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Defining High-Sensitivity Cardiac Troponin Concentrations in the Community

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BACKGROUND: High-sensitivity cardiac troponin (hs-cTn) assays are now available that can detect measurable troponin in significantly more individuals in the general population than conventional assays. The clinical use of these hs-cTn assays depends on the development of proper reference values. Therefore, our objective was to define hs-cTnI reference values and determinants in the general community, in a healthy reference cohort, and in subsets with diseases.

MATERIALS AND METHODS: A well-characterized community-based cohort of 2042 study participants underwent clinical assessment and echocardiographic evaluation. Baseline hs-cTnI measurements were obtained in 1843 individuals. A healthy reference cohort (n = 565) without cardiac, renal, or echocardiographic abnormalities was identified.

RESULTS: Measurable hs-cTnI was identified in 1716 (93%) of the community-based study cohort and 499 (88%) of the healthy reference cohort. Parameters that significantly contributed to higher hs-cTnI concentrations in the healthy reference cohort included age, male sex, systolic blood pressure, and left ventricular mass. Glomerular filtration rate and body mass index were not independently associated with hs-cTnI in the healthy reference cohort. Individuals with diastolic and systolic dysfunction, hypertension, and coronary artery disease (but not impaired renal function) had significantly higher hs-cTnI values than the healthy reference cohort.

CONCLUSIONS: We assessed an hs-cTnI assay with the aid of echocardiographic imaging in a large, well-characterized community-based cohort. hs-cTnI is remarkably sensitive in the general population, and there are important sex and age differences among healthy reference individuals. These results have important implications for defining hs-cTnI reference values and identifying disease.

Cardiac troponin is the biomarker of choice to detect myocardial injury in patients with acute coronary syndrome (ACS)[6] *(1)*. Beyond ACS, cardiac troponin has demonstrated predictive utility for cardiovascular morbidity and mortality in individuals in the general community as well as several specific subsets of patients with cardiovascular disease including heart failure (HF) *(2–4)*.

Although presently available cardiac troponin assays lack the sensitivity to detect cardiac troponin in most healthy individuals in the general population *(5, 6)*, novel high-sensitivity troponin assays are now available that can detect measurable troponin concentrations in significantly more individuals in the general population without cardiovascular disease *(6–8)*. Although many studies have focused on the use of these assays in patients with ACS *(9–11)*, these assays may also facilitate the prediction of subsequent events such as the development of HF *(7, 12, 13)*. The use of cardiac troponin I (cTnI) in ACS and to a lesser extent in prognostic studies depends on the development of proper reference values. However, there is substantial controversy over how best to establish these reference values because it is clear that cardiac comorbidities increase cardiac troponin concentrations *(6, 14, 15)*. Most high-sensitivity cardiac troponin reference interval studies have relied on community-based general population cohorts or putatively healthy controls identified through outpatient clinic patient lists and screening checklists *(7, 16–19)*. Thus, although the independent predictors and distribution of high-sensitivity cardiac troponin have been reported, there is the po-

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[6] Nonstandard abbreviations: ACS, acute coronary syndrome; HF, heart failure; cTnI, cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; CAD, coronary artery disease; LV, left ventricle; REP, Rochester Epidemiology Project; NT-proBNP, amino-terminal pro-B-type natriuretic peptide; LA, left atrium; EF, ejection fraction; GFR, glomerular filtration rate; BP, blood pressure; BMI, body mass index; URL, upper reference limit.

tential for bias due to inadequate exclusion of cardiovascular, renal, or pulmonary disease or other potentially confounding comorbidities. Indeed, it has been shown that as the rigor of exclusion criteria for reference cohorts is increased, the reference interval for troponin narrows *(15)*. Only one study using high-sensitivity cTnT (hs-cTnT) has, to our knowledge, employed in-depth clinical phenotyping and imaging to select a large community-based healthy reference cohort *(13)*.

