Clinical Chemistry 57:8
1146–1153 (2011)

High-Sensitivity Cardiac Troponin I Measurement for Risk Stratification in a Stable High-Risk Population

Peter A. Kavsak,[1] Liqin Xu,[2] Salim Yusuf,[2,3] and Matthew J. McQueen[1,2*]

BACKGROUND: Past investigations regarding the utility of high-sensitivity cardiac troponin I (cTnI) assays have been focused primarily on the acute coronary syndrome setting. We assessed whether such assays can predict future ischemic cardiovascular events in a stable high-risk population.

METHODS: We quantified serum cTnI using an investigational high-sensitivity assay (hs-cTnI IUO, Beckman Coulter) in 2572 participants from the Heart Outcomes Prevention Evaluation (HOPE) study. The derived ROC curve cutoff and the 99th percentile for the hs-cTnI assay were assessed by Kaplan–Meier and Cox analyses for the primary outcome [composite of myocardial infarction (MI), stroke, and cardiovascular death] at 4.5 years of follow-up. We also assessed individual outcomes (MI, stroke, cardiovascular death) and the combined outcome (MI/cardiovascular death) by regression analyses to determine hazard ratios (HRs) and *c* statistics in models that included established risk factors, C-reactive protein, and N-terminal pro-B–type natriuretic peptide (NT-proBNP).

RESULTS: Participants with hs-cTnI >6 ng/L (ROC cutoff) were at higher risk for the primary outcome (HR 1.38, 95% CI 1.09–1.76; $P = 0.008$, adjusted models). For the individual outcomes, participants with hs-cTnI above the 99th percentile (≥10 ng/L) had higher risk for cardiovascular death (HR 2.15, 95% CI 1.32–3.52; $P = 0.002$) and MI (HR 1.49, 95% CI 1.05–2.10; $P = 0.025$) but not stroke (HR 1.38, 95% CI 0.76–2.47; $P = 0.288$, adjusted models). Addition of hs-cTnI to an established risk model with NT-proBNP also yielded a higher *c* statistic for the combined outcome of MI/cardiovascular death.

CONCLUSIONS: The investigational Beckman Coulter hs-cTnI assay provides prognostic information for future MI and cardiovascular death in a stable high-risk population.

Cardiac troponin assay sensitivity has an important role in the diagnosis of myocardial infarction (MI)[4] *(1, 2)*. Assay sensitivity also has a role in prognostication, in that low troponin concentrations exceeding the 99th percentile in and following an episode of acute coronary syndrome (ACS) identify patients at higher risk for an adverse event *(3–7)*. Recent studies have indicated that detectable concentrations of cardiac troponin below the 99th percentile may be used for long-term risk stratification *(8–13)*. This finding was observed in both acute and nonacute settings, suggesting that low levels of cardiac troponin may have prognostic importance across the spectrum of cardiovascular disease (CVD).

Confusion remains, however, as to what constitutes a sensitive vs a high-sensitivity cardiac troponin assay *(14–16)*. One metric that may be used to gauge analytical differences between cardiac troponin assays is the percentage of healthy individuals with measurable concentrations *(14)*. Specifically, in the proposed scorecard, a high-sensitivity assay can be stratified based on the percentage of measureable normal values below the 99th percentile *(14)*. This approach is in contrast to linking assay sensitivity with the precision at the 99th percentile, with assays (either contemporary or high-sensitivity) obtaining a 10% CV at the 99th percentile concentration being designated as guideline acceptable *(14)*. However, a recent statement from the biochemistry subcommittee of the Joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation task force for the definition of MI has suggested widening this estimate, allowing imprecision up

[1] Departments of Pathology and Molecular Medicine and [3] Medicine, McMaster University; [2] Population Health Research Institute, Hamilton Health Sciences, Hamilton, Ontario, Canada.
* Address correspondence to this author at: Lipid Clinic, Hamilton General Hospital, 237 Barton St. East, Hamilton, ON L8L 2X2, Canada; Fax 905-577-8027; email mcquemat@hhsc.ca.
Received February 22, 2011; accepted June 7, 2011.
Previously published online at DOI: 10.1373/clinchem.2011.164574

[4] Nonstandard abbreviations: MI, myocardial infarction; ACS, acute coronary syndrome; CVD, cardiovascular disease; hs-cTnT, high-sensitivity cardiac troponin T; IUO, investigational-use-only; cTnI, cardiac troponin I; HOPE, Heart Outcomes Prevention Evaluation; PVD, peripheral vascular disease; CRP, C-reactive protein; NT-proBNP, N-terminal pro–B-type natriuretic peptide; LoB, limit of the blank; LoD, limit of detection; AUC, area under the curve; HR, hazard ratio; PEACE, Prevention of Events with Angiotensin-Converting Enzyme Inhibition.

to 20% at the 99th percentile concentration *(17)*. Thus, there is continued interest in understanding the prognostic implications of low cardiac troponin concentrations measured with these new, more analytically sensitive assays.

