Journal of the American College of Cardiology
© 2014 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 63, No. 14, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2013.12.032

Biomarkers

Age- and Sex-Dependent Upper Reference Limits for the High-Sensitivity Cardiac Troponin T Assay

M. Odette Gore, MD, MSCS,* Stephen L. Seliger, MD, MS,† Christopher R. deFilippi, MD,† Vijay Nambi, MD, PhD,‡§ Robert H. Christenson, PhD,‖ Ibrahim A. Hashim, PhD,¶ Ron C. Hoogeveen, PhD,‡ Colby R. Ayers, MS,*# Wensheng Sun, MS,‡ Darren K. McGuire, MD, MHSc,* ** Christie M. Ballantyne, MD,‡ James A. de Lemos, MD* **

Dallas and Houston, Texas; and Baltimore, Maryland

Objectives The study sought to determine the 99th percentile upper reference limit for the high-sensitivity cardiac troponin T assay (hs-cTnT) in 3 large independent cohorts.

Background The presently recommended 14 ng/l cut point for the diagnosis of myocardial infarction using the hs-cTnT assay was derived from small studies of presumably healthy individuals, with relatively little phenotypic characterization.

Methods Data were included from 3 well-characterized population-based studies: the Dallas Heart Study (DHS), the Atherosclerosis Risk in Communities (ARIC) Study, and the Cardiovascular Health Study (CHS). Within each cohort, reference subcohorts were defined excluding individuals with recent hospitalization, overt cardiovascular disease, and kidney disease (subcohort 1), and further excluding those with subclinical structural heart disease (subcohort 2). Data were analyzed stratified by age, sex, and race.

Results The 99th percentile values for the hs-cTnT assay in DHS, ARIC, and CHS were 18, 22, and 36 ng/l (subcohort 1) and 14, 21, and 28 ng/l (subcohort 2), respectively. These differences in 99th percentile values paralleled age differences across cohorts. Analyses within sex/age strata yielded similar results between cohorts. Within each cohort, 99th percentile values increased with age and were higher in men. More than 10% of men 65 to 74 years of age with no cardiovascular disease in our study had cardiac troponin T values above the current myocardial infarction threshold.

Conclusions Use of a uniform 14 ng/l cutoff for the hs-cTnT assay may lead to over-diagnosis of myocardial infarction, particularly in men and the elderly. Clinical validation is needed of new age- and sex-specific cutoff values for this assay.
(J Am Coll Cardiol 2014;63:1441–8) © 2014 by the American College of Cardiology Foundation

The recently developed high-sensitivity assay for cardiac troponin T (hs-cTnT) has been implemented in many countries for the diagnosis of acute myocardial infarction (MI), and will likely be introduced in the United States in the near future (1–5). By convention, the upper reference limit for high sensitivity troponin assays is defined as the 99th percentile value from a

From the *Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; †Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland; ‡Department of Medicine, Baylor College of Medicine, Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart and Vascular Center, Houston, Texas; §Michael E. DeBakey Veterans Affairs Hospital, Houston, Texas; ‖Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland; ¶Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas; #Department of Clinical Sciences, University of Texas Southwestern Medical Center, Dallas, Texas; and the **Donald W. Reynolds Cardiovascular Clinical Research Center, University of Texas Southwestern Medical Center, Dallas, Texas. The Dallas Heart Study was supported by the Donald W. Reynolds Foundation (Las Vegas, Nevada) and by grant UL1-TR000451 from the National Center for Advancing Translational Sciences, National Institutes of Health. The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute (NHLBI) contracts HHSN268201100005C through HHSN268201100012C. The Cardiovascular Health Study was supported by NHLBI grant HL080295 and by NHLBI contracts N01-HC-85239, N01-HC-85079 through N01-HC-85086; N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, and N01-HC-45133, with additional support from the National Institute of Aging (AG-023629, AG-15928, AG-20098, and AG-027058). Dr. Gore is supported by training grant T32-HL007360 from the NHLBI. Drs. Seliger, deFilippi, Nambi, Christenson, Ballantyne, Hoogeveen, and de Lemos have received grant funding from Roche Diagnostics. Drs. Seliger and de Lemos have received consulting fees from Roche Diagnostics. Dr. deFilippi has received honoraria and consulting fees from Roche Diagnostics. Dr. Nambi has conducted research in collaboration with GE and Tomtec. Drs. Ballantyne, Nambi, and Hoogeveen have a provisional patent filed with Roche Diagnostics. Dr. de Lemos has received grant support and consulting income from Abbott Diagnostics. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received September 22, 2013; revised manuscript received November 30, 2013, accepted December 23, 2013.

Abbreviations and Acronyms

hs-cTnI = high-sensitivity cardiac troponin I

hs-cTnT = high-sensitivity cardiac troponin T

MI = myocardial infarction

NT-proBNP = N-terminal pro–B-type natriuretic peptide

normal reference population (6,7). However, no clear consensus exists regarding the composition of a "normal population" in this context (3,5,8). Given the central role of troponin measurement in MI diagnosis, accurate determination of the upper reference limit is critically important for the use and interpretation of troponin assays.

