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Article

Analytical Error of Cardiac Troponin and Impact on the Performance of Accelerated Diagnostic Protocols in Patients with Acute Chest Pain

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BACKGROUND: This study simulates how assay-specific bias influences the diagnostic performance of 0/1-h accelerated diagnostic protocols (ADPs) for 3 different high-sensitivity cardiac troponin (hs-cTn) assays.

METHODS: We included 1493 patients presenting with chest pain. hs-cTnT (Roche Diagnostics), hs-cTnI from Abbott Diagnostics (hs-cTnI-A), and Siemens Healthineers (hs-cTnI-S) were measured at admission. The absolute total error observed in a state-of-the-art EQA study were added to the admission concentrations, producing 6 new variables being adjusted for maximum possible bias (if analytical variation is 0) ($+bias_{mean}$, $+bias_{max95\%CI}$, $+bias_{min95\%CI}$, $-bias_{mean}$, $-bias_{max95\%CI}$, $-bias_{min95\%CI}$). The influence of this "worst-case scenario" bias was compared after calculating sensitivity, specificity, negative and positive predictive values, and rule-out proportion for 30-day myocardial infarction or death for the observed and bias-adjusted hs-cTn concentrations.

RESULTS: For 0-h rule-out, hs-cTnI-S and hs-cTnT had a sensitivity of >99.0%, compared to 97.7% for hs-cTnI-A. After adding the bias, sensitivity was unchanged for hs-cTnI-S (99.5%), but lower for hs-cTnT (95.5%), and hs-cTnI-A (96.2%). For the 0-/1-h algorithm, adding bias reduced sensitivity to 95.5% for hs-cTnT, while both hs-cTnI algorithms were unchanged (100.0%). Rule-out proportions for 0 h ranged from 0% to 60.0% for hs-cTnT, 28.2%–62.7% for hs-cTnI-A, and 3.5%–35.5% for hs-cTnI-S. For the 0-/1-h algorithm, ranges were 57.7%–75.8% (hs-cTnT), 52.8%–67.5% (hs-cTnI-A), and 45.7%–61.2% (hs-cTnI-S),

CONCLUSION: Analytical bias of hs-cTn assays affects the clinical rule-out rate of the 0/1-h ADPs more than the diagnostic sensitivity. Bias may have a greater influence on the proportion of patients requiring hospital admission and may contribute to the heterogeneity of the reported rule-out rates of current ADPs.

ClinicalTrials.gov Registration Number: NCT02620202

Introduction

Chest pain is one of the most common complaints in the Emergency Department (ED), contributing to overcrowding, increased mortality and morbidity, and affecting resource utilization (1, 2). After clinical evaluation and investigations, most chest pain patients are not

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diagnosed with non-ST elevation myocardial infarction (NSTEMI) (3–9). To rapidly confirm or exclude patients at risk of myocardial infarction (MI) in the ED, clinical guidelines suggest using accelerated diagnostic protocols (ADPs) for early risk stratification of patients into low, intermediate, and high risk (10–12). Risk stratification is based on absolute cutoff concentrations at presentation and subsequent delta values at 1 or 2 h. Studies have demonstrated the ADPs to be safe for the early risk stratification of patients with acute chest pain (3–9, 13). However, studies provide different estimates of the rule-out rate of the algorithms (13, 14). Earlier studies indicate that the rule-out rate might in part be dependent on the analytical error of troponin assays (4, 15–18). To rely on consistent performance of guideline-recommended ADPs, the analytical performance of high-sensitivity cardiac troponin (hs-cTn) should be consistent over time, especially around the low-risk clinical threshold for ruling out MI (19). Recently, an External Quality Assessment (EQA) study using monthly native plasma sample pools at the relevant concentrations made high-quality data available reporting the total analytical error (TE) for a range of cardiac troponin assays (20, 21). The study exhibited higher analytical error compared to what is usually reported by manufacturers or observed by routine laboratories (22), most likely as it included multiple reagent lots, and between-instrument and between-laboratory variation. Between-laboratory bias might be an overlooked reason for differences in ADP performance and efficacy as the 95% confidence interval (95%CI) for rule-out rates reported by different studies range from 45% to 62% (14).

The aim of the current study was to simulate the effect of bias on 3 different 0-/1-h cardiac troponin rule-out ADPs and its maximum influence on safety and rule-out rate for clinical performance. The bias modeled were derived using TE data for the assays from Kavsak et al. (21) and exceeds the worst-case expected bias in routine use [in the current simulation, 100% of the TE in Kavsak et al. (21) was allocated to bias and the analytical variation (CV_A) was equaled to 0]. Original data used in the simulation were retrieved from a large observational study of patients with suspected acute coronary syndrome (ACS) (23).

Materials and Methods

STUDY DESIGN AND POPULATION

The study included 1496 patients from the prospective observational WESTCOR-study (ClinicalTrials.gov ID: NCT02620202). The study design has been described in detail previously (23). Briefly, 1493 patients who were clinically evaluated in an ED for suspected ACS were included. Admission and 1-h measurements were available for $n = 1493$ patients for hs-cTnT (Roche Diagnostics), $n = 1488$ for hs-cTnI from Abbott Diagnostics, and $n = 1493$ for hs-cTnI Atellica from Siemens Healthineers. Patients were managed according to standard care with electrocardiogram, clinical examination, echocardiography, and coronary angiography when indicated. All patients provided written informed consent and the study and biobank were approved by the Regional Committee for Medical Research Ethics (2014/1365 REK vest and 2014/1905 REK vest). Laboratory procedures and analyses, clinical adjudication, and the ADPs from the European Society of Cardiology (ESC) applied in the current study are described in detail in the Supplemental Material along with a detailed overview of reagent and calibrator lots, assay platforms, and product numbers.

