

Direct comparison of clinical decision limits for cardiac troponin T and I

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Direct comparison of clinical decision limits for cardiac troponin T and I

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ABSTRACT

Objective The 99th percentile upper reference limit of high-sensitivity cardiac troponin (hs-cTn) from a healthy reference population is used for diagnosing acute myocardial infarction (AMI). Accepted current thresholds of hs-cTnT (Roche) and hs-cTnI (Abbott) are 14 and 26 ng/L, respectively. Since thresholds for hs-cTnT and hs-cTnI were derived from different reference cohorts it is unclear whether they are biologically equivalent. We directly assessed sex-specific and age-specific 99th percentile upper reference limits of hs-cTnT and hs-cTnI in a single reference cohort, to investigate whether current divergent thresholds of hs-cTnT and hs-cTnI stem from intrinsic assay differences or reflect cohort variation.

Methods A healthy reference population was derived from a population-based cohort (the Maastricht Study: n=3451; age: 40–75 years). Individuals with diabetes mellitus, a history of cardiovascular disease, cardiac ischaemia on ECG, N-terminal pro-brain natriuretic peptide >125 ng/L or estimated glomerular filtration rate <60 mL/min/1.73 m² were excluded. Non-parametric analyses were performed to assess 99th percentile upper reference limits.

Results 1540 individuals were included in the healthy reference population (age 57±8 years, 52.4% women). Overall 99th percentile upper reference limits of hs-cTnT and hs-cTnI were 15 and 13 ng/L, respectively. Upper reference limits were higher in men than women (hs-cTnT: 16 vs 12 ng/L), (hs-cTnI: 20 vs 11 ng/L) and increased with age.

Conclusions Direct comparison reveals numerically similar thresholds for hs-cTnT and hs-cTnI assays. This finding is in line with recently reported underdiagnosis of AMI with the current decision limit of 26 ng/L for hs-cTnI, especially among women. Downwards adjustment of the hs-cTnI threshold, differentiated for sex, would equalise clinical decision limits for both assays, and may prevent further underdiagnosis of AMI.

INTRODUCTION

Cardiac troponin is the preferred biomarker for the diagnosis of acute myocardial infarction (AMI).^{1–2} The definition of AMI requires a significant ‘rise and/or fall’ of high-sensitivity cardiac troponin (hs-cTn) between serial measurements with at least one value above the 99th percentile upper reference limits of hs-cTn from a healthy reference population.² The consensus requires that the 99th

percentile upper reference limit of hs-cTn should not be lower than the total imprecision, as described by 10% coefficient of variation (10% CV).¹ There are two clinical troponin assays that meet all high-sensitivity criteria: the Roche high-sensitivity cardiac troponin T (hs-cTnT) assay and the Abbott Architect high-sensitivity cardiac troponin I (hs-cTnI) assay.³ The presently recommended 99th percentile upper reference limit for hs-cTnI is almost twice as high as that for hs-cTnT (26 vs 14 ng/L, respectively, package inserts). Since troponin T and I are different molecules, it is not surprising that they have different upper reference limits. More intriguing, however, is the observation that the median troponin value of a healthy reference population is lower for hs-cTnI than hs-cTnT, which is opposite to what one would expect from the 99th percentile values of both assays.⁴ The causes of divergent 99th percentile threshold values between troponin T and I, and opposite effects on the median values of both assays, are unknown. A first possibility is that current upper reference limits for hs-cTnT and hs-cTnI were derived from different reference cohorts, and it is well established that different selection and screening procedures substantially affect the composition of presumably ‘healthy’ reference cohorts.^{5–7} Previous studies recognised this issue and several approaches were developed for defining a healthy reference population.^{3–7,9} Recently, a standardised selection approach is reported for establishing accurate 99th percentile values of cardiac troponin T and I.¹⁰ Second, intrinsic assay differences may play a role, in particular the frequency and distribution of extreme values, where differences across assays can profoundly impact the robustness of the 99th percentile estimation. Third, and of high clinical importance, sex and age differences across reference cohorts may have contributed to substantial heterogeneity.^{6 11–18}

The present study was designed to directly compare sex-specific and age-specific 99th percentile upper reference limits for hs-cTnT and hs-cTnI, in relation to their median value, in a single, large, well-phenotyped healthy reference population. This approach allows to distinguish between cohort variation and intrinsic assay differences as factors that may contribute to divergent 99th percentile upper reference limits, and apparently opposite effects on the median values.

