Determination of 19 Cardiac Troponin I and T Assay 99th Percentile Values from a Common Presumably Healthy Population

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BACKGROUND: Between-assay comparability of 99th percentiles for cardiac troponin concentrations has not been assessed systematically in a single population for a large number of assays.

METHODS: We determined 99th percentiles for 19 cardiac troponin assays in heparin plasma samples from a population of 272 and 252 presumably healthy males and females, respectively. The assays evaluated included 1 cardiac troponin T (cTnT) assay from Roche and 18 cTnI assays from Abbott, Alere, Beckman, bioMerieux, Instrumentation Laboratory, OrthoClinical Diagnostics, Singulex, Siemens, and Roche. Five of these assays were categorized as high-sensitivity, 9 as sensitive-contemporary, and 5 as point-of-care (POC) assays.

RESULTS: For high-sensitivity cTnI (hs-cTnI) assays 99th percentiles varied from 23 to 58 ng/L. At least 80% of individuals had measurable hs-cTnI, whereas only 25% had measurable high-sensitivity cTnT. All high-sensitivity cardiac troponin assays had 99th percentiles that were 1.2–2.4-fold higher in males than females. For the 9 sensitive-contemporary cTnI assays, 99th percentiles varied from 12 to 392 ng/L, and only the Beckman assay gave measurable concentrations in a substantial portion of the population (35% vs ≤6% for the others). Seven of these 9 assays had 1.3–5.0-fold higher 99th percentiles for males than females. For 5 cTnI POC assays, 99th percentiles varied from <10 to 40 ng/L. The Instrumentation Laboratory assay gave measurable results in 27.8% of study participants vs ≤6% for the others. Correlations were generally poor among assays.

CONCLUSIONS: Among cardiac troponin assays 99th percentile concentrations appear to differ. High-sensitivity assays provide measurable cardiac troponin results in a substantially greater fraction of presumably healthy individuals.

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The Global Task Force is in the process of finalizing the third version of the Universal Definition of Myocardial Infarction. The term acute myocardial infarction (MI) is defined as evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia. One of the key conditions that must be met is the detection of a rise and/or fall of cardiac troponin with at least 1 concentration above the 99th percentile value as determined from a reference population (1, 2). However, there is no universal consensus of how to define a reference population according to age, sex, ethnicity, race, or the number of study participants needed in each category for a total reference population (3). The large majority of 99th percentile values published in the literature and in manufacturers' package inserts for their respective cardiac troponin assays are derived from diverse and often poorly defined study populations (4–9). Often there are fewer than the 325 men and 325 women that have been proposed to be necessary for the appropriate calculation of a 1-tailed 99th percentile value (3, 9). No definitive number of individuals that should be included in a reference population has been defined on the basis of evidence. Furthermore, it is well recognized that there is only poor harmonization and no standardization within cardiac troponin I (cTnI) assays or between cTnI and cTnT assays (10, 11). This situation holds true for both sensitive-contemporary cardiac troponin assays and the newer high-sensitivity assays. There is a perception among clinicians that some type of “conversion table” could be created to translate results from 1 cardiac troponin assay to another. Measurement of cTnI seems to be too complex...
to allow for a simple method to develop the equivalent of an international normalized ratio for cTnI values.

The primary goal of the current study was to determine 99th percentile values for cardiac troponin measured by a large number of sensitive-contemporary, high-sensitivity (hs), and point-of-care (POC) cTnI and cTnT assays using common specimens from a large, presumably healthy population. A secondary goal was to compare and contrast the findings from male and female study participants.