ENDPOINTS

The primary endpoint was 30-day MI [including index MI (defined as MI at first presentation)] and all-cause mortality. The secondary endpoint was an index diagnosis of MI. MI was adjudicated by 2 independent cardiologists based on all clinical, imaging, and laboratory data (using hs-cTnT from Roche Diagnostics as the routine test) that were available in the patient files.

SIMULATING THE TOTAL ERROR

The measured hs-cTn concentrations (i.e., raw data from the analyzer) were designated the original (target) value. The maximum possible bias (allocating CV_A to 0%) from the original value was calculated based on the TE data reported by Kavsak et al. (21), see Table 1. Kavsak et al. reported the TE based on 12 monthly measurements of commutable plasma pools with target concentrations corresponding to the baseline cutoffs used in 0-/1-h ADPs. Samples were distributed to 36 laboratories in Canada and therefore include between-institution and long-term analytical imprecision and bias for different cTn assays. The maximum possible bias was added to the original admission concentrations measured in the WESTCOR-study (raw data) to produce new variables simulating the maximum bias with 95%CI (if CV_A was 0%) for the assay-specific troponin concentrations. The absolute concentrations corresponding to the minimum TE (lower bound of the 95%CI), the mean TE and the maximum TE (upper bound of the 95%CI) reported by Kavsak et al. (21) were used in the current simulations (Table 1). This produced 6 new variables named $+bias_{mean}$, $+bias_{max95\%CI}$, $+bias_{min95\%CI}$, $-bias_{mean}$, $-bias_{max95\%CI}$, $-bias_{min95\%CI}$, as applicable for each assay. The resulting concentrations after adding the bias were rounded to the nearest integer, consistent with current recommendations and

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Table 1. The concentrations representing the maximum TE for the corresponding hs-cTn assays/algorithms added to the original values obtained from the WESTCOR-study. The TE (in absolute values) is concentration dependent; applying the estimated TE at concentrations corresponding to the cutoffs used in the 0-/1-h algorithms. Δ is absolute change between admission and 1-h hs-cTn samples.

ESC 0-/1-h algorithm (11)	Concentration used for estimating total error (21)	Mean total error	Maximum total error (bounds of 95% CI) (21)
hs-cTnT$_0$ (Roche) <5 ng/L	6.2 ng/L	±3.8 ng/L	±4.7 ng/L
hs-cTnT$_0$ (Roche) <12 ng/L, Δ < 3 ng/L	11.7 ng/L	±3.6 ng/L	±3.7 ng/L
hs-cTnI$_0$ (Abbott) <4 ng/L	4.5 ng/L	±1.5 ng/L	±1.6 ng/L
hs-cTnI$_0$ (Abbott) <5 ng/L, Δ < 2 ng/L	4.5 ng/L	±1.5 ng/L	±1.6 ng/L
hs-cTnI$_0$ (Siemens) <3 ng/L	4.9 ng/L	±1.3 ng/L	±1.4 ng/L
hs-cTnI$_0$ (Siemens) <6 ng/L, Δ < 3 ng/L	4.9 ng/L	±1.3 ng/L	±1.4 ng/L

clinical practice (19). The decision point for rounding was defined to one decimal place (<5.0, 12.0 etc.). Taking as an example, Kavsak et al. report that the mean TE for hs-cTnT at a concentration close to 5 ng/L is ±3.8 ng/L (21). For a hs-cTnT concentration of 5 ng/L (original value) we would add ±3.8 ng/L to simulate the mean ±bias producing 2 new variables, where the result would be 9 (8.8) ng/L for +bias$_{mean}$ and 1 (1.2) ng/L for −bias$_{mean}$, respectively. Kavsak et al. report the upper bound 95%CI of maximum TE for hs-cTnT to be ±4.7 ng/L. Accordingly, to simulate the maximum 95%CI for analytical bias we would add ±4.7 to the raw value of 5 ng/L resulting in values of 10 (9.7) ng/L for +bias$_{max95\%CI}$ and 0 (0.3) ng/L for −bias$_{max95\%CI}$, respectively. As the baseline cutoff used in the 0-/1-h algorithm for hs-cTnT is higher (12 ng/L) compared to single-sample cutoff and Kavsak et al. reported a lower TE at 11.7 ng/L we used ±3.6 ng/L for bias$_{mean}$ and ±3.7 ng/L for bias$_{max95\%CI}$ when estimating the maximum possible bias for the 0-/1-h hs-cTnT ADP (Table 1). Similarly, the bias variables produced for the hs-cTnI-A and hs-cTnI-S were based on the raw data from the WESTCOR-study and addition of the ±TE (mean and 95% CI) as reported by Kavsak et al. (21).

The 0-/1-h delta values were not modified (only baseline concentrations were altered) as the 1-h within-series analytical variation might be considered already embedded in the data and is likely to be much smaller compared to the between-laboratory and long-term variation dominating the TE reported by Kavsak et al. (21).

