Sex-Specific Versus Overall Clinical Decision Limits for Cardiac Troponin I and T for the Diagnosis of Acute Myocardial Infarction

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Review

Sex-Specific Versus Overall Clinical Decision Limits for Cardiac Troponin I and T for the Diagnosis of Acute Myocardial Infarction: A Systematic Review

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BACKGROUND: The overall clinical decision limits of high-sensitivity cardiac troponin I (hs-cTnI; 26 ng/L) and T (hs-cTnT; 14 ng/L) may contribute to underdiagnosis of acute myocardial infarction in women. We performed a systematic review to investigate sex-specific and overall 99th percentiles of hs-cTnI and hs-cTnT derived from healthy reference populations.

CONTENT: We searched in PubMed and EMBASE for original studies, and by screening reference lists. Reference populations designed to establish 99th percentiles of hs-cTnI (Abbott) and/or hs-cTnT (Roche), published between January 2009 and October 2017, were included. Sex-specific and overall 99th percentile values of hs-cTnI and hs-cTnT were compared with overall clinical decision ranges (hs-cTnI, 23–30 ng/L; hs-cTnT, 13–25 ng/L). Twenty-eight studies were included in the systematic review. Of 16 hs-cTnI and 18 hs-cTnT studies, 14 (87.5%) and 11 (61.1%) studies reported lower female-specific hs-cTn cutoffs than overall clinical decision ranges, respectively. Conversely, male-specific thresholds of both hs-cTnI and hs-cTnT were in line with currently used overall thresholds, particularly hs-cTnT (90% concordance). The variation of estimated overall 99th percentiles was much higher for hs-cTnI than hs-cTnT (29.4% vs 80.0% of hs-cTnI and hs-cTnT studies reported values within the current overall clinical decision range, respectively).

SUMMARY: Our data show substantially lower female-specific upper reference limits of hs-cTnI and hs-cTnT than overall clinical decision limits of 26 ng/L and 14 ng/L, respectively. The statistical approach strongly affects the hs-cTnI threshold. Downward adjustment of hs-cTn thresholds in women may be warranted to reduce underdiagnosis of acute myocardial infarction in women.

Women have lower 1-year survival rates after an acute myocardial infarction (AMI)[7] than men *(1, 2)*. Twenty-three percent of women vs 18% of men will die within 1 year after a first AMI *(2)*. Atypical symptomatology in women with AMI is a possible cause of their undertreatment, but underdiagnosis also may play a crucial role in the existing gap between women and men *(3)*. Indeed, in a population-based cohort study investigating the incidence of (un)recognized AMIs in women and men, the proportion of incident AMIs remaining clinically unrecognized was higher for women than men (54% for women vs 33% for men) *(4)*. However, the incidence of missed AMIs in women from application of the common diagnostic cardiac troponin algorithm is not well established, as prospective trials on this matter are lacking. The urgent need for conducting research to understand these disparities between women and men has recently been recognized *(3)*.

An algorithm using sex-specific cutoff concentrations for cardiac troponins is recommended, but not required, in the third universal definition of myocardial infarction *(5)*. The European Society of Cardiology guidelines require a defined "rise and/or fall" of high-sensitivity cardiac troponin I (hs-cTnI) or T (hs-cTnT) between serial measurements with at least 1 value above the 99th percentile upper reference limits (URLs) of hs-cTn from a healthy reference population; however, the European Society of Cardiology guidelines do not recommend a sex-specific algorithm *(6)*.

The 99th percentile URL of cardiac troponin from a reference population for diagnosis of AMI is proposed by the Joint European Society of Cardiology/American

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[7] Nonstandard abbreviations: AMI, acute myocardial infarction; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; URL, upper reference limit.

College of Cardiology Committee for the Redefinition of Myocardial Infarction, driven by the demonstration that any amount of detectable troponin release is associated with an increased risk of new adverse cardiac events *(7, 8)*. Defining adequate clinical decision limits of hs-cTnI and hs-cTnT was challenging owing to the lack of a biomarker-independent gold standard for diagnosis of AMI. As a result, common clinical thresholds are based on a statistical approach rather than a biological approach (which would have been preferable). Why the 99th percentile URL was chosen rather than the more common 97.5th percentile URL was probably a result of the much lower sensitivity (and precision) of the assays available around the millennium shift when the first universal definition document was created.