Materials and Methods

After this study received approval from the institutional review board, lithium heparin plasma samples were obtained and banked from approximately 2000 apparently healthy volunteers who had given informed consent for study participation (7). Samples were initially frozen (−70 °C) and then thawed for analysis in a previously published study (7) and then refrozen (−70 °C) a second time. The samples were then rethawed and refrozen (−70 °C) after aliquots were taken for the current study 1.5–2 years before analysis. Each tube was labeled with an alphanumeric code. There were a total of 3 freeze-thaw cycles. Banked samples with sufficient plasma volume (5 mL) for measurements by 19 cTnI or cTnT assays were available from 525 individuals (ages 18–64 years). These included 273 males (ages 19–62 years) and 252 females (ages 18–64 years). The volunteer study participants were defined as a presumably healthy population for this study on the basis of the following criteria determined from a health questionnaire completed by each individual: no history of coronary disease, diabetes, hypertension, arthritis; not pregnant; were not marathon runners or extreme athletes; and were not on any medications for renal disease, muscular skeletal disease, or rheumatoid arthritis; never had symptoms of a heart attack; and cTnI or cTnT assays using common specimens from a healthy population, nor were participants screened with healthy population, nor were participants screened with high-sensitivity (hs), and point-of-care (POC) cTnI and cTnT assays using common specimens from a large, presumably healthy population. A secondary goal was to compare and contrast the findings from male and female study participants.

ASSAYS

Individual sample aliquots were shipped frozen on dry ice to each manufacturer in Europe (Roche, Radiometer) or the US (all other manufacturers) with instructions to thaw and spin the samples before conducting cardiac troponin analysis according to the individual manufacturer guidelines. All manufacturers confirmed that samples arrived frozen, except Radiometer, which precluded their participation in the study. Two low-positive control samples from MI patients, aliquoted and frozen within 24 h of collection, were blindly added to the 525 presumably healthy population samples, so a total of 527 specimens were shipped to each company. Each manufacturer then analyzed the specimens and blindly reported their results back to the laboratory of F.S. Apple for analysis.

The evaluated assays included the Roche Cobas e601 hs-cTnI assay and the following cTnI assays: the Abbott ARCHITECT i2000sr STAT cTnI, Abbott ARCHITECT i2000sr STAT hs-cTnI, and Abbott AxSYM Troponin-I, and Abbott i-STAT 300 cTnI (Abbott Diagnostics); the Alere-Triage Cardio3 cTnI (Alere); the Beckman Access 2 hs-cTnI and Beckman Access 2 modified sensitive cTnI (Beckman Coulter); the bioMerieux Vidas cTnI Ultra; the IL (Instrumentation Laboratories) GEM Immuno cTnI prototype instrument and assay (Instrumentation Laboratories); the OCD (Ortho Clinical Diagnostics) Vitros 3600 cTnI ES (Ortho Clinical Diagnostics); the Singulex ERENNA hs-cTnI (Singulex); the Siemens Centaur Tnl Ultra, Siemens Dimension EXL 200 cTnI, Siemens Dimension Vista hs-cTnI, Siemens Dimension Vista cTnI, Siemens Immulite 2000 XPI cTnI, and Siemens Stratus CS cTnI (Siemens Healthcare Diagnostics); and the Roche Cobas e601 cTnI (Roche Diagnostics). Companies that were approached but unable to participate regarding specific assays were: Beckman Coulter, cTnI on the DXi and Nanosphere, Verigene hs-cTnI.

The 99th percentile values (ng/L) were determined by 1-tailed nonparametric statistics according to CLSI guideline C28-A3. We excluded outliers for each assay cohort using the method of Reed et al. (12), and 99th percentiles were recalculated when appropriate. After initial data review it was noted that 1 individual had increased cardiac troponin measured by 11 of 19 assays. Data for this individual were excluded from the final analyzed cohort (n = 524). Statistical analyses were performed with MedCalc 12.2.1.0 (www.medcalc.org).

Results

Table 1 reports the 99th percentile values, with 90% CIs, for the 4 hs-cTnI assays and 1 hs-cTnT assay, the 9 sensitive-contemporary cTnI assays, and the 5 POC assays studied. Assays from 9 different manufacturers were included. For some manufacturers, several assays from the same manufacturer were tested, including Abbott (n = 4), Siemens (n = 6), and Roche (n = 2). The 5 POC assays were the Abbott i-STAT, Alere Triage, bioMerieux-Vidas cTnI Ultra, IL-Gem Immuno prototype, and Siemens Stratus CS. Both Table 1 and Fig. 1 display the similarities and differences observed.
Table 1. 99th Percentile values in a presumably healthy population measured by high-sensitivity, sensitive-contemporary, and POC cTnI and cTnT assays.

