, R.H. Wang, C.J. Li, L.S. Wang, et al., Age-sex distribution of patients with high-sensitivity troponin T levels below the 99th percentile, *Oncotarget* 8 (43) (2017) 75638–75645.
- [17] D.H. Wilson, D.M. Rissin, C.W. Kan, D.R. Fournier, T. Piech, T.G. Campbell, et al., The simoa HD-1 analyzer: a novel fully automated digital immunoassay analyzer with single-molecule sensitivity and multiplexing, *J. Lab Autom.* 21 (4) (2016) 533–547.
- [18] K.M. Eggers, N. Johnston, S. James, B. Lindahl, P. Venge, Cardiac troponin I levels in patients with non-ST-elevation acute coronary syndrome-the importance of gender, *Am. Heart J.* 168 (3) (2014) 317–24 e1.
- [19] C.J. Salton, M.L. Chuang, C.J. O'Donnell, M.J. Kupka, M.G. Larson, K.V. Kissinger, et al., Gender differences and normal left ventricular anatomy in an adult population free of hypertension. A cardiovascular magnetic resonance study of the Framingham heart study offspring cohort, *J. Am. Coll. Cardiol.* 39 (6) (2002) 1055–1060.
- [20] A.S. Shah, M. Griffiths, K.K. Lee, D.A. McAllister, A.L. Hunter, A.V. Ferry, et al., High sensitivity cardiac troponin and the under-diagnosis of myocardial infarction in women: prospective cohort study, *BMJ* 350 (2015), g7873.
- [21] L. Cullen, J.H. Greenslade, E.W. Carlton, M. Than, J.W. Pickering, A. Ho, et al., Sex-specific versus overall cut points for a high sensitivity troponin I assay in predicting 1-year outcomes in emergency patients presenting with chest pain, *Heart* 102 (2) (2016) 120–126.
- [22] P.M. McKie, D.M. Heublein, C.G. Scott, M.L. Gantzer, R.A. Mehta, R.J. Rodeheffer, et al., Defining high-sensitivity cardiac troponin concentrations in the community, *Clin. Chem.* 59 (7) (2013) 1099–1107.
- [23] Y. Sandoval, F.S. Apple, The global need to define normality: the 99th percentile value of cardiac troponin, *Clin. Chem.* 60 (3) (2014) 455–462.
- [24] M. Reiter, R. Twerenbold, T. Reichlin, B. Benz, P. Haaf, J. Meissner, et al., Early diagnosis of acute myocardial infarction in patients with pre-existing coronary

- artery disease using more sensitive cardiac troponin assays, *Eur. Heart J.* 33 (8) (2012) 988–997.
- [25] M.W. Sherwood, Newby L. Kristin, High-sensitivity troponin assays: evidence, indications, and reasonable use, *J. Am. Heart Assoc.* 3 (1) (2014), e000403.
- [26] M. Than, L. Cullen, S. Aldous, W.A. Parsonage, C.M. Reid, J. Greenslade, et al., 2-hour accelerated diagnostic protocol to assess patients with chest pain symptoms using contemporary troponins as the only biomarker: the ADAPT trial, *J. Am. Coll. Cardiol.* 59 (23) (2012) 2091–2098.