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

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BACKGROUND: We determined the diagnostic accuracy of the Advia Centaur TnI-Ultra assay for detecting myocardial infarction (MI) and assessing risk of adverse events in patients presenting with ischemic symptoms suggestive of acute coronary syndrome.

METHODS: We measured cardiac troponin I (cTnI) on admission and 6–24 h after admission (follow-up) in plasma specimens from 371 consecutive patients. The end point was the first of cardiac event or death within 60 days. We estimated survival curves using the Kaplan-Meier method and compared groups with the log rank statistic.

RESULTS: MI was established in 49 patients (13%). Clinical sensitivities and specificities for MI based on the 99th percentile (0.04 μg/L) were 74% and 84%, respectively, on admission and 94% and 81% at follow-up. ROC curves showed significantly higher accuracy for MI in the follow-up specimen compared with admission ($P = 0.001$). Overall there were 2 cardiac deaths, 1 noncardiac death, 49 MIs, 7 coronary artery bypass grafts, and 36 percutaneous coronary interventions in 59 patients during follow-up. The event rate in those with cTnI <0.006 μg/L was significantly lower than in groups with cTnI 0.006–0.04 μg/L, >0.04–0.10 μg/L, or >0.10 μg/L (2.8% vs 11.1%, 24.1%, 55.1%, respectively; P <0.0001). Relative risks for the increasing cTnI cutoff groups were 3.9 (95% CI 1.2–13), 8.9 (2.4–34), and 25 (7.3–82) after adjustment for age, diabetes, history of hypertension, previous MI, and estimated glomerular filtration rate.

CONCLUSIONS: The TnI-Ultra assay is a sensitive, early diagnostic biomarker for MI and an independent predictor of adverse events at any measurable cTnI in patients with symptoms of acute coronary syndrome.

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The presence of circulating cardiac troponin (cTn)[4] is indicative of myocardial injury (1–3). Increased concentrations of cTn are associated with more frequent thrombus, impaired myocardial tissue perfusion, and higher probability of adverse outcomes following coronary intervention (4–6). In addition, the evidence-based literature shows a consistently strong direct relationship between increased cTn concentration and the risk of both short- and long-term cardiac events and mortality in patients who present with symptoms of acute coronary syndrome (ACS) (2, 7). The prognostic information obtained from the measurement of cTnI or cTnT has been shown to be independent of clinical risk factors such as age, ECG, renal disease, and diabetes (2, 7, 8). International associations in cardiology laboratory medicine and emergency medicine have all designated cTn as the preferred biomarker for aiding in the diagnosis of MI and for risk stratification in patients presenting with suspected ACS, and recommend that independent studies validate all cTn assays after FDA clearance before acceptance for risk assessment of adverse outcomes (1–3). The purpose of this study was to determine both the diagnostic accuracy for detection of myocardial infarction (MI) and the prognostic value for assessing risk of short-term adverse events for the second-generation Advia Centaur TnI-Ultra assay in a nonselected, heterogeneous population of patients presenting with ischemic symptoms suggestive of ACS based on cTnI values at the limit of detection (LoD)

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[4] Nonstandard abbreviations: cTn, cardiac troponin I; ACS, acute coronary syndrome; MI, myocardial infarction; LoD, limit of detection; eGFR, estimated glomerular filtration rate; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; RR, relative risk.

and 99th percentile reference value as well as the first-generation Bayer assay 99th percentile value.

Materials and Methods

After institutional review board approval, plasma (heparin) specimens were prospectively collected from leftover specimens collected at presentation (baseline) and 6–24 h after presentation (follow-up sample) from 381 consecutive patients presenting with symptoms suggestive of ACS admitted through the emergency department at Hennepin County Medical Center (Minneapolis, MN) to rule in or rule out acute MI. The median time from symptom onset to presentation was 5.1 h. Estimated glomerular filtration rate (eGFR) was calculated using the National Kidney Disease Education Program Modification of Diet in Renal Disease (MDRD) equation (mL/min/1.73 m^2) based on plasma creatinine, age, sex, and whether African American.