<table>
<thead>
<tr>
<th>Manufacturer • analyzer • assay</th>
<th>No. of results</th>
<th>LoD, ng/L</th>
<th>Measurable values &gt; LoD, %</th>
<th>99th percentile of all study participants (90% CI), ng/L</th>
<th>Excluded value(s), ng/L</th>
<th>All subjects 99th percentile with exclusions (90% CI), ng/L</th>
<th>Male 99th percentile, ng/L</th>
<th>Female 99th percentile, ng/L</th>
<th>Positive control values, ng/L</th>
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<tr>
<td>Abbott • ARCHITECT i2000 STAT • hs-cTnI</td>
<td>524</td>
<td>1.2</td>
<td>96</td>
<td>23 (16–63)</td>
<td>—</td>
<td>36</td>
<td>15</td>
<td>111</td>
<td>83</td>
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<tr>
<td>Beckman • Access 2 • hs-cTnI</td>
<td>524</td>
<td>2.5</td>
<td>80</td>
<td>32 (22–69)</td>
<td>—</td>
<td>52</td>
<td>23</td>
<td>69</td>
<td>50</td>
</tr>
<tr>
<td>Roche • Cobas e601 • hs-cTnT</td>
<td>524</td>
<td>5</td>
<td>25</td>
<td>15 (13–28)</td>
<td>95</td>
<td>15 (13–21)</td>
<td>20</td>
<td>13</td>
<td>17</td>
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<tr>
<td>Siemens • Dimension Vista • hs-cTnI</td>
<td>503</td>
<td>0.5</td>
<td>86</td>
<td>58 (34–125)</td>
<td>—</td>
<td>81</td>
<td>42</td>
<td>—</td>
<td>112</td>
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<td>524</td>
<td>0.09</td>
<td>100</td>
<td>40 (25–215)</td>
<td>636–215</td>
<td>31 (21–47)</td>
<td>36</td>
<td>30</td>
<td>45</td>
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<tr>
<td>Abbott • ARCHITECT i2000 STAT • cTnI</td>
<td>524</td>
<td>9</td>
<td>2</td>
<td>13 (&lt;9–23)</td>
<td>—</td>
<td>20</td>
<td>&lt;9</td>
<td>96</td>
<td>76</td>
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<tr>
<td>Abbott • AxSYM • Tropinin-l</td>
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<td>20</td>
<td>3</td>
<td>34 (22–39)</td>
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<td>38</td>
<td>29</td>
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<td>85</td>
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<td>524</td>
<td>2.5</td>
<td>35</td>
<td>56 (27–100)</td>
<td>—</td>
<td>48</td>
<td>85</td>
<td>84</td>
<td>61</td>
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<td>OCD • Vitro 3600 • cTnI ES</td>
<td>524</td>
<td>12</td>
<td>2</td>
<td>19 (12–22)</td>
<td>73</td>
<td>18 (12–22)</td>
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<td>86</td>
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<tr>
<td>Roche • Cobas e601 • cTnI</td>
<td>524</td>
<td>160</td>
<td>1</td>
<td>184 (&lt;160–706)</td>
<td>2083</td>
<td>&lt;160 (&lt;160–515)</td>
<td>300</td>
<td>60</td>
<td>102</td>
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<td>Siemens • Centaur • Tnl Ultra</td>
<td>523</td>
<td>6</td>
<td>6</td>
<td>12 (10–16)</td>
<td>22</td>
<td>12 (10–14)</td>
<td>14</td>
<td>11</td>
<td>91</td>
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<td>Siemens • Dimension EXL 200 • cTnI</td>
<td>524</td>
<td>17</td>
<td>2</td>
<td>34 (17–44)</td>
<td>—</td>
<td>39</td>
<td>22</td>
<td>71</td>
<td>55</td>
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<tr>
<td>Siemens • Dimension Vista • cTnI</td>
<td>523</td>
<td>15</td>
<td>1</td>
<td>21 (&lt;15–39)</td>
<td>—</td>
<td>30</td>
<td>&lt;15</td>
<td>89</td>
<td>62</td>
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<tr>
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<td>479</td>
<td>100</td>
<td>5</td>
<td>392 (190–520)</td>
<td>—</td>
<td>394</td>
<td>451</td>
<td>110</td>
<td>70</td>
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<tr>
<td><strong>POC</strong></td>
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<tr>
<td>Abbott • i-STAT 300 • cTnI</td>
<td>524</td>
<td>20</td>
<td>6</td>
<td>39 (27–53)</td>
<td>—</td>
<td>37</td>
<td>41</td>
<td>110</td>
<td>50</td>
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<tr>
<td>Alere • Triage • Cardio3 cTnI</td>
<td>521</td>
<td>10</td>
<td>1</td>
<td>12 (&lt;10–16)</td>
<td>35</td>
<td>10 (&lt;10–16)</td>
<td>11</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>bioMerieux • Vidas • cTnI Ultra</td>
<td>524</td>
<td>10</td>
<td>1</td>
<td>&lt;10 (&lt;10–40)</td>
<td>—</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>80</td>
<td>50</td>
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<tr>
<td>IL • GEM Immuno • cTnI</td>
<td>524</td>
<td>1.3</td>
<td>28</td>
<td>15 (10–26)</td>
<td>189–26</td>
<td>14 (8–17)</td>
<td>12</td>
<td>14</td>
<td>27</td>
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<tr>
<td>Siemens • Stratus CS • cTnI</td>
<td>498</td>
<td>30</td>
<td>2</td>
<td>40 (&lt;30–40)</td>
<td>90</td>
<td>30 (&lt;30–40)</td>
<td>40</td>
<td>&lt;30</td>
<td>60</td>
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between the 99th percentile values and the percentages of measurable cardiac troponin values above each assay’s limit of detection (LoD) for the 19 assays. In addition, Table 1 shows the outlier concentrations that were statistically excluded as well as the concentrations measured by each assay on positive control samples 1 and 2.