Accordingly, the objective of the current study was to define hs-cTnI values using a novel hs-cTnI assay (Siemens) in a large cohort of individuals known by history, physical examination, blood testing, and imaging to be without cardiovascular or renal disease. In addition, we sought to compare hs-cTnI concentrations among these healthy reference individuals to those for specific cohorts of patients with cardiovascular comorbidities, including renal insufficiency, hypertension, coronary artery disease (CAD), diastolic dysfunction, and left ventricular (LV) hypertrophy. These findings have important implications for clinicians who will soon be using these assays in the care of patients.

Materials and Methods

The Mayo Foundation and Olmsted Medical Center institutional review boards approved this study.

STUDY POPULATION

We used the resources of the Rochester Epidemiology Project (REP) to identify a random sample of 2042 Olmsted County, MN, residents age ≥45 years. The design and selection criteria of this community-based cohort study as well as the characteristics of the Olmsted County population have been previously described *(20, 21)*. We obtained baseline samples for hs-cTnI in 1843 individuals (199 of the 2042 individuals did not have samples to run for hs-cTnI), and only data from these individuals are included in this analysis.

Participants were characterized as healthy (n = 565) if they had no clinical risk factors, no echocardiographic abnormalities, normal renal function, and an amino-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration within the reference interval. Clinical risk factors were defined as documented CAD, hypertension, diabetes mellitus, prior myocardial infarction, chronic obstructive pulmonary disease, prior or active smoking history, cardiovascular drug use, peripheral vascular disease, hyperlipidemia, and absence of normal sinus rhythm. Echocardiographic abnormalities included in the analysis were LV hypertrophy, left atrial (LA) enlargement, regional wall motion abnormalities, valvular dysfunction, ejection fraction (EF) <50%, and/or any diastolic dysfunction. Normal NT-proBNP for females was defined as ≤150 ng/L for age 45–69 years and ≤200 ng/L for age 70 years and above; for males ≤100 ng/L for age 45–69 years and ≤150 ng/L for age 70 years and above *(22, 23)*. Normal renal function was defined as a calculated glomerular filtration rate (GFR) >60 mL / min.

MEDICAL RECORD REVIEW

All Olmsted County, MN, healthcare providers have maintained a unified medical record, which is indexed by the REP. Each participant underwent a focused physical examination that included measurement of blood pressure (BP), height, and weight. Trained nurse abstractors reviewed each individual's medical record and each study participant completed medication questionnaires. Body mass index (BMI), myocardial infarction, and CAD were defined with established criteria as previously described *(20)*. Diabetes was defined as a fasting glucose >126 mg/dL (7.0 mmol/L) or a diagnosis in the medical record. Criteria for hypertension including at least 1 of the following: systolic BP >140 mmHg, diastolic BP >90 mmHg, and diagnosis in the medical record with concomitant antihypertensive medical therapy. Hyperlipidemia was defined as a total cholesterol >200 mg/dL or a diagnosis of hyperlipidemia in the medical record. Smoking status was defined as never, prior, or active. Estimated GFR was calculated using the Modification of Diet in Renal Disease Study *(24)* formula.

DOPPLER ECHOCARDIOGRAPHY

All echocardiograms were interpreted by a single echocardiologist (M.M. Redfield), who was blinded to the clinical and biomarker data. In each participant, EF, LV mass/hypertrophy, LA size/enlargement, diastolic function, and valvular stenosis/regurgitation were assessed and categorized as previously described *(20, 25–27)* (see Methods in the Data Supplement that accompanies the online version of this report at http://www.clinchem.org/content/vol59/issue7).

BIOMARKER ASSESSMENT

Blood was collected in heparin (hs-cTnI and creatinine) and EDTA (total cholesterol, HDL cholesterol, and NT-proBNP) vacutainers, placed on ice, centrifuged within 2 h, separated into multiple aliquots, and placed in a freezer at −80 °C. A new, never-thawed aliquot was used for each assay.