Plasma was initially stored refrigerated at $-4\ ^\circ$C, and then frozen at $-80\ ^\circ$C within 48–72 h. We measured cTnI by use of the second-generation Bayer (now Siemens) Advia Centaur TnI-Ultra assay following manufacturer's guidelines. cTnI values used for diagnostic and risk assessment calculations were those designated by the manufacturer's claims in their FDA-cleared package insert as the LoD of 0.006 μg/L and the 99th percentile reference value of 0.040 μg/L. Assay total imprecision was reported by Siemens to be 10% at 0.03 μg/L. Total imprecision in the study laboratory was 3.5% for our lowest quality control material, mean 0.26 μg/L over 19 days.

We obtained patient demographics and determination of clinical diagnoses from chart review after patient enrollment into the study. Record review included up-to-date medical history of previous medical conditions and was carried out blinded to biomarker results. Criteria for acute MI were defined along the European Society of Cardiology/American College of Cardiology redefinition of myocardial infarction guidelines (1). This was based on evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia at presentation. A diagnosis of MI was made based on detection of an increase of cTnI (Dade Behring Dimension or Stratus CS as used by Hennepin in their laboratories) above the 99th percentile reference value (<0.1 μg/L; total imprecision 12% at 0.2 μg/L) with at least one of the following: symptoms of ischemia, new ST-T changes on ECG, development of Q waves on ECG, or imaging evidence of new loss of viable myocardium.

We performed chart reviews or telephone follow-up interviews to determine clinical outcomes over 60 days (without knowledge of the cTnI finding). Follow-up information was not available for 10 patients,

Table 1. Patient demographics.

N	371
Mean age, years (range)	54 (23–95)
Males	60
Ethnicity	
Caucasian	41
African-American	48
Native American	8
Hispanic	1
Other/multiple	2
Medical history	
Coronary artery disease	37
Myocardial infarction	22
Diabetes	31
Renal disease	8
Hypertension	66

Data are % unless noted otherwise.

who are excluded from further analysis. The primary end point for this study was a combined end point: first cardiac event (MI, percutaneous coronary intervention [PCI], coronary artery bypass graft [CABG]) or death within 60 days. Exposure was computed from date of blood draw until date of first event, with censoring at time of last contact if <60 days. Cumulative event rates were estimated using the Kaplan-Meier method and compared using the log rank statistic. We estimated relative risks (RRs) and 95% CIs using Cox proportional hazards models. Statistical significance was accepted at the 0.05 level, and all statistical tests were 2 sided. Statistical analyses were performed with MedCalc 9.3.0.0 (www.medcalc.be) and SPSS for Windows version 11.5 (SPSS).

Results

Patients included in this study were diverse in racial make-up and had an average age of 54 years (Table 1). About one-third had a history of coronary artery disease, and nearly two-thirds had a history of hypertension. cTnI concentrations ranged from <0.006 μg/L (LoD) to 53.6 μg/L. Seventy-six percent of patients (n = 282) had cTnI concentrations at or below the 99th percentile value (0.04 μg/L) at baseline, with 29% (n = 108) below the LoD (0.006 μg/L). During the follow-up period, 49 MIs, 36 PCIs, 7 CABGs, 2 cardiac deaths, and 1 noncardiac death occurred in 59 patients.

MI was established in 49 patients (13.2%) during initial hospitalization. Clinical sensitivities and specificities based on the 99th percentile (0.04 μg /L) were

Table 2. Clinical sensitivities and specificities for detection of MI in 371 patients using the Siemens Ultra cTnI assay.

	cTnI cutoff, μg/L		
	>0.006	>0.04	>0.1
Baseline sample			
Sensitivity	96 (86–99)	74 (59–85)	55 (40–69)
Specificity	33 (28–38)	84 (79–87)	93 (89–95)
Follow-up sample			
Sensitivity	98 (89–100)	94 (83–99)	86 (73–94)
Specificity	34 (29–44)	81 (77–86)	90 (87–93)

Data are % (95% CI).

