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Determination of sex-specific 99th percentile upper reference limits for a point of care high sensitivity cardiac troponin I assay

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Abstract

Objectives: High sensitivity (hs) cardiac troponin (cTn) assays are defined per the IFCC Committee on Clinical Application of Cardiac Biomarker (C-CB) by the ability to measure ≥ 50% of concentrations greater than the limit of detection (LoD) with an impression of ≤10% at sex-specific 99th percentiles. Our study determined the sex-specific 99th percentile upper reference limits for males and females utilizing heparinized plasma from AACC universal sample bank for the Siemens point of care (POC) Atellica® VTLi hs-cTnI immunoassay.

Methods: Apparently healthy subjects, included overall 693, males 363, and females 330, following exclusionary surrogate biomarker use of hemoglobin A_{1c}, NT-proBNP, and eGFR, along with statin medication. hs-cTnI was measured in a central laboratory, on multiple POC Atellica® VTLi immunoassay analyzers. The LoD was 1.24 ng/L and limit of quantitation (CV 20%) was 6.7 ng/L. 99th percentile URLs were determined by the nonparametric (NP) method.

Results: Histograms of the hs-cTnI concentrations (ng/L) for males and females were used to visualize the distributions and concentrations in men and women and differed significantly (pre- and post-exclusion, both p <0.001). 99th percentile URLs were: overall 23 ng/L (90% CI 20–32 ng/L); male 27 ng/L (CI 21–37 ng/L); female 18 ng/L (CI 9–78 ng/L). The percentages of subjects having a measurable concentration ≥ the LoD were: overall 83.7%, male 87.3%, female 79.7%.

Conclusions: Our findings show the novel POC Atellica® VTLi hs-cTnI assay meets the designation of a 'high-sensitivity' assay using heparinized plasma.

Keywords: 99th percentile upper reference limit; cardiac troponin; high sensitivity cardiac troponin assays; point of care assay.

Introduction

Manufacturers pursuing the development of point of care (POC) cardiac troponin (cTn) assays, predicated on using whole blood as a specimen, hope to fulfill the criteria as a high-sensitivity (hs) designation [1–3]. High sensitivity cTnI and hs-cTnT assays are currently defined based on two analytical criteria per the IFCC Committee on Clinical Application of Cardiac Biomarkers (C-CB) and the American Association for Clinical Chemistry (AACC) Academy [1, 2]: (a) an imprecision of ≤10% CV (coefficient of variation) at the 99th percentile upper reference limit (URL) for both males and females independently, and (b) the ability to measure ≥50% of concentrations greater than or equal to the assay's limit of detection (LoD). Recently, multiple hs-cTnI and hs-cTnT assays were studied [4] using the plasma specimen set of the AACC's Universal Sample Bank (USB) [5] to determine male and female 99th percentile URLs, allowing for comparative observations based on the sample reference set. In addition, a developmental, POC hs-cTnI assay also recently described its 99th percentile URLs for male and females using plasma from the USB [6]. The purpose of the current study was to determine the sex-specific 99th percentile URLs for males and females utilizing plasma from AACC USB for the Siemens POC Atellica® VTLi hs-cTnI immunoassay along with demonstrating that >50% of normal subjects have

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measurable concentrations >LoD for both sexes to be designated as a high sensitivity assay.

