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Proteomics and Protein Markers

Rapid Rule-Out of Acute Myocardial Injury Using a Single High-Sensitivity Cardiac Troponin I Measurement

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BACKGROUND: Rapid rule-out strategies using high-sensitivity cardiac troponin assays are largely supported by studies performed outside the US in selected cohorts of patients with chest pain that are atypical of US practice, and focused exclusively on ruling out acute myocardial infarction (AMI), rather than acute myocardial injury, which is more common and associated with a poor prognosis.

METHODS: Prospective, observational study of consecutive patients presenting to emergency departments [derivation (n = 1647) and validation (n = 2198) cohorts], where high-sensitivity cardiac troponin I (hs-cTnI) was measured on clinical indication. The negative predictive value (NPV) and diagnostic sensitivity of an hs-cTnI concentration <limit of detection (LoD) at presentation was determined for acute myocardial injury and for AMI or cardiac death at 30 days.

RESULTS: In patients with hs-cTnI concentrations <99th percentile at presentation, acute myocardial injury occurred in 8.3% and 11.0% in the derivation and validation cohorts, respectively. In the derivation cohort, 27% had hs-cTnI < LoD, with NPV and diagnostic sensitivity for acute myocardial injury of 99.1% (95% CI, 97.7–99.8) and 99.0% (97.5–99.7) and an NPV for AMI or cardiac death at 30 days of 99.6% (98.4–100). In the validation cohort, 22% had hs-cTnI <LoD, with an NPV and diagnostic sensitivity for acute myocardial injury of 98.8% (97.9–99.7) and 99.3% (98.7–99.8) and an NPV for AMI or cardiac death at 30 days of 99.1% (98.2–99.8).

CONCLUSIONS: A single hs-cTnI concentration <LoD rules out acute myocardial injury, regardless of etiology, with an excellent NPV and diagnostic sensitivity, and identifies patients at minimal risk of AMI or cardiac death at 30 days.

CLINICAL TRIAL REGISTRATION: Use of Abbott High Sensitivity Troponin I Assay In Acute Coronary Syndromes (UTROPIA), NCT02060760.

Cardiac troponin (cTn)[7] is the preferred biomarker to establish the diagnosis of both acute myocardial injury and acute myocardial infarction (AMI) *(1)*. Determining which patients should undergo cTn testing to rule in and rule out AMI remains controversial, since up to one-third of patients ultimately diagnosed with AMI do not have chest pain on presentation, and because of the potential consequences of missing AMI, particularly among individuals with atypical presentations *(2–4)*. Consequently, in clinical practice cTn testing is performed in a heterogeneous cohort of patients, contributing to overcrowding, unnecessary resource utilization and cost, and potential delays in the evaluation and management of alternative pathologies responsible for the patient's hospital presentation. Most studies assessing rule-out strategies with cTn focus on select cohorts of patients with chest pain *(5–10)*. However, in clinical practice cTn measurements are done in various clinical circumstances, regardless of the presence or absence of chest pain.

Most studies have focused on ruling out AMI *(5–10)*. In contrast, myocardial injury, defined by cTn concentrations above the 99th percentile *(1)*, is more common and has a similar or worse prognosis than AMI *(11–14)*. Challenges in determining whether myocardial

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[7] Nonstandard abbreviations: cTn, cardiac troponin; AMI, acute myocardial infarction; hs, high sensitivity; ED, emergency department; LoD, limit of detection; ECG, electrocardiogram; NPV, negative predictive value; High-STEACS, High-Sensitivity Troponin in the Evaluation of Patients with Acute Coronary Syndrome.

injury is a consequence of type 1 or type 2 MI, or due to other cardiac and noncardiac conditions, have been well documented *(15, 16)*. Hence, rather than developing strategies that rule out AMI in isolation, it may be more practical to rule out acute myocardial injury.

We conducted a prospective, observational cohort study using a high-sensitivity (hs)-cTnI assay in an unselected, heterogeneous population presenting to our inner city emergency department (ED), in whom cTnI measurements were obtained on clinical indication. Subsequently, we sought to examine our findings in a large external validation cohort. Our goals were 2-fold. First, we determined whether a single hs-cTnI measurement at presentation with concentrations below the limit of detection (LoD) of an hs-cTnI assay could rule out acute myocardial injury, regardless of the underlying mechanism of injury, alone or in combination with a normal electrocardiogram (ECG). Second, we examined the safety of this rule-out strategy by assessing AMI and cardiac death at 30 days.