cTnI data were collected over 9 test days covering 11 calendar days with a prototype hs assay on the Dimension Vista® 1500 System (Siemens Healthcare Diagnostics). This assay uses 3 different monoclonal antibodies for detection of cardiac troponin epitopes: 1 to amino acids 30–35, 1 to amino acids 41–56, and 1 to

amino acids 171–190 *(19)*. In preliminary studies of this novel hs-cTnI assay in 304 putative healthy individuals, the limit of blank was 0.35 ng/L, the limit of detection was 0.8 ng/L, and the 99th percentile value was 48 ng/L. Total imprecision (CV) was 8.5% at 4.4 ng/L and 4.6% at 11.8 ng/L [*(28)*; Roger Bauer, personal communication, November 2, 2012]. We ran 2 commercial QC materials daily with the following results: QC1 (Bio-Rad, Liquichek Low), mean 174 ng/L (CV 4.2%), and QC2 (Bio-Rad Liquichek Level 1), mean 403 ng/L (CV 5.0%). In addition, 2 noncommercial QC checks were performed daily with stored samples at −70C with the following results: QC1, mean 102 ng/L9 (CV 2.5%), and QC2 mean 190 ng/L (CV 2.2%). Data on the stability of cTnI in long-term frozen storage with the hs-cTnI assay used in his study are not currently available. However, previous studies suggest the variability in cardiac troponin attributable to a single thaw is small in magnitude *(29)*. Upper reference limits (URL) (95th, 97.5th, and 99th percentile) for hs-cTnI in the current study were obtained from the healthy reference cohort (n = 565).

Plasma NT-proBNP was measured with the Elecsys proBNP electrochemiluminescence immunoassay run on the Elecsys 2010 (Roche Diagnostics) *(30)*. The lower limit of detection is 5 pg/mL, with interassay and intraassay variabilities of 3.1% and 2.5%, respectively. Creatinine, total cholesterol, and HDL cholesterol were assessed by blinded laboratory personnel in the Immunochemical Core Laboratory of the Mayo Clinic (see online Supplemental Methods; online Supplemental Table 1).

STATISTICAL ANALYSIS

Descriptive statistics were used to summarize demographic and clinical characteristics. Continuous numeric variables are reported as median and lower/upper quartiles. Categorical data are summarized with number and percentage. Groups studied included the total study population (n = 1843; all participants with an hs-cTnI value), a healthy reference cohort (n = 565, see methods above), and specific diseased cohorts [hypertension, CAD, impaired renal function (GFR <60 mL/min), systolic LV dysfunction (EF <50%), mild and moderate/severe diastolic dysfunction, and LV hypertrophy]. Linear regression models were used to examine the association of hs-cTnI with age, sex, and clinical characteristics. The distribution of hs-cTnI was examined, and a log transformation was used to satisfy normality assumptions for regression analyses. Variables were initially examined for association with hs-cTnI with only age and sex adjustment. From this analysis, variables that met a 0.20 level of significance were included as candidate variables for the multivariate model. Variables assessed in the study population (n = 1843) included BMI, systolic BP, diastolic BP, hypertension, heart rate, prior and active smoking history, creatinine, estimated GFR, diabetes, EF, prior myocardial infarction, CAD, total cholesterol, HDL cholesterol, LV mass index, LA volume index, and moderate/severe diastolic dysfunction. Variables assessed in the healthy reference cohort (n = 565) included, BMI, systolic BP, diastolic BP, heart rate, creatinine, estimated GFR, EF, total cholesterol, HDL cholesterol, LV mass index, and LA volume index. NT-proBNP was not included in multivariable models due to potential for colinearity with LV mass, age, and BMI. Age was examined per 10-year change. BMI, systolic BP, diastolic BP, heart rate, creatinine, estimated GFR, EF, total cholesterol, and HDL cholesterol were assessed as continuous variables. For hypertension, smoking status, diabetes, prior myocardial infarction, CAD, LA volume index, LV mass index, and total cholesterol, each was fit as categorical with categories abnormal/high and normal. The final multivariate model was chosen via backward elimination and included variables that met a 0.05 level of significance. Continuous variables are modeled per 1 SD change. R^2 is provided for each model. The statistical analysis was performed using SAS version 9.3 computer software.

Results

TOTAL POPULATION

Baseline characteristics of the total study cohort (n = 1843, all participants with an hs-cTnI measurement) and the healthy reference cohort (n = 565) are shown in Table 1.