Most studies to date that have assessed both analytical and clinical issues related to high-sensitivity assays have considered only the high-sensitivity cardiac troponin T (hs-cTnT) assay *(2, 7, 11–13, 18)*. Preliminary reports for hs-cTnI assays have focused primarily on the analytical characteristics/performance *(19–23)*, with only a few studies assessing health outcomes based on hs-cTnI concentrations in the ACS setting *(24, 25)*. No studies to date have assessed long-term cardiovascular outcomes with a hs-cTnI assay in a stable high-risk population. To address these issues, we evaluated and measured an investigational-use-only high-sensitivity cardiac troponin I assay (hs-cTnI IUO) in a high-risk stable CVD population: the Heart Outcomes Prevention Evaluation (HOPE) study.

Materials and Methods

STUDY POPULATION AND OUTCOMES

The HOPE study population has been described *(26)*. Briefly, women and men who were stable were enrolled provided they met the following criteria: ≥55 years of age with a history of coronary artery disease, stroke, diabetes, or peripheral vascular disease (PVD) and at least 1 additional risk factor (e.g., smoking, hypertension, microalbuminuria, increased total cholesterol, or low HDL cholesterol) and without heart failure *(26)*. For this study, the study cohort comprised only those individuals (n = 2572) in whom baseline serum was available stored frozen (−80 °C) *(27)*. The outcomes used in this study were the primary outcome for the HOPE study: a composite of MI, stroke, or cardiovascular death (mean follow-up 4.5 years) as well as the individual outcomes: MI, stroke, and cardiovascular death alone as well as the combined outcome of MI/cardiovascular death. Both C-reactive protein (CRP) and N-terminal pro–B-type natriuretic peptide (NT-proBNP) were previously measured in this population, with information regarding the laboratory analysis reported elsewhere *(27)*. The study received Research Ethics Board approval.

hs-cTnI ANALYTICAL MEASUREMENTS

The Beckman Coulter hs-cTnI IUO assay used in this study (reagent lot 915790, calibrator lot 915791) has undergone modifications compared with the hs-cTnI prototype assay, similar to what has been done to their commercial assay *(28)*. Specifically, assay design was modified with a reduction in incubation time, use of recombinant alkaline phosphatase instead of native alkaline phosphatase, and a new buffer for the reagent pack. The preliminary 99th percentile for the hs-cTnI IUO assay (n = 125 healthy individuals) was 9.5 ng/L (serum), 10.3 ng/L (heparin), and 9.5 ng/L (EDTA) *(28)*, consistent with previous estimates that listed the 99th percentile as 10 ng/L in those <60 years of age *(24)*. The precision (CV) for the hs-cTnI assay over the course of the study was 14.7% at a mean concentration of 14 ng/L (serum pool; n = 86); 10.7% at 53 ng/L, and 8.5% at 650 ng/L (Bio-Rad Liquichek Cardiac Markers Plus Control LT; n = 64).

The analytical characteristics of the Beckman Coulter prototype hs-cTnI assay have been reported *(21)*. Because the assay has been modified, however, the analytical characteristics of this assay were also investigated. Specifically, we also determined the limit of the blank (LoB), limit of detection (LoD), sample matrix comparison, and freeze–thaw effect on concentrations. For the LoB and LoD analyses, another lot of hs-cTnI (reagent lot 019032, calibrator lot 010737) was also tested (over 1 month) with the results combined to the previous lot data. During this time frame, the CV for the serum pool was 9.6% with the same mean concentration of 14 ng/L (n = 17). We used 40 measurements of the zero calibrator to determine the LoB using the 95th percentile concentration (nonparametric) *(21)*. The overall mean for the serum pool was 14 ng/L, SD 1.6 ng/L (n = 101 results). The LoD using these data was as follows: LoD = LoB + $c\beta$(SD), as previously done for the prototype research hs-cTnI assay and consistent with Clinical Laboratory Standards Institute guidelines *(21)*.

We also compared the different matrices. Briefly, we measured 20 fresh matched samples for serum, lithium heparin, and EDTA plasma with the hs-cTnI assay (n = 60) and performed Passing–Bablok regression analysis (Analyse-it software, version 2.12). After this analysis, the serum samples with sufficient volume remaining were subjected to 3 freeze–thaw cycles (n = 18 samples; −70 °C storage, 54 measurements) followed by Kruskal–Wallis testing to assess whether there were differences per freeze–thaw, with P values <0.05 considered significant (StatsDirect statistical software, version 2.7.7).