The 2 clinical troponin assays that meet the analytical properties of a high-sensitivity assay are the Abbott ARCHITECT STAT hs-cTnI assay and the Roche hs-cTnT assay with recommended overall clinical decision limits of 26 ng/L and 14 ng/L, respectively (package insert) *(9)*.

Two concerns have been raised that may hamper the diagnosis of AMI in women. First, the clinical decision limits of hs-cTnI and hs-cTnT are overall thresholds and, therefore, make no distinction between women and men. The introduction of high-sensitivity troponin assays has led to detection of troponin concentrations in a lower range and revealed the presence of female/male differences when assessing the 99th percentile values of hs-cTn *(10, 11)*. These data imply that the overall clinical decision limits of hs-cTn are too high for women, which may contribute to underdiagnosis of AMI *(12)*. The second concern is that the overall clinical decision limits of hs-cTnI and hs-cTnT might not be biologically equivalent, as they are not derived from a single reference population *(12, 13)*. Wildi et al. showed that 1 of 5 diagnoses of AMI is inconsistent with the overall clinical decision limits of hs-cTnI and hs-cTnT. Their data suggested that the overall cutoff of 26 ng/L for hs-cTnI should be lowered to 9 ng/L to become biologically equivalent to the overall hs-cTnT threshold of 14 ng/L *(13)*. Given the lower circulating troponin concentrations in women than men, the effect of a higher diagnostic threshold for the hs-cTnI assay is possibly more problematic for women than men.

Several studies have used healthy reference populations to establish sex-specific and overall 99th percentile URL of hs-cTnI and/or hs-cTnT. Evaluating these sex-specific and overall cut-offs could (*a*) provide direction on whether sex-specific clinical decision limits of hs-cTn should be considered for further investigation in randomized controlled trials, and (*b*) assess whether the currently used overall clinical decision limits of 26 ng/L for hs-cTnI and/or 14 ng/L for hs-cTnT need critical reinvestigation. We performed a systematic review to investigate sex-specific and overall 99th percentile URL of hs-cTnI and hs-cTnT derived from healthy reference populations.

Materials and Methods

SEARCH STRATEGY AND ELIGIBILITY CRITERIA

The study was conducted according to the principles of the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) guidelines *(14)*. We searched in PubMed and EMBASE for original studies published between January 2009 and October 2017 (see supplemental data in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue7). Additionally, we screened the reference lists of relevant articles.

Reference cohorts designed to assess sex-specific and/or overall 99th percentile URL of hs-cTnI and/or hs-cTnT from healthy reference individuals ≥18 years were included. Only studies written in English were included. Exclusion criteria were reference populations with a sample size <300 *(15)* and conventional troponin assays, i.e., assays other than Abbott ARCHITECT STAT hs-cTnI assay or Roche hs-cTnT assay.

STUDY SELECTION AND DATA EXTRACTION

Studies were selected by 2 independent reviewers (DMK and EBNJJ). Initial screening of all identified records was performed on title and abstract. Of potentially eligible studies, full texts were obtained and assessed for inclusion. In case of duplicate studies or overlapping reference populations, the most recently published article was selected. Data were extracted by 2 independent reviewers (DMK and EBNJJ). All disagreements were resolved in the presence of a third reviewer (SJRM). Using a standardized form, the following information was collected: author, year of publication, troponin assay, population and setting, sample size, statistical approach, and 99th percentile values of hs-cTn (with their 90% CI or 95% CI). Previously, Sandoval et al. recommended a set of criteria for defining a reference population *(16)*. According to these criteria, we collected sex-specific and overall 99th percentile URL from the most stringently screened selection of a reference population. In cases when the 99th percentile values of hs-cTn from the most stringently screened reference cohort were not reported, this additional information was requested from the study authors by e-mail.