Materials and methods

Plasma (lithium heparin) specimens from apparently healthy subjects (n=821) were obtained from the AACC USB, which has previously been described as a diverse group of apparently healthy subjects, including their demographics and the health questionnaire used for subject enrollment and exclusions [5]. One clinical outlier from the dataset, a female with a result of 328 ng/L, was removed as an outlier following the Dixon-Reed method as described in CLSI EP28-A3c, resulting in our final n=820 subjects. As this subject sample set was used in the multiple hs-cTn assay study cited in ref. [4], this specimen outlier was likely either a preanalytical specimen quality issue or a potential analytical assay issue [4]. Subjects included 422 men and 398 women who were screened using a health questionnaire. Participants who provided consent along institution review guidelines, were over 18 years of age, without any known prior history of hypertension, renal failure, diabetes, congestive heart failure, heart disease, cancer, deep vein thrombosis/pulmonary embolism, warfarin use, or treatment with cardiovascular medications for known disease, and were symptom free. As previously described in USB studies [4, 6], hemoglobin A_{1c} (>URL 6.5%), NT-proBNP (>URL 125 ng/L) and eGFR (<60 mL/min), along with statin use, were used as surrogate exclusionary biomarkers to further assist in verifying subject normality [7]. Surrogate biomarker exclusion screening removed 127 individuals overall, of which 59 were men and 68 were women. The 125 ng/L NT-proBNP exclusion concentration was chosen by the expert opinions of all coauthors [7] predicated on the Roche's package insert lowest reference limit. The observation of 15% exclusion rate of subjects is not unusual and will vary along the number of surrogate biomarkers or imaging tools used for screening as previously demonstrated [8, 9].

hs-cTnI measurements on the POC Atellica® VTLi immunoassay analyzer were performed in a core laboratory in Eindhoven (The Netherlands), randomly distributed across 25 instruments. The POC Atellica® VTLi system consists of a handheld instrument and plastic disposable cartridge. The system is based on the precisely controlled motion of magnetic particles in a small sample volume. The same magnetic particles also serve as labels that are detected using frustrated total internal reflection (FTIR) imaging [10]. The traditional liquid manipulation steps of an immunoassay have been replaced by magnetically controlled movements of magnetic nanoparticles within a stationary liquid. The magnetic beads carry antibodies directed against cTnI whereas the other side of the sandwich is formed by antibodies printed on the bottom of the cartridge (the sensor surface), as shown in Figure 1. A droplet (~30 µL) of whole blood or plasma is applied to the cartridge and the reaction chamber fills by capillary action. Red blood cells are retained by an integrated separation membrane. In the first phase of the assay, beads coated with antibody capture cTnI molecules in the sample. Subsequently, magnetic fields gradients are engaged to transport the particles rapidly to the sensor surface towards immobilized antibodies able to capture the troponin-bearing nanobeads. After the beads reacted with the sensor surface, un-bound and non-specifically bound beads are rapidly removed with a magnetic wash by applying a magnetic field gradient oriented away from the detection surface. The total analytical turn-around time, from the time the sample is applied on the cartridge to result on screen is <8 min. The primary anti-cTnI mouse-monoclonal antibody is directed against the stable region of the cTnI molecule (amino acid [AA] 41–49) and has been covalently bound to the magnetic beads. A mixture of three secondary antibodies have been attached to the sensor surface by physisorption, which consists of two anti-cTnI antibodies directed at epitopes 23–29 and 87–91) and a single anti-cTnC antibody, to optimize measurement of total cTnI including cTnI–TnC complexes.

The LoD of the assay was determined to be 1.24 ng/L and the limit of quantitation (LoQ) (20% CV) was determined to be 6.7 ng/L. Using low-concentration quality control (QC) materials (n=80) with mean concentrations between 12.2 and 14.0 ng/L (a concentration between the LoD and the female 99th percentile), the assay's within-lab imprecision, expressed %CV, ranged from 7.1 to 9.5%. 99th percentile URLs were determined by the nonparametric (NP) method using R version 4.0.2. and 10.000 bootstrap repeats to calculate 90% confidence intervals [11]; as recommended by the IFCC C-CB. Analyses were performed for the overall group and individually for each sex. Subgroup analysis was performed on those meeting our stricter definition of 'normality/healthy' subjects following exclusions. The Mann–Whitney *U* test was used to determine differences in sex-specific 99th percentiles.