STATISTICAL ANALYSIS FOR HEALTH OUTCOMES

The primary outcome occurred in 406 of the 2572 study participants (15.7%), which is the same rate as was originally reported in the larger study *(27)* (see Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue8). ROC curve analysis was performed for the primary outcome (mean follow-up 4.5 years). For this, the hs-cTnI concentrations were logarithmically transformed and the Youden index used to iden-

Table 1. Baseline characteristics of the study participants with hs-cTnI measurements.[a]

	Men	Women	*P*	All
Population	1979 (100)	593 (100)		2572 (100)
Mean age (SD), years	65 (6)	66 (7)	0.002	65 (7)
Mean body mass index (SD), kg/m^2	28 (4)	29 (5)	<0.001	28 (4)
Hypertension	766 (39)	340 (57)	<0.001	1106 (43)
Diabetes	599 (30)	316 (53)	<0.001	915 (36)
Nonsmoker	369 (19)	229 (39)	<0.001	598 (23)
Former smoker	1337 (68)	276 (47)	<0.001	1613 (63)
Current smoker	272 (14)	88 (15)	0.500	360 (14)
Lipid-lowering agent	611 (31)	190 (32)	0.591	801 (31)
Ramipril	962 (49)	292 (49)	0.788	1254 (49)
Coronary artery disease	1780 (90)	426 (72)	<0.001	2206 (86)
PVD	763 (39)	315 (53)	<0.001	1078 (42)
CVD	1894 (96)	527 (90)	<0.001	2421 (94)
Mean systolic blood pressure (SD), mmHg	136 (18)	140 (19)	<0.001	137 (19)
Mean diastolic blood pressure (SD), mmHg	78 (10)	76 (9)	<0.001	78 (10)
Mean creatinine, μmol/L (SD)	103 (20)	88 (21)	<0.001	100 (21)
Median cholesterol, mmol/L (IQR)	5.34 (4.76–5.99)	5.76 (5.10–6.39)	0.0000	5.43 (4.83–6.09)
Median HDL, mmol/L (IQR)	1.00 (0.84–1.19)	1.17 (0.98–1.40)	<0.001	1.03 (0.86–1.24)
Median triglycerides, mmol/L (IQR)	1.89 (1.37–2.59)	2.10 (1.55–2.85)	<0.001	1.94 (1.39–2.65)
Median glucose, mmol/L (IQR)	5.7 (5.2–7.2)	6.3 (5.3–9.4)	<0.001	5.8 (5.2–7.8)
Median hs-cTnI, ng/L (IQR)	6 (4–10)	5 (4–8)	<0.001	6 (4–9)

[a] Data are n (%) unless noted otherwise.

tify the optimal value (i.e., the point that maximizes sensitivity and specificity). For consistency and comparability with other reported high-sensitivity cardiac troponin assays, we used only integers (i.e., whole numbers) for these analyses *(29)*. We used integer values from ROC analyses (derived from both study population and published concentrations) and 99th percentile (≥10 ng/L) as the cutoffs, with both Kaplan–Meier (with log-rank statistic) and Cox proportional hazard analyses. Cox models were adjusted for sex and age (model 2), for variables that were univariately significant for the primary outcome [i.e., age, sex, diabetes, current smoker, HDL cholesterol (log-transformed) and PVD] (model 3), as well as for other important variables including age, sex, diabetes, smoking, HDL (log-transformed), PVD, lipid-lowering agent, ramipril, systolic blood pressure, CRP (log-transformed), and NT-proBNP (log-transformed) (model 4).

We performed a secondary analysis for each outcome alone based on the following 3 groups classified by hs-cTnI concentrations: <6 ng/L (below the ROC cutoff); ≥10 ng/L (above 99th percentile); and in between (i.e., 6 to <10 ng/L). Kaplan–Meier and Cox proportional hazard analyses were performed over the study timeframe (models 3 and 4) for each outcome, with the <6 ng/L group being the referent group. Multivariate logistic models (including variables in model 3) with and without hs-cTnI (log-transformed) were performed to obtain the *c* statistic for the primary outcome (i.e., composite outcome), as well as for MI alone, stroke alone, and cardiovascular death alone, to assess the discriminative value of hs-cTnI for these outcomes.

We also performed a tertiary analysis on the combined endpoint of MI/cardiovascular death. For this, we used Cox proportional hazard analysis for those study participants who had NT-proBNP, CRP, and hs-cTnI measurements. The biomarkers were evaluated based on previous derived cutoffs (NT-proBNP >156 ng/L and CRP >6 mg/L) *(27)* and the 99th percentile for hs-cTnI (≥10 ng/L), in an established risk factor model (variables included age, systolic blood pressure, antihypertensive treatment, lipid-lowering treatment, diabetes, smoking status, body mass index, log total cholesterol, and log HDL *(30)*; n = 2491). This model was chosen because both CRP and NT-proBNP were previously evaluated in this model for the prediction of

