

Evaluation of the AccuTnI Cardiac Troponin I Assay for Risk Assessment in Acute Coronary Syndromes, David A. Morrow,^{1*} Nader Rifai,² Marc S. Sabatine,¹ Shake Ayanian,² Sabina A. Murphy,¹ James A. de Lemos,³ Eugene Braunwald,¹ and Christopher P. Cannon¹

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Effective risk assessment guides appropriate triage and therapy for patients with suspected unstable angina or non-ST-elevation myocardial infarction (MI) (1, 2). Cardiac biomarkers play a valuable role in risk stratification in non-ST-elevation acute coronary syndromes (NSTE ACS). In particular, the cardiac troponins have been identified as the preferred biomarkers for this purpose (1). Clinical application of cardiac troponin I (cTnI) has been complicated by a lack of standardization across the multiple commercially available assays, which has produced substantial variation in the reported clinical decision limits. As such, clinical appraisal of the prognostic performance of each cTnI assay is important to providing an evidence-based guide to its use for risk assessment.

The most recent generation cTnI assay from Beckman Coulter (AccuTnITM) uses antibodies directed at a stable region (amino acids 30–110) of the NH₂ terminus of cTnI and delivers very good analytic performance (3, 4). We evaluated this assay for the assessment of the short-term risk of death and recurrent ischemic events among patients with suspected NSTE ACS enrolled in the Orbofiban in Patients with Unstable Coronary Syndromes (OPUS)-Thrombolysis in Myocardial Infarction (TIMI) 16 trial.

OPUS-TIMI 16 was a multicenter, randomized, parallel-group trial comparing an oral glycoprotein IIb/IIIa inhibitor with placebo for patients with ACS. The design and results of OPUS-TIMI 16 have been reported (5). The protocol was approved by the Institutional Review Board of each participating hospital, and all patients signed written informed consent. Patients were included if they presented within 72 h of symptom onset and had at least one of the following: dynamic electrocardiographic changes; increased cardiac markers; history of coronary artery disease; or age ≥ 65 with diabetes or vascular disease. Patients were randomized to placebo or one of two orbofiban doses. The present substudy was conducted in all patients with NSTE ACS who were allocated to receive placebo and provided a baseline serum specimen. All clinical events were adjudicated by an independent Clinical Events Committee.

At the time of enrollment, blood specimens were collected by study personnel. Serum samples were frozen at

–20 °C or colder within 60 min of collection and were later shipped in a batch to the TIMI Biomarker Marker Core Laboratory (Boston, MA), where they were stored at –80 °C. For this study, cTnI was measured by the Access AccuTnI (Beckman Coulter, Chaska, MN) by personnel who were blinded to clinical outcomes. The AccuTnI assay is a two-site sandwich immunoassay that uses paramagnetic particles coated with murine monoclonal antibody directed at amino acids 41–49 and alkaline phosphatase-conjugated murine monoclonal antibody directed at amino acids 24–40. The detection limit of this assay is $<0.01 \mu\text{g/L}$. The 97.5th and 99th percentiles in an apparently healthy reference population are 0.03 and 0.04 $\mu\text{g/L}$, respectively (4). The total CVs at 0.03, 0.04, and 0.06 $\mu\text{g/L}$ are 20%, 14% and 10%, respectively (4). In our laboratory, the total CV was comparable at the low end (14% at 0.08 $\mu\text{g/L}$) with total imprecision of 6.5% at 0.37 $\mu\text{g/L}$ and 4.0% at 0.86 $\mu\text{g/L}$. The primary analysis for this study was planned at a decision limit of 0.04 $\mu\text{g/L}$, corresponding to the 99th percentile upper reference limit. Additional analyses were prespecified at 0.01 $\mu\text{g/L}$, the 97.5th percentile (0.03 $\mu\text{g/L}$), and the cutoff of 10% total imprecision (0.06 $\mu\text{g/L}$) from previous work (4).