Detectable hs-cTnI concentrations (≥0.8 ng/L) were present in 1716 of the 1843 (93%) participants with a median (25th, 75th percentile) value of 3 (2,5) ng/L and a mean (SD) of 7 (20) ng/L. hs-cTnI was higher in men than in women ($P < 0.001$) and increased with age ($P < 0.001$) (Figs. 1 and 2; Table 2). The 95th, 97.5th, and 99th percentile values (with 95% CI) for the population as a whole were 21 (18,25), 36 (31,50), and 72 (58,116) ng/L, respectively. For females the 95th, 97.5th, and 99th percentile values (with 95% CI) were 16 (13,25), 32 (25,41), and 51 (40,74) ng/L and for males were 23 (20,34), 49 (32,77), and 111 (71,234) ng/L, respectively. Distributions of hs-cTnI values in the total population and according to sex are shown in Fig. 1. Twelve percent had values above the 95th percentile URL and 2% above the 99th percentile URL (see online Supplemental Table 2) (URLs defined from the healthy reference cohort, Fig. 2).

The clinical and echocardiographic findings that were correlated with hs-cTnI concentrations after adjustment for age and sex included BMI, systolic and diastolic BP, EF, LA size, LV mass, total cholesterol,

Table 1. Characteristics of the total population and healthy reference cohort.

	Total population[a] (n = 1843)	Healthy reference cohort (n = 565)
Age, years	62 (53, 71)	54 (50, 61)
Women, %	52	54
BMI	28 (25, 31)	27 (24, 30)
Systolic BP, mmHg	131 (116, 146)	121 (111, 134)
Diastolic BP, mmHg	73 (67, 80)	72 (66, 78)
Serum creatinine, mg/dL	0.9 (0.7, 1.0)	0.8 (0.7, 0.9)
GFR, mL/min	81 (71, 89)	86 (76, 90)
Total cholesterol, mg/dL	201 (179, 225)	205 (187, 225)
HDL cholesterol, mg/dL	43 (36, 55)	45 (37, 56)
NT-proBNP, pg/mL	69 (28, 149)	41 (18, 82)
hs-cTn I, ng/L	3 (2, 5)	2 (1, 3)
EF, %	65 (60, 68)	65 (60, 65)
LA volume index, mL/m^2	44 (36, 55)	39 (33, 47)
LVmass index,	94 (82, 109)	88 (77, 97)

[a] The total community-based cohort included 2042 individuals, of whom 1843 had assessment of cTnI using a high-sensitivity assay. Data are presented as median (25th, 75th percentile).

and the presence of CAD or prior myocardial infarction. Variables associated with higher values of hs-cTnI by multivariable analysis (Table 2) were age, BMI, systolic (but not diastolic) BP, LA size, LV mass, and presence of CAD. Female sex and total cholesterol concentrations were associated with lower hs-cTnI concentrations. EF, diastolic dysfunction, and estimated GFR <60 mL/min were not significantly associated with hs-cTnI in multivariable models.

HEALTHY REFERENCE COHORT

Baseline characteristics of the healthy reference population (n = 565) are shown in Table 1. Detectable hs-cTnI concentrations (≥0.8 ng/L) were present in 499 (88%) of the 565 individuals in the healthy reference population, with a median (25th, 75th percentile) value of 2 (1,3) ng/L and a mean (SD) value of 4 (10) ng/L. The 95th, 97.5th, and 99th percentile URL values (with 95% CI) for the healthy reference group as a whole (females and males combined) were 10 (7,20), 27 (14,40), and 48 (32, 124) ng/L, respectively; URL values for females were 6 (4,13), 13 (7,36), and 33 (22,155) ng/L and for males were 17 (9,36), 32 (19,77), 55 (32,124) ng/L, respectively. When we further restricted the healthy reference cohort to an NT-proBNP <100 ng/L there was no meaningful change in the 95th or 99th percentiles for females or males (see online Supplemental Table 3). hs-cTnI concentrations according to age and sex in this well-characterized, healthy reference cohort are detailed in Fig. 2. Overall, hs-cTnI was significantly higher among men compared to women and there were significant incremental increases in hs-cTnI with increasing age among both women and men (Fig. 3).