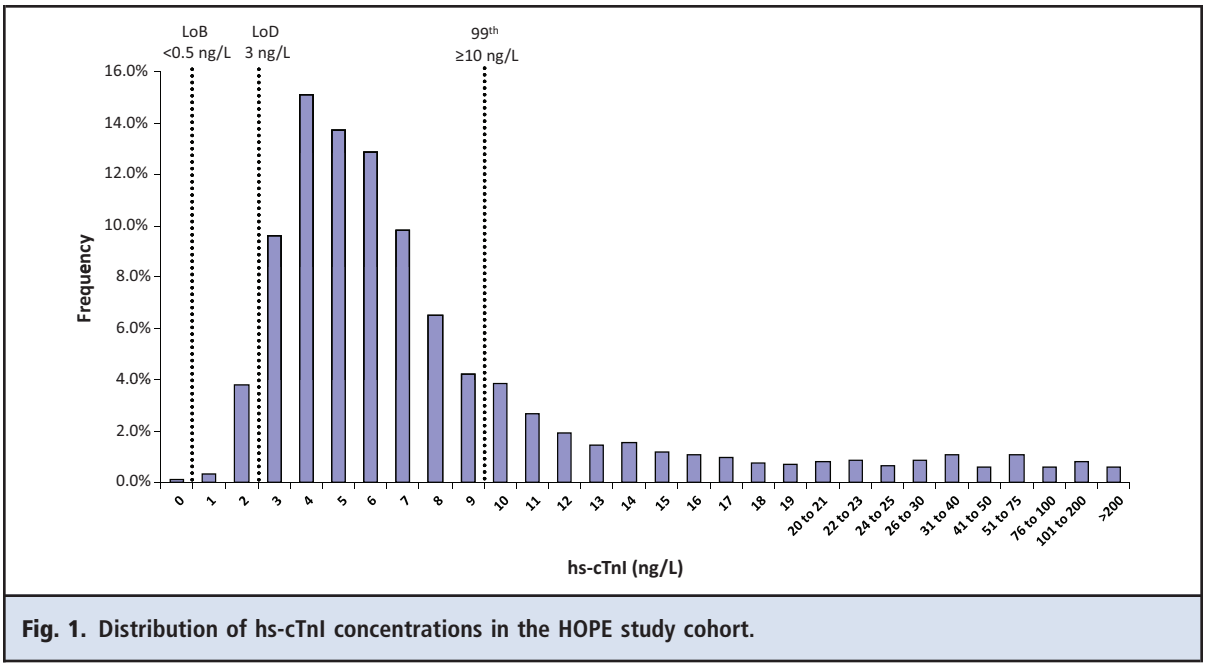

Fig. 1. Distribution of hs-cTnI concentrations in the HOPE study cohort.

cardiovascular death in an older community cohort. Estimates of the *c* statistic (Harrell's *c*) were then derived from these models, but including only the biomarkers (CRP, NT-proBNP, and hs-cTnI) that were significant. Categorical variables were tested by Pearson χ^2 test and continuous variables with nonparametric methods. Analyses were performed in SAS version 9.1 (SAS Institute) and STATA 10.0 (Stata Corp), with *P* values <0.05 considered significant.

Results

In our laboratory, the hs-cTnI IUO assay LoB was determined to be <0.5 ng/L (0.44 ng/L) with the derived LoD of 3 ng/L (3.08 ng/L). Passing–Bablok regression analyses yielded the following relationships between the matrices: EDTA hs-cTnI = 1.06(serum hs-cTnI) − 0.17; heparin hs-cTnI = 1.19(serum hs-cTnI) − 0.98). There was no significant effect on hs-cTnI (range of concentrations 3–32 ng/L) after 3 freeze–thaw cycles ($P = 0.22$). In the HOPE study cohort, men had a higher prevalence of CVD and higher hs-cTnI concentrations than women (Table 1). Concentrations of hs-cTnI were not normally distributed (Shapiro–Wilk $P < 0.001$), with approximately 4% of the results below the limit of detection (i.e., <3 ng/L) (Fig. 1). The area under the ROC curve (AUC) for hs-cTnI for the primary outcome (composite of MI/stroke/cardiovascular death) was 0.625 (95%CI 0.595–0.655). The optimal concentration for predicting the primary outcome in the HOPE cohort was 6 ng/L, with those below this concentration having a lower probability for the outcome based on Kaplan–Meier analysis ($P < 0.001$) (Fig. 2).

Next, we explored the utility of using the published 99th percentile concentration cutoff for risk stratification in this population (i.e., 10 ng/L). Based on the 99th percentile, there was also a significant difference in rates for the primary outcome ($P < 0.001$) (Fig. 2). Cox proportional hazard models assessing the ROC cutoff (6 ng/L) and 99th percentile (10 ng/L) indicated that those above the cutoffs were at significant risk for the primary outcome compared with those below the cutoffs, after adjusting for clinical variables including both CRP and NT-proBNP [hazard ratio (HR) 1.4, $P < 0.01$] (Table 2).