See page 1449

The presently accepted 99th percentile upper reference limit for the hs-cTnT assay (14 ng/l) was initially derived from a study of 616 "apparently healthy" volunteers and blood donors, with little information reported regarding subject selection (6), and then confirmed by a study of 533 individuals selected based primarily on a standardized questionnaire (9). Other studies in smaller cohorts, with varying degrees of phonotypical characterization, reported 99th percentiles ranging from 14.4 to 16.9 ng/l (8,10–12).

Clinical use of the present cutoff for the hs-cTnT assay does not take into account patient sex, age, and race. However, sex differences in the 99th percentile values for both hs-cTnT and high-sensitivity cardiac troponin I (hs-cTnI) assays have been reported in a number of small studies (8–15), with a trend for higher values in men, leading to a statement in the most recent Universal Definition of MI that sex-dependent upper reference limits for high-sensitivity cardiac troponin assays may be recommended in the future (7). A numeric trend towards higher 99th percentiles for the high-sensitivity cardiac troponin assay in older individuals was also reported in some, but not all studies, based on very few subjects in older age strata (9,11,13,14). Finally, whether the 99th percentiles for high-sensitivity cardiac troponin assays are influenced by race is unclear. Presently available data are insufficient to derive sex-, age-, or race-specific upper reference limits.

To address this critical knowledge gap, we analyzed cTnT values measured with the hs-cTnT assay in 3 large independent community-based cohorts: the DHS (Dallas Heart Study) study (16), the ARIC (Atherosclerosis Risk in Communities) study (17), and the CHS (Cardiovascular Health Study) study (18). We determined 99th percentile values for the hs-cTnT assay in clearly defined subcohorts of the DHS, ARIC, and CHS studies, with sequential exclusion of "nonhealthy" participants and those with subclinical cardiovascular disease, and with additional stratification by age, sex, and race. Compared with previous studies of the hs-cTnT assay upper reference limit, the present study of 12,618 adults benefits from a much larger size, unambiguous selection process, detailed phenotypic characterization, and broader representation of different age ranges.

Methods

Study design and populations. Cross-sectional analyses were performed in 3 independent community-based cohorts: the DHS study, the ARIC study, and the CHS study. The DHS is a probability-based sample of 6,101 adults enrolled between 2000 and 2002 from Dallas County, Texas, with intentional oversampling of black individuals to constitute approximately 50% of the cohort (19). cTnT was measured in 3,546 DHS study participants between 30 and 65 years of age. The cohort component of the ARIC study comprises a total of 15,792 individuals 45 to 64 years of age, randomly selected from Forsyth County, North Carolina; suburban Minneapolis, Minnesota; Jackson, Mississippi; and Washington County, Maryland (approximately one quarter of the cohort from each region) (20). For the ARIC study, cTnT was measured during visit 4 of the study (1996 to 1998), in 11,271 eligible individuals (17). The CHS study is a sample of 5,888 community-dwelling adults 65 years of age or older recruited from 4 communities: Forsyth County, North Carolina; Hagerstown, Maryland; Pittsburgh, Pennsylvania; and Sacramento, California (21). Of the CHS study cohort, 5,201 individuals were enrolled in 1989 to 1990 (main cohort), and 687 additional black subjects were enrolled in 1992 to 1993 (supplemental cohort). cTnT was measured in 4,221 participants from the CHS study. Detailed descriptions of the design, objectives, and examinations performed for each of the 3 cohort studies have been previously published (19–21). The study complied with the Declaration of Helsinki, all participants in the DHS, ARIC, and CHS studies provided written informed consent, and approval for the study was obtained from the institutional review boards at all participating institutions.

Definition of subcohorts/exclusion criteria. Because the 99th percentile upper reference limit for cTnT is by convention established in a normal reference population (6,7), we restricted our analyses to 2 prospectively defined subcohorts selected from each of the 3 studies. Subcohort 1 was defined as individuals free from recent hospitalization of any cause (6 months prior to blood collection for the study), with no clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke) or stage III or greater chronic kidney disease (estimated glomerular filtration rage <60 ml/min/1.73m^2). Subcohort 2 further excluded from subcohort 1 those individuals with subclinical structural heart disease, defined as left ventricular hypertrophy by electrocardiography (ARIC and CHS) or magnetic resonance imaging (DHS), left ventricular ejection fraction $<55\%$ by echocardiogram (CHS, ARIC) or magnetic resonance imaging (DHS), or N-terminal pro–B-type natriuretic peptide (NT-proBNP) >450 ng/l. Subjects with missing or exhausted biorepository samples were also excluded

from both subcohorts, and subjects with missing imaging or electrocardiography data were excluded from subcohort 2.

Laboratory measurements. cTnT was measured in sample aliquots previously stored at −70°C using a highly sensitive automated immunoassay (Troponin T hs STAT, Elecsys-2010, Roche Diagnostics, Indianapolis, Indiana), with a limit of detection of 5 ng/l and a limit of blank of 3 ng/l. The lowest cTnT concentration that can be measured with a coefficient of variation ≤ 10% with this assay is 13 ng/l (22). The assay lot numbers used were 153401 for the DHS study, 153401 and 154102 for the ARIC study, and 153401 for the CHS study. None of these lots were affected by problems reported by the manufacturer with other lot numbers (23,24). NT-proBNP levels were measured as described (25). Glomerular filtration rate was estimated using the Modification of Diet in Renal Disease formula (26).