For the hs-cTnI assays, there was a 2.5-fold difference in 99th percentile values, ranging from 23 to 58 ng/L, without the exclusion of any values. None of the hs-cTnI assays had the same concentrations for the 99th percentile. When statistical criteria for exclusion were applied, data for 2 study participants were excluded (Singulex assay), which lowered the 99th percentile to 31 ng/L from 40 ng/L. The hs-cTnT 99th percentile value was 15 ng/L, and this value did not change following the statistical exclusion of 1 study participant. All hs-cTnI assays provided measurable cardiac troponin concentration results in at least 80% of the reference individuals compared with 25% for the hs-cTnT assay (Fig. 1). Ratios of the hs-cTnI 99th percentile values divided by the LoDs of the corresponding assays ranged from 12.8 to 344. This ratio for the hs-cTnT assay was 3.0. All 5 high-sensitivity cardiac troponin assays had 99th percentile values for males that were 1.2 to 2.4 times higher than those for females. For all assays, the concentrations for control 1 were higher than for control 2. For hs-cTnI, the control concentrations ranged from 45 to 111 ng/L (control 1) and 35 to 112 ng/L (control 2). Although the absolute concentrations for each control varied by assay, the ratios of control 1 to control 2 values were similar (range 1.29–1.42) for all 5 high-sensitivity assays. Poor correlations were observed among the 4 high-sensitivity assays ($r < 0.28$), with the exception of a modest correlation between the Abbott and Siemens assays ($r = 0.72$).