Methods

STUDY DESIGN AND POPULATION

Following institutional review board approval, we prospectively included consecutive, unselected patients presenting from February 4, 2014, through May 9, 2014, in whom initial preset serial cTnI measurements at 0, 3, 6, and 9 h were ordered on clinical indication at Hennepin County Medical Center (Minneapolis, MN) to rule in and rule out AMI [Use of Abbott High Sensitivity Troponin I Assay In Acute Coronary Syndromes (UTROPIA); NCT02060760]. For the derivation cohort, patients needed a baseline hs-cTnI measurement at presentation and at least one additional hs-cTnI measured within 24 h of presentation, before discharge. Patients <18 years old, with evidence of acute ST-segment elevation myocardial infarction on presentation, pregnancy, trauma, declined to participate in research as documented on information disclosure, without death date available, did not present through ED, or were transferred from an outside hospital were excluded. For patients with more than one presentation during the study period, we included only the first.

The external validation cohort included consecutive patients presenting to the ED of secondary and tertiary care hospitals in Scotland in whom the primary clinician suspected acute coronary syndrome and serial plasma hs-cTnI measurements were obtained (baseline hs-cTnI measurement at presentation and at least one additional hs-cTnI measurement at >6 h). Patients were excluded if they had a previous presentation during the study period or were not residents in Scotland.

CARDIAC TROPONIN I ASSAYS

Fresh EDTA plasma samples were simultaneously measured with both the contemporary cTnI (clinically used) and hs-cTnI (investigational) assays on the Abbott ARCHITECT $i1000_{SR}$ or $i2000_{SR}$ analyzers. Only the hs-cTnI assay data were used for the current study. Sex-specific 99th percentiles cutoffs for the hs-cTnI assay used were: females 16 ng/L and males 34 ng/L *(12, 17, 18)*. Total imprecision (%CV) (n = 29 days) was 5.3% at 15 ng/L *(12)*. The hs-cTnI assay has a LoD of 1.9 ng/L, %CV 20%. An interlaboratory %CV has been reported to be 12.6% at 3.5 ng/L *(18)*.

MYOCARDIAL INJURY AND ISCHEMIA

Myocardial injury was defined as any hs-cTnI concentration above the sex-specific 99th percentile *(1)*. All 12-lead ECGs in the derivation cohort were interpreted and coded by an independent expert reviewer (author S.W. Smith). A normal ECG was defined as an entirely normal ECG (including those with normal variant ST elevation) or where there were nondiagnostic ST-T wave abnormalities. Sinus bradycardia, prolonged PR interval, low voltage, right or left atrial hypertrophy, right ventricular conduction delay, and occasional premature atrial beats were all within normal for the purposes of this study. All ECGs with atrial fibrillation, sinus tachycardia, high-grade atrioventricular block, premature ventricular contractions, bundle branch block, intraventricular conduction delay (above 120 ms), paced rhythm, left ventricular hypertrophy, pathologic Q-waves, ST-segment depression (horizontal or down-sloping ST depression ≥0.05 mV in 2 contiguous leads) *(1)*, T-wave inversion (≥0.15 mV in 2 contiguous leads with prominent R wave or R/S ratio >1) *(1)*, or ST-elevation were considered abnormal. Nonspecific ST-T wave abnormalities were slight variations in ST or T that were <1.5 mm of abnormal T-wave inversion in 2 consecutive leads or up to 0.5-mm ST depression in 2 consecutive leads or both, or T-wave flattening.

STUDY OUTCOMES

The diagnostic outcome was acute myocardial injury during the index hospitalization. The safety outcome was a composite of AMI or cardiac death at 30 days, including events occurring during the index hospitalization.

STATISTICAL ANALYSES

Categorical variables are shown as percentages. Continuous variables are shown as mean values with correspondent 95% CIs. The χ^2 test was used to compare categorical measures. ANOVA was used to compare continuous variables. Negative predictive values (NPVs) and diagnostic sensitivity for acute myocardial injury during the index hospitalization and 30-day AMI or cardiac death were determined using the LoD alone or in combination

with a normal ECG. For the purpose of evaluating the LoD to rule-out myocardial injury, acute myocardial injury was defined as any increase in hs-cTnI above the sex-specific 99th percentile during the index hospitalization following an initial hs-cTnI concentration under the LoD. CIs for diagnostic sensitivity and NPV were calculated using the MedCalc Version 16.2.1. Analyses were performed for the entire population and for men and women separately. Statistical significance was at *P* values <0.05.

