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Justin L. Grodin
Heart and Vascular Institute

Sarah Neale
Heart and Vascular Institute

Yuping Wu
Cleveland State University, y.wu88@csuohio.edu

Stanley L. Hazen
Cleveland State University, S.HAZEN@csuohio.edu

W.H. Wilson Tang
Heart and Vascular Institute

CLINICAL RESEARCH STUDY

Prognostic Comparison of Different Sensitivity Cardiac Troponin Assays in Stable Heart Failure

Justin L. Grodin, MD, Sarah Neale, MS, Yuping Wu, PhD, Stanley L. Hazen, MD, PhD, W.H. Wilson Tang, MD

Increasing levels of circulating cardiac troponin (cTn) are highly specific for ongoing myocardial damage and are utilized traditionally as markers for defining myocardial infarction.[1] Circulating cTn levels also can be elevated in other cardiac conditions such as acute and advanced chronic heart failure,[2,3] where they may be related to acute or chronic supply and demand mismatch[4] and may signify increased cardiomyocyte turnover in the setting of progressive myocardial dysfunction.[5]

With technological advances, cTn levels measured by high-sensitivity assays have been developed recently, and can detect levels nearly one-tenth that of standard assays.[6] High-sensitivity cTn (hs-cTn) assays are well suited for detecting sub-clinical cardiac structural abnormalities and detect them in patients with chronic heart failure more frequently than standard assays.[7] In patients with heart failure, circulating hs-cTn is associated with adverse cardiovascular events and with both cardiac and all-cause mortality.[7-11] High-sensitivity assays expand the range of cTn detection, and there is likely significant overlap with standard assays in patients with heart failure. Yet, there are few head-to-head comparisons of the prognostic utility of these 2 assays. As such, we hypothesize that circulating high-sensitivity cTn will be associated with mortality and have increased prognostic accuracy compared with circulating cTn measured by a standard assay in patients with chronic stable heart failure.

CLINICAL SIGNIFICANCE

- Circulating cardiac troponin (cTn) was detectable in more stable heart failure patients via the high-sensitivity assay, compared with the standard assay.
- Heart failure with reduced ejection fraction was associated with higher cTn levels than heart failure with preserved ejection fraction.
- Although high-sensitivity cTn levels can risk-stratify lower-risk heart failure patients, there is no added prognostic value when circulating cTn is detectable by a standard assay.

METHODS

Study Population

We enrolled 504 consecutive subjects with a medical history of chronic heart failure who were undergoing elective diagnostic coronary angiography at the Cleveland Clinic between 2001 and 2007. We excluded patients who had an acute coronary syndrome, recent (<30 days) coronary revascularization, or history of heart transplantation. All participants gave their written informed consent and the study was approved by the Cleveland Clinic Institutional Review Board.

Study Design

Arterial blood samples were collected at the time of coronary angiography, after an overnight fast, after arterial sheath placement, but before the catheterization procedure or any therapies that were administered (including anticoagulation medications). Estimated glomerular filtration rate (eGFR) was calculated via the Modification of Diet in Renal Disease equation.[12] Left ventricle ejection fraction was determined via transthoracic echocardiography via biplane Simpson's method by the Cleveland Clinic echocardiography laboratory, and the results were collected via chart review of the electronic medical record, EPIC (EPIC, Verona, WI). Heart failure with preserved or reduced ejection was defined as left ventricular ejection fraction ≥40% or <40%, respectively. Adjudicated outcomes including mortality, death, myocardial infarction, and stroke were collected prospectively over the 5 years by dedicated research personnel and by Social Security Death Index after enrollment for all cohort subjects.

Cardiac Biomarkers Measurement

All biomarkers were measured at a central core laboratory; hs-cTnT was measured by a high-sensitivity (5th generation) assay on a Roche Cobas e411 platform (Roche Diagnostics, Basel, Switzerland). The limit of detection (LOD) was 3 ng/L and there were no values measured below this level in this cohort. The 99th percentile cutoff was 14 ng/L with an average coefficient of variation <10% at 13 ng/L. Amino-terminal pro B-type natriuretic peptide (NT-proBNP) was measured on the same Roche platform. Cardiac troponin I (cTnI) was measured by a standard sensitivity assay on the Abbott Architect platform (*STAT* Troponin I, Abbott Laboratories, Abbott Park, IL) with analytical sensitivity at 0.01 ng/mL. Troponin I values below the LOD were considered "undetectable." Creatinine and fasting lipid profiles were measured on the same Abbott platform.