DATA HANDLING AND STATISTICAL ANALYSIS

Baseline characteristics were calculated using mean ± standard deviation for continuous variables in normally distributed data, while the median and 25–75th percentile values are reported for skewed data. Categorical variables are presented as numbers (percentages). Diagnostic performance and the proportion of patients designated as low-risk/rule-out of the single-sample and hs-cTn 0-/1-h ESC algorithms were analyzed and compared for the original and bias-adjusted variables using receiver operating characteristic area under the curve (ROC-AUC) plots and DeLongs test, sensitivity, specificity, negative predictive value (NPV), positive predictive values (PPV), and rule-out proportions. We excluded early presenters (patients with symptom duration <3 h) for the 0-h rule-out analyses while all-comers were included in the serial 0-/1-h algorithm. A comparison of sensitivity and rule-out percentage between all-comers and patients after excluding the early-presenter group is presented in Supplemental Table 2. *P* values of <0.05 were interpreted as statistically significant in all analyses. All statistical analyses were performed with R (v.4.2.1).

Results

Baseline characteristics are outlined in Table 2. The median age of the population was 62 years [interquartile range (IQR) 52–73], and 40% were women. Of all

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Table 2. Baseline characteristics of the study population.

	Total population ($N = 1493$)
Age, median years (IQR)	62 (52–73)
Female, *n* (%)	592 (39.6)
Chest pain characteristics	
Early presenters (≤3 h), *n* (%)	308 (20.6)
Symptom duration, median hours (IQR)	9.4 (3.6–53.3)
Risk factors, *n* (%)	
Hypertension	612 (40.9)
Hypercholesterolemia	300 (20.0)
Diabetes	180 (12.1)
Current smoking	282 (18.9)
History, *n* (%)	
Prior MI	287 (19.2)
Prior PAD	29 (1.9)
Prior stroke	42 (2.8)
Heart failure	52 (3.5)
ECG findings, *n* (%)	
ST-segment depression	46 (3.1)
T-wave inversion	45 (3.0)
Vital signs	
Heart rate, median (IQR)	72 (64–82)
SBP, median mmHg (IQR)	144 (130–159)
DBP, median mmHg (IQR)	83 (75–92)
BMI, median kg/m^2 (IQR)	26.9 (24.5–30.1)
Median time between 0- and 1-h sample, min (IQR)	62 (60–69)
Biomarker concentration, median (IQR)	
hs-cTnT$_0$ (Roche), ng/L	6.8 (4.0–14.0)
hs-cTnI$_0$ (Abbott), ng/L	2.9 (1.5–7.3)
hs-cTnI$_0$ (Siemens), ng/L	5.0 (2.9–13.6)
hs-cTnT$_{1\ h}$ (Roche), ng/L	7.0 (4.1–14.5)
hs-cTnI$_{1\ h}$ (Abbott), ng/L	3.4 (1.8–9.0)
hs-cTnI$_{1\ h}$ (Siemens), ng/L	6.1 (3.7–16.2)
eGFR, median mL/min/1.73 m^2	86 (72–97)
Myocardial injury at 0 h or 1 h, *n* (%)	
hs-cTnT >99th percentile upper reference limit	445 (30.9)
hs-cTnI (Abbott) >99th percentile upper reference limit	217 (14.4)
hs-cTnI (Siemens) >99th percentile upper reference limit	228 (15.2)
Adjudicated index diagnosis, *n* (%)	
NSTEMI	174 (11.7)
Type 1 MI	167 (96)
Type 2 MI	7 (4)
UAP	202 (13.5)
NCCC	110 (7.4)
NCCP (specified)	103 (6.9)
NCCP (unspecified)	904 (60.5)
Number of endpoints following discharge from hospital, *n* (%)	
30-day MI or death (primary endpoint)	183 (12.3)
Index MI (secondary endpoint)	174 (11.7)

Abbreviations: PAD, peripheral artery disease; ECG, electrocardiogram; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; UAP, unstable angina pectoris; NCCC, noncoronary cardiac conditions; NCCP, noncardiac chest pain.

patients, 308 (20.6%) were early presenters with symptom onset <3 h prior to the first sample. Approximately 40% of patients had previous hypertension, 20% had known hypercholesterolemia, and another 12.1% had a prior diagnosis of diabetes. Twenty percent of patients had a prior MI. Median baseline hs-cTn concentrations were <7 ng/L for all assays, but 445 (30.9%) of patients had myocardial injury (>99th percentile URL at 0 h or 1 h) by the hs-cTnT assay, 217 (14.4%) by the hs-cTnI-A, and 228 (15.2%) by hs-cTnI-S (Table 2). Of patients, 12% were diagnosed with MI within the 30-day observation period (of whom 96% had type 1 MI, and 4% type 2 MI) and 60% were diagnosed with noncardiac chest pain. The rates of 30-day MI or death was 12.3% and the prevalence of index MI was 11.7% (Table 1).

