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FOCUS ISSUE: BIOMARKERS IN CARDIOVASCULAR DISEASE

Prospective Evaluation of the Prognostic Implications of Improved Assay Performance With a Sensitive Assay for Cardiac Troponin I

Marc Bonaca, MD,* Benjamin Scirica, MD, MPH,* Marc Sabatine, MD, MPH,* Anthony Dalby, MD,‡ Jindrich Spinar, MD,§ Sabina A. Murphy, MPH,* Peter Jarolim, MD, PHD,† Eugene Braunwald, MD,* David A. Morrow, MD, MPH*

Boston, Massachusetts; Johannesburg, South Africa; and Pekarska, Czech Republic

Objectives The purpose of this study was to investigate the prognostic implications of low-level increases in cardiac troponin I (cTnI) using a current-generation sensitive assay in patients with suspected acute coronary syndrome (ACS).

Background Recent enhancements in troponin assays have enabled resolution of the 99th percentile reference limit at progressively lower concentrations. However, the clinical significance of low-level increases with sensitive assays is still debated.

Methods We measured cTnI using a sensitive assay (TnI-Ultra, Siemens Healthcare Diagnostics, Deerfield, Illinois) at baseline in 4,513 patients with non–ST-segment elevation ACS randomly assigned to ranolazine or placebo. We applied decision limits at the 99th percentile reference limit (0.04 μg/l), the cut point of the predecessor assay (0.1 μg/l), and 1 equivalent to elevation of creatine kinase–myocardial band (1.5 ng/ml).

Results Patients with baseline cTnI ≥0.04 μg/l (n = 2,924) were at higher risk of death/myocardial infarction (MI) at 30 days than were patients with a negative cTnI (6.1% vs. 2.0%, p < 0.001). After adjusting for the TIMI (Thrombolysis In Myocardial Infarction) risk score, cTnI ≥0.04 μg/l was associated with a 3-fold (95% confidence interval: 2.0 to 4.4, p < 0.001) higher risk of death/MI at 30 days. Moreover, patients with low-level increases (0.04 μg/l to <0.1 μg/l), were at significantly higher risk of death/MI at 30 days (5.0% vs. 2.0%, p = 0.001) and death at 12 months (6.4% vs. 2.4%, p = 0.005) than were patients with cTnI <0.04 μg/l.

Conclusions Low-level increases in cTnI using a sensitive assay identify patients at higher risk of death or MI. These findings support current American College of Cardiology/American Heart Association recommendations defining MI, and the incremental value of newer, more sensitive assays in identifying high-risk patients with ACS. (J Am Coll Cardiol 2010;55:2118–24) © 2010 by the American College of Cardiology Foundation

Cardiac troponin (cTn) is the preferred cardiac biomarker of necrosis for diagnosis and risk assessment in patients presenting with suspected acute coronary syndromes (ACS) (1–3). More than 30 studies have demonstrated a strong relationship between cTn and prognosis independent of other clinical and laboratory risk markers (3–6). However, clinical decision limits for cTn have moved progressively