Results

A total of 1647 patients were included in the derivation cohort and 2198 in the external validation cohort. Baseline characteristics according to sex for the derivation and validation cohorts are shown in Table 1 (also see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/63/issue1). In patients with hs-cTnI concentrations <99th percentile at presentation (derivation 81%, validation 73%), acute myocardial injury occurred subsequently in 8.3% and 11.0% of patients in the derivation and validation cohort, respectively.

In the derivation cohort, 27% (n = 448) of patients had hs-cTnI concentrations <LoD at presentation, 54% (n = 892) had measurable hs-cTnI concentrations between the LoD and the 99th percentile, and 19% (n = 307) had concentrations above sex-specific 99th percentiles. Women were more likely to have baseline hs-cTnI concentrations <LoD than men (32% vs 24%, $P = 0.005$). Both men and women with hs-cTnI concentrations <LoD at presentation were younger and had fewer comorbidities compared to patients with hs-cTnI concentrations above the LoD (Table 1).

In patients with hs-cTnI concentrations <LoD at presentation, regardless of ECG findings, the NPV and diagnostic sensitivity for acute myocardial injury was 99.1% (95% CI, 97.7–99.8) and 99.0% (95% CI, 97.5–99.7), respectively (Table 2). The NPV for AMI or cardiac death at 30 days was 99.6% (95% CI, 98.4–100). Only 4 patients had acute myocardial injury (0.9%, 4/448), of whom 2 had an AMI and none had a cardiac death at 30 days (0.45%, 2 out of 448) corresponding to a miss rate of 1 in 224 (see online Supplemental Tables 2 and 3). The 4 patients with false-negative results were female and the maximum hs-cTnI concentrations for each of these patients were 36, 38, 42, and 48 ng/L (see online Supplemental Table 2). In patients with hs-cTnI concentrations <LoD and a normal ECG (n = 283, 17%) the NPV and diagnostic sensitivity for acute myocardial injury was 99.6% (95% CI, 98.1–100) and 99.8% (95% CI, 98.6–100), respectively; with an NPV for AMI or cardiac death at 30 days of 99.6% (95% CI, 98.1–100).

Among men with baseline hs-cTnI concentrations <LoD (218 out of 922, 24%), the NPV and diagnostic sensitivity for acute myocardial injury was 100% (95% CI, 99.3–100) and 100% (95% CI, 98.1–100), respectively. At 30 days, none of these patients had an AMI or cardiac death; corresponding to an NPV of 100% (95% CI, 98.3–100) (Table 2). Among women with baseline hs-cTnI concentrations <LoD (230 out of 725, 32%), the NPV and diagnostic sensitivity for acute myocardial injury was 98.3% (95% CI, 95.6–99.5%) and 98.1% (95% CI, 95.2–99.5), respectively. At 30 days, 2 out of 230 women with concentrations <LoD had an AMI, without any cardiac deaths. An hs-cTnI concentration <LoD combined with a normal 12-lead ECG improved the NPV and diagnostic sensitivity for acute myocardial injury to 99.3% (95% CI, 96.4–100) and 99.5% (95% CI, 97.4–100), respectively, with an NPV of 99.3% (95% CI, 96.4–100) for AMI or cardiac death at 30 days.

In the derivation cohort, 8.2% (n = 135) were deemed early presenters (≤2 hours) (Supplemental Table 4). In early presenters, a baseline hs-cTnI under the LoD yielded a NPV and diagnostic sensitivity for acute myocardial injury of 97.5% (95% CI 86.8–99.9) and 97.8% (95% CI 88.5–99.9) respectively, yet when combined a baseline hs-cTnI under the LoD was combined with a normal ECG, the NPV and diagnostic sensitivity increased to 100% (95% CI, 84.6–100) and 100% (95% CI, 92.3–100), respectively.