Figure 1 depicts the rates of a range of hs-cTn concentrations at presentation for all 3 assays and the percentage who were measured under the applicable cutoffs. Here, the maximum bias (+bias$_{max95\%CI}$ and −bias$_{max95\%CI}$) is displayed with the original value to illustrate the shift in rule-out at 0 h, depending on analytical bias. Figure 1 (lower panel) shows the data in patients with MI or death within 30 days, indicating that a similar percentage would be falsely ruled out for

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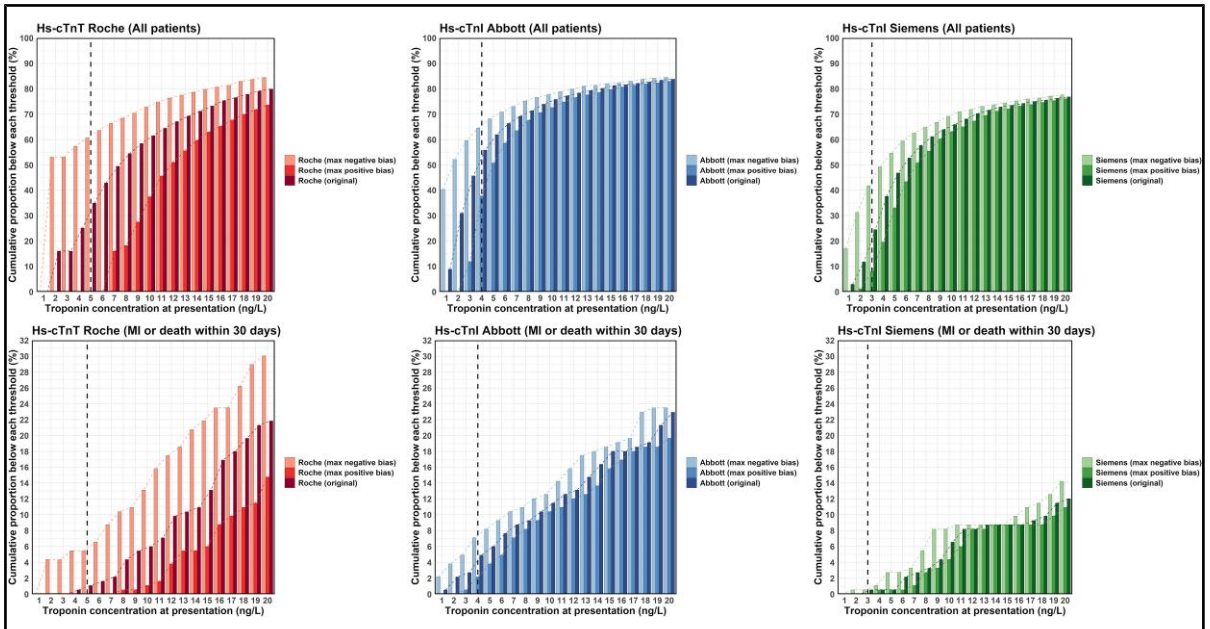

Fig. 1. hs-cTn concentrations at admission in all patients (upper panel) and in patients with the primary endpoint (lower panel) for original, positive (max upper 95%CI) and negative bias (max lower 95%CI) values, for hs-cTnT, hs-cTnI (Abbott Diagnostics), and hs-cTnI (Siemens Healthineers). The 0-h cutoffs for the respective hs-cTn assay are shown as dashed lines.

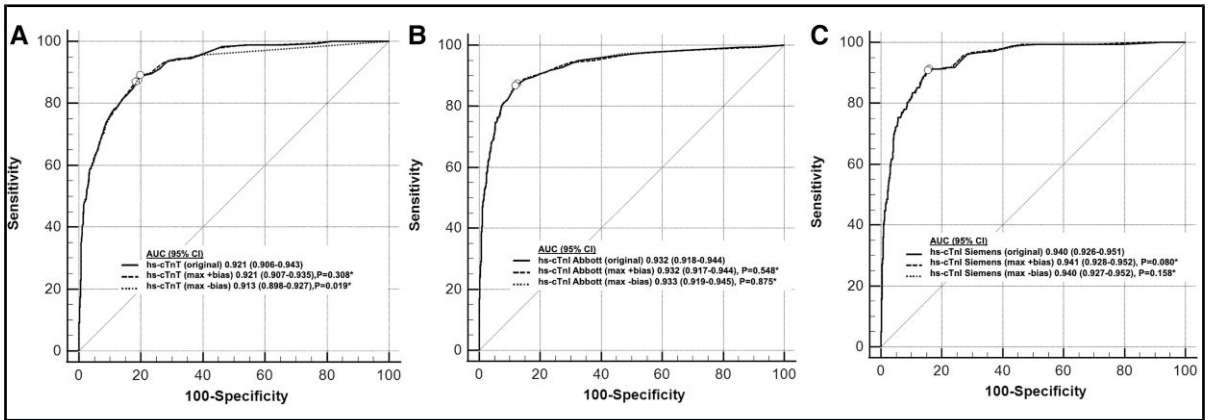

Fig. 2. ROC-AUC plots showing discriminatory performance for the primary endpoint for hs-cTn concentrations for original, positive (max upper 95%CI), and negative bias (max lower 95%CI) values. (A) hs-cTnT, (B) hs-cTnI Abbott Diagnostics, and (C) hs-cTnI Siemens Healthineers. *Comparison by DeLong test with original hs-cTn concentration of the respective assay.

the 0-h cutoff values for hs-cTnT and hs-cTnI-A assays for a maximum negative bias ($-\text{bias}_{\text{max95\%CI}}$), while fewer false negatives were observed for hs-cTnI-S. Figure 2 shows discriminatory prediction of the primary endpoint by ROC-AUCs. hs-cTnT with negative maximum bias had significantly lower AUCs than hs-cTnT neutral (Fig. 2A) whereas no differences were seen for the hs-cTnI assays.

