ORIGINAL ARTICLE

Determination of the 99th percentile upper reference limit for high-sensitivity cardiac troponin I in Malaysian population

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Abstract

Introduction of high-sensitivity cardiac troponin I (hscTn I) assays for routine clinical use in Malaysia requires determination of the 99th percentile upper reference limit (URL) for each assay to suit local context. Hence, this study aimed to determine the 99th percentile URL for hscTn I in the Malaysian population. A total of 250 (120 males and 130 females) healthy Malaysian blood donors aged 18 to 60 years old were recruited. Blood samples for hscTn I were measured using Abbott Diagnostics hscTn I assay on Architect i2000sr analyser. The 99th percentile was calculated using a non-parametric method and gender specific results were compared. The 99th percentile URL for hscTn I for the overall population was 23.7 ng/L, with gender specific values being 29.9 ng/L and 18.6 ng/L for male and female, respectively. Females had significantly lower hscTn I compared to males. This study confirms the use of gender specific 99th percentile URL for hscTn I for clinical use in a multi-ethnic Malaysian population.

Keywords: high-sensitivity, troponin I, 99th percentile, Malaysian, gender

INTRODUCTION

Cardiac troponin (cTn) is the biomarker of choice for diagnosing acute myocardial infarction (AMI). Elevated cTn is defined as a value exceeding the 99th percentile of a normal reference population or the upper reference limit (URL), with this value chosen as the decision limit for AMI diagnosis.[1] Increasing demand for an earlier AMI diagnosis has led to the introduction of many high-sensitivity cardiac troponin (hscTn) assays. To be defined as a high-sensitivity assay, it must have a coefficient variation (CV) of ≤10% at the 99th percentile URL and a measurable concentration above the assay's limit of detection (LoD) in at least 50% of the healthy population.[2] The 99th percentile URL for each assay thus needs to be determined when introduced into clinical practice. This includes gender specific 99th percentile URL as differences in cTn concentrations have been increasingly recognised.[3-8] There is also a possibility of age and ethnicity difference although these are still debatable.[5-8] In view of this, we aimed to determine the 99th percentile URL of hscTn I in Malaysian population.

MATERIALS AND METHODS

Self-proclaimed healthy Malaysian blood donors (with no known history of hypertension, diabetes, cardiac, muscle, liver or renal diseases) aged between 18 to 60 years old were recruited at the National Blood Centre or other mobile blood donation sites organised by the centre. Subjects were interviewed with regards to their current health and past medical history. Blood pressure was measured as part of routine screening of blood donors. Informed consent was obtained from individual donors. The research was approved by the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia [(JKEUPM)UPM/TNCPI/RMC/JKEUPM/1.4.18.1/F2] and the Medical Research Ethical Committee (MREC), Ministry of Health, Malaysia (NMRR-14-1943-22884). Three (3) mls of unanticoagulated whole blood sample obtained from the blood donor diversion pouch bag were immediately transferred to a lithium heparin tube. The serum was aliquot and stored at -70°C until analysis.

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hscTn I analysis

hscTn I was assayed on the Architect i2000sr in the Chemical Pathology Laboratory, Hospital Serdang, Malaysia in batch analysis. Precision study was performed prior to the sample analysis of research subjects. Using three levels of quality control (QC) materials, the precision across the assay range was verified. This precision study was performed in accordance to the Clinical and Laboratory Standards Institute (CLSI) document guideline for precision study (CLSI GUIDELINE EP15 - A2)[9] and Guideline for Method Verification of Quantitative Measurement in Medical Laboratories, Pathology Services Ministry of Health Malaysia.[10] All three QC levels were performed daily for five consecutive days with each level assayed in triplicates with a minimum of two hours apart, generating in total 45 data. The CVs were 5.8%, 3.0% and 2.4% respectively for QC concentrations of 19.8, 200.7 and 15886.9 ng/L. These values were in line with the manufacturer's claim of CV < 10%. The assay's limit of blank (LoB) (ng/L) and assay limit of detection (LoD) (ng/L) have been verified in previous studies.[5,7,11,12]