METHODS

Study design and populations

In this study, we used data from the Maastricht Study, an ongoing observational prospective population-based cohort study. The rationale and methodology have been described previously.¹⁹ In brief, the Maastricht Study focuses on the aetiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus and is characterised by an extensive phenotyping approach. All participants gave written informed consent. The study was conducted according to the principles of the Declaration of Helsinki.²⁰ Further details regarding the Maastricht Study cohort are provided in online supplementary appendix. The healthy reference cohort was derived from the Maastricht Study cohort (n=3451) and defined as described in the online supplementary appendix.

Biomarker measurements

Morning blood samples were obtained from all individuals. Samples were centrifuged according to the manufacturer's instructions and serum samples were stored at -80°C in aliquots. The storage time ranged from 1–4 years. Prior to measurement, samples were thawed at room temperature, mixed and centrifuged at $2500\times g$ for 2 min. Serum cTnT was measured, using the Roche Cobase601 hs-cTnT assay (Roche) on the Cobas6000 analyser. This method has a limit of blank (LoB) of 3 ng/L, limit of detection (LoD) of 5 ng/L and 10% CV of 13 ng/L (package insert). hs-cTnI was measured after an additional freeze–thaw step. Potential preanalytical bias was investigated by reassessment of 3% of the hs-cTnI measurements. Reassessed values correlated strongly with the original data (Pearson's correlation coefficient: 0.998, 95% CI 0.996 to 0.999, $p < 0.0001$; intercept: -0.81 , 95% CI -1.71 to 0.09), making preanalytical bias unlikely. Serum cTnI was measured with ARCHITECT i2000SR STAT hs-cTnI assay (Abbott). According to the package insert, LoB ranged between 0.7 and 1.3 ng/L and LoD ranged between 1.1 and 1.9 ng/L. The hs-cTnI assay has a 10% CV of 4.7 ng/L (package insert).

Statistical analysis

Continuous variables are expressed as mean and SD or median and IQR when not normally distributed. Categorical data are reported as number and percentage. The correlation between log-transformed hs-cTnT and log-transformed hs-cTnI was assessed by Pearson's correlation test. The relationship between hs-cTn and age was examined with Spearman's correlation test. Non-parametric analyses were performed to determine 99th percentile upper reference limits of hs-cTnT and hs-cTnI, stratified by sex and age. Bias corrected and accelerated bootstrapped percentile method (resampling with replacement: 5000 bootstrap replicates) was used to determine 95% CIs for the stratified 99th percentile upper reference limits. Uncorrected and outlier-adjusted 99th percentiles according to Dixon's outlier detection method were calculated. Briefly, Dixon's outlier method is based on D/R ratio whereby D is the absolute difference between the most extreme value and the preceding value and R is the range of the values (maximum–minimum).²¹ If the D/R ratio is $>1/3$, the extreme value is considered an outlier and excluded from analyses. Dixon's method further dictates that a group of extreme values can be evaluated as a whole, by testing the least extreme value of this group according to the D/R ratio criterion. If this is considered an outlier (D/R ratio $>1/3$), also the more extreme values of this group are excluded. Sensitivity analyses with the non-

parametric Tukey's outlier detection method were performed to verify robustness of the calculated 99th percentile upper reference limits, independent of the outlier detection method applied. The Tukey method is based on 25th quartile (Q1), 75th quartile (Q3) and IQR.²² For the Tukey method the hs-cTn values were Box–Cox transformed. By means of these values lower and upper fences were defined: lower fence= $Q1-3(IQR)$ and upper fence= $Q3+3(IQR)$. Values above the upper fence and under the lower fence were defined as outliers and excluded from analyses. Finally, 97.5th percentile upper reference limits were calculated for both troponin assays. This analysis is an even more stringent approach than the classical outlier detection methods against the influence of extreme values in the right tail of the troponin distribution. Hence, differences in the dispersion of values in the right tail of the troponin distributions, and their effect on the upper reference value of both assays, can be identified.

Statistical tests were performed using SPSS V.20 (SPSS, Chicago, Illinois, USA) and STATA V.13 (Stata, College Station, Texas, USA).

RESULTS

Detection and dispersion of hs-cTnT and hs-cTnI in the healthy reference cohort

A total of 1540 individuals from the Maastricht Study cohort were included in the healthy reference population (figure 1).