The association between troponin status and outcome at 30 days was tested with the χ^2 test. Multivariable analysis was performed for this time point by logistic regression including other known important predictors and potential confounders in this data set (age, ST-deviation at presentation, history of coronary disease, and smoking) as covariates. Evaluation of the association between cTnI status and outcome through 10 months was performed with Cox proportional hazard modeling. Comparison of decision limits was performed by the likelihood ratio test based on the logistic regression model. All statistical testing was performed with STATA v7-intercooled (STATA Corp.). *P* values <0.05 (two-sided) were considered to indicate statistical significance.

This substudy from OPUS-TIMI 16 included 1736 patients with NSTE ACS. The study population was composed of patients with a median age of 61 years. Seventy percent of the patients were men; 58% of the patients had previous coronary artery disease, 22% had diabetes mellitus, and 45% had ST-segment depression. cTnI concentrations were $\geq 0.04 \mu\text{g/L}$ at study entry in 64% of patients, including 45% of patients with creatine kinase MB (CK-MB) concentrations less than 1 \times the upper limit of the reference interval. Of patients with samples collected within 48 h of symptom onset, 61% had a cTnI concentration $\geq 0.04 \mu\text{g/L}$. Patients with cTnI $\geq 0.04 \mu\text{g/L}$ were more likely to present with ST-segment deviation (*P* <0.01) and were less likely to have a previous history of coronary artery disease (*P* <0.01) compared with patients with cTnI $<0.04 \mu\text{g/L}$. There was no difference in other important clinical predictors of outcome such as age (*P* = 0.3) or prevalence of heart failure at presentation (*P* = 0.2) among patients with vs without increased cTnI.

Patients presenting with NSTE ACS and a cTnI concentration $\geq 0.04 \mu\text{g/L}$ were at significantly higher risk of death or recurrent ischemic events at 30 days than those

with cTnI $<0.04 \mu\text{g/L}$ (Fig. 1A). A cTnI concentration $\geq 0.04 \mu\text{g/L}$ was an independent predictor of the 30-day risk of death [odds ratio (OR), 4.1; 95% confidence limit (CI), 1.2–13.8], death and MI (OR, 3.4; 95% CI, 1.8–6.7), and death, MI, or urgent revascularization (OR, 2.3; 95% CI, 1.5–3.6). Moreover, a cTnI concentration above this decision limit maintained a strong association with the risk of death (OR, 2.1; 95% CI, 1.1–4.2) as well as death or MI (OR, 2.6; 95% CI, 1.6–4.2) through 10 months of follow-up (Fig. 1B).

When the analysis was limited to patients from whom the baseline sample was obtained within 48 h of symptom onset ($n = 1097$), the outcomes were qualitatively similar, with a greater than twofold higher risk of death, MI, or urgent revascularization at 30 days among those with cTnI $\geq 0.04 \mu\text{g/L}$ (9.8% vs 4.5%; $P = 0.001$).

In analyses of other prespecified alternative decision limits, similar prognostic capacity was maintained at cut-points ranging from 0.01 to 0.06 $\mu\text{g/L}$ (Table 1). Specifically, in this study a decision limit of 0.01 $\mu\text{g/L}$ provided the highest negative predictive value with comparable overall discriminatory capacity compared with the primary decision limit. When dichotomized at 0.06

Table 1. Exploratory evaluation of additional decision limits.

Decision limit, $\mu\text{g/L}$	% positive	D/MI ^a at 30 days, %		Adjusted OR (95% CI)	χ^2
		cTnI Pos	cTnI Neg		
≥ 0.01	75	5.2	0.7	8.0 (2.5–26)	13.0
≥ 0.03	66	5.4	1.6	3.8 (1.9–7.8)	13.4
≥ 0.04	64	5.4	1.8	3.4 (1.8–6.7)	13.3
≥ 0.06	61	5.6	1.6	4.0 (2.0–7.7)	16.6

^a D/MI, composite of death or MI; Pos, (positive), above the decision limit; Neg, below the decision limit.