Statistical Analysis

Statistical analyses were performed using JMP Pro version 10 (SAS Institute, Inc, Cary, NC) and R software, version 3.0.2. Continuous variables were expressed as either mean ± standard deviation or median (interquartile range) and analyzed by the Student's unpaired *t* test or the Wilcoxon or Kruskal-Wallis tests where appropriate. Categorical variables were expressed as percentage (%) and analyzed by Fisher's exact test. Spearman's correlations were performed to assess relationship between hs-cTnT and clinical characteristics characterized by continuous variables. This cohort was split into 2 groups, split by the LOD of cTnI in a normal reference population: subjects with cTnI <0.01 ng/mL ("undetectable cTnI") or with cTnI ≥0.01 ng/mL ("detectable cTnI"). The subgroups above and below the cTnI LOD were each split into tertiles of hs-cTnT levels. Independent variables were cTnI ≥ or <0.01 ng/mL (n = 302 and n = 202, respectively), hs-cTnT tertiles overall, and hs-cTnT tertiles in each cTnI subgroup. Dependent variables were mortality at 5 years. Two-sided *P*-values of ≤ .05 were considered significant to reject the null hypothesis that there are no differences in mortality at 5 years of follow-up between cTn levels. Survival analyses were completed via the Kaplan-Meier method and log-rank analysis to compare survival curves between cTnI and hs-cTnT groups. Cox proportional hazards models were used to compare time-to-event analysis to determine hazard ratios (HR) and 95% confidence intervals (CI) for 5-year mortality across tertiles of cTnI and hs-cTnT. Multivariate adjustment (base model for mortality) was for age, sex, systolic blood pressure, diabetes mellitus, smoking history, high-density lipoprotein, and low-density lipoprotein cholesterol. In contrast to the area under the curve (AUC), which is a measure of discrimination for the predictive separation of a model based on risk, we included the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) as methods to compare the relative performance of 2 prognostic models.[13] NRI reflects the proportion of cases that are reclassified to a higher risk category between models. The term IDI is based on the difference of average predicted risks for the cases and controls between models. Risk-prediction and net-reclassification methods were used to compare Cox hazard models for mortality by the Pencina method.[14]

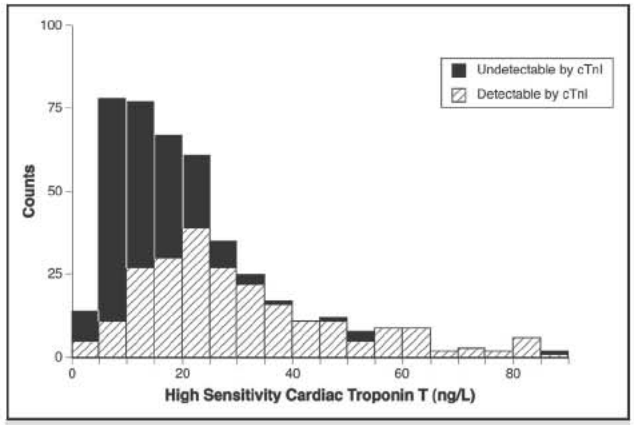

Figure 1 Distribution of high sensitivity cardiac troponin T levels. All values are greater than or equal to the limit of detection for the high sensitivity cardiac troponin T assay, 3 ng/L, and 302 (59.9%) subjects had detectable cardiac troponin I.

RESULTS

Baseline Characteristics

Baseline characteristics for our cohort (all with detectable hs-cTnT and 302 [59.9%] with detectable cTnI) were representative of a patient population with chronic heart failure and are described in Table 1. High-sensitivity cTnT levels were nonparametrically distributed with a right skew (Figure 1). The median hs-cTnT level was 21.2 (12.3-40.9) ng/L. Median hs-cTnT levels across increasing tertiles of hs-cTnT for the whole cohort were 9.6 (7.1-12.2), 21.1 (17.9-24.6), and 63.2 (40.7-189.9) ng/L, respectively. Both cTnI and hs-cTnT levels were higher in subjects with left ventricular ejection fraction <40% in comparison with subjects with a left ventricular ejection fraction ≥40% ($P < .001$ and $P = .02$, respectively; Figure 2). Median hs-cTnT levels were higher in men than women (23 [14-49] ng/L and 18 [10-31] ng/L, $P = .0005$, respectively).