DATA SYNTHESIS

Owing to substantial heterogeneity in screening criteria and applied methods for determination of 99th percentile URL, a qualitative data synthesis was performed. Female-specific, male-specific, and overall 99th percentile URL of hs-cTnI and hs-cTnT were depicted in

Table 1 Characteristics of reference populations in which package insert clinical decision limits of hs-cTnI and hs-cTnT were derived.[a]

Study characteristics	hs-cTnI	hs-cTnT
Publication	Rogers et al. 2013 *(17)*	Saenger et al. 2011 *(18)*
Country	US	US/Europe
Description cohort	US population	3 US and 5 European sites
Women/men, n (%)	765 (50)/766 (50)	265 (49.7)/268 (50.3)
Age range, years	21–75	20–71
Statistical approach	Robust	NP[b]/Dixon
Female-specific 99th percentile	16 [14–18]	9
Male-specific 99th percentile	34 [29–39]	16
Overall 99th percentile	26 [23–30]	14 (13–25)

[a] The 99th percentile URLs are reported in ng/L and are presented with their 90% CI (in brackets) or 95% CI (in parentheses).
[b] NP, nonparametric.

graphs. Outcomes were compared with the package insert-defined 90% or 95% CI ranges for the overall clinical decision thresholds of hs-cTnI and hs-cTnT from 23 to 30 ng/L and from 13 to 25 ng/L, respectively. Such graphs demonstrate to what extent reported sex-specific 99th percentile URLs of cardiac troponins match with currently used decision limits, and whether reconsideration of the overall clinical decision limits is warranted.

To investigate the hypothesis that reference population heterogeneity contributes to discrepancy between 99th percentile values of hs-cTnI and hs-cTnT, we selected the studies that directly compared the 99th percentile URL of hs-cTnI and hs-cTnT derived from a single reference population. Additionally, we investigated the differences between the 99th percentile URLs of hs-cTnI and hs-cTnT, also stratified by sex.

Results

Full-text screening of 67 studies revealed that 37 studies did not meet the inclusion criteria (see Fig. 1 of the online Data Supplement). The 2 original studies that were the basis for currently used decision limits of hs-cTnI and hs-cTnT were also excluded (Table 1) *(17, 18)*. Twenty-eight studies were included in the qualitative synthesis *(10–12, 19–43)*. Six studies derived 99th percentile values for both hs-cTnI and hs-cTnT (Table 2; see also Table 1 in the online Data Supplement) *(11, 12, 19–22)*, 11 studies determined 99th percentile values of hs-cTnI (Table 3; see also Table 2 of the online Data Supplement) *(23–33)*, and 11 studies determined 99th percentile values of hs-cTnT (Table 3; see also Table 3 of the online Data Supplement) *(10, 34–43)*.

Table 2 Characteristics of included studies that reported sex-specific and overall 99th percentiles of hs-cTnI and hs-cTnT (n = 6).

Publication	Country	Cohort description	Female/male, n (%)	Age range, years	Statistical approach
Apple et al. 2012 *(11)*	US	Healthy volunteers	252 (48)/273 (52.0)	18–64	NP[a]/Dixon
Hickman et al. 2017 *(19)*	Australia	CHS and a younger cohort of population-based individuals	hs-cTnI 262 (54.1)/222 (45.9) hs-cTnT 262 (53.5)/228 (46.5)	48–95	NP/Dixon
Kimenai et al. 2016 *(12)*	the Netherlands	Healthy individuals from The Maastricht Study	806 (52.5)/729 (47.5)	40–75	NP/Dixon
Ko et al. 2017 *(20)*	Korea	Individuals seen for health checks	338 (52.7)/303 (47.3)	22–86	NP/Tukey
Mueller et al. 2016 *(21)*	Austria	Blood donors	143 (36)/259 (64)	<65	NP
Ungerer et al. 2016 *(22)*	Australia	Blood donors	705 (35.2)/1299 (64.8)	Not reported	NP

[a] NP, nonparametric; CHS, Canberra Heart Study.

Table 3 Characteristics of included studies that reported sex-specific and overall 99th percentiles of hs-cTnI or hs-cTnT (n = 22).