Assessing the distribution of hs-cTnI values based on these cutoffs identified nearly 50% of the study participants (n = 1279) with concentrations <6 ng/L, with 22% (n = 567) having concentrations above the 99th percentile (≥10 ng/L) and the remainder having hs-cTnI concentrations 6 to <10 ng/L (n = 726). Kaplan–Meier analysis was undertaken to determine the event rate between the 3 groups for each outcome (i.e., MI alone, stroke alone, and cardiovascular death alone; see Fig. 3). There were significant differences between the 3 groups for MI ($P < 0.001$), stroke ($P = 0.007$), and cardiovascular death ($P < 0.001$). Cox proportional hazard analyses were performed to assess the risks for the 6 to <10 ng/L group and ≥10 ng/L group compared with the <6 ng/L group (Table 3). After adjustment for the important variables, study participants with hs-cTnI concentrations 6 to <10 ng/L or ≥10 ng/L were at the same risk for MI (HR 1.5, $P <$

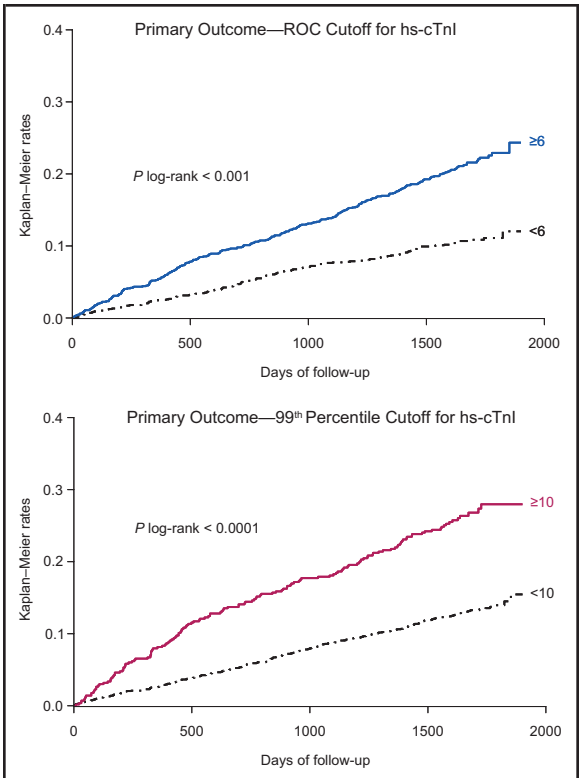

Fig. 2. Kaplan–Meier curves for primary outcome (MI/stroke/cardiovascular death) based on the ROC cutoff (6 ng/L) and 99th percentile (10 ng/L) for hs-cTnI.

Table 2. Cox proportional hazard models for the primary outcome.[a]

	HR (95% CI)	*P*
ROC cutoff, 6 ng/L hs-cTnI		
Model 1	2.13 (1.73–2.62)	<0.001
Model 2	1.94 (1.57–2.39)	<0.001
Model 3	1.86 (1.50–2.29)	<0.001
Model 4	1.38 (1.09–1.76)	0.008
99th percentile cutoff, 10 ng/L hs-cTnI		
Model 1	2.22 (1.81–2.72)	<0.001
Model 2	2.07 (1.69–2.54)	<0.001
Model 3	1.97 (1.60–2.42)	<0.001
Model 4	1.36 (1.08–1.72)	0.009

[a] Model 1, crude; model 2 adjusted for age and sex; model 3 adjusted for age, sex, diabetes, smoking, HDL, and PVD; model 4 adjusted for age, sex, diabetes, smoking, HDL, PVD, lipid-lowering agent, ramipril, systolic blood pressure, CRP, and NT-proBNP.

0.03); those with hs-cTnI ≥10 ng/L were at a higher risk for cardiovascular death (HR 2.2, 95% CI 1.3–3.5; $P = 0.002$). Neither hs-cTnI group was at higher risk for stroke compared with those with hs-cTnI <6 ng/L (Table 3).

Next we determined the discriminative value of hs-cTnI for the different outcomes. The *c* statistic for the primary outcome was increased when hs-cTnI was included in the model ($c = 0.672$ with hs-cTnI vs 0.653 without; $P = 0.004$). Assessing the individual outcomes showed that inclusion of hs-cTnI significantly increased the *c* statistic compared with models without for cardiovascular death (0.742 vs 0.709; $P < 0.001$) and MI (0.637 vs 0.597; $P < 0.001$), but not stroke (0.664 vs 0.661; $P = 0.683$).

As hs-cTnI appeared to provide prognostic information for only MI and cardiovascular death, we next evaluated hs-cTnI for the combined outcome MI/cardiovascular death in the context of established risk factors and biomarkers for cardiovascular disease *(30)*. In these models, after adjusting for the established risk factors, only NT-proBNP (HR 2.14, 95% CI 1.69–2.73; $P < 0.001$) and hs-cTnI (HR 2.16, 95% CI 1.73–2.70; $P < 0.001$) remained significant, whereas CRP was not (HR 1.13, 95% CI 0.88–1.44; $P = 0.346$) (see Supplemental Table 2). Inclusion of hs-cTnI in the model that included these established risk factors and NT-proBNP increased the *c* statistic compared with the model without hs-cTnI for the combined outcome (0.680 vs 0.659; $P = 0.020$).

Discussion

The present study is the first report demonstrating the prognostic significance of measuring hs-cTnI in a high-risk, stable population. Importantly, this study demonstrates for the hs-cTnI assay that concentrations below the reported 99th percentile from a healthy population can identify individuals at risk for an ischemic event. These data are different from the other studies in the literature assessing hs-cTnT, in that either MI or stroke was not used as an outcome in those populations *(12, 13)* or the study assessed MI and failed to show a risk for this event with respect to hs-cTnT concentrations *(11)*. Additional studies are warranted to address the role of the high-sensitivity assays in predicting ischemic cardiovascular outcomes in the nonacute, stable CVD population.

