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The 99th percentile values of six cardiac troponin assays established for a reference population using strict selection criteria

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ABSTRACT

Background: Since the 99th percentile reference limit for cardiac troponin (Tn) can vary depending on the reference population, Sandoval et al. published systematic selection criteria. In this study, these systematic criteria were applied for the first time to obtain the 99th percentile reference limits for 6 Tn tests.
Methods: The reference population was selected in accordance with the systematic criteria, and reference limits were set with respect to the six types of Tn assays. The coefficient of variation (CV) at the reference limit was determined using 3–4 concentrations of frozen serum.
Results: In total, 641 South Koreans (303 males, 338 females) were selected as the reference population. The 99th percentile reference limit of Tn in the six assays ranged from 13.4 to 34.2 pg/ml. The measurable fractions among the reference population ranged from 1.3% to 80.5%. The CVs at the reference limit ranged from 5.3% to 43.0%, and three were <10%.
Conclusions: In this study, a reference population was selected for the first time in accordance with the systematic criteria of Sandoval et al., and the reference limit for South Koreans was established. The values obtained in this study are different from those proposed by manufacturers, which confirms the importance of having a reference population. Four out of six assays did not fulfill the criteria for high-sensitivity tests.

1. Introduction

According to the third universal definition of myocardial infarction (MI) published in 2012 by the European Society of Cardiology, the American College of Cardiology Foundation, the American Heart Association, and the World Heart Federation, cardiac troponin (Tn) concentration is the most important factor for the diagnosis of MI. A Tn value is considered abnormal if it is above the 99th percentile of the healthy population [1]. The coefficient of variation (CV) at the reference limit is ideally 10% or less, and although values up to 20% are clinically suitable, those higher than 20% are not recommended for use [1,2].

In previous studies, high-sensitivity Tn (hsTn) assays from several companies, including Roche TnT [3–5], Abbott TnI [5–9], Beckman TnI [5,10–12], and Siemens TnI [5,13,14], were assessed. However, the reference limits differed among these studies, and were different from those provided by the manufacturers. These differences could be attributable to the influence of ethnicity or sample type, but the use of different selection criteria for reference populations is considered the most important factor. The importance of reference population selection criteria was well highlighted in a previous study [15].

Sandoval and Apple [16] previously presented guidelines for selecting reference populations for Tn assays. Moreover, the International Federation of Clinical Chemistry (IFCC) Task Force on Clinical Applications of Cardiac Bio-Markers also recommended similar criteria for establishing reference intervals [17]. However, to date, no study has established reference limits according to these guidelines.

2. Materials and methods

This study was conducted after being reviewed and approved by the Institutional Review Board of Asan Medical Center.

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Table 1
Inclusion criteria for samples collected for the establishment of the 99th percentile reference limits.

Parameters	Inclusion criteria
eGFR	>60 ml/min/1.73 m^2
HbA1c	≤6.0%
NT-proBNP	Within reference intervals[a]
Clinical history for known cardiovascular disease and medication usage	No
Diverse distribution of ages	From 18 to >70 y

Abbreviations: eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.
[a] Suggested by Roche package insert. See text for details.

2.1. Reference population selection and sample preparation

To calculate the 99th percentile reference limit of Tn, residual plasma (lithium heparin) samples from patients who visited Asan Medical Center for general health checks from August 25 to September 26, 2014 were included. The inclusion criteria for selecting the reference population are shown in Table 1. The estimated glomerular filtration rate (eGFR) cut-off was set at 60 ml/min/1.73 m^2 since we used the Modification of Diet in Renal Disease eGFR equation and because values > 60 were not reported [18]. The HbA1c cut-off was set at 6.0% according to the recent American Diabetes Association guidelines [19]. The NT-proBNP cut-off was set at the age- and sex-specific values provided by the manufacturer [18–44 y: 85.8 (male) or 130 (female) pg/ml; 45–54 y: 121 (male) or 249 (female) pg/ml; 55–64 y: 210 (male) or 287 (female) pg/ml; 65–74 y: 376 (male) or 301 (female) pg/ml; ≥75 y: 486 (male) or 738 (female) pg/ml]. Since this study focused on the South Korean population, we did not use the race/ethnicity criteria. Other explanations regarding minor concerns were added in the manuscript.