In the external validation cohort, 22% (n = 477) of patients had hs-cTnI concentrations <LoD at presentation, 51% (n = 1131) had measurable hs-cTnI concentrations between the LoD and the 99th percentile, and 27% (n = 590) had concentrations above sex-specific 99th percentiles (see online Supplemental Table 1). Among patients with hs-cTnI concentrations <LoD at presentation, regardless of ECG findings, the NPV and diagnostic sensitivity for acute myocardial injury were 98.8% (95% CI, 97.9–99.7) and 99.3% (95% CI, 98.7–99.8), respectively (Table 3). The NPV for AMI or cardiac death at 30 days was 99.1% (95% CI, 98.2–99.8). For those with hs-cTnI <LoD (n = 477), 5 had acute myocardial injury and 4 patients had AMI or cardiac deaths at 30 days (see online Supplemental Table 5). The probability of 30-day AMI or cardiac death was 0.8% (n = 4/477) corresponding to a miss rate of less than one in a hundred.

In the validation cohort, 23% (n = 502) were deemed early presenters. In comparison to a baseline hs-cTnI alone, a similar improvement was observed in the validation cohort, when combining baseline hs-cTnI <LoD with a normal ECG (see online Supplemental Table 6). Among early presenters with baseline hs-cTnI <LoD, the NPV and diagnostic sensitivity for acute myocardial injury were 97.5% (95% CI, 94.5–99.8) and 98.8% (95% CI, 97.3–99.9) respectively, with 2 false

Table 1. Patient characteristics according to sex and baseline hs-cTnI concentrations in the derivation cohort.

	Entire cohort, n = 1647				Men, n = 922				Women, n = 725			
Derivation cohort	<1.9 ng/L, n = 448	1.9th–99th, n = 892	>99th, n = 307	*P* value	<1.9 ng/L, n = 218	1.9–34 ng/L, n = 561	>34 ng/L, n = 143	*P* value	<1.9 ng/L, n = 230	1.9–16 ng/L, n = 331	>16 ng/L, n = 164	*P* value
Age, years, mean (SD)	50 (13)	60 (15)	61 (16)	0.005	48 (13)	57 (14)	58 (14)	0.005	51 (13)	65 (15)	64 (16)	0.005
Hypertension, n (%)	199 (44)	644 (72)	238 (78)	<0.001	100 (46)	389 (69)	109 (76)	<0.001	99 (43)	255 (77)	129 (79)	<0.001
Diabetes mellitus, n (%)	99 (22)	284 (32)	119 (39)	0.002	48 (22)	160 (29)	53 (37)	0.03	51 (22)	124 (38)	66 (41)	0.002
Dyslipidemia, n (%)	121 (27)	425 (48)	156 (51)	<0.001	59 (27)	252 (45)	67 (47)	<0.001	62 (27)	173 (52)	89 (55)	<0.001
Renal insufficiency (nondialysis), n (%)	12 (3)	122 (14)	112 (36)	<0.001	5 (2)	73 (13)	51 (36)	<0.001	7 (3)	49 (15)	61 (37)	<0.001
End-stage renal disease on hemodialysis, n (%)	0 (0)	31 (4)	50 (16)	<0.001	0 (0)	21 (4)	25 (18)	<0.001	0 (0)	10 (3)	25 (15)	<0.001
History of coronary artery disease, n (%)	27 (6)	172 (19)	69 (23)	<0.001	16 (7)	115 (20)	35 (24)	<0.001	11 (5)	57 (17)	34 (21)	<0.001
Prior myocardial infarction, n (%)	24 (5)	125 (14)	43 (14)	<0.001	16 (7)	78 (14)	22 (15)	0.04	8 (4)	47 (14)	21 (13)	<0.001
Prior percutaneous coronary intervention, n (%)	16 (4)	101 (11)	34 (11)	<0.001	7 (3)	64 (11)	17 (12)	<0.001	9 (4)	37 (11)	17 (10)	<0.001
Prior coronary artery bypass graft surgery, n (%)	3 (1)	53 (6)	18 (6)	<0.001	1 (1)	39 (7)	13 (9)	<0.001	2 (1)	14 (4)	5 (3)	0.07
History of congestive heart failure, n (%)	9 (2)	129 (15)	92 (30)	<0.001	5 (2)	78 (14)	39 (27)	<0.001	4 (2)	51 (15)	53 (33)	<0.001
History of atrial fibrillation, n (%)	8 (2)	82 (9)	41 (13)	<0.001	5 (2)	51 (9)	17 (12)	<0.001	3 (1)	31 (9)	24 (15)	<0.001
Peripheral vascular disease, n (%)	3 (1)	30 (3)	9 (3)	0.005	1 (1)	18 (3)	4 (3)	0.09	2 (1)	12 (4)	5 (3)	0.13
Cerebrovascular disease, n (%)	16 (4)	94 (11)	42 (14)	0.005	8 (4)	51 (9)	12 (8)	0.05	8 (4)	43 (13)	30 (18)	0.005
Baseline hs-cTnI concentration, mean (95% CI)	0.8 (0.7–0.8)	8.3 (7.8–8.7)	391 (98–684)	<0.001	0.8 (0.7–0.9)	9.5 (8.8–10.1)	491.4 (0–1075.2)	<0.001	0.8 (0.7–0.8)	6.3 (5.8–6.7)	301.5 (95.4–507.6)	<0.001
Creatinine, mg/dL, mean (95% CI)	0.9 (0.8–0.9)	1.5 (1.4–1.6)	3.4 (2.6–4.2)	<0.001	1.0 (0.9–1.0)	1.6 (1.4–1.8)	4.4 (2.7–6.0)	<0.001	0.8 (0.8–0.9)	1.4 (1.2–1.6)	2.5 (2.0–2.9)	<0.001
Estimated glomerular filtration rate, $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$, mean (95% CI)	104 (100–107)	85 (82–88)	61 (56–66)	<0.001	110 (105–115)	89 (85–92)	59 (52–66)	<0.001	98 (93–103)	79 (74–83)	63 (55–70)	<0.001
Chest pain on presentation, n (%)	276 (62)	447 (50)	118 (38)	0.005	129 (59)	284 (51)	61 (43)	0.093	147 (64)	163 (49)	57 (35)	0.005
Shortness of breath on presentation, n (%)	176 (39)	364 (41)	146 (48)	0.19	82 (38)	227 (40)	69 (48)	0.289	94 (41)	137 (41)	77 (47)	0.605