Table 3 summarizes the diagnostic performances of 0/1-h algorithms across all hs-cTn assays for the discrimination of the primary endpoint (30-day MI or death). After the 0-h measurement, the original values (raw

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Table 3. Summary of the performance of 0/1-h rule-out algorithms for identifying primary endpoint of 30-day MI and death in patients hospitalized for acute chest pain after adding the bias[a] (mean, upper and lower 95% CI) of hs-cTn assays. Early presenters were excluded for the calculations of 0-h cutoff performance. Δ values define change between 0-h and 1-h hs-cTn concentrations.

Algorithm	Total error applied	Sensitivity, % (95% CI)	Specificity, % (95% CI)	NPV, % (95% CI)	PPV, % (95% CI)	Rule-out, %
hs-cTnT (Roche Diagnostics)						
hs-cTnT$_0$ <5 ng/L	**Original data**	**99.2 (95.9–100.0)**	**33.1 (30.2–36.0)**	**99.7 (98.4–100.0)**	**15.8 (13.4–18.5)**	**29.4**
Positive bias[a]	+bias$_{min95\%CI}$ (+2.8 ng/L)	100.0 (97.3–100.0)	17.3 (15.1–19.8)	100.0 (98.0–100.0)	13.3 (11.3–15.6)	15.4
	+bias$_{mean}$ (+3.8 ng/L)	100.0 (98.0–100.0)	NC[b]	NC[b]	11.3 (9.5–13.2)	NC[b]
	+bias$_{max95\%CI}$ (+4.7 ng/L)	100.0 (97.3–100.0)	NC[b]	NC[b]	11.3 (9.5–13.2)	NC[b]
Negative bias[a]	−bias$_{min95\%CI}$ (−2.8 ng/L)	97.0 (92.5–99.2)	57.2 (54.1–60.2)	99.3 (98.3–99.8)	22.3 (19.0–25.9)	51.1
	−bias$_{mean}$ (−3.8 ng/L)	95.5 (90.4–98.3)	62.2 (59.2–65.5)	99.1 (98.0–99.7)	24.3 (20.7–28.2)	55.8
	−bias$_{max95\%CI}$ (−4.7 ng/L)	95.5 (90.2–98.3)	67.0 (64.1–69.9)	99.2 (98.2–99.7)	26.8 (22.9–31.1)	60.0
hs-cTnT$_0$ <12 ng/L, Δ < 3 ng/L	**Original data**	**100.0 (96.7–100.0)**	**75.8 (72.8–78.6)**	**100.0 (99.4–100.0)**	**34.3 (29.1–39.7)**	**67.3**
Positive bias[a]	+bias$_{min95\%CI}$ (+3.3 ng/L)	100.0 (96.7–100.0)	66.4 (61.1–67.6)	100.0 (99.3–100.0)	26.2 (22.0–30.7)	57.2
	+bias$_{mean}$ (+3.6 ng/L)	100.0 (96.7–100.0)	63.1 (59.8–66.4)	100.0 (99.3–100.0)	25.5 (21.5–29.9)	56.1
	+bias$_{max95\%CI}$ (+3.7 ng/L)	100.0 (96.7–100.0)	62.7 (59.4–65.9)	100.0 (99.3–100.0)	25.3 (21.3–29.6)	55.7
Negative bias[a]	−bias$_{min95\%CI}$ (−3.3 ng/L)	97.3 (92.2–99.4)	83.6 (81.0–86.0)	99.6 (98.8–99.8)	42.8 (36.6–49.2)	74.5
	−bias$_{mean}$ (−3.6 ng/L)	96.4 (91.0–99.0)	84.0 (81.4–86.4)	99.5 (98.6–99.9)	43.3 (37.0–49.7)	75.0
	−bias$_{max95\%CI}$ (−3.7 ng/L)	95.5 (89.7–98.5)	84.8 (81.4–86.4)	99.3 (98.4–99.8)	43.0 (36.7–49.5)	75.1
hs-cTnI (Abbott Diagnostics)						
hs-cTnI$_0$ <4 ng/L	**Original data**	**97.7 (93.5–99.5)**	**58.4 (55.3–61.4)**	**99.5 (98.6–99.9)**	**23.0 (19.6–26.7)**	**52.1**
Positive bias[a]	+bias$_{min95\%CI}$ (+1.3 ng/L)	98.5 (94.7–99.8)	38.3 (35.3–41.3)	99.5 (98.2–99.9)	16.8 (14.3–19.7)	34.1
	+bias$_{mean}$ (+1.5 ng/L)	98.5 (94.7–99.9)	35.0 (32.1–38.0)	99.5 (98.1–99.9)	16.1 (13.7–18.8)	31.2
	+bias$_{max95\%CI}$ (+1.6 ng/L)	98.5 (94.7–99.8)	31.6 (28.8–34.5)	99.4 (97.8–99.9)	15.4 (13.1–18.1)	28.2
Negative bias[a]	−bias$_{min95\%CI}$ (−1.3 ng/L)	99.4 (98.6–99.8)	68.2 (65.3–71.0)	99.4 (98.6–99.8)	27.9 (23.9–32.3)	60.9
	−bias$_{mean}$ (−1.5 ng/L)	96.2 (91.4–98.8)	69.7 (66.8–72.4)	99.3 (98.4–99.8)	28.7 (24.5–33.1)	62.2
	−bias$_{max95\%CI}$ (−1.6 ng/L)	96.2 (91.4–98.8)	70.1 (67.3–72.9)	99.3 (98.4–99.8)	29.0 (24.8–33.5)	62.7