Data analysis

Statistical calculations were performed using the MedCalc software (MedCalc Software bvba, Ostend, Belgium). Outliers were screened with a method by Reed *et al*[13]. The 99th percentile URL was determined in accordance to the CLSI C28-A3c document.[14] It was calculated based on non-parametric method whereby sample results were ranked accordingly and

linear interpolation was carried out due to the estimated rank not being an integer. URL was calculated for the whole population and gender specific reference, respectively. Comparison of hscTn I concentration between male and female was analysed using Mann-Whitney U test. In all statistical analyses, a 'p' value of < 0.05 was considered to be statistically significant.

RESULTS

A total of 250 serum samples from self-declared healthy donors (120 males and 130 females) were obtained. Table 1 shows the demographics of the study population and the median hscTn I value. The median age was 34 years old with the youngest being 19 and the eldest being 56 years old. Majority (75.6%) were ≤ 40 years old and of Malay ethnicity (69%). There were no significant differences in the hscTn I concentrations in terms of age and race.

The hscTn I results ranged from 0 to 31.1 ng/L with a median of 0.8 ng/L (IQR = 1). Seventy-seven participants (30.8%) had concentrations above the assay LoD. The median and the 99th percentile URL for both overall population and gender-specific are shown in Table 2. The 99th percentile URL for the population was 23.7 ng/L (90% CI 16.3-31.1). hscTn I concentrations were significantly higher in men than in women (p = 0.009).

Figure 1 shows the non-parametric distribution of hscTn I results, which was skewed to the right. Results were analysed by MedCalc software (MedCalc Software bvba, Ostend, Belgium) with no outlier noted.

TABLE 1: Demographics of reference population and hscTn I results

Demographics	n (%)	hscTn I		
		Median ng/L (95% CI)	IQR	p value*
Gender				
Male	120 (48)	0.9 (0.8 - 1.3)	1.7	0.009
Female	130 (52)	0.8 (0.7 - 0.9)	1.1	
Age (years)				
≤ 40	189 (75.6)	0.8 (0.7 - 0.95)	1.1	0.17
> 40	61 (24.4)	0.8 (0.7 - 1.56)	1.6	
Race				
Malay	172 (68.8)	0.85 (0.8 - 1.1)	1.1	0.88**
Chinese	53 (21.2)	0.7 (0.6 - 0.94)	1.1	
Indian	25 (10.0)	0.9 (0.61 - 2.3)	3.7	

* p value calculated using Mann - Whitney U test. A p value < 0.05 is considered significant
** Malay vs non-Malay