74% and 84%, respectively, at baseline and 94% and 81% at follow-up (10 h median time to follow-up sample; Table 2). A higher diagnostic accuracy for MI was observed with the follow-up specimen vs the baseline sample as evidenced by a higher area under the ROC (area under curve [AUC] 0.957 vs 0.860, $P = 0.001$; Fig. 1). Using the LoD concentration cutoff (≥ 0.006 μg/L) as the diagnostic threshold increased sensitivity for both baseline and follow-up samples to >95% but reduced specificity to <35%. For comparison, at the 99th percentile–derived cutoff of 0.1 μg/L as determined for
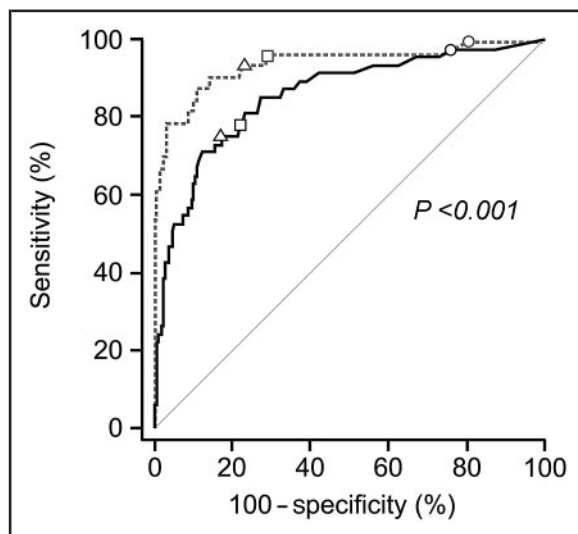
Fig. 1. ROC curves representing baseline (solid line) and follow-up (dashed line) samplings for detection of MI using the Siemens Ultra cTnI assay.

Open circles, 99th percentile value; open boxes, 10% CV value; open triangle, first-generation Bayer assay 99th percentile value.

the first-generation Bayer Centaur assay, sensitivities were lower at both baseline (55%) and follow-up (86%) sampling compared with the 99th percentile cutoff, whereas specificities were >90%. Based on the Dade cTnI <0.1 μg/L 99th percentile value used at the hospital lab, the clinical sensitivity was 58% (CI 43% to 72%) and specificity was 89% (CI 85% to 92%) for the baseline sample (n = 359). For the follow-up specimen (n = 346), the sensitivity was 96% (CI 85% to 99%) and specificity was 87% (CI 83% to 91%). The area under the ROC was significantly higher at baseline for Siemens cTnI vs Dade cTnI (0.86 vs 0.77, $P = 0.01$; n = 359) but not at follow-up (0.97 vs 0.96, $P = 0.58$; n = 346). Further examination of cTnI sensitivities and specificities broken down for samplings in the 6- to 12-h range (n = 249) and 12- to 18-h range (n = 106) demonstrated the following sensitivities and specificities: 6- to 12-h sensitivity, Siemens 90%, Dade 86%; 6- to 12-h specificity, Siemens 83%, Dade 92%; 12- to 18-h sensitivity, Siemens 100%, Dade 100%; 12- to 18-h specificity, Siemens 79%, Dade 87%. As there were only 12 draws in the 18+ h group, no data are provided.

Increasing 60-day event rates (cardiac event and death) were observed with increasing cTnI concentrations (Table 3, Fig. 2). The 60-day event rate was significantly lower in the <LoD group (2.8%) than in either the intermediate group (11.1%) ($P = 0.01$) or the increased cTnI (>99th percentile) group (42%) ($P < 0.0001$). In the 89 patients with cTnI concentration above the 99th percentile cutoff (0.04 μg/L), patients with a concentration up to 0.1 μg/L (n = 38) had a 60-day event rate of 24% vs 55% for patients (n = 51) with a concentration >0.10 μg/L ($P = 0.004$). Compared with the <LoD cTnI group, the relative risk of an event was 3.9 (CI 1.2–13) in the intermediate group and 8.9 (2.4–34) in the >99th percentile group and 24.5 (7.3–82) in the >0.10 μg/L group after adjustment for age, eGFR, diabetes, history of hypertension, and previous MI. Table 3 also demonstrates the 60-day event rate for MI and cardiac death alone. Similar significant RRs are shown at all cutoff concentrations examined, and ranged from 3.3 at <0.006 μg/L to 34.9 at >0.10 μg/L ($P < 0.001$). No MIs were related to PCI or CABG procedures for outcomes assessment.