Results

Subjects excluded from all 820 subjects enrolled following screening by the health questionnaire were as

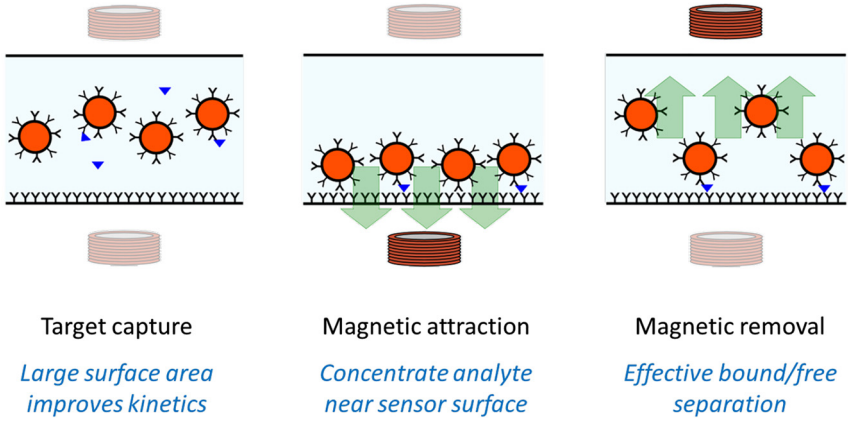

Figure 1: Schematic of POC Atellica® VTLi hs-cTnI immunoassay technology.

follows: 19 by statin use, 79 by NT-proBNP, 19 by hemoglobin A_{1c}, five by eGFR, one for both NT-proBNP and HbA_{1c}, and four for both eGFR and NT-proBNP. There were 481 Caucasians, 212 African or African Americans, 91 Asians and Pacific Islanders, and 24 individuals described as "others," including Native American Indians. Ages ranged from 18–91 years, with a median of 39 years.

Figure 2 panels shows the histograms of the subjects (A, all; B, males, C, females), by cTnI concentration based on the number of subjects at each concentration (density). The post-exclusion subjects', overall n=693 (A), males n=363 (B), and females n=330 (C), 99th percentile URLs were: overall – 23 ng/L (90% confidence interval [CI]) 20–32 ng/L); males – 27 ng/L (90% CI 21–37 ng/L); females – 18 (90% CI 9–78 ng/L). Violin plots shown in Figure 3 illustrate the post-exclusion VTLi hs-cTnI measurements (median ± 1.5 × interquartile range) for all subjects, and by sex, with females showing lower hs-cTnI concentrations compared to males. Concentrations in men and women differed significantly for both pre-exclusion and post-exclusion (p<0.001). The percentages of subjects having a measurable concentration ≥ LoD for the post exclusion subjects were: overall 83.7%, male 87.3%, female 79.7%; with no statistical differences compared to the non-exclusion subjects.

Discussion

Our findings provide new, unique, information for a high sensitivity POC assay for cTnI. First, we show that the POC Atellica® VTLi hs-cTnI assay meets the IFCC C-CB and AACC Academy recommendation for a high sensitivity cTnI assay using plasma specimens based on the AACC's USB. Independently, male subjects and female subjects each measured >50% of subjects with >80% measurable above the assay's LoD. This complements the <10% CV for the assay using patient plasma and quality control materials at concentrations less than the female's 99th percentile URL. Second, we observed minimal changes in 99th percentile