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From the *TIMI Study Group and the †Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts; ‡South African Cardiology Clinical Trials Group, Johannesburg, South Africa; and the §University Hospital St. Ann, Pekarska, Czech Republic. Dr. Scirica has received research support from Merck, Novartis, AstraZeneca, Daiichi-Sankyo, and Bristol-Myers Squibb; honoraria for educational presentations from CV Therapeutics, Novartis, Merck, Schering-Plough, Sanofi-Aventis, Lilly, and Daiichi-Sankyo; has served as a consultant for AstraZeneca, Novartis, Congentus, and Gilead; and is the recipient of an unrestricted grant from the Michael Lerner Foundation. Dr. Sabatine has received research grants from BRAHMS, diaDexus, and Ortho-Clinical Diagnostics, and is a consultant for Singulex. Dr. Jarolim has received research support from Amgen, Beckman-Coulter, Ortho Clinical Diagnostics, Roche Diagnostics, and Siemens Healthcare Diagnostics, and honoraria for educational presentations from Ortho Clinical Diagnostics. Dr. Braunwald is the chairman of the TIMI Study Group, who receives grant support from Beckman-Coulter, CV Therapeutics (now Gilead Sciences), and Roche Diagnostics. Dr. Morrow has received research/educational grant support through the TIMI Study Group from Accumetrics, AstraZeneca, Bayer Healthcare, Beckman Coulter, Bristol-Myers Squibb, CV Therapeutics, Daiichi Sankyo, Eli Lilly and Co., Genentech, Johnson & Johnson, Merck and Company, Nanosphere, Novartis Pharmaceuticals, Ortho-Clinical Diagnostics, Pfizer, Roche Diagnostics, Sanofi-Aventis, Siemens, Singulex, and Schering-Plough; has received honoraria for educational presentations from CV Therapeutics and Eli Lilly and Co.; and is a consultant/on the advisory board of AstraZeneca, Beckman-Coulter, Cardiokinetix, Gilead, Ikaria, Menarini, Molecular Insight, OrthoClinical Diagnostics, Sanofi-Aventis, Schering-Plough Research Institute, Siemens, and Roche Diagnostics.

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toward lower concentrations. Professional guidelines have recommended a diagnostic limit based on the 99th percentile of a reference population instead of higher cut points defined by comparison to creatine kinase-myocardial band (CK-MB) (3,7). At the same time, enhancements to the analytic performance of commercially available assays for cTn have enabled reliable detection of the 99th percentile at lower concentrations of cTn (2). Recently, several such sensitive assays for cTn were shown to improve significantly the overall diagnostic accuracy over prior generation assays (8,9). Nevertheless, the accompanying substantial increase in the proportion of patients presenting with chest pain who are found to have elevated troponin continues to raise doubt for many practitioners as to the clinical significance of such low-level increases in cTn identified by these newer, more sensitive assays and the appropriateness of the 99th percentile cut point (10–13). Therefore, we investigated the prognostic performance of 1 such sensitive commercial assay for cTnI (8,9) at the 99th percentile decision limit in a robustly sized population with suspected ACS, with a specific interest in subjects with low-level increases.

Abbreviations and Acronyms

ACS = acute coronary syndrome
CI = confidence interval
CK-MB = creatine kinase-myocardial band
cTn = cardiac troponin
HR = hazard ratio
MI = myocardial infarction
NSTE-ACS = non–ST-segment elevation acute coronary syndrome

Methods

Patient population. In the MERLIN–TIMI 36 (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST Elevation Acute Coronary–Thrombolysis In Myocardial Infarction 36) trial, 6,560 patients with non–ST-segment elevation acute coronary syndrome (NSTE-ACS) were enrolled between October 2004 and May 2006. They were assigned randomly to treatment with either ranolazine or placebo in a 1:1 ratio and followed up for a median of 348 days (14). The median time from symptom onset to randomization was 23 h (25th, 75th percentiles: 13 h, 24 h). The study design and primary results have been published previously (15). Eligible patients were 18 years of age or older, had symptoms consistent with myocardial ischemia at rest, and had at least 1 of the following indicators of moderate to high risk of death or recurrent ischemic events: elevated biomarker of necrosis, ST-segment depression of at least 0.1 mV, diabetes mellitus, or an intermediate or high (3) TIMI risk score for unstable angina/non–ST-segment elevation MI. Major exclusion criteria included cardiogenic shock, persistent ST-segment elevation, successful revascularization of the culprit lesion before randomization, and life expectancy <12 months. The MERLIN–TIMI 36 trial, including this substudy, was approved by the relevant institutional review boards at all participating centers. Written informed consent was obtained from all patients.