Statistical analyses. The 99th percentile values and corresponding 95% confidence intervals were calculated for subcohorts 1 and 2 of the DHS, ARIC, and CHS studies, with further stratification by sex, age and race. Distribution-free confidence intervals were used because of the skewed distribution of cTnT. Specifically, rank order statistics were used for the bounds on the confidence limit such that the difference in the cumulative binomial probabilities satisfied the coverage probability requirement of 0.95. The 95% confidence interval for a given 99th percentile indicates that the 99th cTnT percentile of a general population sample with similar baseline characteristics as the respective subcohort or sex/age/race stratum has a 95% probability of falling within the calculated confidence interval. Within the DHS cohort only, sensitivity analyses were performed to determine the impact of assumptions made in determining the composition of subcohort 2. In these sensitivity analyses, the impact of various inclusion/exclusion criteria (including imaging, electrocardiography, and NT-proBNP criteria) on the 99th percentile cTnT value was assessed.

Results

Characteristics of the study population. Baseline demographic, clinical, and laboratory characteristics of the study subcohorts are presented in Table 1. A total of 12,618 adults were analyzed across the 3 studies. The median age was lowest in DHS and highest in CHS.

The 99th percentile values for the hs-cTnT assay. The 99th percentile values for subcohort 1 (representing adults without clinically overt cardiovascular disease or impaired renal function) are presented in Table 2 and Figure 1, with data for subcohort 2 (those additionally free from subclinical structural heart disease or an elevated NT-proBNP) correspondingly shown in Table 3 and Figure 2. The 99th percentile values were significantly higher than 14 ng/l (95% confidence intervals do not cross 14 ng/l) in subcohort 1 for all 3 studies, and in subcohort 2 for all studies except DHS. Moreover, the 99th percentile values were significantly >14 ng/l in all strata of men ≥50 years and women ≥65 years. Importantly, 99th percentile values were consistent across cohorts within age and sex strata.

Table 1 Baseline Characteristics of the Study Subcohorts

	DHS		ARIC		CHS	
	Subcohort 1* (n = 2,955)	Subcohort 2† (n = 1,978)	Subcohort 1 (n = 7,788)	Subcohort 2 (n = 7,575)	Subcohort 1 (n = 1,875)	Subcohort 2 (n = 1,374)
Age, yrs	43.3 (9.8)	43.2 (9.6)	61 (9)	61 (9)	73 (6)	72 (6)
Female	54.5	55.9	61.2	60.8	62.9	64.4
Race/ethnicity						
Black	49.4	41.5	21.1	20.9	18.2	20.6
White	30.5	36.1	78.9	79.1	81.5	79.8
Hispanic/Latino	17.9	20.1	N/A‡	N/A	N/A‡	N/A
Asian/Pacific Islander	1.9	2.1	0	0	0.1	0.1
Native American	0.3	0.2	0	0	0.1	0.2
Other	0.1	0.1	0	0	0.1	0.2
Smoking	27.8	24.7	12.8	12.8	10.5	10.1
Hypertension	29.8	24.4	41.6	40.8	53.4	52.3
Diabetes mellitus	9.9	8.3	9.9	9.8	12.4	11.6
GFR <90 ml/min/1.73 m^2	33.2	35.6	20.9	20.7	90.3	89.2
LVH or LVEF <55%	10.3	0	2.42	0	7.7	0
NT-proBNP >450 ng/l	0.8	0	1.1	0	5.4	0
Detectable cTnT (hs-cTnT assay)	23.9	21.1	37.9	38.2	56.1	52.3

Values are median (interquartile range) or %. *Subcohort 1: Subjects free from recent hospitalization (6 months), clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke), and stage III or greater chronic kidney disease (estimated glomerular filtration rate [GFR] <60 ml/min/1.73 m^2). †Subcohort 2: Subjects free from recent hospitalization (6 months), clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke), subclinical cardiovascular disease (left ventricular hypertrophy [LVH] or left ventricular ejection fraction [LVEF] <55% by echo or magnetic resonance imaging, left ventricular hypertrophy by electrocardiography, N-terminal pro–B-type natriuretic peptide [NT-proBNP] >450 ng/l), and stage III or greater chronic kidney disease (estimated GFR <60 ml/min/1.73 m^2). ‡In the ARIC (Atherosclerosis Risk in Communities) and CHS (Cardiovascular Health Study) studies race and ethnicity were recorded separately, and thus Hispanic/Latino subjects self-reported their race separately from their Hispanic/Latino ethnicity.

cTnT = cardiac troponin T; DHS = Dallas Health Study; GFR = glomerular filtration rate; hs-cTnT = high-sensitivity cardiac troponin T; LVEF = left ventricular ejection fraction; LVH = left ventricular hypertrophy; NT-proBNP = N-terminal pro–B-type natriuretic peptide.