Publication	Country	Cohort description	Female/male, n (%)	Age range, years	Statistical approach
hs-cTnI studies (n = 11)					
Abe et al. 2018 (*23*)	Japan	Individuals who visited the Japanese Red Cross Medical Center for health checkup	385 (55.2)/313 (44.8)	23–86	Smoothed empirical likelihood/ Dixon
Aw et al. 2013 (*24*)	Singapore	Individuals participating in a health screening program	523 (46.7)/597 (53.3)	35–65	NP
Collinson et al. 2015 (*25*)	UK	Individuals from 7 representative local community practices in Harrow, North London	195 (53.3)/171 (46.7)	>45	NP
Eggers et al. 2016 (*26*)	Sweden	Community inhabitants of the PIVUS study	255 (48.9)/266 (51.1)	70	NP/Dixon
Ji et al. 2016 (*27*)	South Korea	Individuals participating in health screening program	428 (50.1)/426 (49.9)	18–90	NP/Dixon
Koerbin et al. 2012 (*28*)	Australia	CHS and a younger cohort of population-based individuals	276 (55.5)/221 (44.5)	20–84	Not reported
Krintus et al. 2014 (*29*)	Poland	Apparently healthy individuals and blood donors recruited in 7 European countries	776 (43.9)/993 (56.1)	18–91	NP/Dixon
Krintus et al. 2015 (*30*)	Poland	Presumably healthy individuals from 9 European countries (health checkups, GHS, NOBIDA, healthy volunteers)	354 (55.8)/280 (44.2)	GHS 35–74 NOBIDA ≥18	NP/Dixon
Krintus et al. 2016 (*31*)[a]	Poland	Presumably healthy individuals recruited in various workplaces in Bydgoszcz	208 (51.0)/200 (49.0)	18–70	NP/Dixon
Li et al. 2017 (*32*)	China	Individuals seen for health checkup	754 (50.8)/731 (49.2)	18–85	NP
Zeller et al. 2015 (*33*)	Germany	GHS	1316 (50.4)/1292 (49.6)	35–74	Hajek's method

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Table 3 Characteristics of included studies that reported sex-specific and overall 99th percentiles of hs-cTnI or hs-cTnT (n = 22) (*Continued from page 1037*).

Publication	Country	Cohort description	Female/male, n (%)	Age range, years	Statistical approach
hs-cTnT studies (n = 11)					
Aw et al. 2017 *(34)*	Singapore	Individuals participating in a health screening program	543 (50)/543 (50)	40–65	NP
Collinson et al. 2012 *(35)*	UK	Individuals from 7 representative local community practices in Harrow, North London	170 (54.1)/144 (45.9)	>45	NP
Fan et al. 2014 *(36)*[a]	China	Individuals seen for health check	305 (44.9)/374 (55.1)	18–89	NP
Franzini et al. 2015 *(37)*	Italy	G. Monasterio Tuscany Foundation, San Maurizio Regional Hospital, MHELP, CAMELIA, and MAREA	368 (43.0)/488 (57.0)	20–64	NP/Tukey
Gaggin et al. 2014 *(38)*	US/Vietnam	NSEW Trial	US 312 (55.2)/253 (44.8) Vietnam 292 (49.3)/300 (50.7)	>18	Not reported
Giannitsis et al. 2010 *(39)*	Germany	Apparently healthy volunteers and blood donors	307 (49.8)/309 (50.2)	20–71	Not reported
Gore et al. 2014 *(40)*	US	DHS/ARIC/CHS	DHS 1105 (55.9)/873 (44.1) ARIC 4603 (60.8)/2972 (39.2) CHS 885 (64.4)/489 (35.6)	DHS 30–65 ARIC 45–64 CHS >65	NP
Gunsolus et al. 2017 *(41)*	US	Subjects from the AACC Universal Sample Bank	339 (48.8)/355 (51.2)	19–91	NP/Dixon
Hammarsten et al. 2012 *(42)*[a]	Sweden	MONICA study	315 (78.2)/88 (21.8)	25–64	NP
Mingels et al. 2009 *(10)*	the Netherlands	Individuals seen for health check	215 (44.9)/264 (55.1)	≥18	NP
Yang et al. 2016 *(43)*[a]	China	Individuals from northern China, covering cities and villages	661 (53.0)/585 (47.0)	18–<70	NP/Dixon

[a] No female-specific and/or male-specific 99th percentile URLs of hs-cTn reported.