Troponin testing. Baseline blood samples were to be collected in all patients at all sites in countries participating in the biomarker substudy. Serum samples were isolated on site and stored at −20°C or colder for <6 weeks. Samples were subsequently shipped frozen to the TIMI Clinical Trials Laboratory (Boston, Massachusetts) where they were stored at −80°C or colder. Cardiac troponin I was measured in batches using the TnI-Ultra assay (ADVIA Centaur, Siemens Healthcare Diagnostics, Deerfield, Illinois) by personnel blinded to treatment allocation and clinical events. This assay is a sandwich immunoassay using monoclonal capture antibodies directed against the stable central region of the TnI molecule and 1 polyclonal detecting antibody (10). The lower limit of detection is 0.006 μg/l. The 99th percentile reference limit has been established at 0.04 μg/l (10). The total imprecision is 10% at a concentration of 0.03 μg/l.

End points. Commensurate with previous studies evaluating the prognostic performance of troponin, the primary end point for this analysis was death or myocardial infarction (MI). The primary efficacy end point for the MERLIN–TIMI 36 trial was a composite of cardiovascular death, MI, or recurrent ischemia through the end of the trial. The definitions for each component of the end point have been described previously (15). Each element was adjudicated by an independent clinical end points committee, blinded to treatment allocation.

Statistical methods. Baseline characteristics were compared using the chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous ones. We analyzed the prognostic implications of this assay using contemporary guidelines (99th percentile cut point at 0.04 μg/l), as well as divided into 3 strata defined by the 99th percentile (0.04 μg/l), the cut point defined for the prior generation assay (0.1 μg/l), and a cut point (1.5 μg/l) equivalent to that of CK-MB (3,7). An exploratory analysis was performed examining the cohort with cTnI values below the 99th percentile (<0.04 μg/l) and above the lower limit of detection (0.006 μg/l). The assessment of the relationship between cTnI and outcome was performed using Cox regression. Adjusted analyses were performed including all elements of a well-validated risk model in ACS (TIMI risk score) (16). There was no interaction with the randomized therapy, and therefore, treatment allocation was not included in the model. Event rates presented are proportions at 30 days, and Kaplan-Meier estimates of the cumulative incidence at 12 months. The increased discriminative value of cTnI was further examined with the method described by Pencina et al. (17) to determine the net reclassification improvement and the integrated discrimination improvement, which evaluates the change in the estimated risk as a continuous variable. For net reclassification improvement, patients were separated into risk tertiles according to a clinical risk score using the TIMI risk score, creatinine clearance <60 ml/min, and a history of heart failure, and reclassification was determined by the same model with the addition of biomarker data. All analyses

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Table 1 Baseline Characteristics by Troponin Status

	cTnI <0.04 μg/l (n = 1,589)	cTnI ≥0.04 μg/l (n = 2,924)	p Value
Demographics			
Age, yrs, median	63	65	< 0.001
Female, %	43	31	< 0.001
White race, %	97	97	0.704
Weight, kg, median (IQR)	82 (73–93)	81 (72–92)	0.008
Risk factors, %			
Diabetes mellitus	37	30	< 0.001
Current smoker	20	28	< 0.001
History of hypertension	84	67	< 0.001
History of dyslipidemia	77	63	< 0.001
Previous MI	44	31	< 0.001
Previous CHF	29	16	< 0.001
Previous PCI or CABG	34	22	< 0.001
Creatinine clearance <60 ml/min	18	21	0.004
Presentation			
TIMI risk score ≥4, %	13	26	< 0.001
ST-segment depression ≥0.1 mV, %	26	41	< 0.001
Time from symptom onset, h, median (IQR)	20 (10.2–30.2)	24 (14.4–35.1)	< 0.001
Treatment, %			
Coronary angiography during index hospitalization	40	63	< 0.001
Thienopyridine during index hospitalization	47	73	< 0.001
GPIIb/IIIa inhibitor during index hospitalization	4	19	< 0.001
Randomization group (ranolazine)	49	50	0.507

CABG = coronary artery bypass graft surgery; CHF = congestive heart failure; cTnI = cardiac troponin I; GP = glycoprotein; IQR = interquartile range; MI = myocardial infarction; PCI = percutaneous coronary intervention; TIMI = Thrombolysis In Myocardial Infarction.

were performed using STATA version 9.2 (StataCorp LP, College Station, Texas).