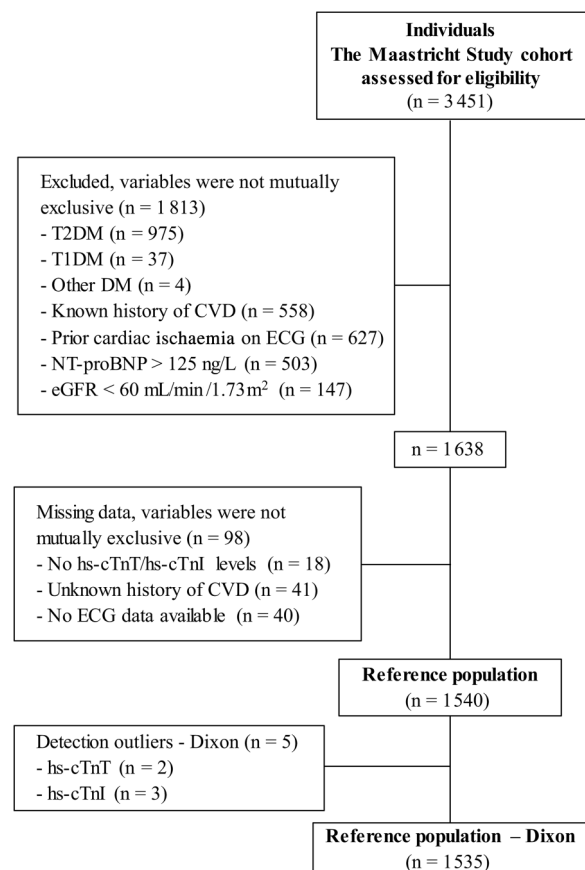


Figure 1 Study flow diagram. CVD, cardiovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

Table 1 Baseline characteristics of the entire reference population and subpopulations

Characteristics	Entire reference population (n=1540)	Men (n=733)	Women (n=807)	Men, 40–49 years (n=120)	Men, 50–64 years (n=443)	Men, 65–75 years (n=170)	Women, 40–49 years (n=163)	Women, 50–64 years (n=503)	Women, 65–75 years (n=141)
Age (years)	57 (8)	58 (8)	57 (8)	45 (3)	58 (4)	68 (3)	45 (3)	57 (4)	68 (3)
Sex (male)	733 (47.6%)	733 (100%)	0 (0%)	120 (100%)	443 (100%)	170 (100%)	0 (0%)	0 (0%)	0 (0%)
Weight (kg)	75 (66–85)	83 (76–90)	67 (61–75)	84 (77–91)	83 (77–92)	80 (74–86)	67 (62–78)	67 (61–76)	67 (61–74)
Height (cm)	171 (9)	178 (6)	165 (6)	179 (6)	179 (6)	176 (6)	167 (6)	165 (6)	163 (5)
BMI (kg/m ²)*	26 (23–28)	26 (24–28)	25 (23–27)	26 (24–28)	26 (24–29)	26 (24–27)	25 (22–28)	25 (22–27)	25 (23–27)
Hypertension (yes)	604 (39.3%)	356 (48.7%)	248 (30.7%)	34 (28.3%)	218 (49.2%)	104 (61.9%)	26 (16.0%)	157 (31.2%)	65 (46.1%)
Smoking*									
Never	602 (39.1%)	281 (38.4%)	321 (39.8%)	62 (51.7%)	168 (37.9%)	51 (30.2%)	86 (52.8%)	178 (35.4%)	57 (40.4%)
Former	737 (47.9%)	350 (47.8%)	387 (48.0%)	38 (31.7%)	207 (46.7%)	105 (62.1%)	46 (28.2%)	266 (52.9%)	75 (53.2%)
Current	200 (13.0%)	101 (13.8%)	99 (12.3%)	20 (16.7%)	68 (15.3%)	13 (7.7%)	31 (19.0%)	59 (11.7%)	9 (6.4%)
BP lowering medication (yes)*	301 (19.6%)	169 (23.1%)	132 (16.4%)	6 (5.0%)	105 (23.7%)	58 (34.3%)	15 (9.2%)	86 (17.1%)	31 (22.0%)
Lipid lowering medication (yes)*	226 (14.7%)	131 (17.9%)	95 (11.8%)	9 (7.5%)	80 (18.1%)	42 (24.9%)	5 (3.1%)	54 (10.7%)	36 (25.5%)
Total cholesterol (mmol/L)	5.6 (1.0)	5.5 (1.0)	5.8 (1.0)	5.4 (1.0)	5.5 (1.0)	5.5 (1.0)	5.2 (0.9)	5.9 (1.0)	5.9 (1.0)
HDL-cholesterol (mmol/L)	1.6 (1.3–1.9)	1.4 (1.2–1.6)	1.8 (1.5–2.1)	1.3 (1.0–1.5)	1.4 (1.2–1.6)	1.5 (1.3–1.7)	1.7 (1.4–1.9)	1.8 (1.5–2.1)	1.8 (1.5–2.1)
LDL-cholesterol (mmol/L)	3.4 (0.9)	3.4 (0.4)	3.4 (0.9)	3.5 (1.0)	3.4 (0.9)	3.4 (0.9)	3.1 (0.8)	3.5 (1.0)	3.5 (0.9)
Triglycerides (mmol/L)	1.1 (0.8–1.5)	1.2 (0.9–1.7)	1.1 (0.8–1.5)	1.2 (0.9–1.6)	1.2 (0.9–1.7)	1.1 (0.9–1.6)	1.0 (0.7–1.3)	1.1 (0.8–1.5)	1.1 (0.9–1.5)
eGFR (mL/min/1.73 m ²)	91 (12)	91 (12)	91 (12)	100 (11)	91 (11)	85 (12)	100 (12)	91 (11)	82 (10)
BP, systolic (mm Hg)*	131 (17)	137 (15)	127 (16)	131 (13)	137 (15)	140 (16)	119 (13)	127 (16)	132 (19)
BP, diastolic (mm Hg)*	76 (10)	79 (10)	73 (9)	79 (10)	80 (10)	77 (9)	72 (9)	74 (9)	72 (9)