Circulating hs-cTnT and Mortality in the Overall Cohort

At 5 years, there were a total of 170 deaths, with an estimated cohort 5-year survival of 66%. Detectable cTnI was associated with higher incident mortality than undetectable

Table 1 Baseline Characteristics (n = 504)

Variable	Total Cohort (n = 504)	Detectable cTnI (n = 302)	Undetectable cTnI (n = 202)	*P*-Value
Age, years	68 ± 10	69 ± 11	66 ± 10	.01
Male (%)	63.1	67.2	56.9	.02
Diabetes (%)	36.5	43.1	26.5	.0001
Coronary artery disease (%)	78.0	83.3	70.0	.0006
Hypertension (%)	76.4	79.7	71.6	.04
eGFR (mL/min/1.73 m^2)	74.8 ± 26.2	69.3 ± 27.5	83.3 ± 21.7	<.0001
Ever smoker (%)	70.2	68.5	72.8	.3
BMI (kg/m^2)	29 ± 6	29 ± 7	30 ± 7	.1
Beta-blocker (%)	63.3	61.3	66.3	.3
ACEI/ARB (%)	68.3	65.9	71.8	.2
Loop diuretic (%)	63.9	67.2	58.9	.06
Left ventricular ejection fraction (%)	40 (27-55)	35 (25-50)	45 (35-55)	<.0001
NT-proBNP (pg/mL)	1057 (400-2715)	1787 (798-4720)	531 (197-1110)	<.0001
hs-cTnT (ng/L)	21.2 (12.3-40.9)	32.7 (20.3- 75.2)	12.5 (8.6-18.1)	<.0001

Continuous values are expressed mean ± standard deviation or median (interquartile range).

For comparison between cTnI levels ≥ or < 0.01 ng/mL, *P* values calculated via Student's *t* test or Wilcoxon for continuous variables and Fisher's exact test for categorical variables.

Abbreviations: ACEI angiotensin converting enzyme inhibitor; ARB angiotensin receptor blocker; BMI body mass index; cTnI cardiac troponin I measured by standard assay; DASI Duke Activity Status Index; eGFR estimated glomerular filtration rate; hs cTnT cardiac troponin T measured by a highly sensitive assay; NT proBNP amino terminus pro B type natriuretic peptide.

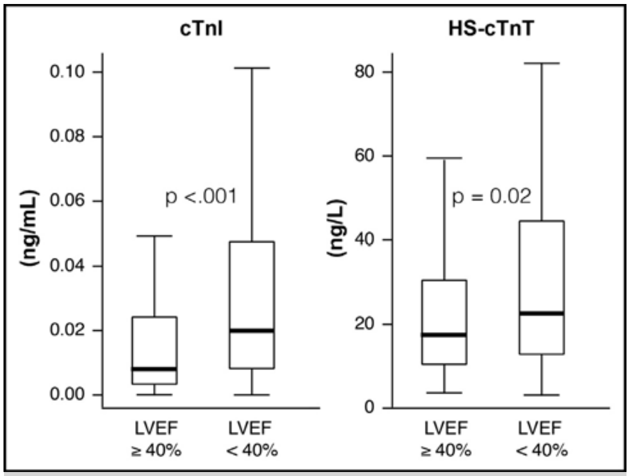

Figure 2 Cardiac troponin levels stratified by left ventricular ejection fraction. *P* value calculated by Wilcoxon test. LVEF = left ventricular ejection fraction; cTnI = cardiac troponin I; hs cTnT = high sensitivity cardiac troponin T.

cTnI (57% vs 80%, respectively; log-rank chi-squared 28.0 and $P < .0001$; Figure 3A).