Table 2 The 99th Percentile Values and Corresponding 95% CIs for hs-cTnT and Percentiles Corresponding to cTnT = 14 ng/l in Subcohorts 1* of the DHS, ARIC, and CHS Studies, With Further Stratification by Sex, Age, and Race

	DHS			ARIC			CHS	
	n	99th hs-cTnT Percentile [95% CI] (ng/l)	Percentile for hs-cTnT 14 ng/l	N	99th hs-cTnT Percentile [95% CI] (ng/l)	Percentile for hs-cTnT 14 ng/l	n	99th hs-cTnT Percentile [95% CI] (ng/l)	Percentile for hs-cTnT 14 ng/l
Entire subcohort 1	2,955	18 [16–23]	98.3	7,788	22 [20–24]	95.8	1,875	36 [30–42]	90.9
Stratified by sex									
Men	1,346	23 [19–47]	97.1	3,023	28 [24–35]	91.7	695	39 [34–44]	83.5
Women	1,609	12 [9–18]	99.2	4,765	16 [15–17]	98.4	1,180	34 [24–41]	95.3
Stratified by sex and age (yrs)									
Men <50	992	19 [14–50]	98.2	0	N/A	N/A	0	N/A	N/A
Men 50–64	339	28 [23–83]	94.0	2,030	24 [21–31]	93.3	0	N/A	N/A
Men 65–74	15	N/A	N/A	992	35 [27–49]	88.4	404	36 [28–59]	87.5
Men ≥75	0	N/A	N/A	1	N/A	N/A	291	77 [34–173]	77.7
Women <50	1,149	9 [7–43]	99.3	0	N/A	N/A	0	N/A	N/A
Women 50–64	448	14 [12–21]	99.2	3,246	14 [13–17]	99.0	0	N/A	N/A
Women 65–74	12	N/A	N/A	1,519	18 [15–21]	88.4	695	25 [17–45]	96.9
Women ≥75	0	N/A	N/A	0	N/A	N/A	485	40 [24–79]	92.7
Stratified by sex, age (yrs), and race									
Men <50, Black	445	20 [17–87]	97.4	0	N/A	N/A	0	N/A	N/A
Men <50, non-black	547	14 [11–17]	99.0	0	N/A	N/A	0	N/A	N/A
Men 50–64, black	175	28 [23–31]	91.0	389	31 [24–53]	89.7	0	N/A	N/A
Men 50–64, non-black	164	29 [12–83]	97.5	1,641	22 [19–29]	94.2	0	N/A	N/A
Men 65–74, black	8	N/A	N/A	119	37 [37–79]	78.2	66	35 [19–35]†	83.5
Men 65–74, non-black	7	N/A	N/A	873	30 [24–47]	89.9	338	36 [28–59]	88.3
Men ≥75, black	0	N/A	N/A	1	N/A	N/A	52	73 [26–73]†	80.5
Men ≥75, non-black	0	N/A	N/A	0	N/A	N/A	239	46 [32–71]	77.1
Women <50, black	584	15 [8–20]	99.0	0	N/A	N/A	0	N/A	N/A
Women <50, non-black	565	7 [5–19]	99.4	0	N/A	N/A	0	N/A	N/A
Women 50–64, black	241	13 [12–21]	99.2	837	14 [13–50]	99.0	0	N/A	N/A
Women 50–64, non-black	207	14 [9–15]	99.0	2,409	14 [13–17]	99.0	0	N/A	N/A
Women 65–74, black	7	N/A	N/A	298	17 [15–21]	96.6	131	58 [15–72]	95.9
Women 65–74, non-black	5	N/A	N/A	1,221	18 [16–24]	97.5	564	24 [17–36]	97.1
Women ≥75, black	0	N/A	N/A	0	N/A	N/A	93	79 [20–79]†	91.0
Women ≥75, non-black	0	N/A	N/A	0	N/A	N/A	392	35 [23–53]	93.0

*Subcohort 1: Subjects free from recent hospitalization (6 months), clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke), and stage III or greater chronic kidney disease (estimated glomerular filtration rate <60 ml/min/1.73 m^2). †99th percentile is equivalent to maximum observed value.

ARIC = Atherosclerosis Risk in Communities; CHS = Cardiovascular Health Study; CI = confidence interval; DHS = Dallas Health Study.

The 99th percentile cut points were higher in men compared with women, and increased in subgroups of increasing age among both men and women. Analyses stratified by race showed generally higher 99th percentile values for black versus non-black individuals, particularly among men and older women (≥65 years of age).

Percentiles corresponding to cTnT = 14 ng/l. Tables 2 and 3 also include the percentiles corresponding to a cTnT level of 14 ng/l in each subcohort and sex/age/race stratum. These percentiles were numerically lower than the 99th percentile in all overall subcohorts except subcohort 2 of DHS. Moreover, the percentile values corresponding to 14 ng/l were consistently lower in men compared with women, decreased with increasing age in both men and women, and were lower in black versus non-black men and older women.