*Variable consisted missing values (maximum 2 missing values), percentage is calculated over the valid values. Continuous variables are mean (SD) in case of normal distribution, otherwise reported as median (IQR). Categorical data are reported as n (%).

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Baseline characteristics of the entire reference population and subpopulations are shown in [table 1](#).

The proportion of subjects with a measurable troponin T level (LoD ≥ 5 ng/L) in this healthy reference population was 43.4%, in line with previously reported findings (24%–58%).^{8 12–15} The proportion with detectable troponin I concentrations ranged between 43.1% and 79.1%, depending on the LoD that is employed (troponin I has an LoD range of 1.1–1.9 ng/L). Previous studies applied a fixed LoD of 1.9 ng/L and reported a range of detectable troponin I concentrations between 64% and 81%.^{6 16} Percentages of measurable troponin levels stratified by sex are shown in online supplementary table S1. The proportion of subjects with measurable concentrations with both assays ranged between 29.1% and 41.3%, depending on the troponin I LoD applied (see online supplementary table S1). The median (IQR) was 5(3.4–6.3) ng/L for hs-cTnT and 2(1.1–2.5) ng/L for hs-cTnI. The dispersion of values directly around the median was smaller for hs-cTnI than for hs-cTnT, however, both the frequency and the absolute value of extreme values were higher for hs-cTnI than hs-cTnT ([figure 2](#)). To verify that heterophilic antibodies did not underlie the relatively extended right tail of the troponin I distribution, all samples of the Maastricht Study cohort with an hs-cTnI >11 ng/L (n=102) were reassessed in a dilution series (1:2; 1:4). No evidence for assay interference was found in any of these samples (data not shown), suggesting that the values representing the stretched right tail of the hs-cTnI distribution are analytically correct. The Pearson's correlation coefficient between log-transformed hs-cTnT and log-transformed

hs-cTnI was 0.545 (95% CI 0.509 to 0.579, $p < 0.0001$) ([figure 3](#)).

The 99th percentile upper reference limits of hs-cTnT and hs-cTnI

By direct comparison, uncorrected 99th percentile upper reference limits of hs-cTnT and hs-cTnI assays were in the same range with overlapping 95% CIs: hs-cTnT, 15 (95% CI 14 to 16) ng/L versus hs-cTnI, 16 (95% CI 12 to 21) ng/L ([table 2](#)).

Two hs-cTnT values and three hs-cTnI values were considered outliers according to Dixon's outlier detection method, leaving 1535 individuals for refined outlier-adjusted analyses. The calculated 99th percentile threshold of hs-cTnT was unaffected after outlier removal ([table 2](#)). Unlike hs-cTnT, outlier-correction decreased the overall hs-cTnI upper reference limit with 3 ng/L (hs-cTnI, uncorrected: 16 ng/L; hs-cTnI, outlier-adjusted: 13 ng/L) ([table 2](#)), which further equalised the 99th percentile upper reference limits of hs-cTnT and hs-cTnI.

To verify that the 99th percentile upper reference limits were robust and independent of the outlier detection method applied, sensitivity analysis was performed to compare results from Dixon's and Tukey's outlier detection methods. Two hs-cTnT values were outliers according to Dixon, no hs-cTnT measurements were considered outliers according to Tukey. Three hs-cTnI values were outliers according to Dixon's method and 10 hs-cTnI values were outliers according to Tukey's method. Calculated 99th hs-cTnI percentile upper reference limits were largely independent of the outlier detection method applied ([figure 4](#)).