Grouped by hs-cTnT level, hs-cTnT tertiles 1, 2, and 3 had 29, 55, and 86 deaths, respectively, with significant decrements in survival for increasing tertiles (Figure 3B, log-rank chi-squared 44.9 and $P < .0001$). Increased hs-cTnT was associated with nearly a 3.7-fold increase in 5-year mortality (tertile 1 vs 3, HR 3.74; 95% CI, 2.49-5.79; $P < .0001$). After adjustment for traditional risk factors in addition to hypertension history, and coronary artery disease history, eGFR, NT-proBNP, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker use, beta-blocker use, history of chronic obstructive pulmonary disease, serum sodium, left ventricular ejection fraction, and blood urea nitrogen, increased hs-cTnT remained independently associated with 5-year mortality (tertile 1 vs 3, HR 2.14; 95% CI, 1.24-3.79, $P = .006$).

In comparison with the base model for mortality with cTnI, the prognostic accuracy of the model with hs-cTnT (Table 2) was improved modestly (AUC 66.1% and AUC 69.4%, respectively, $P = .03$) with a 9.0% IDI ($P < .001$) and 13.6% NRI ($P < .001$). In contrast, there was no increase in prognostic accuracy when cTnI and hs-cTnT were both added to the base model for mortality (AUC 69.4% and AUC 69.2%, respectively, $P = .9$), although there was continued IDI (9.0%, $P < .001$) and NRI (3.6%, $P < .001$).

Circulating hs-cTnT and Mortality in the Detectable cTnI Subgroup

In the subgroup with detectable cTnI (n = 302), cTnI and hs-cTnT were correlated (Spearman's rho 0.74, $P < .0001$). The highest hs-cTnT tertile was associated with a 2.1-fold increase in 5-year mortality risk when compared with the lowest hs-cTnT tertile (HR 2.1; 95% CI, 1.4-3.3; $P = .0009$, Figure 4). After multivariate adjustment for traditional risk factors, the association between high hs-cTnT and mortality persisted (HR 2.0; 95% CI, 1.3-3.2; $P = .003$). In a sensitivity analysis with additional adjustment for hypertension history and coronary artery disease history, the association between high hs-cTnT and mortality persisted (HR 1.96; 95% CI, 1.22-3.20, $P = .005$). In further sensitivity analyses with additional adjustment for eGFR to traditional risk factors, there was a similar association with high hs-cTnT and mortality (HR 2.1; 95% CI, 1.1-4.0; $P = .03$), but the effects were not significant when NT-proBNP was further added to the model.

In comparison with the base model for mortality with cTnI (AUC 70.8%), the prognostic accuracy of base model with hs-cTnT or with both hs-cTnT and cTnI was not different (AUC 71.2%, $P = .8$ and AUC 70.9%, $P = .9$; respectively; Table 2). However, both models had sustained IDI at 11.0% ($P < .001$, for both) and had 8.1% ($P < .001$) and 6.8% ($P < .001$) event-specific NRI,