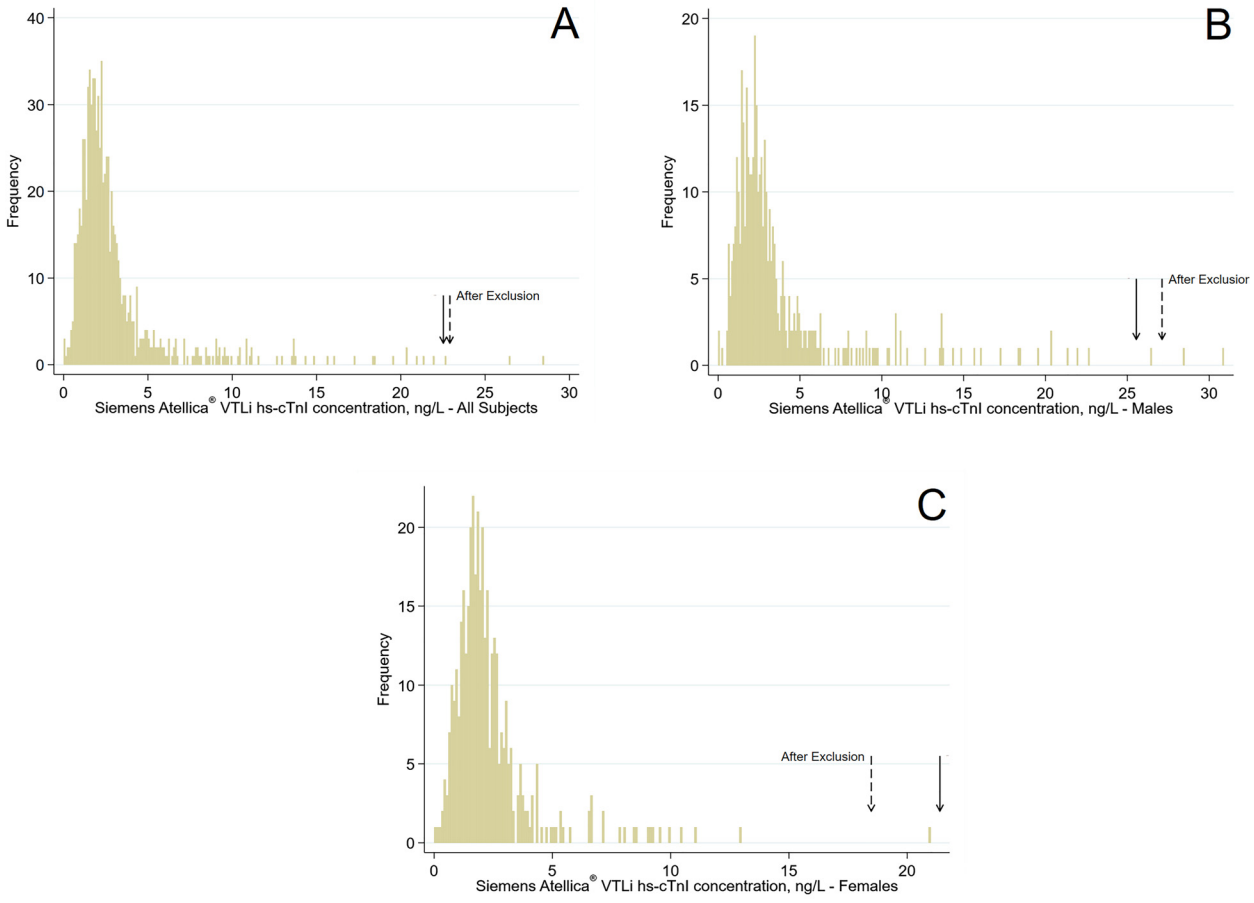

Figure 2: Histograms of the distribution of the POC Atellica® VTLi hs-cTnI immunoassay for apparently healthy subjects, with indications of the sex-specific 99th percentile upper reference limits (ng/L) before and after exclusions. (A) Overall subjects; (B) males; (C) females.

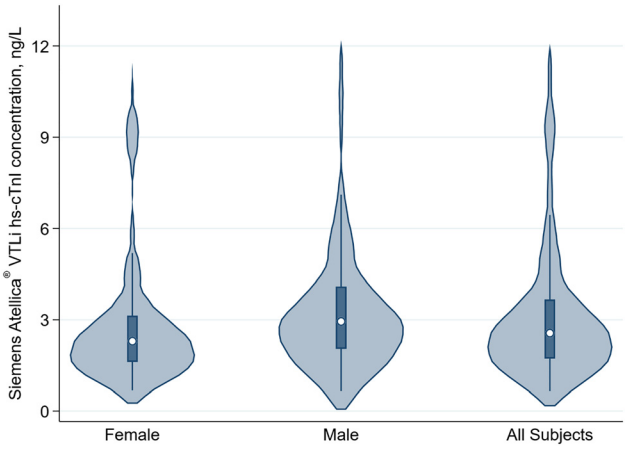

Figure 3: Violin plot illustrating the POC Atellica® VTLi hs-cTnI measurements (median ± 1.5× interquartile range) for females, males, and for all subjects.