$\mu\text{g/L}$, at which the total imprecision was $\sim 10\%$, the baseline concentration of cTnI was also strongly associated with cardiovascular outcomes (Table 1). A total of 255 (15%) patients had a cTnI concentration between 0.01 and 0.06 $\mu\text{g/L}$, and 91 (5%) patients had a concentration between 0.03 and 0.06 $\mu\text{g/L}$. Overall, there was no detectable difference in the prognostic discriminatory capacity of decision limits at 0.03 and 0.04 $\mu\text{g/L}$ ($P = 0.9$) or 0.04 and 0.06 $\mu\text{g/L}$ ($P = 0.08$). However, patients with a cTnI concentration ≥ 0.01 to 0.06 $\mu\text{g/L}$ were at significantly higher risk of death or MI compared with those with cTnI $<0.01 \mu\text{g/L}$ (3.2% vs 0.7%; $P = 0.01$).

Our results provide a basis for evidence-based application of the AccuTnI assay for risk stratification among patients presenting with NSTEMI ACS. Specifically, this study demonstrates a strong prognostic performance of the AccuTnI assay with respect to the short-term risk of death, as well as the composite of death or recurrent ischemic events in this population. The magnitude of the risk relationships for death and death or MI observed in this study are strikingly consistent with the fourfold higher risk associated with increased cardiac troponin documented in a comprehensive meta-analysis (6). Our results provide a direct clinical evaluation of a decision limit at the 99th percentile upper reference limit (0.04 $\mu\text{g/L}$) recommended by the European Society of Cardiology (ESC)/American College of Cardiology (ACC) Committee for the Redefinition of Myocardial Infarction, as well as at the acceptable limit of imprecision ($\sim 10\%$) defined in that document (7, 8).

The robust prognostic performance of the AccuTnI at a cut-point corresponding to the 97.5th percentile (0.03 $\mu\text{g/L}$) provides important independent confirmation of previous work from Venge et al. (9), as well as our group (10), demonstrating that among patients with a high clinical probability of ACS, a low-level increase in cardiac troponin detected with current generation assays confers important prognostic information. Taken together, our present study and previous work (9–11) suggest that such low-level increases identify an additional 5–15% of patients with ACS who are at significantly higher risk for death and recurrent ischemic events than “troponin-negative” patients. Moreover, the introduction of assays with increased analytic detection limits has enhanced the clinician’s ability to reliably categorize patients with suspected ACS who are at very low short-term risk of death and recurrent ischemic events (negative predictive value).