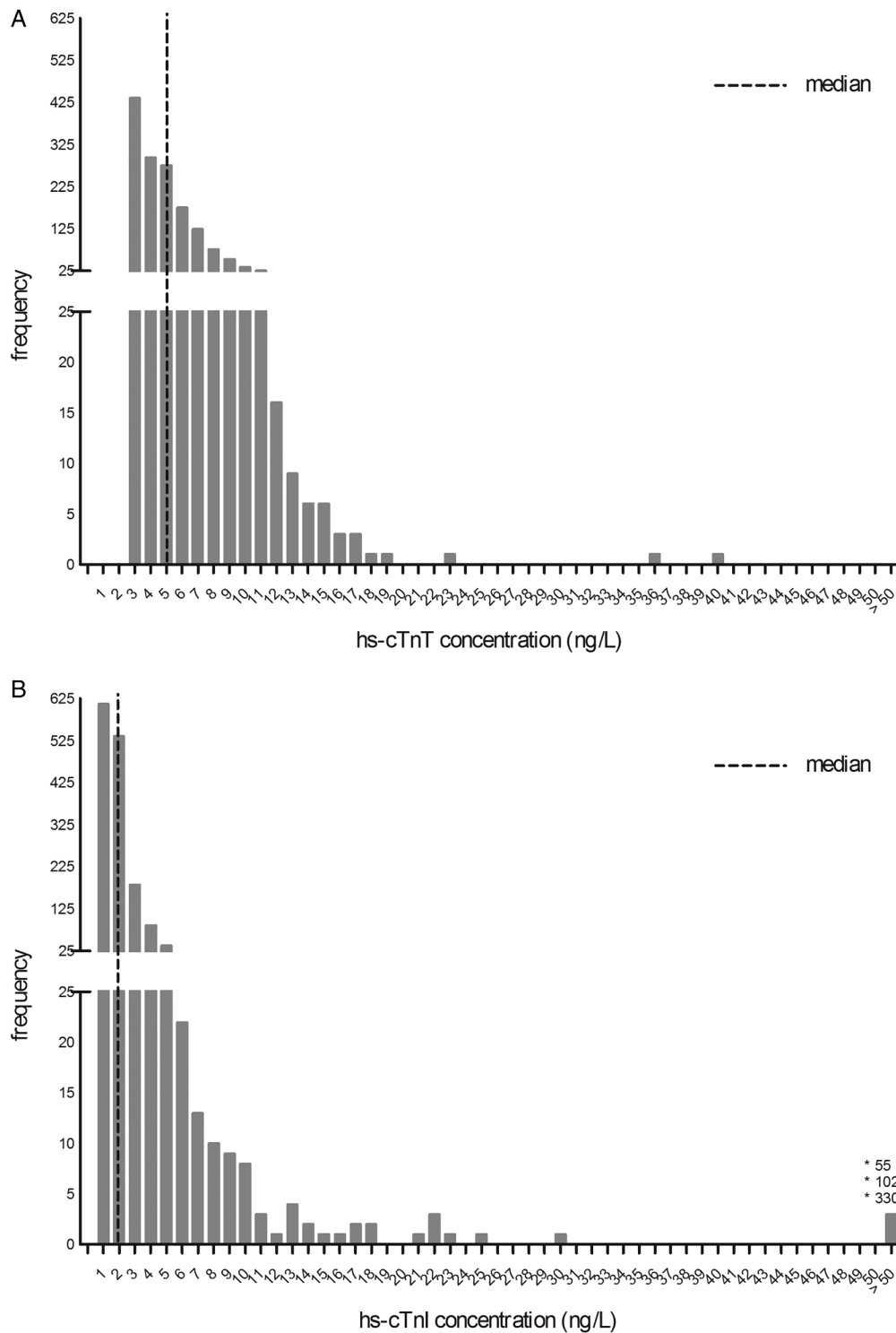


Figure 2 Distribution of hs-cTnT (A) and hs-cTnI (B) of healthy reference population. hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T.

The 97.5th percentile upper reference limits of hs-cTnT and hs-cTnI

The troponin I distribution is characterised by a more extended right tail than the troponin T distribution, in particular when compared with the median value of both distributions (hs-cTnT: 5 ng/L, hs-cTnI: 2 ng/L; [figure 2](#), compare A to B). This suggests that variation in a very small proportion of the reference cohort (1%–2%) strongly influences calculated upper reference limits of troponin I, whereas troponin T is more

robust in terms of variation in the extremity of the right tail of the distribution.