Sensitivity analyses in the DHS study. To determine the sensitivity of our findings to components used for the definition of the subcohort free from clinical or subclinical cardiovascular disease, we performed sensitivity analyses

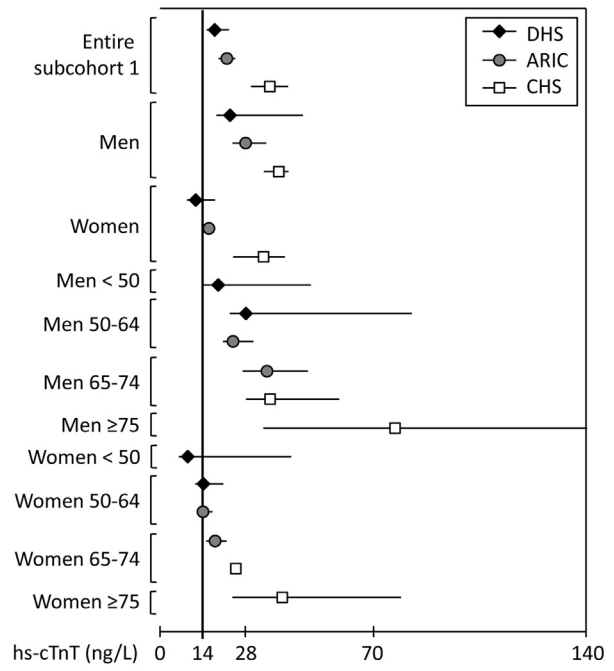

Figure 1 **The 99th Percentile Values for the hs-cTnT Assay in Subcohorts 1 of the DHS, ARIC, and CHS Studies, With Further Stratification by Sex and Age**

Subcohorts 1 include subjects free from recent hospitalization (6 months), clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke), and stage III or greater chronic kidney disease (estimated glomerular filtration rate <60 cc/min/1.73m^2). Whiskers represent 95% confidence intervals. ARIC = Atherosclerosis Risk in Communities; CHS = Cardiovascular Health Study; DHS = Dallas Health Study.

by modifying individual exclusion criteria for DHS subcohort 2, with additional stratification by sex, race and age. As shown in Table 4, no major differences were observed in the 99th percentile cTnT values when the NT-proBNP exclusion cutoff for subcohort 2 was changed from 450 to 125 ng/l, or when the NT-proBNP or electrocardiography exclusion criteria were removed. In contrast, when exclusions based on cardiac imaging for left ventricular hypertrophy or left ventricular systolic dysfunction were removed, the 99th percentile values increased among men, older individuals, and black participants (Table 4).

Discussion

The key finding from this study is that a uniform upper reference limit for the hs-cTnT assay of 14 ng/l, as currently recommended for MI diagnosis, does not reflect the 99th percentile value of a reference population with diverse demographic characteristics. This study unequivocally demonstrates in a very large and well-characterized population that 99th percentile values for the hs-cTnT assay are greater in men and rise notably with increasing age in both men and women. New sex- and age- specific cutoff values for the hs-cTnT assay are proposed, and will require clinical validation. We also found that 99th percentile values are generally higher in black compared with non-black individuals. Although no race-specific cutoff values can be reliably derived from the present dataset, our findings clearly indicate that race may influence hs-cTnT assay cutoff values.

The upper reference limit for the hs-cTnT assay. By convention, an increased cardiac troponin concentration is defined as a measurement exceeding the 99th percentile value for the specific assay within a normal reference population (6,7). However, there is no consensus with regard to the criteria for selecting a reference population, and the definition of "normal" in this context remains a matter of continuing debate (3,5,8).

A recent statement by the International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Cardiac Biomarkers (3) recommended that "normal" populations used to derive the 99th percentile of high-sensitivity cardiac troponin assays should ideally be selected by detailed physician evaluation, including electrocardiogram, echocardiogram and NT-proBNP measurement, and should include both younger and older subjects. The present report is the first to meet (and surpass) these recommendations, far exceeding the size and thus the statistical power of any previous study that determined the 99th percentile upper reference limit of the hs-cTnT assay.

Importantly, 99th percentile values for the hs-cTnT assay were lower in this study when participants with subclinical structural heart disease were excluded (subcohort 2), compared with the use of less stringent exclusion criteria (subcohort 1). These differences were magnified in older individuals, who are more likely to have subclinical cardiovascular disease. Taken together with the results of our sensitivity analyses, these findings support assessment of subclinical structural heart disease to further characterize the reference population in future studies.

The case for sex- and age-specific hs-cTnT cutoff values. Several small studies have previously reported trends towards higher 99th percentile values for both the hs-cTnT and hs-cTnI assays in men (8–15) and in the elderly (9,11,14), but small sample sizes and inconsistent phenotype characterization preclude the derivation of sex- and age-specific clinical cutoff values from these studies. The most recent update to the consensus definition of MI suggested that sex-dependent cutoff values may be endorsed in the future for high-sensitivity cardiac troponin assays (7), but in the absence of reliable data, no specific numeric recommendation was made.

Based on our findings, the cutoff value for the hs-cTnT assay should remain 14 ng/l only for men younger than 50 years of age and for women younger than 65 years of age. Of note, the true 99th percentile cTnT value for women younger than 50 may be lower than 14 ng/l, but validating a lower cutoff is not feasible with the current assay because the coefficient of variation of the assay exceeds 10% at values of 13 ng/l and lower. Utilizing the most conservative

Table 3 The 99th Percentile Value for the hs-cTnT Assay, With 95% CIs, and Percentiles Corresponding to cTnT = 14 ng/l in Subcohorts 2* of the DHS, ARIC, and CHS Studies, With Further Stratification by Sex, Age, and Race