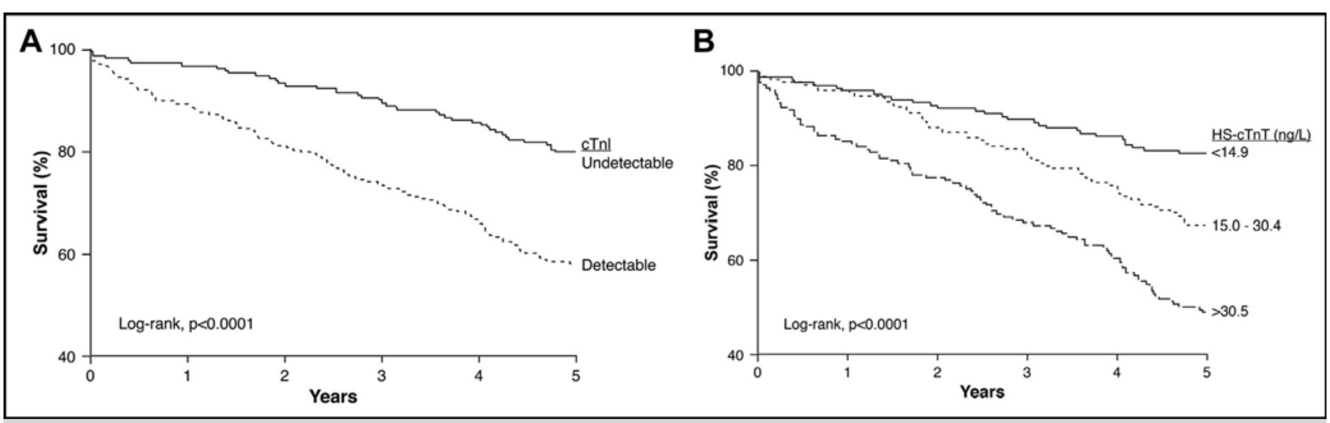

Figure 3 (**A**) Kaplan Meier estimates of 5 year survival rates according to cTnI levels above or below the limit of detection (n = 504). *P* value calculated by the log rank test. (**B**) Kaplan Meier estimates of 5 year survival rates according to hs cTnT levels (n = 504). *P* value calculated by the log rank test. cTnI = cardiac troponin I; hs cTnT = high sensitivity cardiac troponin T.

Table 2 Risk Prediction and Net Reclassification for Cox Multivariate Hazard Models for Mortality

	Whole Cohort (n = 504)						Detectable cTnI (n = 302)					
	Integrated Discrimination Improvement		Event-specific Reclassification		ROC Analysis		Integrated Discrimination Improvement		Event-specific Reclassification		ROC Analysis	
Covariate	IDI (%)	*P*-Value	NRI (%)	*P*-Value	AUC (%)	*P*-Value	IDI (%)	*P*-Value	NRI (%)	*P*-Value	AUC (%)	*P*-Value
With hs-cTnT*	9.0	<.001	13.6	<.001	69.4	.03	11.0	<.001	8.1	<.001	71.2	.8
With hs-cTnT over cTnI*	9.0	<.001	3.6	<.001	69.2	.9	11.0	<.001	6.8	<.001	70.9	.9

Abbreviations: AUC area under the curve; cTnI cardiac troponin I; hs cTnT cardiac troponin T measured by a highly sensitive assay; IDI integrated discrimination improvement; NRI net reclassification improvement; ROC receiver operating curve.

*Base model for 5 year mortality includes: age, sex, systolic blood pressure, low density lipoprotein cholesterol, high density lipoprotein cholesterol, smoking, and diabetes.

respectively, when compared with the base model for mortality with cTnI.

Circulating hs-cTnT Mortality in the Undetectable cTnI Subgroup

High-sensitivity cTnT levels in this subgroup were modestly correlated with age ($r = 0.30$, $P < .0001$) and NT-proBNP ($r = 0.25$, $P = 0.0001$), but negatively correlated with eGFR ($r = -0.33$, $P < .0001$). For the undetectable cTnI subgroup (n = 202), tertiles of circulating hs-cTnT were <9.7, 9.7-12.4, and >12.4 ng/L. In Cox proportional hazards models, the highest hs-cTnT tertile was associated with a 3.8-fold increase in 5-year mortality compared with the lowest hs-cTnT tertile (HR 3.8; 95% CI, 1.6-10.3; $P = .002$; Figure 4). This relationship persisted after multivariate adjustment for traditional risk factors (HR 3.1; 95% CI, 1.2-9.1; $P = .02$). In a sensitivity analysis with additional adjustment for hypertension history and coronary artery disease history, the association between high hs-cTnT and mortality persisted (HR 3.12; 95% CI, 1.21-9.16; $P = .02$). In further sensitivity analyses with additional adjustment for eGFR to traditional risk factors, higher hs-cTnT had a similar association with mortality (HR 3.6; 95% CI, 1.4-10.4; $P = .008$), yet the effects were also not significant when NT-proBNP was further added to the model.