Table 2. Diagnostic performance of baseline hs-cTnI concentrations <LoD alone and in combination with an initial normal 12-lead electrocardiogram for ruling out acute myocardial injury (diagnostic outcome) and a 30-day safety outcome of acute myocardial or cardiac death in the derivation cohort.

	All, n = 1647		Men, n = 922		Women, n = 725	
Derivation cohort	Baseline hs-cTnI <1.9 ng/L (n = 448)	Baseline hs-cTnI <1.9 ng/L and normal ECG (n = 283)	Baseline hs-cTnI <1.9 ng/L (n = 218)	Baseline hs-cTnI <1.9 ng/L and normal ECG (n = 130)	Baseline hs-cTnI <1.9 ng/L (n = 230)	Baseline hs-cTnI <1.9 ng/L and normal ECG (n = 153)
Diagnostic outcome, acute myocardial injury						
NPV, mean (95% CI)	99.1 (97.7–99.8)	99.6 (98.1–100.0)	100.0 (99.3–100.0)	100.0 (97.2–100.0)	98.3 (95.6–99.5)	99.3 (96.4–100.0)
Diagnostic sensitivity, mean (95% CI)	99.0 (97.5–99.7)	99.8 (98.6–100.0)	100.0 (98.1–100.0)	100.0 (94.1–100.0)	98.1 (95.2–99.5)	99.5 (97.4–100.0)
Safety outcome, 30-day acute myocardial infarction or cardiac death						
NPV, mean (95% CI)	99.6 (98.4–100.0)	99.6 (98.1–100.0)	100.0 (98.3–100.0)	100.0 (97.2–100.0)	99.1 (96.9–99.9)	99.3 (96.4–100.0)
Diagnostic sensitivity, mean (95% CI)	98.8 (95.8–99.9)	99.4 (96.8–100.0)	100.0 (95.7–100.0)	100.0 (95.7–100.0)	97.7 (91.9–99.7)	98.8 (93.7–100.0)

Table 3. Diagnostic performance of baseline hs-cTnI concentrations below the LoD alone and in combination with an initial normal 12-lead electrocardiogram for ruling out acute myocardial injury (diagnostic outcome), and a 30-day safety outcome of acute myocardial or cardiac death in the validation cohort.