Our findings also raise important questions regarding what cutoff should be used in the non-ACS setting for long-term risk stratification *(31)*. For the hs-cTnI assay, analysis of data from 3 different populations (including the present study) using ROC analyses suggests a possible lower cutoff than the 99th per-

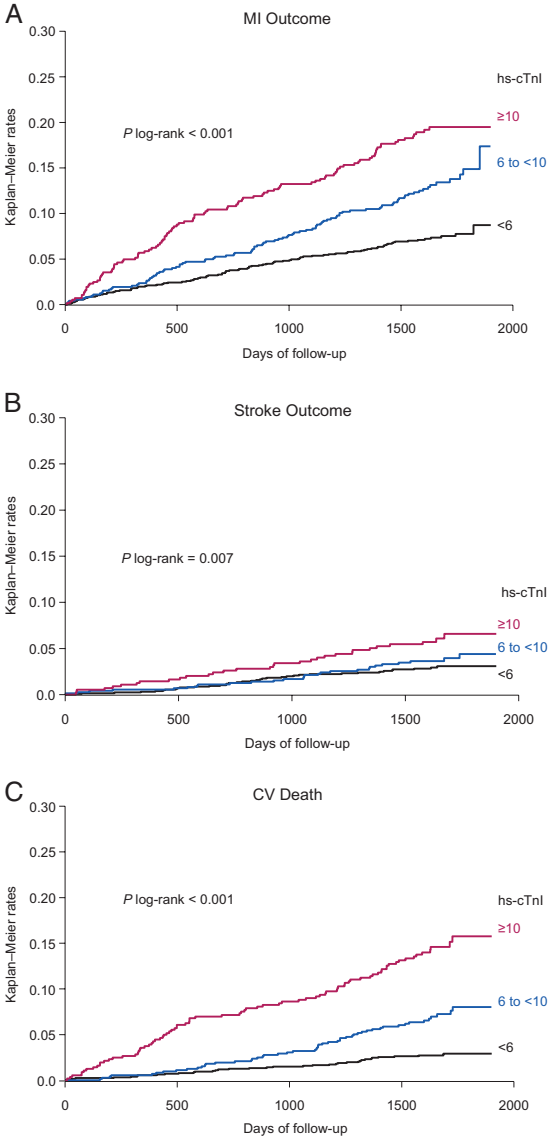

Fig. 3. Kaplan–Meier curves for MI outcome (A), stroke (B), and cardiovascular death (C) based on the following 3 groups: <6 ng/L (below ROC cutoff); 6 to <10 ng/L; and ≥10 ng/L (above 99th percentile).

centile *(24, 32)*. For example, Venge et al. *(24)* identified 6.4 ng/L as the optimal cutoff to discriminate healthy individuals from those with ACS. Furthermore, individuals with hs-cTnI concentrations ≥6 ng/L had a higher rate of death at 1 year (8.1%) than those with concentrations <6 ng/L (3.0%), as did those with concentrations ≥10 ng/L *(24)*. However, the risk for those ACS patients with hs-cTnI concentrations above the ROC cutoff and below the 99th percentile (i.e., 6–10 ng/L) was not assessed in that study *(24)*.

Table 3. Cox proportional hazard models for MI, stroke and cardiovascular death comparing the 6 to <10 ng/L and ≥10 ng/L groups to the group with hs-cTnI concentrations <6 ng/L (referent) at the end of study.[a]

	HR (95% CI)	*P*
Model 3		
6 to <10 ng/L group		
MI	1.72 (1.29–2.30)	<0.001
Stroke	1.14 (0.69–1.89)	0.612
Cardiovascular death	2.33 (1.50–3.61)	<0.001
≥10 ng/L group		
MI	2.37 (1.78–3.16)	<0.001
Stroke	1.77 (1.08–2.88)	0.023
Cardiovascular death	4.43 (2.95–6.64)	<0.001
Model 4		
6 to <10 ng/L group		
MI	1.49 (1.07–2.07)	0.017
Stroke	1.11 (0.64–1.93)	0.716
CV death	1.73 (1.03–2.90)	0.037
≥10 ng/L group		
MI	1.49 (1.05–2.10)	0.025
Stroke	1.38 (0.76–2.47)	0.288
Cardiovascular death	2.15 (1.32–3.52)	0.002

[a] Model 3 adjusted for age, sex, diabetes, smoking, HDL, and PVD. Model 4 adjusted for age, sex, diabetes, smoking, HDL, PVD, lipid lowering agent, ramipril, systolic blood pressure, CRP, and NT-proBNP.