Of the 3 deaths that occurred, none were in patients with a cTnI below the LoD. One death occurred in a patient with a concentration below the 99th percentile cutoff, and the other 2 above 0.010 μg/L.

Discussion

The current study demonstrates the ability of the FDA-cleared second-generation Siemens Ultra assay to provide acceptable clinical sensitivity for detection of MI

Table 3. Sixty-day cardiac event and/or death rates based on the Siemens cTnI-Ultra limit of detection and 99th percentile values and the first-generation Bayer assay 99th percentile value.

cTnI, μg/L	n (%)	No. of events	Cumulative event rate, %	RR
Cardiac event and death				$P < 0.0001$
<0.006	108 (29)	3	2.8	Reference
0.006 to 0.04	174 (47)	19	11.1[a]	3.9 (1.2–13)
>0.04 to 0.10	38 (10)	9	24.1[a,b]	8.9 (2.4–34)
>0.10	51 (14)	28	55.1[a,b,c]	24.5 (7.3–82)
MI and cardiac death				$P < 0.0001$
<0.006	108 (29)	2	1.9	Reference
0.006 to 0.04	174 (47)	11	6.4[d]	3.3 (0.7–15)
>0.04 to 0.10	38 (10)	9	24.1[e,f]	13.0 (2.7–61)
>0.10	51 (14)	28	55.1[e,f,g]	34.9 (8.2–150)

Relative risk adjusted for age, estimated GFR, diabetes, history of hypertension, and previous MI.
Significantly different from [a] <0.006 group; [b] 0.006 to 0.04 group; [c] >0.04 to 0.10 group; [d] <0.006 group at $P = 0.08$; [e] <0.006 group; [f] 0.006 to 0.04 group; [g] >0.04 to 0.10 group.

as well as risk stratification for short-term adverse events. First, the clinical sensitivity for detection of MI predicated on the presenting blood specimen was 74% based on the 99th percentile value of ≤0.04 μg/L. This is a substantial improvement compared to first-generation cTnI and cTnT assays, including Bayer's initial first-generation cTnI assay, which demonstrated clinical sensitivities of 3% to 33% that were not different from sensitivities for creatine kinase MB assays (9–11). Further, our findings demonstrated a 94% sensitivity in the 6- to 24-h follow-up sample, again a substantial improvement compared with first-generation cTn assays, which showed substantially lower sensitivities of

50% to 92% in specimens collected 6–24 h after baseline (9–11).

Surprisingly few published studies have clearly demonstrated this improvement in diagnosing MI using second-generation cTnI assays. Our data add to the evidence-based literature supporting reliable triage capabilities based on the use of the second-generation Siemens assay. The improved sensitivity that coincides with lowering the diagnostic cutoff to the 99th percentile value comes with a specificity trade-off, however. Because first-generation cTn assays were not as robust analytically, cutoff concentrations based on higher WHO (ROC-derived cutoff optimized for clinical sensitivity and specificity) biomarker criteria demonstrated clinical specificities >90% (12). The clinical specificity of 84% in the present study at baseline using the analytically sensitive second-generation Siemens assay demonstrates that other pathology mechanisms besides ischemic MI were responsible for myocardial injury. It is now well described that increases in cardiac troponin occur in the absence of overt ischemic heart disease (1, 12). Although 6 of 10 patients who had increased cTnI in the current study did not have an MI, prognostic value is still added with this biomarker information; the clinician must frame the results accordingly with the patient's clinical presentation. These non-MI cTnI increases, therefore, do not represent false-positive findings, although these do occur rarely (13). To aid the clinician in determining the etiology of an increased cTnI in a questionable MI clinical presentation, a temporal, rising pattern of cTnI also assists in the diagnosis of MI (1, 2). Increased cTnI concentrations that do not change over time are very unlikely due
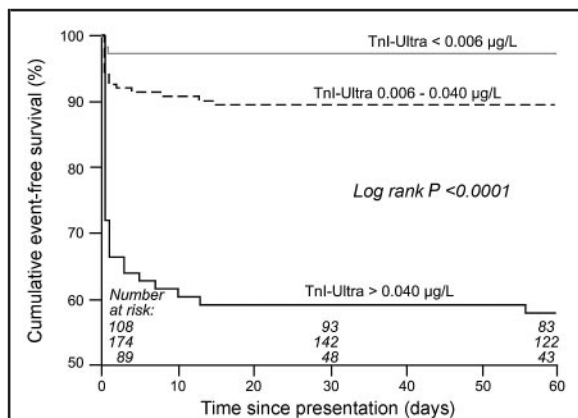