To quantitate the effect of this phenomenon on calculated upper reference limits, 97.5th percentile upper reference limits were assessed for both assays. The 97.5th percentile eliminates the undesirable effect of outliers on the upper reference limit, and also reduces the disproportional influence of widely dispersed tails, which are often not representative for the vast majority of measurements in a healthy reference cohort. The

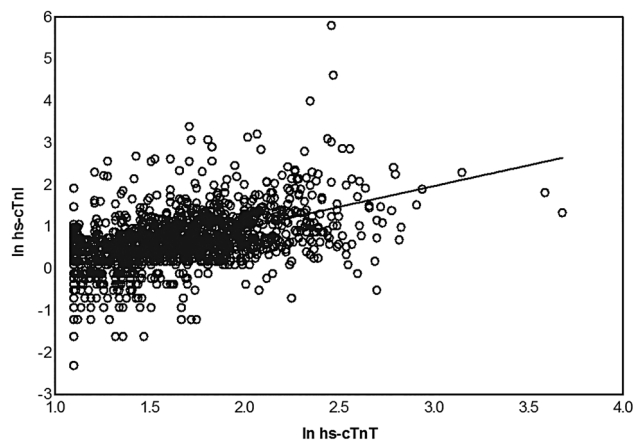


Figure 3 Scatterplot of the relationship between log-transformed hs-cTnT and log-transformed hs-cTnI ($\beta=0.983$ (95% CI 0.908 to 1.059), $R^2=0.297$). hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T.

calculated 97.5th percentile upper reference limits were 12 ng/L for hs-cTnT and 9 ng/L for hs-cTnI, a decrease of respectively ~20% versus ~60% compared with the uncorrected 99th percentile upper reference limits of both assays (table 3).

The much larger numerical difference between the 99th and 97.5th percentile for troponin I compared with troponin T, confirm the much wider dispersed tails of troponin I, which makes the 99th percentile upper reference limit of troponin I less robust, and more subject to variation and outliers.

Table 2 The 99th percentile upper reference limits (ng/L, 95% CI) for hs-cTnT and hs-cTnI

	n	hs-cTnT (ng/L) (95% CI)	hs-cTnI (ng/L) (95% CI)
<i>Uncorrected 99th percentile</i>			
Reference population	1540	15 (14 to 16)	16 (12 to 21)
Stratified by sex			
Men	733	16 (15 to 18)	22 (16 to 46)
Women	807	12 (10 to 15)	11 (8 to 13)
Stratified by sex, age			
Men, 40–49 years	120	16 (10 to 17)	13 (5 to 15)
Men, 50–64 years	443	14 (13 to 16)	23 (16 to 55)
Men, 65–75 years	170	28 (19 to 40)	113 (17 to 330)
Women, 40–49 years	163	12 (7 to 16)	12 (10 to 14)
Women, 50–64 years	503	12 (9 to 15)	9 (6 to 14)
Women, 65–75 years	141	27 (12 to 36)	13 (10 to 13)
<i>Outlier-adjusted 99th percentile</i>			
Reference population	1535	15 (13 to 16)	13 (11 to 18)
Stratified by sex			
Men	729	16 (15 to 17)	20 (14 to 22)
Women	806	12 (10 to 14)	11 (8 to 13)
Stratified by sex, age			
Men, 40–49 years	120	16 (10 to 17)	13 (5 to 15)
Men, 50–64 years	441	14 (13 to 16)	22 (13 to 23)
Men, 65–75 years	168	20 (17 to 23)	20 (13 to 25)
Women, 40–49 years	163	12 (7 to 16)	12 (10 to 14)
Women, 50–64 years	503	12 (9 to 15)	9 (6 to 14)
Women, 65–75 years	140	13 (11 to 14)	13 (10 to 13)

Uncorrected and outlier-adjusted 99th percentiles according to Dixon's outlier detection method were calculated.
hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T.

Sex-specific and age-specific upper reference limits of hs-cTnT and hs-cTnI

For both assays, 99th percentile upper reference limits were higher in men than women. For hs-cTnT the 99th percentile was 16 ng/L for men and 12 ng/L for women. For hs-cTnI the 99th percentile was 20 ng/L for men and 11 ng/L for women (table 2). The Spearman's correlation coefficient between hs-cTn and age was 0.398 (95% CI 0.354 to 0.440, $p<0.0001$) for hs-cTnT and 0.292 (95% CI 0.244 to 0.338, $p<0.0001$) for hs-cTnI. The 99th percentile upper reference limits progressively increased per age stratum, in particular for the hs-cTnT assay. Similar to the overall 99th percentile upper reference limits, sex-specific and age-specific estimates are less robust for troponin I than troponin T (see CIs table 2).