As seen in the total population, systolic BP, EF, LA size, LV mass, and total cholesterol were correlated with hs-cTnI values after adjustment for age and sex. In contrast BMI was not correlated with hs-cTnI in the total population. Table 2 demonstrates parameters significantly associated with hs-cTnI concentrations in the healthy reference subgroup by multivariable analysis. Specifically, age, female sex, systolic BP, and LV mass were independently associated with hs-cTnI. GFR (as also observed in the total population cohort), BMI, and LA volume were not independently associated with hs-cTnI after multivariable analysis. Importantly, even when GFR was adjusted for only age and sex it was not significantly associated with hs-cTnI values (see online Supplemental Table 4).

DISEASED COHORTS

hs-cTnI was assessed in specific diseased cohorts, including those with systolic LV hypertrophy (n = 184), EF <50% (n = 80), diastolic dysfunction (mild, n = 367; moderate/severe, n = 126), impaired renal function (GFR <60 mL/min, n = 252), hypertension (n = 514), and CAD (n = 221). These results, including the percentage of individuals in each disease cohort with hs-cTnI values above the sex specific 95th percentile and 99th percentile URL, are presented in online Supplemental Table 1 and online Supplemental Fig. 1. hs-cTnI values were higher among these diseased cohorts compared to the healthy reference cohort cohorts ($P < 0.001$) with the exception of GFR <60 mL/min ($P > 0.05$).

Discussion

These data, supported by in-depth clinical and echocardiographic data, represent a comprehensive evaluation of hs-cTnI in a large community-based cohort and are an essential step in the development of appropriate metrics to define reference intervals for hs-cTnI values in the general community. Our results clearly demonstrate the high sensitivity of this novel hs-cTnI assay. Circulating cTnI was measurable in 93% of individuals in this large, well-characterized, community-based cohort of study participants aged 45 years and older. This degree of detection of cardiac troponin in the general population is beyond what has been reported for standard cardiac troponin assays *(5, 31)* and most high-sensitivity assays *(4, 12, 13, 32)*. We defined the metrics for a healthy reference cohort in a subset (n = 565)

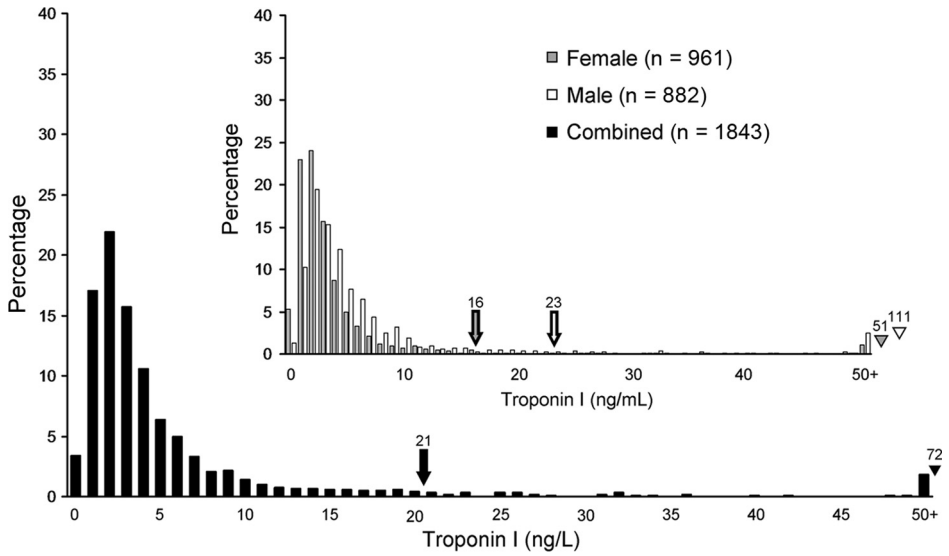

Fig. 1. Distribution of hs-cTnI results in the community-based cohort (n = 1843).
Distribution by female (n = 961) and male (n = 882) sex is also shown. Large arrow denotes the 95th percentile and triangle the 99th percentile. Black, total population; gray, female; white, male.