Results

Baseline characteristics. Baseline samples were available for analysis in 4,513 of the 6,560 patients enrolled in the clinical trial. Their baseline characteristics are listed in Table 1. The baseline concentration of cTnI was ≥0.04 μg/l in 65% of patients. Patients with baseline cTnI ≥0.04 μg/l were older and more frequently male, and current smokers. They were also more likely to have presented later (24 h vs. 20 h) and to have high-risk findings including ST-segment depression or a TIMI risk score for NSTE-ACS >4. Patients with a cTnI ≥0.04 μg/l were more likely to receive thienopyridines and glycoprotein IIb/IIIa inhibitors, and to undergo coronary angiography. The randomized treatment (ranolazine or placebo) was well balanced among patients with and without cTnI ≥0.04 μg/l.

Troponin concentration and prognosis. 99TH PERCENTILE DECISION LIMIT. At 30 days, compared to patients with cTnI <0.04 μg/l, patients with baseline cTnI ≥0.04 μg/l had significantly higher rates of death (2.1% vs. 0.5%, $p < 0.0001$), and death or MI (6.1% vs. 2.0%, $p < 0.0001$). The higher risk of adverse outcomes associated with cTnI ≥0.04 μg/l persisted at 12 months for both death (6.6% vs. 2.4%, $p < 0.0001$), and death or MI (14.1% vs. 5.6%, $p < 0.0001$) (Fig. 1). Patients with an elevated baseline cTnI were also at higher risk of the primary composite end point for the trial of cardiovascular death, MI, or recurrent ischemia at 30 days (9.9% vs. 4.4%, $p < 0.0001$) and at 12 months (21.7% vs. 13.6%, $p < 0.0001$) (15).

When adjusted for elements of the TIMI risk score for NSTE-ACS including age, multiple coronary risk factors, known coronary disease, recent aspirin use, repeated episodes of rest-angina, and ST-segment deviation, patients with increased cTnI ≥0.04 μg/l at presentation were at 3-fold higher risk of death when compared to patients with cTnI <0.04 μg/l (adjusted hazard ratio [HR]: 3.0, 95% confidence interval [CI]: 1.4 to 6.4) and death or MI (adjusted HR: 3.0, 95% CI: 2.0 to 4.4) at 30 days. Similarly, at 12 months, the significant association between cTnI and outcome remained apparent with respect to death (adjusted HR: 2.4, 95% CI: 1.8 to 3.5) and death or MI (adjusted HR: 2.7, 95% CI: 2.1 to 3.4) (Table 2). In addition, adding cTnI to the clinical model resulted in a significant net reclassification improvement of −0.405 ($p < 0.0001$) and an integrated discrimination improvement ($p < 0.0001$) for death or MI. There was no evidence for an interaction between the baseline cTnI result and the efficacy of ranolazine (p value for interaction = 0.76).

LOW-LEVEL ELEVATION. Among patients with cTnI ≥0.04 μg/l, 319 (10.9%) had low-level elevation of cTnI between 0.04 μg/l and 0.1 μg/l. An additional 1,002 (22%) fell