NP, nonparametric; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; CHS, Canberra Heart Study; GHS, Gutenberg Health Study; NOBIDA, Nordic Reference Interval Project Bio-bank and Database; MHELP, Montignoso HEart Lung Project; CAMELIA, CArdiovascular risks, MEtabolic syndrome, Liver, and Autoimmune; MAREA, Metabolic Alterations in Reggio Calabria Adolescents; NSEW, North South East West; DHS, the Dallas Heart Study; ARIC, the Atherosclerosis Risk in Communities study; MONICA, MONItoring of trends and determinants for CArdiovascular disease.

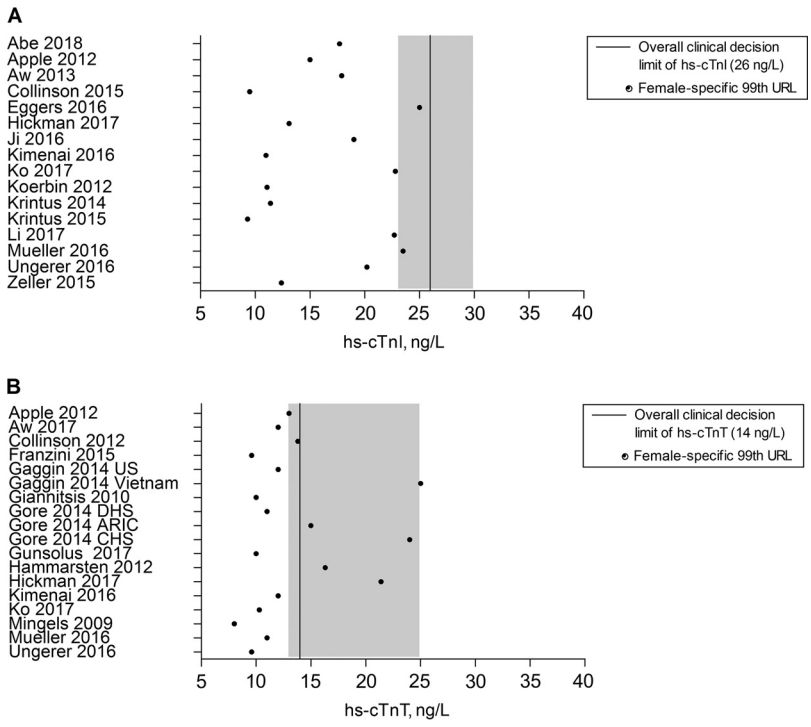

Fig. 1. **Female-specific 99th percentile URLs of hs-cTnI (A) and hs-cTnT (B) compared with the package insert-defined 90% or 95% CI ranges for the overall clinical decision threshold of 23 to 30 ng/L for hs-cTnI and 13 to 25 ng/L for hs-cTnT (shaded area).**

SEX-SPECIFIC 99TH PERCENTILE URL OF hs-cTnI AND hs-cTnT

In almost 90% of studies (14 of 16 studies), female-specific 99th percentile values of hs-cTnI were lower than the overall package insert-defined clinical decision CI ranges (Fig. 1A) *(11, 12, 19–30, 32, 33)*. Regarding the female-specific clinical decision CI range of hs-cTnI (16 ng/L; range, 14–18 ng/L; package insert), 7 (43.8%) studies reported female-specific 99th percentile values below this range. Although somewhat less remarkable, 11 of 18 hs-cTnT studies (61.1%) also reported lower female-specific 99th percentile values than the overall clinical decision CI range (Fig. 1B) *(10, 12, 20, 22, 34, 35, 37–41)*. In men, the 99th percentile values of both hs-cTnI and hs-cTnT are more in line with currently used overall thresholds. For hs-cTnT, almost 90% of the male-specific 99th percentile URL match with current clinical practice *(10–12, 19, 20, 22, 34, 35, 38–43)*, although values of hs-cTnI showed a much wider dispersion around the recommended overall threshold (see Fig. 2 in the online Data Supplement) *(11, 12, 19–30, 32, 33)*.