of this community-based cohort. This large and well-verified healthy reference cohort adds strength to our data. Our data further demonstrate that there are significant differences in hs-cTnI values according to sex, suggesting that there is a need to use different cutoff values for women and men when defining reference intervals. This is consistent with some but not all studies performed to determine reference intervals for hs-

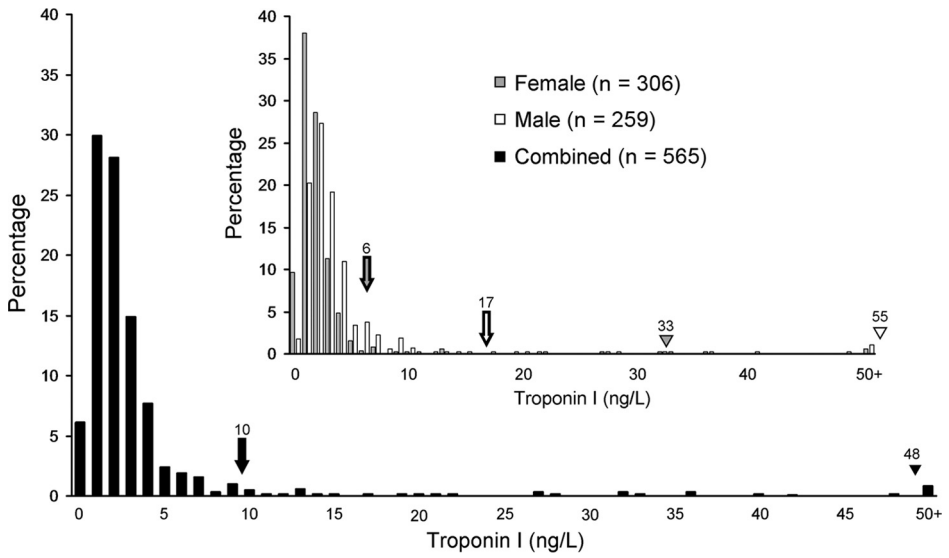

Fig. 2. Distribution of hs-cTnI results in the healthy reference cohort (n = 565).
Distribution by female (n = 306) and male (n = 259) sex is also shown. Large arrow denotes the 95th percentile URL and triangle the 99th percentile URL. Black, total population; gray, female; white, male. These data determined the sex combined and sex specific URL for subsequent analysis.

Table 2. Parameters that significantly contribute to hs-cTnI in multivariable analysis.[a]

Population Parameters included in the model	No.	Regression coefficient (SE)	*P*
Total population (n = 1,843) (R^2 = 0.26)			
Age[b]		0.031 (0.003)	<0.001
Female	961	−0.520 (0.048)	<0.001
BMI[c]		0.091 (0.024)	<0.001
Systolic BP[c]		0.111 (0.026)	<0.001
LA volume index (>33 males, >30 females ml/m^2)	1186	0.132 (0.059)	0.026
LV mass index (>134 males, >110 females g/m^2)	184	0.406 (0.079)	<0.001
Total cholesterol (>200 mg/dL)	941	−0.196 (0.047)	<0.001
CAD	221	0.395 (0.078)	<0.001
Healthy reference cohort (n = 565) (R^2 = 0.16)			
Age[b]		0.015 (0.007)	0.025
Female	306	−0.569 (0.102)	<0.001
Systolic BP[c]		0.130 (0.049)	0.009
Total cholesterol (>200 mg/dL)	121	−0.198 (0.093)	0.034
LV mass index (continuous variable)		0.167 (0.051)	0.001

[a] Dependent variable in all models was log hs-cTnI value + 1. Additional variables for the total population that were evaluated but did not make the final model included diastolic BP, hypertension, heart rate, creatinine, estimated GFR, diabetes, EF, prior myocardial infarction, HDL cholesterol, C-reactive protein, and moderate/severe diastolic dysfunction. Additional variables for the healthy reference cohort that were evaluated but did not make the final model included diastolic BP, heart rate, creatinine, estimated GFR, EF, HDL cholesterol, C-reactive protein, and LA volume index.