For the 9 sensitive-contemporary cTnI assays (Table 1, Fig. 1), there was a 32-fold (12–392 ng/L) range of 99th percentile values without excluding any values. Two of the sensitive-contemporary assays had the same 99th percentile (Abbott AxSYM and Siemens Dimension EXL). The Roche and Siemens Immulite 99th percentiles were substantially higher than all other assays.
(184 ng/L, 392 ng/L, respectively). Following data exclusion based on statistical criteria, 3 study participants for 3 assays were excluded, lowering the 99th percentile values from 19 to 18 ng/L (OCD-Vitros), and 184 to <160 ng/L (Roche); 99th percentiles values for the Siemens Centaur Ultra assay were not changed following data exclusion. Only the Beckman assay determined measurable concentrations of cTnI in a substantial fraction of study participants, 35%. All the other assays gave measured concentrations in ≤6% of reference individuals, a substantially lower fraction than observed for the hs-cTnI assays. Ratios of the measured 99th percentile values divided by the corresponding LoDs ranged from 0.5 to 22.4, results substantially lower than for the hs-cTnI assays. Seven of 9 assays demonstrated 1.3–5.0-fold higher 99th percentiles for males than for females. For all assays, the concentrations for control 1 were higher than for control 2. The control concentrations ranged from 71 to 118 ng/L (control 1) and 55–90 ng/L (control 2). Although the absolute concentrations for each control varied by assay, the ratios of the measured 99th percentile values divided by the corresponding LoDs ranged from 0.5 to 22.4, results substantially lower than for the hs-cTnI assays. Seven of 9 assays demonstrated 1.3–5.0-fold higher 99th percentiles for males than for females. For all assays, the concentrations for control 1 were higher than for control 2. The control concentrations ranged from 71 to 118 ng/L (control 1) and 55–90 ng/L (control 2). Although the absolute concentrations for each control varied by assay, the ratios of the measured 99th percentile values divided by the corresponding LoDs ranged from 0.5 to 22.4, results substantially lower than for the hs-cTnI assays. Seven of 9 assays demonstrated 1.3–5.0-fold higher 99th percentiles for males than for females. For all assays, the concentrations for control 1 were higher than for control 2. The control concentrations ranged from 71 to 118 ng/L (control 1) and 55–90 ng/L (control 2). Although the absolute concentrations for each control varied by assay for control 1, 3 assays gave comparable concentrations for control 2. The ratios of control 1 to control 2 concentrations varied from 1.20 to 2.20. Poor correlations were found among all assays (r < 0.16).

Discussion

The current study is unique in its comparison of 99th percentile values from 19 different cardiac troponin assays in the same large, presumably healthy population. The data of the study have yielded several novel findings. Great diversity was observed for the 99th percentile values for the 4 hs-cTnI assays (2.5-fold variation), the 9 sensitive-contemporary cTnI assays (32-fold variation), and the 5 POC assays (4.0-fold variation). Only 2 of 9 assays within the sensitive-contemporary group had the same 99th percentiles, and 2 POC assays had 99th percentile values that matched those for 2 high-sensitivity assays. Otherwise the determined 99th percentile values were all different. Our data support those from previous studies that compared substantially fewer assays among common reference groups (3–9). The poor correlation observed among the large majority of cTnI assays does not allow for a correction factor that would adequately harmonize all results. We strongly encourage this approach as a method to harmonize assay results. A follow-up study is currently underway in our laboratory to examine specimens from patients with a diagnosis of MI. We expect that the results of this study will further support the diversity and the lack of correlation between cTnI assay concentrations as exemplified by the large variability between the 2 MI control specimens examined (Table 1). Our data suggest that each individual assay must be evaluated independently for clinical use.

No assays besides the high-sensitivity cardiac troponin assays and the modified, sensitive Beckman and POC IL GEM cTnI assays gave a measurable concentration above the assay’s LoD for more than 6% of individuals in our presumably healthy population. This implies that with most assays patients who present to the emergency department very early after a myocardial injury will more likely have an undetectable baseline cardiac troponin concentration (13, 14). These observations support studies that demonstrate clinical sensitivities that are <80% in patients who present early after the onset of acute MI (15, 16).