	DHS			ARIC			CHS		
	N	99th hs-cTnT Percentile [95% CI] (ng/l)	Percentile for hs-cTnT 14 ng/l	N	99th hs-cTnT Percentile [95% CI] (ng/l)	Percentile for hs-cTnT 14 ng/l	N	99th hs-cTnT Percentile [95% CI] (ng/l)	Percentile for hs-cTnT 14 ng/l
Entire subcohort 1	1,978	14 [12–17]	99.0	7,575	21 [19–22]	95.9	1,374	28 [25–35]	93.5
Stratified by sex									
Men	873	17 [13–50]	98.5	2,972	26 [23–30]	91.9	489	34 [26–42]	87.4
Women	1,105	11 [7–15]	99.4	4,603	15 [14–17]	98.6	885	24 [18–35]	96.8
Stratified by sex and age (yrs)									
Men <50	651	14 [12–56]	98.8	0	N/A	N/A	0	N/A	N/A
Men 50–64	216	17 [14–83]	98.1	2,000	23 [21–30]	93.5	0	N/A	N/A
Men 65–74	6	N/A	N/A	971	31 [25–37]	88.7	297	34 [26–42]	89.5
Men ≥75	0	N/A	N/A	1	N/A	N/A	192	39 [26–39]	83.9
Women <50	790	7 [5–43]	99.4	0	N/A	N/A	0	N/A	N/A
Women 50–64	310	12 [11–15]	99.3	3,154	13 [13–17]	99.1	0	N/A	N/A
Women 65–74	5	N/A	N/A	1,449	17 [15–21]	97.6	551	24 [15–73]	97.7
Women ≥75	0	N/A	N/A	0	N/A	N/A	334	24 [18–41]	95.2
Stratified by sex, age (yrs), and race									
Men <50, black	232	23 [14–87]	97.9	0	N/A	N/A	0	N/A	N/A
Men <50, non-black	419	13 [9–56]	99.3	0	N/A	N/A	0	N/A	N/A
Men 50–64, black	90	21 [16–21]†	95.6	378	31 [24–53]	89.7	0	N/A	N/A
Men 50–64, non-black	126	12 [12–83]	99.2	1,622	21 [18–29]	94.5	0	N/A	N/A
Men 65–74, black	1	N/A	N/A	113	37 [37–79]	78.8	50	35 [20–35]†	83.8
Men 65–74, non-black	5	N/A	N/A	858	27 [23–35]	90.1	247	32 [26–42]	90.6
Men ≥75, black	0	N/A	N/A	1	N/A	N/A	49	25 [22–25]†	81.7
Men ≥75, non-black	0	N/A	N/A	0	N/A	N/A	153	39 [26–39]	84.4
Women <50, black	355	6 [5–51]	99.4	0	N/A	N/A	0	N/A	N/A
Women <50, non-black	435	7 [5–51]	99.5	0	N/A	N/A	0	N/A	N/A
Women 50–64, Black	141	12 [11–12]	N/A‡	802	13 [12–49]	99.1	0	N/A	N/A
Women 50–64, non-black	169	15 [9–15]	98.8	2,352	13 [13–17]	99.1	0	N/A	N/A
Women 65–74, black	2	N/A	N/A	288	17 [15–21]	96.5	114	66 [15–73]	95.3
Women 65–74, Non-black	3	N/A	N/A	1,161	17 [15–21]	97.9	437	18 [13–36]	98.3
Women ≥75, black	0	N/A	N/A	0	N/A	N/A	70	20 [13–20]†	96.0
Women ≥75, non-black	0	N/A	N/A	0	N/A	N/A	264	28 [18–41]	94.6

*Subcohort 2: Subjects free from recent hospitalization (6 months), clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke), subclinical cardiovascular disease (left ventricular hypertrophy or left ventricular ejection fraction <55% by echo or magnetic resonance imaging, left ventricular hypertrophy by electrocardiography, N-terminal pro–B-type natriuretic peptide >450 ng/l), and stage III or greater chronic kidney disease (estimated glomerular filtration rate <60 ml/min/1.73 m^2). †99th percentile is equivalent to maximum observed value. ‡There was no high-sensitivity cardiac troponin T (hs-cTnT) value ≥14 ng/l in this subgroup.

ARIC = Atherosclerosis Risk in Communities; CHS = Cardiovascular Health Study; CI = confidence interval; DHS = Dallas Heart Study.

estimates from our study (i.e., the lowest reported 99th percentile value from multiple studies with data for subcohort 2 within a given age/sex strata, as shown in Table 3), we propose that cutoff values for the hs-cTnT assay be increased to 17 ng/l for men 50 to 64 years of age and for women 65 years of age or older, and to 31 ng/l for men 65 years of age or older.

Further research is imperative to determine whether these age- and sex-specific cutoff values improve diagnostic performance for MI, both in prospective studies and in