Fig. 2. Kaplan-Meier curves demonstrating cardiac events plus all-cause 60-day mortality survival rates for the Siemens Ultra cTnI assay.

to an MI *(3, 12, 14)*. These observations are consistent with recent guidelines on the use of cardiac troponin in the initial evaluation of ACS *(1, 2)*. Our observations also demonstrate that the Siemens cTnI assay showed a significantly greater ROC AUC (0.86) compared with the Dade cTnI at baseline (0.77), but not after 6 h in follow-up specimens. We note, as a potential limitation, the modest number of patients studied.

Our findings using the second-generation Siemens Ultra assay complement a recent risk stratification study from Sweden, which demonstrated using the second-generation Beckman cTnI assay that any detectable cTnI was a predictor of coronary artery disease and mortality in 70-year-old men with and without known cardiovascular disease *(15)*. Their findings demonstrated greater risk of adverse events in the group of patients with measurable but normal values compared with the cTnI values less then the LoD, comparable to our findings with the Siemens Ultra assay. Whereas similar findings have been described in patients with high-risk unstable angina and non-ST elevation myocardial infarction *(9, 15)*, our findings are the first to describe risk stratification to rule out MI using this second-generation assay in a heterogeneous nonselected patient group presenting with symptoms suggestive of acute coronary syndrome. We have based our outcomes analysis on the initial plasma sampling, before clinical diagnosis. Thus, including the initial diagnosis for the 49 MI patients in our risk stratification may be considered a study limitation.

Previous risk stratification data were published for the first-generation Bayer assay *(16, 17)*. But the reader must understand that absolute concentrations between the first- and second-generation Bayer/Siemens assays cannot be compared, as different antibodies and calibrators were used in the 2 assays. The first-generation cTnI assay that used the Bayer ACS:180 analyzer had both poor low-end concentration analytical sensitivity and poor low-end precision. Outcomes were initially stratified based on a cTnI concentration of 0.1 μg/L, which was the respective 99th percentile value for this first-generation Bayer assay, a value 2.5-fold higher than the second-generation Siemens Ultra assay 99th percentile (0.04 μg/L) used in the current study. The LoD of this first-generation assay was 0.03 μg/L, 5-fold higher than the Ultra assay LoD (0.006 μg/L) of the present study. Risk stratification using the first-generation assay was demonstrated in the TACTICS TIMI-18 (Treat Angina with Aggrastat and Determine Cost of Therapy with an Invasive or Conservative Strategy—Thrombolysis in Myocardial Infarction) trial, with cTnI increases >0.1 μg/L identifying high-risk patients who derived clinical benefit from early invasive strategy *(6)*. We speculate that, given the increased incidence of 60-day events in patients with detectable TnI levels by the second-generation Ultra assay, the number of patients in TACTICS TIMI-18 who would have been identified as benefiting from invasive therapy might have been greater with use of the second-generation assay.

In conclusion, we demonstrate acceptable clinical sensitivity for detection of MI using the 99th percentile reference value, with increasing sensitivity over time after presentation. Further, our data add to the growing evidence that with improved, analytically robust cTn assays with low LoDs, any measurable cTnI implies a higher risk than cTnI concentrations below an assay's LoD. We believe that this biomarker will assist in early and improved patient triage, management, and outcomes.

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