Samples in which the Tn concentration may have been affected were excluded by reviewing the sex and age distribution, laboratory test results, and medical history. We reviewed 812 cases, of which 171 were excluded. The subjects were divided into the following age groups: 18–39, 40–49, 50–59, 60–69, and ≥70 y. The numbers of subjects in each age group were set to be as similar as possible, with the aim of enrolling at least 300 males and 300 females [16].

To measure Tn using multiple devices, the samples were divided into one 500-μl aliquot and at least 4 aliquots of 250 μl, all of which were kept frozen at −70 °C until testing. The aliquoting and freezing process was completed within 24 h of sample collection.

2.2. Establishment of 99th percentile reference limits

The devices used in this study and their specifications are summarized in Table 2. Tn tests were performed using the samples collected to set the 99th percentile reference limit for each device. Roche C6000 was used to measure TnT, and other devices were used to measure TnI. The Abbott Architect tests were performed at Konkuk University Hospital, the Beckman Coulter DxI test was performed at Chungnam National University Hospital, and the remaining 4 tests were performed at Asan Medical Center.

2.3. Precision profile calculation

Samples for the precision profile calculation were donated from healthy volunteers to make commutable frozen serum. The samples were spiked with high concentrations of Tn. Three to five samples spanning the 99th percentile reference limit for each device were prepared. Sample collection and serum isolation were performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) C37-A guidelines, and samples and serum were aliquoted and kept frozen at −70 °C until use [20]. The prepared specimens were measured using each device for 20 days in accordance with CLSI EP5-A3, with 2 runs per day and 2 repeated measurements per run [21]. Based on the results, precision plots at each concentration were created, and the CV at each reference limit in males and females was obtained.

2.4. Statistical analysis

Excel 2010 (Microsoft) was used to calculate the 99th percentile reference limit, and EP Evaluator 8.0 (Data Innovations) and Variance Function Program 12.0 [22] were used for statistical analysis to generate precision profiles. Outliers were identified via Tukey's method and excluded from the final results.

3. Results

In total, 303 samples from men and 338 samples from women were obtained. The sample distributions according to gender and age are shown in Fig. 1. The patients included in this study were 22–86 y, with a median age of 51 (51 y for males and 50 y for females).

The device- and gender-specific distributions of Tn concentrations are shown in Fig. 2, and the results of the 99th percentile reference limit are shown in Table 3. The Roche hsTnT assay showed values similar to those provided by the manufacturer (14 vs. 13.6 pg/ml), but the other devices showed lower values (Siemens Centaur XP, 40 vs. 16.0 pg/ml; Dimension EXL, 56 vs. 34.2 pg/ml; Dimension VISTA, 45 vs. 21.6 pg/ml; and Beckman Coulter DxI, 20 or 40 vs. 13.4 pg/ml) or higher (Abbott Architect, 26.2 vs. 32.1 pg/ml) than those presented by the manufacturers. The differences between the reference limits obtained from this study and those provided by the manufacturers ranged from −60.0% to +22.5%. The reference limit for males was 9.6–116.0% higher than for females for all of the devices tested.

The precision profiles for each device are shown in Fig. 3. The CVs at the 99th percentile reference limit of the Roche C6000, Abbott Architect, and Beckman Coulter DxI were 5.4%, 5.3%, and 8.8%, respectively. In contrast, the CVs of the Siemens Centaur XP, Dimension EXL, and Dimension VISTA were 43.0%, 14.7%, and 32.7%, respectively (Table 3).

Table 2
Instrument information and claimed specifications evaluated in this study. All the values (pg/ml) are from the manufacturers' package inserts.

Company/platform	LoB	LoD	LoQ	10% CV	99th percentile reference limit
Beckman Coulter DxI Access AccuTnI + 3	NP	10	NP	40	20[a]/40[b]
Siemens Dimension EXL 200 TNI	10	17	50	50	56
Siemens Dimension VISTA 500 CTNI	NP	15	NP	40	45
Siemens ADVIA Centaur XP TnI-Ultra	NP	6	NP	30	40
Abbott Architect *STAT* hs-cTnI	1.3[c]	1.9[c]	10.0[c]	4.7	26.2
Roche C6000/E170 hsTnT	3	5	13	13	14

Abbreviations: LoB, limit of blank; LoD, limit of detection; LoQ, limit of quantitation; NP, not provided.
[a] For US population.
[b] For EU population.
[c] These values are the maximum values in the suggested intervals.