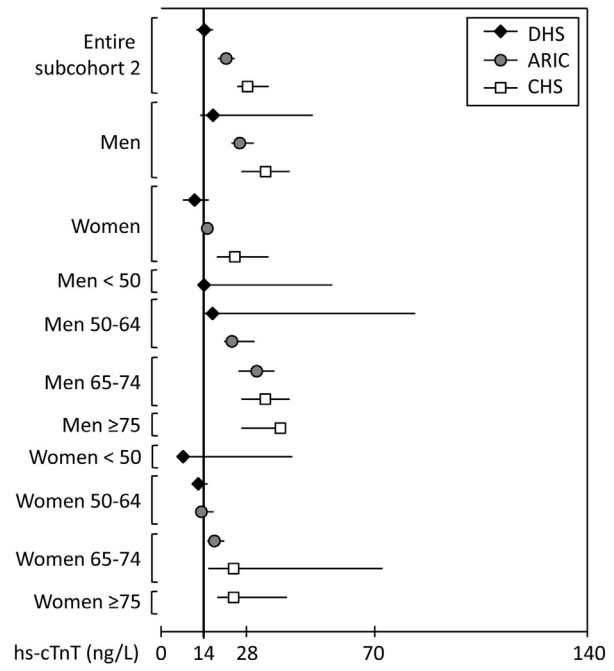

Figure 2 **The 99th Percentile Values for the hs-cTnT Assay in Subcohorts 2 of the DHS, ARIC, and CHS Studies, With Further Stratification by Sex and Age**

Subcohorts 2 include subjects free from recent hospitalization (6 months), clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke), subclinical cardiovascular disease (left ventricular hypertrophy or left ventricular ejection fraction <55% by echo or magnetic resonance imaging, left ventricular hypertrophy by electrocardiography, NT-proBNP >450 pg/ml), and stage III or greater chronic kidney disease (estimated glomerular filtration rate <60 cc/min/1.73m^2). Whiskers represent 95% confidence intervals. Abbreviations as in Figure 1.

post-hoc analyses of existing databases. In addition, further studies are necessary to determine whether age- and sex-specific cutoffs should also be considered for hs-cTnI assays.

Study limitations. This was a retrospective study, but selection bias is unlikely given the population-based nature of the DHS, ARIC, and CHS studies. Nevertheless, prospective validation of the revised cutoff values proposed in this study is critically important. Race and ethnicity were not uniformly recorded for Hispanic/Latino individuals across the 3 cohorts, and there was a low number of black individuals in several age and race strata, thus precluding the derivation of race-specific cutoff values for the hs-cTnT assay. Measurements were performed in sample aliquots stored at −70°C for a variable amount of time, and some cTnT loss is possible with long-term freezing (27). However, any such loss would have led to an underestimation, not overestimation of the 99th percentiles. Because of this potential for underestimation, our recommendations to increase cutoff values for the hs-cTnT assay in men and the elderly may in fact be too conservative, and the values may be upwardly revised in the future as more data become available. For example, a recent study of 406 consecutive patients over 70 years of age with symptoms suggestive of acute MI reported that the optimal hs-cTnT assay cutoff for early diagnosis of MI was 54 ng/l, as determined by receiver-operating characteristic analysis. This value is higher than our age-specific recommendations, and is almost 4 times higher than the presently recommended "one size fits all" upper reference limit (28).

Conclusions

More than 10% of men older than 65 years of age in our study who were free from clinical or subclinical cardiovascular disease had cTnT values above the current MI threshold. This suggests that clinical use of the hs-cTnT assay with the currently recommended cut point may result in over-diagnosis of MI, particularly in elderly men.

The universal definition of MI recommends performing serial measurements of troponins and emphasizes the importance of rising and/or falling levels to distinguish acute MI from other sources of troponin elevation (7). When considered together with baseline levels, moderate changes in cTnT over serial time points improve specificity for MI with the hs-cTnT assay (29). However, it is important to note that the operating characteristics of changes in cTnT values are contingent on whether baseline cTnT is above the MI detection threshold. Thus, an inaccurate upper reference

Table 4 **The 99th Percentile Values for the hs-cTnT Assay (ng/l) Stratified by Sex, Age, and Race in DHS Study Subcohort 2: Sensitivity Analyses**

	DHS Subcohort 2*	Change NT-proBNP Exclusion Cutoff From 450 to 125 ng/l	Remove NT-proBNP Exclusion	Remove ECG LVH Exclusion	Remove Exclusions Based on Cardiac Imaging for LVH or LVEF <55%
Overall	14 (n = 1,978)	14 (n = 1,872)	14 (n = 1,981)	14 (n = 2,121)	14 (n = 2,703)
Men	17 (n = 873)	17 (n = 861)	17 (n = 873)	17 (n = 957)	23 (n = 1,205)
Women	11 (n = 1,105)	9 (n = 1,011)	12 (n = 1,108)	11 (n = 1,164)	11 (n = 1,498)
Age <50 yrs	13 (n = 1,441)	13 (n = 1,384)	13 (n = 1,441)	13 (n = 1,538)	14 (n = 1,985)
Age 50–64 yrs	15 (n = 526)	15 (n = 480)	15 (n = 529)	15 (n = 570)	21 (n = 697)
Black	16 (n = 821)	16 (n = 789)	16 (n = 823)	17 (n = 931)	21 (n = 1,261)
Non-black	13 (n = 1,157)	13 (n = 1,083)	13 (n = 1,158)	13 (n = 1,190)	14 (n = 1,442)

*Subcohort 2: Subjects free from recent hospitalization (6 months), clinical cardiovascular disease (coronary heart disease, chronic heart failure, atrial fibrillation, prior stroke), subclinical cardiovascular disease (left ventricular hypertrophy [LVH] or left ventricular ejection fraction [LVEF] <55% by magnetic resonance imaging, LVH by electrocardiography [ECG], N-terminal pro–B-type natriuretic peptide [NT-proBNP] >450 ng/l), and stage III or greater chronic kidney disease (estimated glomerular filtration rate <60 ml/min/1.73 m^2).