OVERALL 99TH PERCENTILE URL OF hs-cTnI AND hs-cTnT

Several studies (17 and 20, respectively) determined the overall 99th percentiles of hs-cTnI and hs-cTnT (see Tables 1–3 in the online Data Supplement) *(10–12, 19–43)*. The majority of studies (58.8%) that determined overall 99th percentile URL for hs-cTnI reported lower 99th percentile values than the clinically recommended overall decision range in the package insert (29.4% within and 11.8% above the overall clinical decision range) (Fig. 2A). In line with the sex-stratified analysis, the range of overall 99th percentiles was more dispersed for hs-cTnI than for hs-cTnT (Fig. 2B).

DIRECT COMPARISON OF 99TH PERCENTILE URL FOR hs-cTnI AND hs-cTnT

To investigate whether the package insert overall clinical decision limits of 26 ng/L for hs-cTnI and 14 ng/L for hs-cTnT could be discrepant owing to differences in the reference populations from which they were derived, we additionally investigated the 6 studies that derived 99th percentiles of both hs-cTn from a single reference population *(11, 12, 19–22)*. Examining the absolute numeric differences between 99th percentile concentrations of hs-cTnI and hs-cTnT as a measure of dispersion, these values ranged from −6 to 18 ng/L (Fig. 3). The dispersion seems less an issue in women than in men (absolute difference 99th percentile hs-cTnT − hs-cTnI: women, −1 to 13 ng/L; men, 4–30 ng/L) (see Fig. 3 in the online Data Supplement).

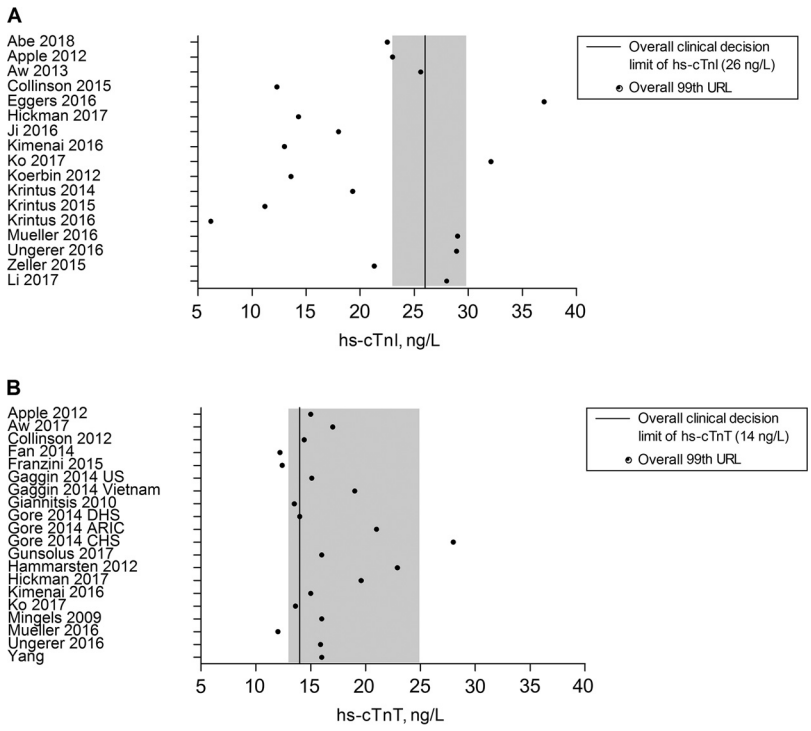

Fig. 2. Overall 99th percentile URLs of hs-cTnI (A) and hs-cTnT (B) as compared with the package insert-defined 90% or 95% CI ranges for the overall clinical decision threshold of 23 to 30 ng/L for hs-cTnI and 13 to 25 ng/L for hs-cTnT (shaded area).