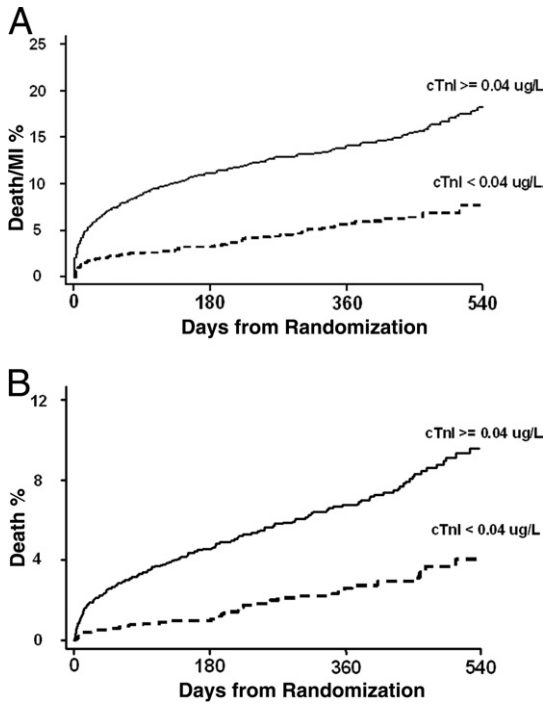

Figure 1 **Risk of Death or MI by Baseline cTnI Result**

Kaplan-Meier estimated cumulative incidence of **(A)** death or myocardial infarction (MI) and **(B)** death stratified by baseline cardiac troponin I (cTnI) concentration using the 99th percentile reference limit (0.04 μg/l [cTnI ≥0.04 μg/l, n = 2,924; cTnI <0.04 μg/l, n = 1,589]).

below the cut point (1.5 μg/l), equivalent to an abnormal increase in CK-MB. When stratified by these specified cut points (0.04 to <0.1, 0.1 to <1.5, and ≥1.5) and compared to patients with cTnI <0.04 μg/l, patients with low-level increases (0.04 to <0.1) had a significantly higher incidence of death or MI both at 30 days and at 12 months (Fig. 2). Patients with cTnI between 0.04 μg/l and 0.1 μg/l had a more than 2-fold higher risk of adverse outcomes at 30 days when compared to patients with cTnI <0.04 μg/l (death or MI 5.0% vs. 2.0%, p = 0.001; death 1.3% vs. 0.5%, p = 0.12). At 12 months, there was a similar increase in the incidence of recurrent events in patients with low-level cTnI elevations compared to patients with no detectable cTnI (death 6.4% vs. 2.4%, p = 0.005). Additional analysis of the group with cTnI <0.04 μg/l showed that patients with cTnI ≥0.006 and <0.04 were at numerically but not significantly higher risk of death or MI at 12 months than were patients with cTnI <0.006 (5.8% vs. 4.0%, p = 0.42).

When adjusted for elements of the TIMI risk score for NSTE-ACS, patients with low-level cTnI elevation (≥0.04 μg/l to <0.10 μg/l) were at increased risk of death (adjusted HR: 1.8, 95% CI: 1.05 to 3.21) and death or MI (adjusted HR: 2.14, 95% CI: 1.48 to 3.10) at 12 months (Fig. 3).

Discussion

The recommended clinical decision limits for cTn for clinical practice have moved toward lower concentrations (13). Newer generations of current commercial assays have enabled measurement of cTn at concentrations not reliably detected with prior generations, improving overall diagnostic accuracy (13) but fostering lingering uncertainty for many practitioners as to the clinical relevance of very low level increases in cTn (13). In this prospectively designed evaluation of 1 such widely employed sensitive assay for cTnI in >4,500 subjects with ACS, we demonstrated excellent prognostic performance at the 99th percentile cut point and found, in particular, that elevated cTnI in the lowest range (~10% of the population) identified with this sensitive assay was indeed associated with an increased short- and intermediate-term risk of death and recurrent major cardiovascular events. This assessment of the clinical significance of low level elevations of cTnI, using the TnI-Ultra assay as an example, confirms the pattern of sustained prognostic relevance in patients with chest symptoms consistent with ACS as assays have evolved toward more sensitive current generations.