	All, n = 2,198		Men, n = 1271		Women, n = 927	
Validation cohort	Baseline hs-cTnI ≤2 ng/L (n = 477)	Baseline hs-cTnI ≤2 ng/L and normal ECG (n = 400)	Baseline hs-cTnI ≤2 ng/L (n = 239)	Baseline hs-cTnI ≤2 ng/L and normal ECG (n = 206)	Baseline hs-cTnI ≤2 ng/L (n = 238)	Baseline hs-cTnI ≤2 ng/L and normal ECG (n = 194)
Diagnostic outcome, acute myocardial injury						
NPV, mean (95% CI)	98.8 (97.9–99.7)	99.1 (98.2–99.9)	99.0 (97.7–99.9)	99.8 (99.1–100)	98.5 (97.0–99.8)	98.6 (96.8–99.9)
Diagnostic sensitivity, mean (95% CI)	99.3 (98.7–99.8)	99.1 (98.2–99.9)	99.4 (98.7–100)	99.8 (99.1–100)	99.0 (98.0–99.9)	99.0 (97.8–99.9)
Safety outcome, 30-day acute myocardial infarction or cardiac death						
NPV, mean (95% CI)	99.1 (98.2–99.8)	99.4 (98.6–100)	99.0 (97.7–99.9)	99.8 (99.1–100)	99.0 (97.7–99.9)	98.6 (96.8–99.9)
Diagnostic sensitivity, mean (95% CI)	99.3 (98.7–99.9)	99.2 (98.3–100)	99.3 (98.4–100)	99.7 (98.9–100)	99.1 (98.1–99.9)	98.8 (97.4–99.9)

negatives. Yet, a baseline hs-cTnI <LoD at presentation in combination with a normal ECG yielded an NPV and diagnostic sensitivity of 99.4% (95% CI, 97.7–100) and 99.5% (95% CI, 98.1–100), respectively, without any false negative events.

Discussion

Several findings are unique to our large study of consecutive patients undergoing hs-cTnI testing in the ED. First, we demonstrate that a single hs-cTnI measurement <LoD at presentation rules out acute myocardial injury, regardless of the underlying etiology and ECG findings, in 27% of patients, with an NPV and diagnostic sensitivity of ≥99%, and a probability of AMI or cardiac death at 30 days of <0.5%. Second, we show that there are sex differences in the proportion of patients qualifying for the rule out strategy, with women being more likely to have hs-cTnI concentrations at presentation <LoD than men. Healthy reference population studies have demonstrated that women are more likely to have hs-cTnI concentrations <LoD *(19)*. These observations suggest that studies evaluating low hs-cTn concentrations to rule out myocardial injury should stratify their analysis by sex. Third, an hs-cTnI concentration below the LoD at presentation alone was able to rule out acute myocardial injury with an NPV and diagnostic sensitivity of 100% in men. Conversely, in women, the NPV and sensitivity for acute myocardial injury was lower at 98.3% and 98.1%, respectively. In women, the combination of an hs-cTnI concentration <LoD and a normal ECG improved the NPV to 99.3% and diagnostic sensitivity to 99.5% for acute myocardial injury. While combining hs-cTn with ECG findings improved the rule out performance in women, it also reduced the proportion of women ruled out from 32% to 21%. Fourth, in early presenters, the NPV and diagnostic sensitivity for acute myocardial injury were suboptimal when using baseline hs-cTnI alone, yet when combined with a normal ECG, the NPV and diagnostic sensitivity were shown to be excellent. Finally, we validated our findings in a large external cohort, and found similar NPVs and diagnostic sensitivities (including early presenters), suggesting our findings are generalizable.

Our purpose of ruling out acute myocardial injury, rather than focusing solely on AMI, is based on the facts that (*a*) patients with myocardial injury have similar or worse prognosis than patients with AMI *(11–14)*, (*b*) it is challenging to distinguish whether patients with increased hs-cTn concentrations >99th percentile have myocardial injury vs type 1 or 2 MI, and (*c*) clinicians are concerned about patients with any hs-cTnI increase due to its prognostic implications, regardless of whether the ultimate diagnosis is AMI. By expediting the safe rule-out of acute myocardial injury, which by definition encompasses all hs-cTn increases >99th percentile regardless of etiology, including AMI, this rule-out strategy may reduce costs and the need for serial hs-cTn testing, facilitate early discharge in selected cases, and reduce delays in the evaluation and management of patients with alternative diagnosis.