DHS = Dallas Health Study; hs-cTnT = high-sensitivity cardiac troponin T.

limit for the hs-cTnT assay would be expected to impact the performance of all algorithms for MI diagnosis.

Use of more accurate as well as sex- and age-specific 99th percentile values for the hs-cTnT assay would be expected to decrease false positive MI diagnosis with the hs-cTnT assay, a problem with major clinical and public health ramifications (30,31).

Acknowledgments

The authors thank the staff and participants of the DHS, ARIC and CHS studies for their important contributions.

Reprint requests and correspondence: Dr. James A. de Lemos, Department of Internal Medicine, Division of Cardiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-8830. E-mail: James.deLemos@UTSouthwestern.edu.

REFERENCES

1. Wu AH, Jaffe AS. The clinical need for high-sensitivity cardiac troponin assays for acute coronary syndromes and the role for serial testing. Am Heart J 2008;155:208–14.
2. Reichlin T, Hochholzer W, Bassetti S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. N Engl J Med 2009;361:858–67.
3. Apple FS, Collinson PO. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem 2012;58:54–61.
4. Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J 2012;33:2252–7.
5. Korley FK, Jaffe AS. Preparing the United States for high-sensitivity cardiac troponin assays. J Am Coll Cardiol 2013;61:1753–8.
6. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clinical Chem 2010;56:254–61.
7. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012;60:1581–98.
8. Collinson PO, Heung YM, Gaze D, et al. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. Clin Chem 2012;58:219–25.
9. Saenger AK, Beyrau R, Braun S, et al. Multicenter analytical evaluation of a high-sensitivity troponin T assay. Clin Chim Acta 2011;412:748–54.
10. Mingels A, Jacobs L, Michielsen E, Swaanenburg J, Wodzig W, van Dieijen-Visser M. Reference population and marathon runner sera assessed by highly sensitive cardiac troponin T and commercial cardiac troponin T and I assays. Clin Chem 2009;55:101–8.
11. Chenevier-Gobeaux C, Meune C, Blanc MC, Cynober L, Jaffray P, Lefevre G. Analytical evaluation of a high-sensitivity troponin T assay and its clinical assessment in acute coronary syndrome. Annals of clinical biochemistry 2011;48:452–8.
12. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. Clin Chem 2012;58:1574–81.
13. Apple FS, Simpson PA, Murakami MM. Defining the serum 99th percentile in a normal reference population measured by a high-sensitivity cardiac troponin I assay. Clin Biochem 2010;43:1034–6.
14. McKie PM, Heublein DM, Scott CG, et al. Defining high-sensitivity cardiac troponin concentrations in the community. Clin Chem 2013;59:1099–107.
15. Keller T, Ojeda F, Zeller T, et al. Defining a reference population to determine the 99th percentile of a contemporary sensitive cardiac troponin I assay. Int J Cardiol 2013;167:1423–9.
16. de Lemos JA, Drazner MH, Omland T, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA 2010;304:2503–12.
17. Saunders JT, Nambi V, de Lemos JA, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. Circulation 2011;123:1367–76.
18. deFilippi CR, de Lemos JA, Christenson RH, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. JAMA 2010;304:2494–502.
19. Victor RG, Haley RW, Willett DL, et al. The Dallas Heart Study: a population-based probability sample for the multidisciplinary study of ethnic differences in cardiovascular health. J Am Coll Cardiol 2004;93:1473–80.
20. The ARIC investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. Am J Epidemiol 1989;129:687–702.
21. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1991;1:263–76.
22. Troponin T hs and troponin T hs STAT product information brochure, Elecsys 2010 System, Roche Diagnostics, 2009.
23. Hallermayer K, Jarausch J, Menassanch-Volker S, Zaugg C, Ziegler A. Implications of adjustment of high-sensitivity cardiac troponin T assay. Clin Chem 2013;59:572–4.
24. Apple FS, Jaffe AS. Clinical implications of a recent adjustment to the high-sensitivity cardiac troponin T assay: user beware. Clin Chem 2012;58:1599–600.
25. Barnes SC, Collinson PO, Galasko G, Lahiri A, Senior R. Evaluation of N-terminal pro-B type natriuretic peptide analysis on the Elecsys 1010 and 2010 analysers. Ann Clin Biochem 2004;41:459–63.
26. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461–70.
27. Basit M, Bakshi N, Hashem M, et al. The effect of freezing and long-term storage on the stability of cardiac troponin T. Am J Clin Pathol 2007;128:164–7.
28. Reiter M, Twerenbold R, Reichlin T, et al. Early diagnosis of acute myocardial infarction in the elderly using more sensitive cardiac troponin assays. Eur Heart J 2011;32:1379–89.
29. Reichlin T, Irfan A, Twerenbold R, et al. Utility of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. Circulation 2011;124:136–45.
30. de Lemos JA, Morrow DA, deFilippi CR. Highly sensitive troponin assays and the cardiology community: a love/hate relationship? Clin Chem 2011;57:826–9.
31. de Lemos JA. Increasingly sensitive assays for cardiac troponins: a review. JAMA 2013;309:2262–9.

Key Words: diagnosis ■ myocardial infarction ■ population ■ troponin.