Evaluation of low-level increases in cardiac troponin. The introduction of cardiac troponin more than a decade ago as a more sensitive tool for detection of myocardial injury than

Table 2 **Outcomes Stratified by Baseline cTnI at the 99th Percentile Cut Point**

Outcome	cTnI <0.04 μg/l	cTnI ≥0.04 μg/l	Adjusted HR* (95% CI)	p Value
30 days				
n	1,589	2,924		
Death (%)	8 (0.5)	60 (2.1)	3.0 (1.4–6.4)	0.004
Death or MI (%)	31 (2.0)	178 (6.1)	3.0 (2.0–4.4)	< 0.001
Primary end point (%)	70 (4.4)	289 (9.9)	2.2 (1.7–2.9)	< 0.001
12 months				
Death (%)	39 (2.4)	198 (6.6)	2.4 (1.8–3.5)	< 0.0001
Death or MI (%)	86 (5.6)	413 (14.1)	2.7 (2.1–3.4)	< 0.0001
Primary end point (%)	218 (13.6)	628 (21.7)	1.6 (1.4–4.8)	< 0.0001

*Adjusted for all elements of TIMI risk score (age, multiple coronary risk factors, known coronary disease, recent aspirin use, recent severe angina, and ST-segment deviation).

CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 1.

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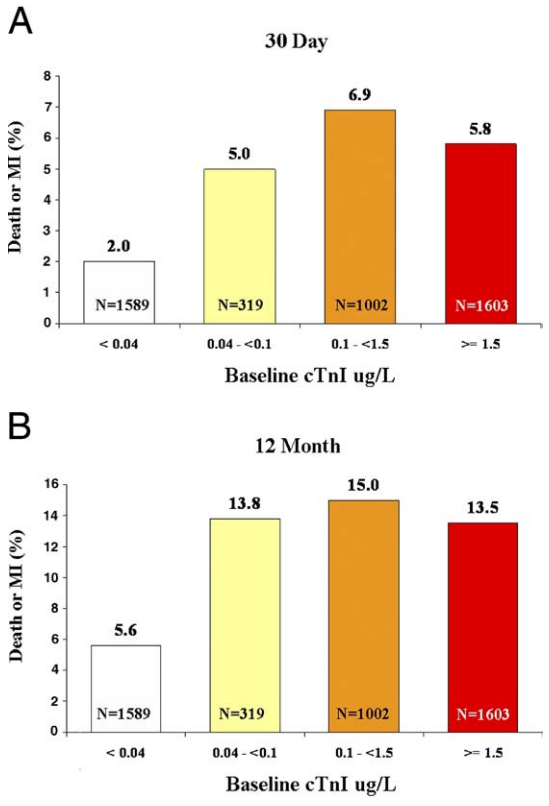

Figure 2 Kaplan-Meier Estimated Event Rates for Death or MI

Kaplan-Meier estimated event rates for death or myocardial infarction (MI) at **(A)** 30 days and **(B)** 12 months by strata of baseline cardiac troponin I (cTnI). Patients who had low-level elevation of cTnI (0.04 μg/l to 0.1 μg/l) were at significantly increased risk of death or MI compared with patients who did not have elevated cTnI. $p < 0.001$.

CK-MB raised appropriate questions regarding the clinical meaning of myocardial necrosis detected with this new, more sensitive biomarker. These questions were addressed by outcomes studies that firmly established that MIs detected with troponin but not CK-MB were associated with a substantial risk of death or recurrent ischemic events. For example, in a study of 359 patients presenting with unstable angina and negative serial CK-MB values, patients with cTnI >0.1 ng/ml were at significantly increased risk of death or MI at both 48 h and 14 days (18). Subsequently, the move to lower decision limits for troponin raised the same questions regarding the clinical significance of quantitatively small increases in cTn above the 99th percentile. Again, this issue was resolved by consistent findings from several prospective studies nested in clinical trials as well as in community-based populations establishing the prognostic relevance of such "low-level" increases in cTn using prior generation assays that were less sensitive than in those used currently (9). For example, the relationship of outcomes and degree of cTnI elevation was evaluated by Kontos et al. (19) in a study of 4,123 patients presenting with suspected NSTE-ACS in a community-based population. Patients who had a peak cTnI value greater than the lower limit of detection and <99th percentile as well as those with a peak cTnI >99th percentile but less than the manufacturer's suggested upper reference limit had significantly increased risk of death at 30 days and 6 months compared with patients who had nondetectable cTnI (19). Now, the emergence of commercial assays with improved analytical performance, including approximately 10-fold higher sensitivity than prior assays, has enabled the reliable detection of even lower concentrations of cTn and provided enhanced diagnostic accuracy (8,9,13). Nevertheless, this progress again requires the medical community to critically assess the prognostic implications of cTn detected with newer assays.