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Table 3. (continued)

Algorithm	Total error applied	Sensitivity, % (95% CI)	Specificity, % (95% CI)	NPV, % (95% CI)	PPV, % (95% CI)	Rule-out, %
hs-cTnI <5 ng/L, Δ < 2 ng/L	**Original data**	**100.0 (96.6–100.0)**	**68.3 (65.1–71.4)**	**100.0 (99.4–100.0)**	**28.2 (23.7–33.0)**	**60.7**
Positive bias[a]	$+bias_{min95\%CI}$ (+1.3 ng/L)	100.0 (96.6–100.0)	57.0 (53.6–60.3)	100.0 (99.3–100.0)	22.5 (18.8–26.4)	50.7
	$+bias_{mean}$ (+1.5 ng/L)	100.0 (96.6–100.0)	54.3 (50.9–57.7)	100.0 (99.2–100.0)	21.4 (17.9–25.3)	48.3
	$+bias_{max95\%CI}$ (+1.6 ng/L)	100.0 (96.6–100.0)	53.5 (50.1–56.9)	100.0 (99.2–100.0)	21.1 (17.7–24.9)	47.6
Negative bias[a]	$-bias_{min95\%CI}$ (−1.3 ng/L)	100.0 (96.6–100.0)	74.0 (71.0–76.9)	100.0 (99.4–100.0)	32.4 (27.4–37.8)	65.8
	$-bias_{mean}$ (−1.5 ng/L)	100.0 (96.6–100.0)	74.7 (71.7–77.6)	100.0 (99.4–100.0)	33.0 (28.0–38.4)	66.5
	$-bias_{max95\%CI}$ (−1.6 ng/L)	100.0 (96.6–100.0)	74.9 (71.8–77.7)	100.0 (99.4–100.0)	33.1 (28.0–38.5)	66.6
hs-cTnI (Siemens Healthineers)						
$hs\text{-}cTnI_0$ <3 ng/L	**Original data**	**99.2 (95.9–100.0)**	**20.0 (17.6–22.5)**	**99.5 (97.4–100.0)**	**13.6 (11.5–15.9)**	17.8
Positive bias[a]	$+bias_{min95\%CI}$ (+1.1 ng/L)	100.0 (97.3–100.0)	6.5 (5.1–8.1)	100.0 (94.7–100.0)	11.9 (10.1–14.0)	5.7
	$+bias_{mean}$ (+1.3 ng/L)	100.0 (97.3–100.0)	4.8 (3.5–6.2)	100.0 (92.9–100.0)	11.7 (9.9–13.7)	4.2
	$+bias_{max95\%CI}$ (+1.4 ng/L)	100.0 (97.3–100.0)	4.0 (2.9–5.4)	100.0 (91.6–100.0)	11.6 (9.8–13.6)	3.5
Negative bias[a]	$-bias_{min95\%CI}$ (−1.1 ng/L)	99.2 (95.9–100.0)	36.4 (33.5–39.4)	99.7 (98.6–100.0)	16.5 (14.0–19.2)	32.4
	$-bias_{mean}$ (−1.3 ng/L)	99.2 (95.9–100.0)	38.9 (35.9–41.9)	99.8 (98.6–100.0)	17.0 (14.5–19.9)	34.6
	$-bias_{max95\%CI}$ (−1.4 ng/L)	99.2 (95.9–100.0)	39.9 (36.9–43.0)	99.8 (98.7–100.0)	17.3 (14.7–20.1)	35.5
$hs\text{-}cTnI_0$ <6 ng/L, Δ < 3 ng/L	**Original data**	**100.0 (96.7–100.0)**	**59.0 (55.2–62.3)**	**100.0 (99.3–100.0)**	**23.4 (19.7–27.6)**	**52.5**
Positive bias[a]	$+bias_{min95\%CI}$ (+1.1 ng/L)	100.0 (96.7–100.0)	49.4 (46.0–52.7)	100.0 (99.1–100.0)	19.9 (16.6–23.4)	43.9
	$+bias_{mean}$ (+1.3 ng/L)	100.0 (96.7–100.0)	46.8 (43.5–50.2)	100.0 (99.1–100.0)	19.1 (15.9–22.6)	41.6
	$+bias_{max95\%CI}$ (+1.4 ng/L)	100.0 (96.7–100.0)	45.2 (41.9–48.6)	100.0 (99.1–100.0)	18.6 (15.6–22.0)	40.2
Negative bias[a]	$-bias_{min95\%CI}$ (−1.1 ng/L)	100.0 (96.7–100.0)	65.5 (62.2–68.6)	100.0 (99.4–100.0)	26.7 (22.4–31.2)	58.2
	$-bias_{mean}$ (−1.3 ng/L)	100.0 (96.7–100.0)	66.5(63.3–69.6)	100.0 (99.4–100.0)	27.3 (22.9–31.9)	59.1
	$-bias_{max95\%CI}$ (−1.4 ng/L)	100.0 (96.7–100.0)	67.1 (63.9–70.2)	100.0 (99.4–100.0)	27.6 (23.2–32.3)	59.6

Performance as calculated for the original (raw) data is highlighted in bold.
[a]The bias equals the total error assigned based on data from Kavsak et al. (CODE-MI study) (21).
[b]Not calculable (NC) as the cutoff after adding a bias of 3.8 ng/L to original value of 1.5 ng/L (LoB/2) is too high to result in any false or true negatives, nor any role-out proportion.