[b] Age, expressed per 10-year change. For LA volume index, LV mass index, total cholesterol, and CAD, each was fit as categorical with the categories abnormal/high (effect shown) and normal (reference level). R^2 reported is for the final multivariable model.

[c] Per SD change(total population: BMI SD = 5.3 kg/m^2 and systolic BP SD = 21.3 mmHg; healthy reference cohort: systolic BP SD = 16.9 mmHg and LV mass = 13.9 g/m^2).

cTn assays *(19, 32, 33)*. In addition, our results describe the variables that influence hs-cTnI values in the healthy reference cohort and total community-based cohort as well as the altered distribution of hs-cTnI mediated by structural abnormalities.

Although previous studies have assessed cardiac troponin using high-sensitivity assays in healthy reference cohorts, most have done so without the benefit of imaging to assist with the identification of a cohort without cardiac structural abnormalities. Few other studies have used imaging in a nonelderly community-based cohort to identify reference populations *(13, 15)*. A unique aspect of the current study was the additional use of diastolic function to characterize the healthy reference cohort. The use of imaging allows for the identification of a more robust healthy reference cohort, which has a significant impact on the metrics of high-sensitivity assessment. Specifically, when we remove echocardiographic criteria in our definition of the healthy reference cohort, the population increases in size and the hs-cTnI values are significantly greater (data not shown). When we excluded individuals with structural changes of the heart, including diastolic dysfunction, LV hypertrophy, and LA enlargement, our metrics for the healthy reference cohort were more robust as we attempt to define reference hs-cTnI values. This information is critical for defining the 99th percentile myocardial infarction threshold as well as for clinicians who should expect a modest shift in hs-cTnI values among patients with comorbidities that may not be easily appreciable at the bedside.

When we compare the results obtained with this hs-cTnI assay to those of other large studies in which a high-sensitivity assay was assessed with the aid of cardiac imaging *(12, 13, 15)*, there are several important observations. First, 93% of the study participants in the community-based cohort of the current study have detectable troponin (hs-cTnI). This is compared to 25% among the younger cohort (age 30–65 years) studied by de Lemos et al. *(13)* and 66% in the older cohort (age above 65 years) studied by deFilippi et al. *(12)*. Both of these previous studies assessed hs-cTnT, whereas the current study assessed hs-cTnI. Demographic differences between the 2 populations alone are unlikely to account for the large discrepancy in detectable troponin, thereby suggesting that the hs-cTnI assessed in the current study is a more sensitive assay than the hs-cTnT assay. However, previous studies, al-

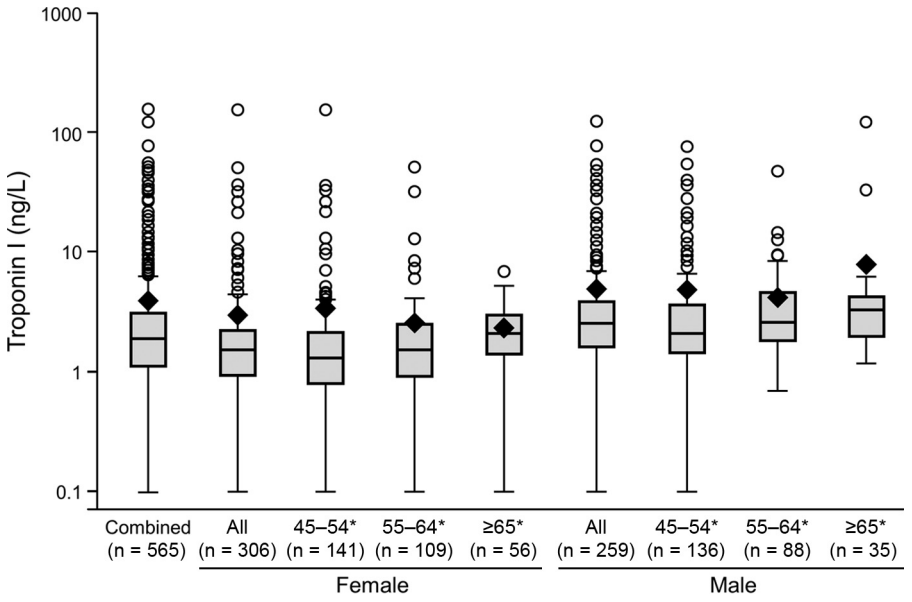

Fig. 3. hs-cTnI in the healthy reference cohort according to age and sex.