To address this issue, we studied a current-generation sensitive assay (TnI-Ultra). In prior work with this assay, Apple et al. (11) measured cTnI at 2 time points in 371 patients with suspected ACS who were followed up for 60 days and found that results >99th percentile (>0.04 μg/l to

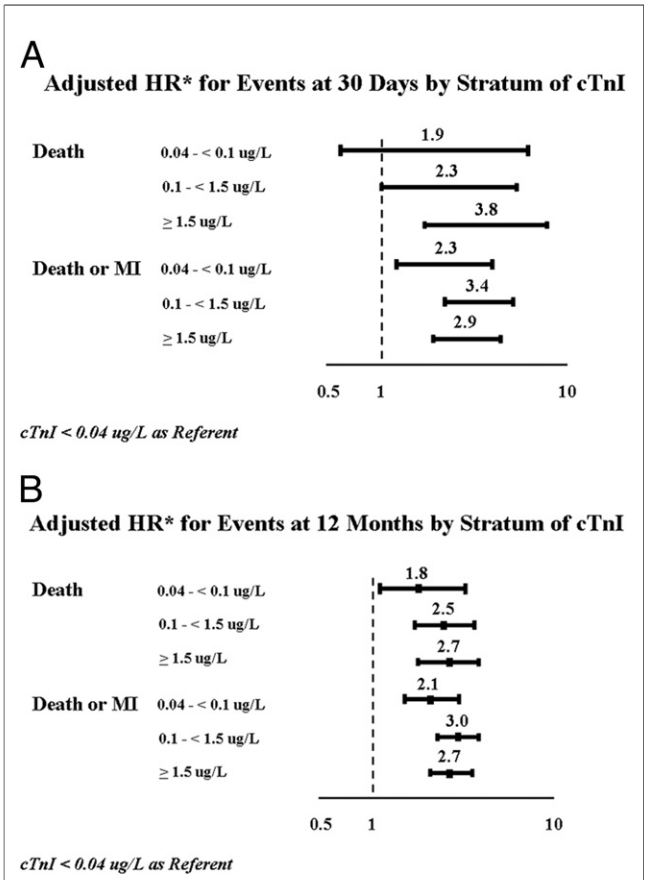

Figure 3 Adjusted HR for Death and Death or MI

Adjusted hazard ratio (HR) for death and death or myocardial infarction (MI) at **(A)** 30 days and **(B)** 12 months stratified by baseline cardiac troponin I (cTnI). After adjusting for established clinical predictors of outcome, patients who had low-level elevation of cTnI were consistently at higher risk of recurrent cardiovascular events compared with patients who had cTnI <0.04 μg/l. *Adjusting for all elements of TIMI risk score (age, multiple coronary risk factors, known coronary disease, recent aspirin use, recent severe angina, and ST-segment deviation).

0.10 μg/l) were predictive of the risk of death or first cardiac event (MI or revascularization) with an adjusted HR of 8.9 (95% CI: 2.4 to 34). Those with TnI between 0.006 μg/l and 0.04 μg/l (n = 174) were also at significantly increased risk of the composite end point (adjusted HR: 3.9, 95% CI: 1.2 to 13). Notably, more than one-third of the end point events were revascularization procedures that appeared to occur during the index hospitalization, and the study overall was not powered to examine death or MI (11).