For the Roche cTnI assay our findings substantially differed from the FDA-cleared package insert for this assay, which reports a 99th percentile value of 160 ng/L, the same as the assay’s reported LoD. We found that the 99th percentile was 184 ng/L without excluded values and <160 ng/L with excluded values, and only 1% of reference individuals had a measurable concentration, based on an LoD of 160 ng/L. The clinical diagnostic accuracy of this assay has been poor compared with other cTnI and hs-cTnT assays for MI diagnosis.
Since the completion of our study, the FDA has removed this assay from the marketplace in the US. The large variation among the 99th percentile values and the poor correlations among the sensitive-contemporary cTnI assays (except for the modest correlation between the 2 Siemens Dimension assays) further suggest that there is a lack of standardization. The lack of correlation among assays was also skewed by the fact that so few individuals had measurable concentrations.

The 4 hs-cTnI assays gave measurable concentrations for at least 80% of individuals within the presumably healthy population we studied, confirming previously published data (4–6). We have noted previously that the use of different presumably healthy or normal reference populations for determination of 99th percentile values can give different 99th percentile concentrations with the use of the same assays used in our study (4–9). Such differences are to be expected owing to the heterogeneity between study groups that can vary by age, sex, ethnicity, and race. In most studies conducted in a reference or presumably healthy population range, the demographics of study participants are poorly described. The findings for several of the 99th percentile values in the current study of a presumably healthy population were different from those previously reported for similar assays. For example, for the Beckman hs-cTnI assay we observed a value of 32 ng/L (80% measurable) compared to 10 ng/L (71.2% measurable) in 542 study participants reported by Venge et al. (4) and 9 ng/L (70% measurable) in 125 study participants reported by Kavask et al. (18), and for the Singulex hs-cTnI assay in a previous study from our laboratory in 348 study participants we found a 99th percentile of 10 ng/L (100% measurable) (6) compared to 31 ng/L (100% measurable) in the current study. For the Roche hs-cTnT assay, our 99th percentile of 15 ng/L was similar to previous reports of 14 ng/L (5, 19, 20), and our finding of measurable concentrations in only 25.4% of presumably healthy study participants was comparable to 32% reported by Saenger et al. in 533 study participants (5). Differences between the reporting of measurable hs-cTnT concentrations may have resulted from the use of heparin plasma in the current study compared with serum in previously published studies. In addition, a recent Roche technical bulletin addressed poor recovery between different reagent lots in the low cTnT concentration range using the hs assay (21). In the current study we used the new reagent lot (lot 167 345) introduced by Roche for improved recovery. These observations demonstrate that numerous parameters are likely to affect findings in different studies of presumably healthy individuals. These include the previously noted diversity of study participants by age, sex, race, and ethnicity, as well as unknown underlying cardiovascular disease that may have gone undetected at study participant enrollment and impaired specimen integrity following freeze–thaw processing. The unique aspect of the current study was that all the variables were constant for all samples and all measurements on all analyzers were made under similar conditions. We are not claiming that these samples are the perfect picture of a normal-range study. However, the results we obtained with these samples do demonstrate how different assays, manufactured by different companies and in some cases using the same antibodies and calibration materials within companies, give substantially different results. Simply put, one needs to know the specifics of both the assay being used in clinical practice and the individuals of the population being studied.

With the LoD concentrations being 12.8–344-fold less for hs-cTnI assays (3.0 for hs-cTnT) than the 99th percentile concentrations, the use of hs-assays in clinical practice will allow the detection of small cardiac troponin changes evolving from low cardiac troponin concentrations within the reference interval to small increases above the 99th percentile value (21–23). Such monitoring with hs assays will reflect real δ changes over a 1–3-h window (22–26) that sensitive-contemporary assays may not detect until the increase in cardiac troponin goes above the 99th percentile after as long as 6 h (15, 25). Monitoring with high-sensitivity assays will be important in improving our understanding of the role of absolute concentration changes vs percentage δ changes regarding improved clinical specificity for MI compared to another myocardial injury mechanism. Given the heterogeneity of assays, it is likely that whatever metric is used for a δ will need to be individualized by assay (25–27). With proper education of both laboratory and clinicians, the transition from sensitive-contemporary and POC assays to high-sensitivity cardiac troponin assays should provide more and different information to numerous clinical specialties. An unexpected increased cardiac troponin value measured by a high-sensitivity assay in clinical practice will challenge the clinician in 2012 or 2013 in a similar manner as occurred when biomarkers transitioned from creatine kinase MB to cardiac troponin in 2000 (28, 29).