Schulz et al. *(32)*, measuring hs-cTnI in patients with stable CVD, identified 6.75 ng/L as the best concentration for predicting the type of chronic heart disease; however, ROC concentrations were not predictive of events in that smaller study. In the present study, 6 ng/L was identified as the optimal concentration for predicting the primary outcome. On further analysis, however, it appears that this effect was driven mainly by MI and cardiovascular death. Assessing CRP, NT-proBNP, and hs-cTnI in this population for the combined outcome (MI/cardiovascular death) revealed that only increased NT-proBNP and hs-cTnI were significant risk factors; addition of hs-cTnI to a model including NT-proBNP resulted in a higher *c* statistic. This is similar to findings for the Prevention of Events with Angiotensin-Converting Enzyme Inhibition (PEACE) study, where addition of hs-cTnT to a model including NT-proBNP resulted in a higher *c* statistic. It is worthy to note that in the HOPE population, increased CRP was not related to future MI, but was associated with future stroke *(27)*, perhaps explaining the nonsignificant findings for CRP for MI/cardiovascular death.

Analytically, this newer version of the hs-cTnI assay (hs-cTnI IUO) appears to have retained attributes similar to those of the prototype assay, although the estimates for both the LoB and LoD are slightly different (example, hs-cTnI IUO assay LoD = 3 ng/L compared to prototype LoD = 2 ng/L). A larger study assessing multiple lots of reagents may be required to obtain a more thorough analytical description of this assay and its precision below the 99th percentile. Importantly, almost all individuals had measurable concentrations using the hs-cTnI IUO assay, which is perhaps the most important aspect, as this allowed health outcome analysis exploring different cutoffs.

Finally, additional work is required in various clinical settings to ascertain the impact of this testing. This is especially important considering the modest, albeit significant, incremental effect of the addition of hs-cTnI to models for MI and cardiovascular death. Furthermore, outcome studies relating treatment to hs-cTnI concentrations are required, as well as understanding what a significant change in concentration would be in this setting for individuals with serial measurement. One approach would be to use biological variation to ascertain what would constitute a difference in serial measurements longitudinally in the chronic setting *(33, 34)*, or alternatively, an assessment of the minimum concentration change that translates to an adverse outcome. These data are beginning to emerge in the ACS setting *(35, 36)*; however, the criteria for change in both chronic and acute settings will most likely be different, as recently demonstrated with hs-cTnT assay *(12)*. Further studies addressing these important points are required before these high-sensitivity assays are used for screening large populations at risk *(37)*.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: P.A. Kavsak, Ortho Clinical Diagnostics.
Stock Ownership: None declared.
Honoraria: P.A. Kavsak, Beckman Coulter and Randox.
Research Funding: P.A. Kavsak, Beckman Coulter; Roche Diagnostics; Canadian Institutes of Health Research (CIHR) grant; an Early Researcher Award, Ministry of Research & Innovation, Ontario Government; and Abbott; M.J. McQueen, Beckman Coulter. Reagents provided through grant from Beckman Coulter.
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

1. Kavsak PA, MacRae AR, Lustig V, Bhargava R, Vandersluis R, Palomaki GE, Yerna MJ, Jaffe AS. The impact of the ESC/ACC redefinition of myocardial infarction and new sensitive troponin assays on the frequency of acute myocardial infarction. Am Heart J 2006;152:118–25.
2. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. N Engl J Med 2009;361:858–67.
3. Kavsak PA, MacRae AR, Palomaki GE, Newman AM, Ko DT, Lustig V, Tu JV, Jaffe AS. Health outcomes categorized by current and previous definitions of acute myocardial infarction in an unselected cohort of troponin-naïve emergency department patients. Clin Chem 2006;52:2028–35.
4. Eggers KM, Lagerqvist B, Venge P, Wallentin L, Lindahl B. Persistent cardiac troponin I elevation in stabilized patients after an episode of acute coronary syndrome predicts long-term mortality. Circulation 2007;116:1907–14.
5. Eggers KM, Lagerqvist B, Oldgren J, Venge P, Wallentin L, Lindahl B. Pathophysiologic mechanisms of persistent cardiac troponin I elevation in stabilized patients after an episode of acute coronary syndrome. Am Heart J 2008;156:85–92.
6. Bonaca M, Scirica B, Sabatine M, Dalby A, Spinar J, Murphy SA, Jarolim P, Braunwald E, Morrow DA. Prospective evaluation of the prognostic implications of improved assay performance with a sensitive assay for cardiac troponin I. J Am Coll Cardiol 2010;55:2118–24.
7. Lindahl B, Venge P, James S. The new high-sensitivity cardiac troponin T assay improves risk assessment in acute coronary syndromes. Am Heart J 2010;160:224–9.
8. Zethelius B, Johnston N, Venge P. Troponin I as a predictor of coronary heart disease and mortality in 70-year-old men: a community-based cohort study. Circulation 2006;113:1071–8.
9. Kavsak PA, Newman AM, Lustig V, MacRae AR, Palomaki GE, Ko DT, Tu JV, Jaffe AS. Long-term health outcomes associated with detectable troponin I concentrations. Clin Chem 2007;53:220–7.
10. Apple FS, Smith SW, Pearce LA, Ler R, Murakami MM. Use of the Centaur TnI-ultra assay for detection of myocardial infarction and adverse events in patients presenting with symptoms suggestive of acute coronary syndrome. Clin Chem 2008;54:723–8.
11. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med 2009;361:2538–47.
12. deFilippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, Seliger SL. 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.
13. de Lemos JA, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, 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.
14. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem 2009;55:1303–6.
15. Jaffe AS, Apple FS. High-sensitivity cardiac troponin: hype, help, and reality. Clin Chem 2010;56:342–4.
16. Wu A, Collinson P, Jaffe A, Morrow D. High-sensitivity cardiac troponin assays: what analytical and clinical issues need to be addressed before introduction into clinical practice? Interview by Fred S. Apple. Clin Chem 2010;56:886–91.
17. Jaffe AS, Apple FS, Morrow DA, Lindahl B, Katus HA. Being rational about (im)precision: a statement from the Biochemistry Subcommittee of the joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation Task Force for the Definition of Myocardial Infarction. Clin Chem 2010;56:941–3.
18. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin Chem 2010;56:254–61.