In our study of 4,513 patients with an average follow-up of 1 year, we found that this current-generation assay identified patients with low-level increases in cTnI (>0.04 μg/l to 0.10 μg/l) who were at more than 2-fold higher risk of death and death or new MI at 30 days and at 1 year. Our findings provide strong evidence for the enhanced prognostic performance of more sensitive current-generation assays for cTn in patients with a clinical syndrome consistent with ACS, and establish the clinical relevance of such low-level increases in cTnI using a current-generation assay at the contemporary cut point (99th percentile) recommended by the universal definition of MI (7). This finding was independent of other routinely used clinical tools for risk assessment in ACS, including electrocardiographic findings, prior coronary artery disease, and the tempo of chest symptoms. In contrast to the study by Apple et al. (11), in our study, we did not find a significant increase in risk in those patients with a detectable cTnI below the 99th percentile (i.e., between 0.006 μg/l and 0.04 μg/l).

Future directions. This trend toward the clinical application of even lower concentrations of cardiac troponin is likely to continue. Other sensitive assays for cTn are commercially available (9,13), and at the same time, research assays for cTn are emerging that provide sensitivity and precision that is increased an order of magnitude over the present sensitive commercial assay, including the currently used cTnI Ultra assay employed in this study (20–22). As an example, using the single-photon fluorescence detection method, a 10% coefficient of variation was reported at 0.0018 μg/l (23). This emerging group of assays offers the possibility of fully characterizing the distribution of cTn in the healthy population, as well as detecting very small changes in cTn concentration over time that may facilitate discrimination of acute myocardial injury from chronic causes of elevated circulating cTn, thereby improving the very early detection of patients with ACS (24,25). Moreover, preliminary investigation of cut points below the 99th percentile has revealed an association between increases in cTn higher than the limit of detection and adverse cardiovascular outcomes (26,27). Implementation of cut points below the 99th percentile may be particularly relevant for emerging applications for cTn, such as risk stratification in patients with stable coronary artery disease or identification of subclinical structural heart disease in patients at risk (27–29).

These trends toward new uses of more sensitive assays for cTn are associated with important challenges that must be navigated to guide any new clinical applications (24). In particular, consensus with regard to what defines an appropriate "normal reference population" has not been reached. Also, it will be necessary to re-evaluate the possible impact of biological variability and assay interference from nonspecific antibody binding that have not been meaningful with assays available to date (30). Adequately powered clinical studies are needed to assess whether the therapeutic implications of low concentrations of cTn using a high-sensitivity assay are the same as those established for current commercial assays. Although use of lower cut points will facilitate identification of additional patients at risk for recurrent ischemic events, it is important to determine whether this risk may be modified by altering therapy, as compared with patients who have normal troponin results.

Study limitations. This study was limited to patients selected for participation in a clinical trial who presented with suspected ACS. As such, these results do not address the question of prognostic significance in the broader group of patients presenting with nontraumatic chest pain, including patients with symptoms atypical for myocardial ischemia or presenting with other acute illnesses. In addition, the landscape of technical advances in assays for troponin is changing rapidly. Although our results provide an important proof-of-principle that is likely to apply qualitatively to other assays with similar sensitivity, it is not possible to confirm the prognostic performance of other assays without direct investigation, including the next generation of research assays presently under study (13). Samples were stored in liquid nitrogen to maximize sample stability; in addition, any degradation of troponin during long-term storage would introduce random noise to our analysis and would be expected to have resulted in our underestimating the strength of the risk relationship with outcome.

Conclusions

We found that low-level increases in cTnI, as detected by a current-generation sensitive commercial assay, in patients presenting with suspected ACS were associated with an adverse prognosis over the short and long term. These results indicate the clinical relevance of cTn detected with newer sensitive assays for troponin. In addition, our findings support current guidelines for use of the 99th percentile cut point established by the universal definition of MI (7).

Reprint requests and correspondence: Dr. Marc P. Bonaca, TIMI Study Group, Cardiovascular Division, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115. E-mail: mbonaca@partners.org.

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