URLs following exclusion of subjects based on the surrogate biomarkers from the IFCC C-CB recommendations [1, 2]. An interesting observation was that 99th percentile URLs do not always decrease after exclusion of subjects. However, other studies using different biomarkers and exclusionary tools have shown different observations [8, 9]. Third, we show distinct female and males URLs using the non-parametric statistic along IFCC-CB and Universal Definition of Myocardial Infarction [12] recommendations for clinical utilization.

Recently, the POC Atellica® VTLi hs-cTnI assay was CE marked approved for clinical use**.** The POC Atellica® VTLi hs-cTnI assay's plasma 99th percentile URLs for males and females add to the growing evidence-base for POC assays, along with central laboratory assays, that qualify as hs-cTn assays that can now be used in practice in both rural hospital and or clinic settings, as well as to use in a busy, urban emergency department for rapid triage of patients [13–15]**.** Utilizing plasma from the AACC's USB, we were able to add to the growing comparative list of central laboratory and POC hs-cTnI and hs-cTnT assays that have evidence for both regulatory approved and for research only assays [16, 17]. The literature shows substantial variations in female and male 99th percentiles between assays, among different assays and even different instruments from the same manufacturer. The lack of standardization is clear; in that each laboratory must understand their own assay's analytical characteristics and avoid use of different platforms in the same hospital or health system without a strong educational effort by laboratory medicine with their clinical partners who will use the POC assays in practice [3, 18].

The following limitations are noted. First, there is a need to validate the assay's 99th percentile URLs in whole blood, that will be essential for gaining acceptance into practice in emergency medicine and other decentralized settings. hs-POC assays in the emergency department will provide objective evidence to clinicians to reduce the chance of missing falsely negative results that occur with contemporary POC assays, that miss 10–15% of true positive results compared to central laboratory assays [19, 20]. Studies are underway to validate the 99th percentile URLs in fresh capillary and venous (whole blood and plasma) specimens from apparently healthy subjects. Second, the question often arises as to what are the drawbacks eliminated using POC testing based on a 'hs' assay. It is our opinion, with the use of a hs-POC assay that provides improved analytical sensitivity at very low concentrations and lack of analytical noise around the 99th percentile, analytical drawbacks are eliminated [9, 20]. As the Atellica VTLi assay cartridges are pre-calibrated, and do not require regular calibration of the operator, even analytical drift at the LoD minimized. Third, as noted above, having different assays that provide different, non-equivalent results, can and will be challenging to both laboratories and clinicians. However, if utilized appropriately, the pros outweigh the cons. Fourth, the study was not designed to assess the %CV at the LoD, as this assessed in a different study [21]. Fourth, we recognize that the 90% CI of the female URL is extraordinarily wide and that range of QC concentrations validated does not include all the theoretical values that the URL can present. However, the 99th percentile per definition is the tail end of the distribution. The ordered highest cTnI concentration were 79, 78, 21, 13, and 11 ng/L. Therefore, the resulting bootstrap determined CI is bound to be large as found in real-world distributions of individuals per each sex. We opine that it is more meaningful to look at the %CV at the actual 99th percentile, than at the edges of the CI.

In summary, our findings are unique, showing the novel POC Atellica® VTLi hs-cTnI assay meets the designation of a 'high-sensitivity' assay using heparinized plasma specimens.

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Competing interests: FSA: Board of Directors, HyTest Ltd.; Honorarium Advisory Boards: Siemens Healthcare, Instrumentation Laboratory, Qorvo; Research PI through Hennepin Healthcare Research Institute (not salaried): Abbott Diagnostics, Abbott Point of Care, Roche Diagnostics, Siemens Healthcare, Quidel/Alere, Ortho-

Clinical Diagnostics, Beckman Coulter; Other: Associate Editor Clinical Chemistry.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board.

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