From a clinical perspective, it is important to emphasize that our strategy of ruling-out acute myocardial injury using the LoD intends to identify a subset of patients at low-risk in whom clinical care may be safely expedited by avoiding lengthy rule-out strategies using serial hs-cTn testing that are widely used in the US. None of the strategies using a single hs-cTn are intended to rule-in disease (such as AMI) and guide therapeutic interventions, but rather to facilitate the rapid identification of patients at low-risk *(20)*. To guide specific therapeutic interventions, clinicians still need to define whether patients with hs-cTn increases >99th percentile have type 1 or 2 MI or myocardial injury without overt ischemia related to other cardiac or noncardiac conditions, and integrate the patient's clinical presentation, including a careful history and exam, as well as serial cTn concentration changes (δ) to determine a specific diagnosis and tailor therapies accordingly.

Our study has several strengths. First, we evaluated hs-cTn testing as used in clinical practice, and hence included an unselected, heterogeneous population rather than a narrow, selected, chest pain population. Second, pending FDA clearance of hs-cTn assays for clinical use, our findings are both timely and important, with the derivation cohort typical of a US ED population. Our large study, including both a US derivation and non-US external validation cohort, supports the global use of this approach. Third, by ruling out acute myocardial injury rather than AMI, we provide an objective, reproducible endpoint, and avoid the controversy related to the adjudication and classification of AMI. Fourth, all patients, including both derivation and validation cohorts, required a baseline hs-cTnI measurement at presentation plus at least one additional measurement, which overcame the possibility of missing myocardial injury, since not all studies examining a single measurement approach have obtained serial sampling *(6)*.

Several limitations are noted. First, despite observational data supporting the use of sex-specific thresholds to improve the diagnosis of AMI in women, whether general vs sex-specific 99th percentiles should be used with hs-cTnI or hs-cTnT assays remains uncertain *(17, 19–22)*. However, expert opinions support sex-specific 99th percentiles *(21)*. In our study, the 4 false negative patients were female and the maximum hs-cTnI concentrations for each of these patients surpassed both the general (26 ng/L) and sex-specific 99th percentile (16 ng/L). Second, there are no guidelines or consensus recommendations addressing what is an acceptable risk

of missed events *(23)*. In our study, a strategy using undetectable hs-cTnI concentrations to rule-out acute myocardial injury and assessing 30-day AMI or cardiac death, had a miss rate of 0.45% or 1 in 224 in the derivation cohort and 0.8% or <1 in 100 in the validation cohort. Third, our observations using one manufacturer's hs-cTnI assay (Abbott) cannot be assumed to be transferable to other hs-cTnI or hs-cTnT assays due to the lack of assay standardization *(24)*. Even for the same Abbott hs-cTnI assay used in the current study, the low-level LoD hs-cTn concentration threshold varies from 1.2 *(25)* to 1.9 ng/L *(26)*. Fourth, in contrast to the High-Sensitivity Troponin in the Evaluation of Patients with Acute Coronary Syndrome (High-STEACS) study that consisted of >95% Scottish patients (5,996 out of 6,305) and had a primary outcome focused on type 1 myocardial infarction *(18)*, our study represents the complete UTROPIA study (NCT02060760), which represents one of the largest cohorts examining a hs-cTn assay in the US. Of note, a small subset of patients (n = 308) enrolled in UTROPIA served as an external cohort in High-STEACS. Finally, in the derivation cohort, the 30-day follow-up information including AMI or cardiac death was obtained by reviewing electronic medical records, Social Security Death Index records, and local newspaper obituaries. However, similar outcomes were observed in the validation cohort, which used regional and national databases to ensure that follow-up was complete for the entire study population, and screened every hospital admission where cTn was measured.

In conclusion, among unselected patients undergoing hs-cTnI measurements on clinical indication, a single hs-cTnI concentration <LoD at presentation may facilitate the rule-out of acute myocardial injury, regardless of the etiology or ECG findings, with an excellent NPV and diagnostic sensitivity. For patients with hs-cTnI concentrations <LoD at presentation, the probability of AMI or cardiac death at 30 days is <0.5%. In early presenters and women, a baseline hs-cTnI under the LoD in combination with a normal ECG provides an excellent rule-out strategy for myocardial injury. Our novel strategy of ruling out any acute myocardial injury, rather than AMI, and as tested in a real-life, heterogeneous cohort of patients, may be more practical for clinicians, reduce costs, expedite patient care, and facilitate early discharge in selected patients.

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