DISCUSSION

In a direct comparison of troponin T and I upper reference limits, the present study reveals remarkably similar 99th percentile upper reference limits of 15 and 13 ng/L for the hs-cTnT (Roche) and hs-cTnI (Abbott) assay, respectively. The 99th percentile upper reference limit of 13 ng/L for hs-cTnI contrasts with the currently employed decision limit of 26 ng/L. One previous study performed a direct comparison between troponin T and I but focussed mainly on the effect of population selection on the 99th percentile upper reference limits.⁷

An important strength of this study is that the 99th percentile upper reference limits of hs-cTnT and hs-cTnI were obtained from a single healthy reference population. Although proposed hs-cTnT thresholds are relatively reproducible in different healthy reference populations, it seems that hs-cTnI thresholds are less robust across studies, and decrease substantially in reference populations that applied more stringent selection criteria.⁵ Our approach eliminated cohort variation as an uncontrollable source of heterogeneity, and enabled the estimation of biologically identical clinical decision limits for troponin T and I. Current non-bioequivalent 99th percentile upper reference limits of hs-cTnT and hs-cTnI are a worrying source of misdiagnosis of AMI and harm to patients.²³ The clinical importance of our key finding—a lower clinical decision limit for troponin I—is recently illustrated in a study by Wildi *et al.*²³ They showed that one out of five patients with AMI has an inconsistent diagnosis when using the hs-cTnT or hs-cTnI assays with currently approved upper reference limits of 14 and 26 ng/L, respectively. This inconsistency was driven by a too stringent hs-cTnI clinical decision limit of 26 ng/L, and led to underdiagnosis of AMI with the troponin I assay.²³ Importantly, the percentage of inconsistent diagnoses could be halved by downwards adjustment of the hs-cTnI threshold to 9 ng/L.²³ The present study therefore corroborates and extends these observations, and strengthens the evidence of two studies who reported lower hs-cTnI thresholds than currently employed.^{6,7} Our in-depth exploration of the dispersion of measurements with both hs-cTn assays showed that the 99th percentile upper reference limit of the hs-cTnT assay is less susceptible to variation and outliers in the extremity of the right tail of the distribution than the hs-cTnI assay. Importantly, this finding is robust and unrelated to the lower LoD of troponin I relative to troponin T: when a troponin I LoD threshold at the right side of the reported troponin I LoD range is employed (1.9 ng/L instead of 1.1 ng/L), which brings the proportion of subjects with measurable concentrations to an identical percentage as the troponin T assay (43%), the number of outliers remained similar. These intrinsic assay differences, along with cohort variation, may have

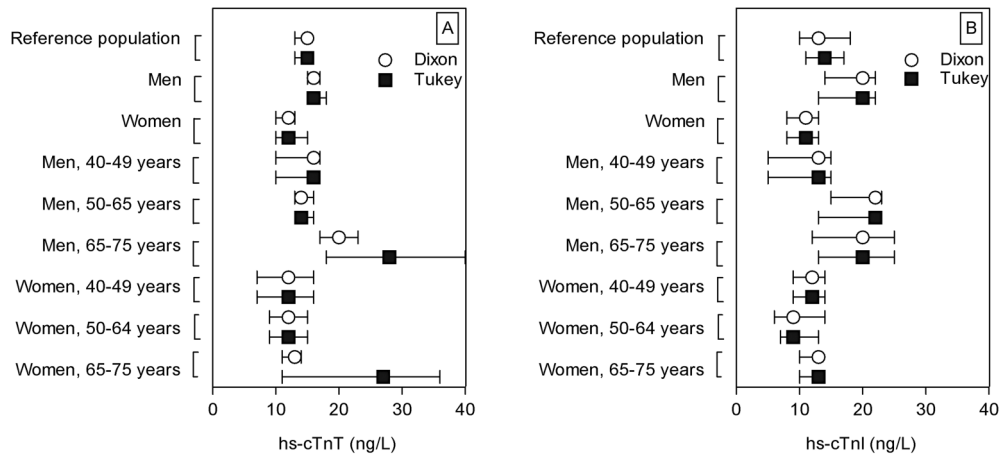


Figure 4 Sensitivity analyses of the 99th percentile values and 95% CIs for hs-cTnT (ng/L) (A) and hs-cTnI (ng/L) (B) according to different outlier detection methods (Dixon and Tukey). hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T.

contributed to rather inconsistent 99th percentile upper reference limits for troponin I compared with troponin T across studies,⁵ and underlie current non-bioequivalent clinical decision limits for troponin T and I.