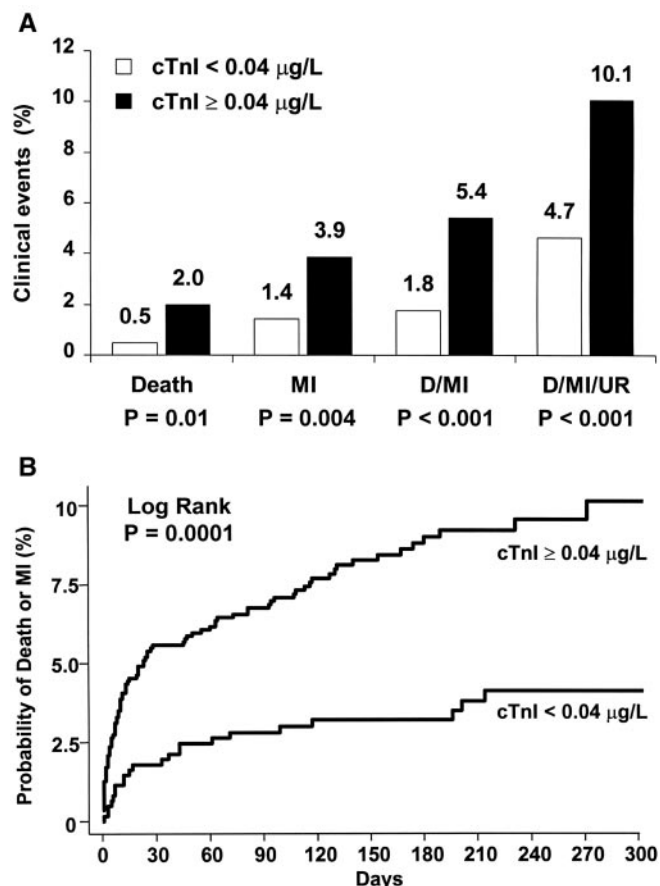


Fig. 1. Risk of recurrent cardiac events through 30 days stratified by cTnI result (A) and Kaplan-Meier probability estimates for death or MI through 10 months of follow-up (B).

D, death; UR, urgent revascularization.

Because each of these studies was performed among patients with a strong clinical suspicion for ACS enrolled in clinical trials, the results regarding cut-points at concentrations with >10% imprecision should not be extrapolated to the general population of patients with nontraumatic chest pain presenting to the emergency department. In addition, the measurement of troponin in this study was conducted in a batch with a single lot of reagent and may underrepresent the impact of imprecision experienced as a result of lot-to-lot and instrument-to-instrument variability across routine clinical practice in different institutions. As recommended by the ESC/ACC, higher precision seems warranted for application in the latter population and for establishing the diagnosis of MI (7, 12). Nevertheless, our results should alert clinicians to the potential importance of low-level increases in cardiac troponin among patients with a clinical history strongly suggestive of ACS.

The AccuTnI assay is an effective tool for risk assessment among patients with NSTEMI ACS at a decision limit (0.06 µg/L) commensurate with the most recent recommendations from the ESC/ACC. Lower-level increases in cTnI detected with this assay seem clinically important among patients with a high clinical probability of ACS.

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Quantitative Evaluation of Alternative Promoter Usage and 3' Splice Variants for Parathyroid Hormone-related Protein by Real-Time Reverse Transcription-PCR, Virgile Richard,¹ Alexander Luchin,¹ Romulo M. Brena,² Christoph Plass,² and Thomas J. Rosol^{1*} (¹ Department of Veterinary Biosciences, College of Veterinary Medicine, and ² Division of Human Cancer Genetics, Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Columbus, OH 43210; * address correspondence to this author at: The Ohio State University, College of Veterinary Medicine, Department of Veterinary Biosciences, 1925 Coffey Rd., Columbus, OH 43210; fax 614-292-6473, e-mail rosol.1@osu.edu)

Parathyroid hormone-related protein (PTHrP) was originally isolated from specific cancers as the primary cause of humoral hypercalcemia of malignancy, a paraneoplastic syndrome occurring in humans with a wide variety of malignancies (1). PTHrP also has been reported to be overexpressed by many types of neoplasms not associated with hypercalcemia (2). PTHrP is a polypeptide hormone with structural similarities to parathyroid hormone (PTH) (3,4). Amino-terminal fragments of PTHrP exert PTH-like actions in bone and kidney by binding to a common receptor for PTH/PTHrP (PTH1 receptor), producing hypercalcemia (5–8). High expression of PTHrP by cancer cells also has been proposed to play a role in the progression of breast cancer metastasis to bone (9–11).

The human PTHrP gene is composed of nine exons (Fig. 1A). Products of exons 5 and 6 are present in all PTHrP transcripts and encode for the prepro region and the majority of the mature peptide. Alternative splicing of the 3' end produces three PTHrP isoforms 139-, 173-, and 141-amino acids in length. Transcriptional regulation of the PTHrP gene is achieved by three distinct promoters located at the 5' end and identified as P1, P2, and P3, respectively. Alternative promoter usage has been evaluated previously by reverse transcription (RT)-PCR based on 5' alternative splicing, and previous studies showed that P3-initiated transcripts were detectable in most tumors, whereas transcripts initiated by either P1 or P2 were present in only a subset of tumors (12–16).

We describe a novel real-time RT-PCR assay for the specific quantification and characterization of PTHrP