TABLE 2: The 99th percentile URL for overall population and according to gender

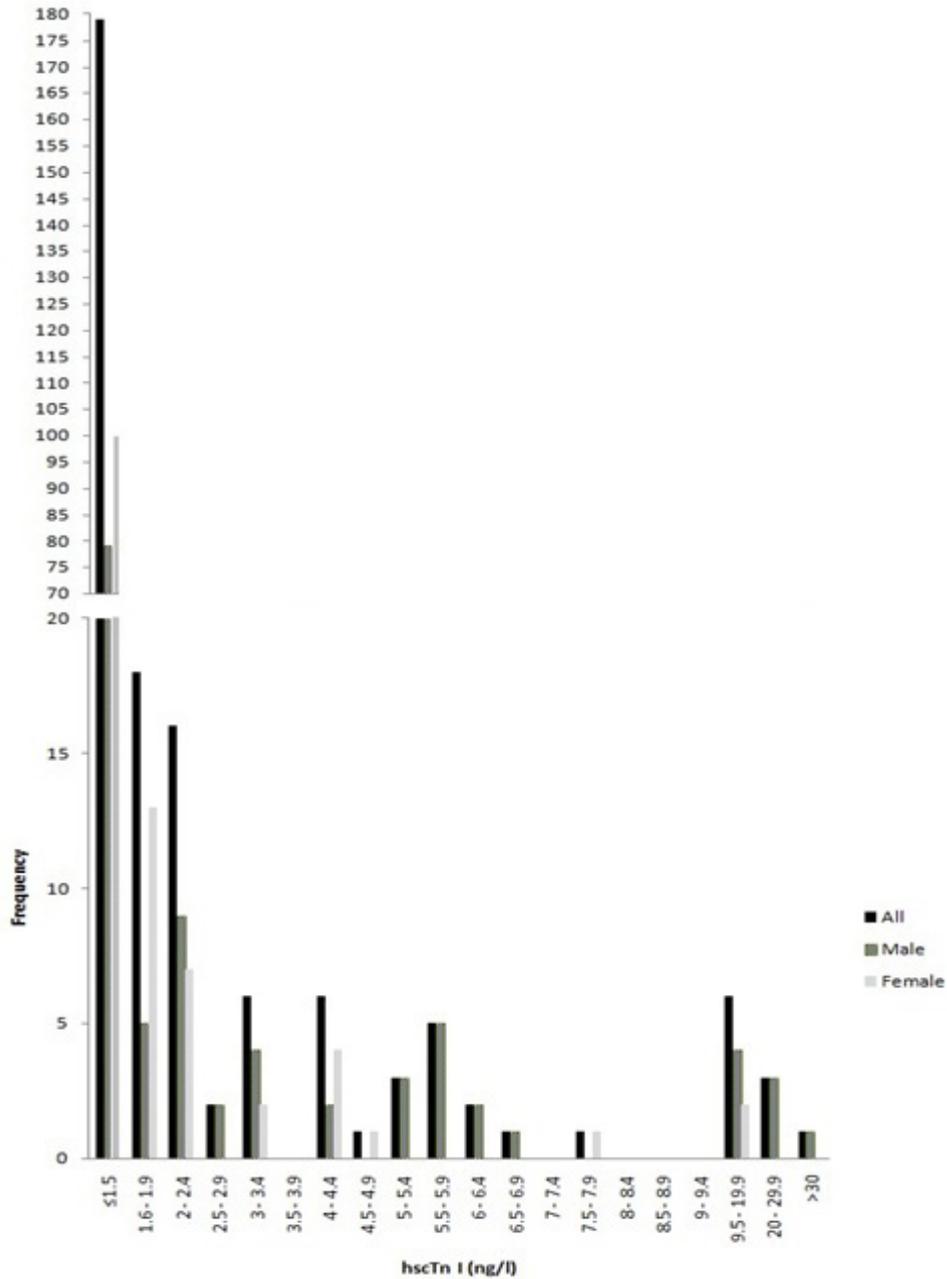|	Median (ng/L)	99th percentile (ng/L)
All samples (250)	0.8	23.7 (90% CI 16.3 - 31.1)
Male (120)	0.9	29.9 (90% CI 10.0 - 31.1)
Female (130)	0.8	18.6 (90% CI 4.6 - 19.6)

FIG. 1: Distribution of hscTn I concentration measured in all population, male and female