The back diamonds represent the mean value. Box plots represent median (horizontal line) and interquartile range (25th to 75th percentile) (shaded box). The whiskers represent values within 1.5 times the interquartile range from the box. The open circles represent values outside 1.5 times the interquartile range. *, Years of age.

beit in discrepant populations, have identified higher sensitivity for the hs-cTnT *(8, 32)* assay, suggesting that technical differences may also account for the sensitivity discrepancy between the 2 assays.

The current data from the healthy reference and total community cohorts suggest that hs-cTnI values are influenced by age, sex, and LV mass in multivariable analyses in a similar manner to previous studies with cTnI *(4)*. Further, specific disease cohorts are associated with higher hs-cTnI values including diastolic dysfunction. These data are of notable clinical benefit because they alert clinicians to parameters and clinical situations that are apt to increase hs-cTnI. In addition, our data have important implications in defining 99th percentile myocardial infarction thresholds and also suggest there is a need to use different values for men and women. In addition, even in participants we defined as healthy (based on clinical, biochemical, and imaging criteria), hs-cTnI increased with age. These data suggest that perhaps some of the increases seen in other studies in association with aging *(34)* may be due to more subtle changes than we were able to detect.

Importantly, in the current study, GFR did not significantly influence hs-cTnI in the total community-based or healthy reference cohorts. Although this finding contrasts with previous studies that suggest an association between the presence of renal and cardiovascular disease *(35, 36)*, most studies that have evaluated cardiac troponin in patients with renal failure have reported on patients with marked renal impairment in contrast to our study population. In addition, although the differences with hs-cTn assays are smaller, cTnT is more frequently increased than cTnI in renal failure *(37, 38)*. Also, it is now established that skeletal muscle pathologies can influence cTnT *(39)*.

Large, well-characterized, healthy reference cohorts, as studied in the investigation we report here, are essential to help identify hs-cTnI reference values. With this information, it may be possible to use hs-cTnI values to help identify asymptomatic individuals who are at increased risk of future events. cTnI has previously proved helpful in this endeavor *(2, 3)*, in part because troponin release into circulation represents a common pathway for multiple disease states, thereby incorporating multiple comorbidities associated with structural heart disease into one measure. It is likely that the more robust hs-cTnI assay will further enhance the ability to identify populations who are at increased risk and may be superior and more cost-effective than models based on clinical and echocardiographic parameters. Alternatively, the models may suggest synergism between echocardiographic measures and hs-cTnI whereby hs-cTnI would be combined with

other blood tests such as the natriuretic peptides and clinical and/or echocardiographic parameters.

The current study has several limitations. First, the cohort was primarily white individuals *(40)* age 45 years and older, and caution should be observed when drawing conclusions regarding other age and ethnic groups. The healthy reference cohort was free of echocardiographic abnormalities and risk factors for CAD. However, these individuals did not undergo coronary angiography, cardiac magnetic resonance imaging, or computed tomography and we cannot exclude the possibility of preclinical CAD among the healthy reference cohort. Finally, relatively few individuals, particularly females, had systolic dysfunction and therefore caution must be used in assessing the relationship between hs-cTnI and systolic HF.

In summary, we report the first study to assess hs-cTnI in a large, well-characterized community-based cohort aided by in-depth echocardiographic imaging. Our data suggest that hs-cTnI as measured by a current assay is remarkably sensitive in the general population and that there are important sex and age differences in cTnI concentrations among healthy reference individuals as well as diseased cohorts. These results have important implications for defining hs-cTnI reference values.

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