Discussion

The main finding of our systematic review is that female-specific 99th percentile URLs of hs-cTn are lower than the package insert values currently recommended in clinical practice, especially for hs-cTnI. In contrast with female-specific 99th percentile values, the male-specific 99th percentile values are more in line with those in current clinical practice. Furthermore, we found a much wider range of reported overall 99th percentile URL for hs-cTnI than for hs-cTnT.

Although sex differences in basal concentrations of circulating troponin were first recognized in 2009 *(10)*, the question of whether this finding requires a change in clinical practice is an ongoing topic of debate. Lower troponin concentrations in women are probably the re-

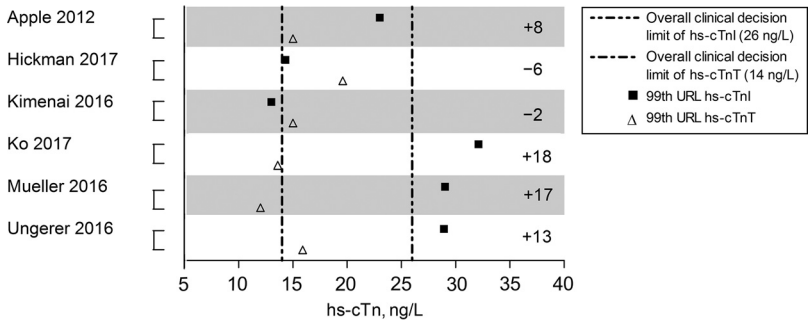

Fig. 3. Direct comparison of overall 99th percentile URL of hs-cTnI and hs-cTnT derived from a single reference population.
The right column shows the absolute numeric differences between 99th percentile values of hs-cTnI and hs-cTnT from a single reference population.

sult of several factors *(44)*. The difference in left ventricular mass between sexes is most likely the main contributor to the differences in baseline troponin concentration between sexes *(45–47)*. Better understanding of the pathogenesis of coronary artery disease also revealed differences between women and men *(48)*. The sex hormone estrogen seems to have a protective role in the development of coronary artery disease for women by the attenuation of several processes of cardiac remodeling, reflected in lower troponin concentrations for women than men *(49–51)*.

As our findings strongly suggest that the female-specific troponin 99th percentile URL of hs-cTnI is lower than the overall clinical decision limit of 26 ng/L, it is urgently recommended to further investigate the clinical relevance of this observation. The choice to implement sex-specific analysis of hs-cTn should be carefully weighed, and potential clinical benefit should be evaluated in relation to the already advanced diagnostic algorithms of AMI using cardiac troponins. According to the guidelines, the 99th percentile URL of hs-cTn should be above the 10% CV thresholds (hs-cTnI, 5 ng/L; hs-cTnT, 13 ng/L) to ensure that detected differences are within analytical error margins to avoid misclassification of AMI diagnosis *(52)*. For hs-cTnI, all reported CIs of female-specific 99th percentile URL of hs-cTnI were above the 10% CV threshold of 5 ng/L, making downward adjustment of hs-cTnI threshold for diagnosis of AMI in women seem feasible. Examining the analytical properties of the hs-cTnT assay, the CIs for the female-specific 99th percentile URL of hs-cTnT were predominantly below the 10% CV threshold of 13 ng/L. A plausible argument against sex-specific algorithms is that women suspected of AMI are, on average, 10 years older than men; therefore, the age effect on troponin concentrations might neutralize the sex difference *(53)*. Indeed, Eggers et al. determined 99th percentile URL from a reference cohort of 70 years of age, and reported a substantially higher female-specific 99th percentile of hs-cTnI as compared with the other studies *(26)*. In addition, age-adjusted 30-day mortality after AMI is similar between women and men, suggesting that for the consideration of sex-specific thresholds of hs-cTn, age is an important confounding factor that should be considered *(2)*. On the other hand, lowering the clinical decision limit of hs-cTnI might detect the subgroup of women who are at high risk and currently missed *(54)*. This hypothesis is reinforced by Shah et al., who showed that sex-specific thresholds for hs-cTnI resulted in a similar prevalence of AMI diagnoses between women and men *(55)*. Whether reclassification by sex-specific thresholds leads to better treatment—and, accordingly, improved prognosis after AMI for women—needs to be confirmed by future prospective randomized trials.