DISCUSSION

The 99th percentile URL hscTn I obtained in this Malaysian population study was 23.7 ng/L for the overall population; 29.9 ng/L for males and 18.6 ng/L for females. These cut-offs were similar to a study using the same assay performed in Singapore by Aw *et al*,[5] which had obtained a 99th percentile cut-off of 25.6, 32.7 and 17.9 ng/L for overall, male and female gender, respectively. Table 3 shows the list of gender specific 99th percentile URL for Abbott hscTn I assays in preceding studies, supporting the use of gender specific references for AMI diagnosis.[3-8,11] There are however, variations in the 99th percentile URL values obtained between studies, emphasising the importance of establishing a local reference value. Based on these studies, the male 99th percentile URL ranged from 13.2 to 36 ng/L and female between 9.3 to 19.0 ng/L. Furthermore, although all studies demonstrated a higher male 99th percentile URL compared to females, the magnitude of difference between male and female cut-offs varied. The greatest difference between male and female cut-offs was 22.3 ng/L[6] whilst the narrowest difference was 1 ng/L.[7] We had obtained a difference of 11.3 ng/L. Apart from age and ethnicity, the differences could also be contributed by selection method of a healthy reference population. We had selected the reference population based on subjects' self-declaration of no known medical illness, normotensive and being well on the day of blood sampling. In two other studies that had used a similar pre-selection criteria, the hscTn I values obtained were similar.[3,5] In contrast, studies, which had used biomarker pre-screening with or without additional cardiac imaging for confirmation of healthy reference population had obtained lower hscTn I values.[4,6-8] Using parameters such as estimated glomerular filtration rate (eGFR) for detection of mild renal impairment, HbA1c or fasting serum glucose for diabetes and either B-type natriuretic peptide (BNP), N-terminal-proBNP (NT-proBNP) or echocardiogram for sub-clinical cardiac disease resulted in decreased 99th percentile values for both men and women. BNP-based inclusion criterion was shown to be the most effective in selection of a healthy cohort with exclusion of 21% of presumably healthy population (from health questionnaire).[6] Further screening for dyslipidaemia and exclusion of these subjects during analysis, resulted in further reduction in the 99th percentile URL.[6] Progressive filtering of the healthy population resulted in decreased 99th percentile URL.[4,6,8,15] Collinson *et al* 2015 also showed that the 99th percentile URL varied depending on how normality was defined (presumably healthy population, after applying health questionnaire, and after application of surrogate biomarkers) as shown in Table 3. However, they generally had one of the lowest 99th percentile URL even without exclusion of subjects by surrogate biomarkers, stressing the importance of local population cut-off values.

In terms of age, our subjects were mostly in the middle-age group with a median age of 34 years old. Only 6% of the study population were above 50 years old, limiting the ability to establish an age-based 99th percentile URL. Cardiac troponin increases in the elderly population even in the absence of ACS or other comorbidities such as hypertension or diabetes.[15] The Gutenberg Health Study found clear effect of age on the 99th percentile URL.[16] Similarly using hscTn I, the median values were significantly lower in those ≤50 years old compared to those older than 50 years old.[5,7] Although age was predominantly associated with an elevated hscTn I, the impact of age decreased after adjustments for gender and when a more strict definition of healthy population was used.[6,8]

The main limitation of this study is the comparatively small sample size, which consisted of a younger population. This may have resulted in the inability to detect hsTn I concentration above the assay's LoD in at least 50% of the healthy population. However, this particular characteristic of the assay had been previously verified.[5,7,11,12] Secondly, no subjects above the age of 60 were included in the study, due to rarity of blood donors among this age group. Hence, this 99th percentile cut-off may not be reflective of the elderly population. However, this issue may be remedied by performing serial testing and observing the cTn kinetics.

In conclusion, the overall 99th percentile cut-off of Abbott hscTn I assay in our Malaysian population was 23.7 ng/L, with significantly lower values obtained for females. The male and female hscTn I 99th percentile cut-offs were 29.9 ng/L and 18.6 ng/L, respectively.

TABLE 3: Comparison of overall and gender specific 99th percentile URL hscTn I on Abbott assay and the pre-selection method of their reference population