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data) of hs-cTnT resulted in a sensitivity of 99.2% (95.9%–100.0%), a specificity of 33.1% (30.2%–36.0%), NPV of 99.7% (98.4%–100.0%), PPV of 15.8% (13.4%–18.5%), and 29.4% were ruled out. Adding the bias with 95%CI altered the clinical performance. In particular, the negative bias (reducing all baseline data with 3.8 ng/L for $-bias_{mean}$ and 4.7 ng/L for $-bias_{max95\%CI}$) resulted in a nonsignificant lower point estimate for sensitivity of 95.5% (90.2%–97.3%) and higher specificity 67.0% (64.1%–69.9%). Similar observations were also noted for the secondary endpoint of index MI (Table 4). The sensitivity of the higher cutoff used in the 0-/1-h algorithm was less influenced. The NPV was less influenced by bias at 0 h and 0-/1-h. For the 0-h rule-out, the rule-out rate was clearly affected by bias as the proportion ruled out varied from 0 ($+bias_{max95\%CI}$, increasing all results by 4.7 ng/L would increase all concentrations above the rule-out cutoff of 5 ng/L) to 60.0% ($-bias_{max95\%CI}$). Again, adding the bias was less likely to affect the rule-out proportion of the higher cutoff used in the 0-/1-h algorithms, with the proportions varying from 55.7% to 75.1%.

For the hs-cTnI-A assay, the original values at 0-h provided a sensitivity of 97.7% (93.5%–99.5%), specificity of 58.4% (55.3%–61.4%), NPV of 99.5% (98.6%–99.9%), PPV of 23.0% (19.6%–26.7%), and the proportion ruled out was 52.1%. Adjusting a bias of mean −1.5 ng/L lowered the sensitivity nonsignificantly to 96.2% (91.4%–98.8%). Similar results were seen for the secondary endpoint of index MI (Table 4). After serial measurements at 0-/1-h, performance was less influenced by adding bias. The sensitivity did not change when adding different levels of bias [100.0% (96.6%–100.0%) for all], and the NPV was stable. For the hs-cTnI-A, the estimated proportion of patients ruled out varied from 28.2% to 62.7% (0-h) and 47.6%–66.6% (0-/1-h), the lowest rule-out rates were seen for the bias that leads to increased baseline values ($+bias_{mean}$ and $+bias_{max95\%CI}$), while the highest rule-out rates were seen when the baseline data were reduced, for the $-bias_{mean}$ and $-bias_{max95\%CI}$.

The original values measured by hs-cTnI-S assay had a sensitivity of 99.2% (95.9%–100.0%), a specificity of 20.0% (17.6%–22.5%), a NPV of 99.5% (97.4%–100.0%), a PPV of 13.6% (11.5%–15.9%), and the proportion of rule-out was 17.8%. The sensitivity and NPV were largely unaffected by adding the bias, although increasing the baseline concentrations by +1.4 ng/L ($+bias_{max95\%CI}$) lowered the proportion ruled out at 0 h to 3.5%. Reducing the baseline concentrations by −1.4 ng/L ($-bias_{max95\%CI}$) increased the proportion ruled out to 35.5%. Corresponding proportions after serial measurements at 0-/1-h ranged from 40.2% to 59.6%; again, the lowest rule-out rates were seen for the TE that increased baseline concentrations ($+bias_{mean}$ and $+bias_{max95\%CI}$), while the highest rule-out rates were seen when the baseline concentrations were reduced ($-bias_{mean}$ and $-bias_{max95\%CI}$).

For all assays similar results were seen for the secondary endpoints (Table 4).

Discussion

In this study, we estimate the effect of maximum potential analytical bias on hs-cTn measurements on clinical performance of 0-/1-h algorithms used in ADPs, for a diagnosis of 30-day MI and death, and index MI. Assay bias has a larger effect on clinical performance than changes in assay precision when absolute cutoffs are used (4, 15–17). Furthermore, the effect will be dependent on the proportion of patients who present with concentrations close to the cutoff. Since most patients investigated for possible ACS have hs-cTn concentrations close to the LoD of the assays (3–9, 11), rule-out metrics, and the proportion of patients ruled out using a single sample are most likely to be affected. Our findings are consistent and show that bias has a substantial influence on the proportions of patients ruled out but a smaller influence on sensitivity and no appreciable influence on the NPV, and aligns with the consistently high-sensitivity and NPV values reported from clinical studies (3–9).