We observed a large dispersion in the range of reported overall 99th percentile values of hs-cTnI, which might have led to nonbiologically equivalent clinical decision limits of hs-cTnI and hs-cTnT. To investigate the influence of reference cohort variation on estimated 99th percentile URL, we compared the 99th percentile URL of hs-cTnI and hs-cTnT derived from a single reference population. Even when derived from a single cohort, absolute differences up to 18 ng/L were observed between troponin I and T thresholds. Hence, this indicates that the divergence between the clinical decision limits of hs-cTnI and hs-cTnT is not simply a result of heterogeneity across reference populations. We believe that the dispersion seems rather to be a result of the higher proportion of outliers for hs-cTnI as previously observed *(12)*. A small proportion of hs-cTn values in the extreme right tail of the distribution determines the 99th percentile URL for hs-cTnI and hs-cTnT. The hs-cTnI distribution is more profoundly affected by extreme outliers than hs-cTnT distribution *(12)*. Thus, the 99th percentile URL is highly sensitive to outliers, and a sufficiently large sample size is an absolute requirement. Most studies that directly compared thresholds of hs-cTnI and hs-cTnT included only a limited number of reference individuals. Hence, the play of chance may have resulted in the discrepant 99th percentiles for hs-cTnI and hs-cTnT. Therefore, handling of outliers seems a critical factor for the determination of an appropriate clinical cutoff for hs-cTnI. For hs-cTnI, much wider CIs around the 99th percentile values have been reported than for hs-cTnT. As most of the CIs of hs-cTn are derived from bootstraps, the wider intervals for hs-cTnI could be explained by the higher number of hs-cTnI outliers, rather than biological variation. In addition to the outlier detection method, the heterogeneity in the applied statistical approaches for calculating the 99th percentile values also may have resulted in the large dispersion of values for hs-cTnI. Indeed, 2 studies recently showed that the applied statistical approach highly influences the 99th percentile URL of hs-cTn *(19, 26)*. They both conclude that the nonparametric approach in combination with a conservative outlier detection method (e.g., Dixon) is the preferred method for determination of the 99th percentile URL for hs-cTn *(19, 26)*.

The following limitations of our study require attention: (*a*) Only the hs-cTnI assay from Abbott was investigated, precluding the extrapolation of our findings to other troponin I assays. (*b*) Although Sandoval et al. recommended criteria for defining a healthy reference population, we believe that it was not appropriate to translate this into "hard" quality criteria owing to the wide variety of population differences globally (e.g., European vs US). Therefore, we reported the 99th percentile URL derived from the most stringently screened selection of a reference population according to the Sandoval recommen-

dations. (*c*) The statistical approach used for estimation of the 99th percentile URL was highly variable across studies, preventing the calculation of numerical 99th percentiles. We chose to use a less quantitative approach and evaluated whether the reported 99th percentile URL matched with common practice. The clinical decision ranges of hs-cTnI and hs-cTnT derived from the package inserts were pragmatically defined as "common practice," as a gold standard diagnostic threshold for both hs-cTn assays is not established. (*d*) Furthermore, the focus in this study was on the reported 99th percentile URL for hs-cTnI and hs-cTnT from healthy reference populations. The relation of downward adjustment of the diagnostic cutoff of hs-cTnI in relation to clinical outcome should be the subject of future research.

In conclusion, this systematic review shows that the female-specific 99th percentile URL of hs-cTnI is lower than the overall clinical decision limit of 26 ng/L. A similar, but less profound, effect is seen for hs-cTnT. Direct comparison of 99th percentile URL of hs-cTnI and hs-cTnT revealed that the statistical approach, rather than reference population heterogeneity, contributes to current discrepant clinical decision limits. Handling of outliers seems particularly critical for the hs-cTnI threshold. Our study results suggest that future research is needed on this topic, particularly to investigate whether downward adjustment of hs-cTn thresholds, particularly hs-cTnI, can close the diagnostic gap between women and men and improve prognosis for women.

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