Author	Population studied	Median age (years)	Method for screening reference population	hscTn I 99th percentile URL (ng/L)		
				Overall	Male	Female
	Western					
Apple 2012[3]	United States	NA	Health questionnaire	23	36	15
Koerbin 2012[11]	Australian	NA	Health questionnaire & biochemical markers (NT-proBNP, eGFR)	13.6	14.0	11.1
Krintus 2015[6]	Europeans	51	(1) Health questionnaire	23.7	35.2	12.9
			(2) (1) + biochemical markers (BNP, HbA1c, FSL, eGFR)	11.2	13.2	9.3
Collinson 2015[8]	British	58	(1) Apparently healthy population	21	28.5	9.9
			(2) (1) + health questionnaire	12.3	18.3	9.5
			(3) (2) + biochemical markers (fasting serum glucose, eGFR) & spirometry, ECG, echocardiogram	8.7	–	–
	Asian					
Aw 2013[5]	80% Singaporeans (75% Chinese, 15% Malay, 10% Indian) and 20% foreigners from China Philippines, Malaysia, India and Indonesia	50.4	Health questionnaire	25.6	32.7	17.9
Ji 2016[7]	Korean	49.8	Health questionnaire, biochemical markers (BNP, HbA1c)	18	20	19
Current study	Malaysian	34	Health questionnaire	23.7	29.9	18.6

BNP: B-type natriuretic peptide; NT-proBNP: N-terminal-pro BNP; FSL: fasting serum lipid; eGFR: estimated glomerular filtration rate; NA: not available

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REFERENCES

1. Thygesan K, Alpert JS, Jaffe AS, *et al*. Third universal definition of myocardial infarction. J Am Coll Cardiol. 2012; 60: 1581-98.
2. Apple FS, Collinson PO; IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem; 2012; 58: 54-61.
3. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably health population. Clin Chem. 2012; 58:1574-81.
4. Koerbin G, Abhayaratna WP, Potter JM, *et al*. Effect of population selection on 99th percentile values for a high sensitivity cardiac troponin I and T assays. Clin Biochem. 2013; 46: 1636-43.
5. Aw TC, Phua SK, Tan SP. Measurement of cardiac troponin I in serum with a new high-sensitivity assay in a large multi-ethnic Asian cohort and the impact of gender. Clin Chim Acta. 2013; 422: 26-8.
6. Krintus M, Kozinski M, Boudry P, *et al*. Defining normality in a European multinational cohort: critical factors influencing 99th percentile upper reference limit for high sensitivity cardiac troponin I. Int J Cardiol. 2015; 187: 256-63.
7. Ji M, Moon HW, Hur M, Yun YM. Determination of high-sensitivity cardiac troponin I 99th percentile upper reference limits in a healthy Korean population. Clin Biochem. 2016; 49: 756-61.
8. Collinson PO, Gaza D, Goodacre S. The clinical and diagnostic performance characteristics of the high sensitivity Abbott cardiac troponin I assay. Clin Biochem. 2015; 48: 275-81.
9. Clinical and Laboratory Standards Institute (CLSI). User verification of performance for precision and trueness: approved guideline – second edition. CLSI document EP15-A2. Wayne, PA, USA: CLSI; 2005.
10. Guideline for method verification of quantitative measurement in medical laboratories version 2015. Pathology Services Ministry of Health Malaysia.
11. Koerbin G, Tate J, Potter JM, Cavanaugh J, Glasgow N, Hickman PE. Characterisation of a highly sensitive troponin I assay and its application to a cardio-healthy population. Clin Chem Lab Med. 2012; 50: 871-8.
12. Krintus M, Kozinski M, Boudry P, *et al*. European multicenter analytical evaluation of the Abbott ARCHITECT STAT high sensitive troponin I immunoassay. Clin Chem Lab Med. 2014; 52: 1657-65.
13. Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. Clin Chem Lab Med. 1971; 17: 275-84.
14. Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical Laboratory: approved guideline – third edition. CLSI Document EP28-A3c. Wayne, PA, USA: CLSI; 2010.
15. McKie PM, Heublein DM, Scott CG, *et al*. Defining high-sensitivity cardiac troponin concentrations in the community. Clin Chem. 2013; 59: 1099-107.
16. Zeller T, Ojeda F, Brunner FJ, *et al*. High sensitivity cardiac troponin I in the general population - defining reference populations for the determination of the 99th percentile in the Gutenberg Health Study. Clin Chem Lab Med. 2015; 53: 699-706.