19. Wu AH, Fukushima N, Puskas R, Todd J, Goix P. Development and preliminary clinical validation of a high sensitivity assay for cardiac troponin using a capillary flow (single molecule) fluorescence detector. Clin Chem 2006;52:2157–9.
20. Wu AH, Agee SJ, Lu QA, Todd J, Jaffe AS. Specificity of a high-sensitivity cardiac troponin I assay using single-molecule-counting technology. Clin Chem 2009;55:196–8.
21. Kavsak PA, MacRae AR, Yerna MJ, Jaffe AS. Analytic and clinical utility of a next-generation, highly sensitive cardiac troponin I assay for early detection of myocardial injury. Clin Chem 2009; 55:573–7.
22. Wilson SR, Sabatine MS, Braunwald E, Sloan S, Murphy SA, Morrow DA. Detection of myocardial injury in patients with unstable angina using a novel nanoparticle cardiac troponin I assay: observations from the PROTECT-TIMI 30 Trial. Am Heart J 2009;158:386–91.
23. 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.
24. Venge P, Johnston N, Lindahl B, James S. Normal plasma levels of cardiac troponin I measured by the high-sensitivity cardiac troponin I access prototype assay and the impact on the diagnosis of myocardial ischemia. J Am Coll Cardiol 2009;54: 1165–72.
25. Kavsak PA, Wang X, Ko DT, MacRae AR, Jaffe AS. Short- and long-term risk stratification using a next-generation, high-sensitivity research cardiac troponin I (hs-cTnI) assay in an emergency department chest pain population. Clin Chem 2009; 55:1809–15.
26. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342:145–53.
27. Blankenberg S, McQueen MJ, Smieja M, Pogue J, Balion C, Lonn E, et al. Comparative impact of multiple biomarkers and N-terminal pro-brain natriuretic peptide in the context of conventional risk factors for the prediction of recurrent cardiovascular events in the Heart Outcomes Prevention Evaluation (HOPE) study. Circulation 2006;114: 201–8.
28. Kavsak PA, McQueen MJ. Sensitive and high sensitivity cardiac troponin I concentrations in the Heart Outcomes Prevention Evaluation (HOPE) study: a high risk population. Clin Chim Acta 2010;411:1832.
29. Kavsak PA. Highly sensitive cardiac troponin T assay, cardiac disease, and mortality risk. JAMA 2011;305:1196.
30. Zethelius B, Berglund L, Sundstrom J, Ingelsson E, Basu S, Larsson A, et al. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. N Engl J Med 2008;358: 2107–16.
31. Kavsak PA, McQueen MJ. High sensitivity cardiac troponin concentration cutoffs: is a healthy population the right reference population for those with underlying cardiac disease? Clin Biochem 2010;43:1037–8.
32. Schulz O, Reinicke M, Berghoefer GH, Bensch R, Kraemer J, Schimke I, Jaffe AS. High-sensitive cardiac troponin I (hs-cTnI) values in patients with stable cardiovascular disease: an initial foray. Clin Chim Acta 2010;411:812–7.
33. Wu AH, Lu QA, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. Clin Chem 2009; 55:52–8.
34. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. Clin Chem 2010;56:1086–90.
35. Kavsak PA, Ko DT, Wang X, MacRae AR, Jaffe AS. 2007 universal myocardial infarction definition change criteria for risk stratification by use of a high-sensitivity cardiac troponin assay. Clin Chem 2010;56:487–9.
36. Kavsak PA, Ko DT, Wang X, MacRae AR, Jaffe AS. Increasing cardiac troponin changes measured by a research high-sensitivity troponin I assay: absolute vs. percentage changes and long-term outcomes in a chest pain cohort. Clin Chem 2010; 56:1905–7.
37. Apple FS. High-sensitivity cardiac troponin for screening large populations of healthy people: is there risk? Clin Chem 2011;57:537–9.