DISCUSSION

This head-to-head comparative study of cTn assays has several novel findings that improve our understanding of the clinical utility of cardiac troponin levels measured by highly sensitive assays in chronic stable heart failure. First, circulating cTn was detectable in more (33.1%) patients via the high-sensitivity assay compared with the standard assay. Second, patients with heart failure and reduced ejection fraction had higher cTn levels compared with patients with heart failure and preserved ejection fraction. Third, hs-cTnT yielded independent and incremental prognostic information to traditional risk factors and even to NT-proBNP and

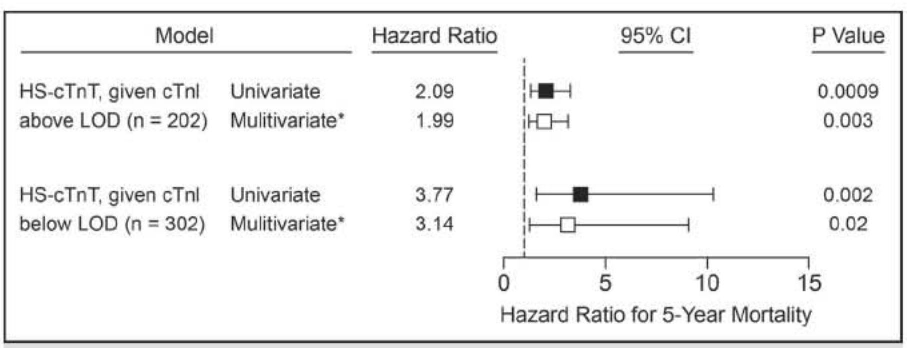

Figure 4 Cox proportional hazards models and forest plot for risk of 5 year mortality. Tertiles 1 vs 3. For the detectable cTnI subgroup, hs cTnT tertiles 1 3 were <23.6, 23.6 52.1, and >52.1 ng/L. For the undetectable cTnI subgroup, hs cTnT tertiles 1 3 were <9.7, 9.7 12.4, and >12.4 ng/L. *Adjustment for age, sex, systolic blood pressure, diabetes, smoking history, high density lipoprotein cholesterol, and low density lipoprotein cholesterol. cTnI = cardiac troponin I; hs cTnT = high sensitivity cardiac troponin T; LOD = limit of detection; for cTnI = 0.01.

eGFR. However, while the analytical performance of hs-cTnT appeared superior, there was overlap in prognostic accuracy of hs-cTnT in subjects with detectable cTnI. These findings highlight the prognostic value of highly sensitive cTn assays in the setting of heart failure, yet also point to the need for future studies to better determine whether the improved sensitivity of cTn assays can translate into incremental clinical benefits.

In contrast to the general population,[15,16] patients with chronic heart failure have more prevalent detectable cTn. The etiology of cTn release in chronic heart failure patients is unclear and likely multifactorial. It may be triggered by acute and chronic myocardial stress, chronic sub-clinical sub-endocardial ischemia, or direct cardiomyocyte injury.[17] It also may result from increased apoptosis in heart failure,[5] thus representing increased cardiomyocyte turnover, which may be indicative of progressive myocardial dysfunction. In heart failure patients, circulating cTn levels have prognostic value independent of renal function[18] and natriuretic peptide levels,[19] in heart failure with either reduced or preserved left ventricular ejection fraction,[20] and in the elderly.[11]

Our results support and add to the growing body of evidence that detectable cTn at any level of assay sensitivity has strong prognostic utility in patients with heart failure.[7-11,21-23] As hypothesized, increased circulating hs-cTnT was independently and incrementally associated with incident 5-year mortality after multivariate adjustment for strong heart failure risk factors. Previously, a retrospective analysis of the Valsartan Heart Failure Trial (Val-HeFT) found a similarly high portion (92.0%; 3728/4053) had detectable hs-cTn by an older assay.[7] In this and another analysis that pooled the Val-HeFT and Gruppo Italiano per lo Studio della Sproavvivenza nell'Insufficienza Cardiaca-Heart Failure cohorts,[9] higher circulating hs-cTn was associated with both incident heart failure hospitalization and mortality, and improved the prognostic accuracy of subjects' clinical risk factors in addition to B-type natriuretic peptide. Unlike our analysis, however, no head-to-head comparisons were made between circulating cTn by both assays.