Adequate selection and screening procedures are of great importance to compose a healthy reference cohort. Our study fulfilled the recent Sandoval criteria for defining a healthy reference population and therefrom sex-specific and age-specific upper reference limits of hs-cTnT and hs-cTnI assays were assessed. In line with previous studies 99th percentile upper reference limits were sex-specific, with lower levels in women than men.^{4 6–8 11–18 24} This finding extends recent clinical data that the generally used ‘one size fits all’ clinical decision limits are too high for women, leading to underdiagnosis of AMI.²⁵ Sex-specific thresholds may tackle this clinical problem. The 99th percentile threshold of hs-cTnT also increased with age, in particular in men ≥ 65 years. Age-dependency of the hs-cTnT upper reference limit was also evident in women, though less pronounced in outlier-adjusted analyses. Troponin I revealed a similar trend of age-dependency, although not as clear as hs-cTnT. This is consistent with the lower correlation coefficients between hs-cTnI and age compared with hs-cTnT. However, the larger CIs confirm that the much wider dispersed tails of troponin I complicate robust assessment of the 99th

percentile for troponin I, in particular in stratified analyses with less individuals.

The requirement that clinically used thresholds should be $>10\%$ CV merits consideration. The women-specific hs-cTnT threshold of 12 ng/L exceeds the 10% CV of 13 ng/L. Especially for women <65 years it is with the current definition not possible to adjust the hs-cTnT threshold downwards, as the 99th percentile upper reference limit would not meet the required precision.

However, adjustment for the hs-cTnI threshold is possible, since the women-specific 99th percentile upper reference limit of 11 ng/L $>10\%$ CV of 5 ng/L. The clinical relevance of sex-specific 99th percentile upper reference limits of hs-cTnI, with in particular a single threshold for women, requires further urgent attention. The next step should be a multicentre randomised controlled trial investigating the performance of the adjusted sex-specific hs-cTnI thresholds. This should clarify whether sex-specific thresholds can effectively reduce the underdiagnosis of AMI.

Limitations of this study merit attention. (1) Individuals from the Maastricht Study were mainly of Caucasian origin and all individuals were between 40 and 75 years of age. Hence, 99th percentile upper reference limits cannot be extrapolated to other ethnicities and age groups. (2) Men and women were not matched or adjusted for characteristics. Hence, we cannot formerly exclude the possibility that differences in upper reference limits are (in part) attributable to differences in patient case mix. (3) Imaging modalities such as cardiac echocardiography were not yet available in the current dataset. It is conceivable that inclusion of imaging modalities, although not formally recommended, would have resulted in even slightly lower upper reference limits. These limitations, however, do not affect the key finding of this study, being that 99th percentile upper reference limits of hs-cTnI should be adjusted downwards to become more equivalent to the clinical decision limit that is used for hs-cTnT, and reduce underdiagnosis of AMI.

In conclusion, direct comparison from a single reference cohort reveals numerically similar 99th percentile upper reference limits for hs-cTnT and hs-cTnI assays. Downwards adjustment of the 99th percentile upper reference limit of hs-cTnI, differentiated for sex, would equalise clinical decision limits for both assays, and may prevent underestimation of AMI in clinical practice. Sex-specific clinical decision limits seems to be crucial to reduce underdiagnosis of AMI in women.

Table 3 The 97.5th percentile upper reference limits (ng/L, 95% CI) for hs-cTnT and hs-cTnI

	N	hs-cTnT (ng/L) (95% CI)	hs-cTnI (ng/L) (95% CI)
Reference population	1540	12 (11 to 13)	9 (8 to 10)
Stratified by sex			
Men	733	14 (12 to 15)	11 (9 to 18)
Women	807	9 (8 to 10)	6 (5 to 9)
Stratified by sex, age			
Men, 40–49 years	120	11 (8 to 17)	5 (4 to 15)
Men, 50–64 years	443	13 (12 to 14)	13 (9 to 22)
Men, 65–75 years	170	18 (15 to 23)	16 (8 to 50)
Women, 40–49 years	163	7 (6 to 16)	10 (3 to 11)
Women, 50–64 years	503	9 (8 to 11)	5 (5 to 8)
Women, 65–75 years	141	12 (9 to 28)	9 (5 to 13)

hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T.

Key messages

What is already known on this subject?

For the diagnosis of acute myocardial infarction current clinical decision limits of high-sensitivity cardiac troponin T and I are 14 and 26 ng/L, respectively. It is unclear whether these thresholds are biologically equivalent, since they are derived from different reference cohorts.

What might this study add?

We directly assessed sex-specific and age-specific 99th percentile upper reference limits of troponin T and I in a single reference cohort of 1540 individuals. Direct comparison reveals numerically similar 99th percentile upper reference limits of 15 and 13 ng/L for troponin T and I, respectively.

How might this impact on clinical practice?

In agreement with recent clinical data, the current troponin I clinical decision limit seems too high, especially in women. Downwards adjustment of the troponin I threshold, may prevent underdiagnosis of acute myocardial infarction.

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Patient consent Obtained.

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