All 4 hs-cTnI assays demonstrated substantially (1.2–2.4-fold) higher 99th percentile values in males vs females. These data support those from other studies (5, 6), albeit there are exceptions (4), which have identified a sex difference with the use of high-sensitivity cardiac troponin assays. We suspect that the studies that do not show sex difference are likely underpowered. Consideration will need to be given to establishing separate decision values according to patient sex in clinical practice, as used for creatine kinase MB. Also,
how male/female differences are implemented in clinical trials or studies during the MI adjudication process will need to be considered. For the sensitive-contemporary assays significant male/female differences were also observed in 7 of the 9 assays and 1 of 5 POC assays. The male/female difference noted for a POC assay (Siemens CS) has not been previously reported in the literature. The lack of sex-defined cutoffs may explain why women have fewer increases in cardiac troponin and may be why women in some studies appear to have different clinical outcomes when they present with acute coronary syndrome (30). We strongly advocate for separate 99th percentile values based on sex, regardless of the assay.

Even for manufacturers that market numerous cardiac troponin assays, different assays showed substantially different 99th percentile values. Therefore, caution needs to be exercised and clinicians educated to the fact that identical cardiac troponin concentrations from 2 different assays marketed by the same manufacturer cannot be expected to have similar interpretations. In our current study, for example, Abbott assays varied 3-fold and Siemens assays varied 33-fold.

Several limitations to the current study should be mentioned. First, the 4th generation Roche cTnT assay was not evaluated owing to a decision of the manufacturer. This assay is being phased out worldwide outside the US with the implementation of the Roche hs-cTnT assay. It is expected that following FDA clearance of the hs-cTnT assay, the hs-cTnT will also replace the 4th generation assay in the US. Second, this study was not designed to determine the total imprecision (%CV) of each assay at the 99th percentile values to determine whether these assays would be clinically acceptable according to the Universal Definition of MI guidelines (1) or be used as part of a scorecard classification (17). %CV information on most assays can be found in a recent review article (3). Third, the presumably healthy study participants used in this study, as for most reference-interval studies, were identified via a health questionnaire interview and not by a clinician using additional screening tools such as imaging, echocardiogram, electrocardiogram, or measurement of a natriuretic peptide (brain natriuretic peptide or N-terminal-pro brain natriuretic peptide) as a surrogate myocardial dysfunction biomarker; the latter tools we feel are often cost prohibitive. It should be noted that in one study in which reference individuals were screened by more stringent criteria, including imaging, the 99th percentile values decreased with increasing screening (9). Fourth, the samples from presumably healthy individuals all went through 3 freeze–thaw cycles before analysis. How this may have affected some results for some assays cannot be quantified. However, our internal data for 2 of the sensitive-contemporary assays have demonstrated less than a 10% cTnI concentration variation from the 3 freeze thaw cycles (data not shown).

In conclusion, our findings underscore the importance of knowing the analytical performance characteristics of the individual cardiac troponin assay used in the clinical setting as well as in research. The large majority of cTnI assays have different 99th percentile values. High sensitivity cTnT and cTn assays will provide the ability to measure cardiac troponin in a substantially greater fraction of the presumably healthy population, providing a baseline that may assist in patient management (31), and in defining true normality, as well as providing the potential for primary prevention (32) for improving long-term outcomes. We support the use of sex-specific 99th percentile values as a guide for clinicians to assist in the diagnosis of acute MI. We emphasize that different populations of presumably healthy individuals may display different values when similar assays are evaluated in different geographical environments.

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References