What is unclear from previous chronic heart failure cohorts, where circulating cTn is measured by both assays,[7,9] is determining whether there is a clinical advantage to prognosticate by measuring hs-cTn when cTn may be detectable by a standard assay; and if such an advantage is related to the increased sensitivity of the hs-cTn assay. In our cohort, circulating hs-cTnT had higher prognostic accuracy when compared with circulating cTnI, thus supporting the use of measuring cTn by high-sensitivity assays instead of standard assays in chronic heart failure. However, there was overlap in incident mortality discrimination of measuring circulating hs-cTnT if cTnI was detectable, which questions the use of measuring cTn by a high-sensitivity assay if cTn is in the detectable range of the standard assay. Therefore, these results suggest that circulating hs-cTn may have a higher prognostic accuracy primarily as a result of their increased sensitivity.[6] In other words, when patients present with a quantifiable cTn level based on standard assay, there appears to be an incremental benefit to stratify risk, although a limited change in endpoint prediction (discrimination) with the addition of a highly sensitive cTn test based on our findings. Risk calibration and endpoint discrimination are often discordant for major disease factors, and excessive reliance on the AUC has been discouraged previously.[24]

One of the biggest advantages of utilizing a more sensitive assay is to expand the lower range of quantifiable cTn. From head-to-head comparisons in non-heart-failure populations, circulating cTn measured by high-sensitivity assays identifies a larger population with cardiac risk factors, structural cardiac abnormalities, increased risk of incident heart failure, and adverse cardiac events than circulating cTn measured by standard assays.[15,16,25] In the undetectable cTnI subgroup, very low levels of circulating hs-cTnT were still associated with 5-year mortality (**Figure 4**). This suggests that very low circulating hs-cTnT, well below the assay's 99th percentile cut-off, yields prognostically important information in patients with heart failure and are supported by similar findings in previous heart failure cohorts.[7,9,11] Taken in aggregate, there appears to be clinically important information embedded in very low cTn levels, thus questioning the clinical utility of using a 99th percentile cut-off for "normal" in patients with heart failure. Although we describe associations of very low cTn levels with age, NT-proBNP, and renal function, further studies are needed to determine the etiology of cTn release and whether similarly low-risk chronic heart failure populations need further risk stratification.

Furthermore, high-sensitivity cTn levels may be viable therapeutic targets for medication titration in similarly low-risk patients with chronic heart failure. In patients with non-ST-segment elevation acute coronary syndromes, antiplatelet therapy escalation guided by circulating cTn has been shown to favorably influence treatment outcomes.[26] Indeed, detectable cTn levels in the setting of receiving high-dose chemotherapy have already demonstrated the ability to identify a patient population with risk of progressive deterioration of cardiac dysfunction that may be ameliorated by initiation of angiotensin-converting enzyme inhibitors.[27] In heart failure, however, whether adjusting chronic heart failure therapy (beta-blockers, renin-angiotensin blockers, or mineralocorticoid receptor antagonists) affects serial hs-cTn levels is unknown. Yet, because changing high-sensitivity cTn levels are associated with prognosis in chronic heart failure,[9,28] future studies to assess associations of medical therapy adjustments and changes in high-sensitivity cTn levels are therefore warranted.

These results must be interpreted in the context of several limitations in our study design. Because cTn levels were measured at only one point in time, we were unable to examine the variability and prognostic value of changing cTn levels by 2 cTn assays over time or the impact of different therapies in the interim. We cannot exclude the presence of selection bias for those undergoing coronary angiography for further evaluation and management of heart failure at a tertiary care center, even though based on baseline clinical characteristics, our cohort is relatively representative of a contemporary patient population with chronic heart failure with both preserved and reduced left ventricular ejection fraction, and we excluded all patients with any suspicion or clinical history of acute coronary syndromes. However, limitations to external validity include a large proportion of patients in this analysis with ischemic cardiomyopathy. Indeed, many noncardiac conditions are associated with detectable circulating troponin,[29] such as sepsis, pulmonary embolism, chronic kidney disease, and myocarditis. With the exception of renal dysfunction, the incidence of these and other acute conditions where troponin is associated with mortality was likely very low as subjects in this study were included before elective coronary angiography. Nevertheless, based on these analyses and because we are in the era when recognizing the potential harms for excessive diagnostics are at the forefront, further investigations continuing to clarify clinical utilities of cTn measured by highly sensitive assays are warranted.

CONCLUSION

In patients with chronic heart failure, when compared with standard assays, high-sensitivity assays identify more patients with detectable circulating cTn. Although plasma hs-cTnT levels provide incremental and independent prognostic value and increased prognostic accuracy in patients with chronic heart failure, there is overlap in this value when both assays measure cTn in the detectable range.

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