CLINICAL IMPLICATIONS OF OUR FINDINGS

Even though sensitivity varied modestly due to analytical bias changes, they were nonsignificant and are unlikely to have a major influence on the safety of patients presenting to the ED with suspected ACS, reflected by the overall low number of false negatives observed (Fig. 1, lower panel). This is due to a very low prevalence of NSTEMI among those presenting with troponin concentrations close to the LoD (3–9). Li et al. recently demonstrated that the likelihood of missing an MI due to assay imprecision is just 1 in 10 000 patients if the CV_A is 10% at the cutoff used for the single-sample rule-out of 5 ng/L for hs-cTnI (17). This is further reflected in our findings as the NPV was unaffected even though the maximum bias resulted in substantial fluctuations in troponin concentrations. hs-cTnT and hs-cTnI-A showed larger variations in sensitivity, with point estimates dropping to approximately 95% at a maximum negative bias, while the hs-cTnI-S demonstrated sensitivity above 99% regardless of analytical error. This could be explained by the very low single-sample cutoff at 3 ng/L applied for the Siemens assay that compels a large "safety-margin," meaning that all NSTEMI patients will present with concentrations higher than 3 ng/L + the upper bound of the 95% CI for the positive TE. It has been advocated that the cutoff of 3 ng/L for

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Table 4. Summary of the performance of 0/1-h rule-out algorithms for identifying the secondary endpoint of index MI in patients hospitalized for acute chest pain after adding the bias[a] (mean, upper, and lower 95% CI) of hs-cTn assays. Early presenters were excluded for the calculations of 0-h cutoff performance. Δ values define change between 0-h and 1-h hs-cTn concentrations. Cutoffs for concentrations in the algorithms were set with one decimal when rounding.

Algorithm	Total error applied	Sensitivity, % (95% CI)	Specificity, % (95% CI)	NPV, % (95% CI)	PPV, % (95% CI)	Rule-out, %
hs-cTnT (Roche Diagnostics)						
hs-cTnT$_0$ <5 ng/L	**Original data**	**99.2 (95.6–100.0)**	**32.8 (30.0–35.8)**	**99.7 (98.4–100.0)**	**14.9 (12.5–17.5)**	**29.4**
Positive bias[a]	+ bias$_{min95\%CI}$ (+2.8 ng/L)	100.0 (97.1–100.0)	17.2 (15.0–19.6)	100.0 (98.0–100.0)	12.5 (10.5–14.7)	15.4
	+bias$_{mean}$ (+3.8 ng/L)	100.0 (97.1–100.0)	0.0 (0.0–0.3)	NC[b]	10.6 (8.9–12.5)	NC[b]
	+bias$_{max95\%CI}$ (+4.7 ng/L)	100.0 (97.9–100.0)	0.0 (0.0–0.3)	NC[b]	10.6 (8.9–12.5)	NC[b]
Negative bias[a]	−bias$_{min95\%CI}$ (−2.8 ng/L)	97.6 (93.1–99.5)	56.9 (53.8–59.9)	99.5 (98.6–99.9)	21.1 (17.8–24.7)	51.1
	−bias$_{mean}$ (−3.8 ng/L)	96.0 (90.9–98.7)	61.9 (58.9–64.8)	99.2 (98.2–99.8)	22.9 (19.4–26.8)	55.8
	−bias$_{max95\%CI}$ (−4.7 ng/L)	96.0 (90.9–98.7)	66.6 (63.7–69.4)	99.3 (98.4–99.8)	25.4 (21.5–29.5)	60.0
hs-cTnT$_0$ <12 ng/L, Δ < 3 ng/L	**Original data**	**100.0 (96.6–100.0)**	**75.4 (72.4–78.2)**	**100.0 (99.4–100.0)**	**33.0 (27.9–38.5)**	**67.3**
Positive bias[a]	+bias$_{min95\%CI}$ (+3.3 ng/L)	100.0 (96.6–100.0)	64.1 (60.8–67.3)	100.0 (99.3–100.0)	25.2 (21.2–29.7)	57.2
	+bias$_{mean}$ (+3.6 ng/L)	100.0 (96.6–100.0)	62.9 (59.6–66.1)	100.0 (99.3–100.0)	24.6 (20.6–28.9)	56.1
	+bias$_{max95\%CI}$ (+3.7 ng/L)	100.0 (99.6–100.0)	62.4 (59.1–65.6)	100.0 (99.3–100.0)	24.4 (20.4–28.7)	55.7
Negative bias[a]	−bias$_{min95\%CI}$ (−3.3 ng/L)	98.1 (93.4–99.8)	83.3 (80.7–85.7)	99.7 (99.0–100.0)	41.6 (35.4–48.0)	74.5
	−bias$_{mean}$ (−3.6 ng/L)	97.2 (92.0–99.4)	83.8 (81.2–86.2)	99.6 (98.8–99.9)	42.0 (35.8–48.5)	75.0
	−bias$_{max95\%CI}$ (−3.7 ng/L)	96.2 (90.6–99.0)	83.8 (81.2–86.2)	99.5 (98.6–99.9)	41.8 (35.5–48.3)	75.1
hs-cTnI (Abbott Diagnostics)						
hs-cTnI$_0$ <4 ng/L	**Original data**	**97.6 (93.1–99.5)**	**58.0 (54.9–61.0)**	**99.5 (98.6–99.9)**	**21.6 (18.2–25.2)**	**52.1**
Positive bias[a]	+bias$_{min95\%CI}$ (+1.3 ng/L)	98.4 (94.3–99.8)	38.0 (35.0–41.0)	99.5 (98.2–99.9)	15.8 (13.3–18.6)	34.1
	+bias$_{mean}$ (+1.5 ng/L)	98.4 (94.3–99.8)	34.8 (31.9–37.7)	99.5 (98.1–99.9)	15.1 (12.8–17.8)	31.2
	+bias$_{max95\%CI}$ (+1.6 ng/L)	98.4 (94.3–99.8)	31.3 (28.6–34.2)	99.4 (97.8–99.9)	14.5 (12.2–17.1)	28.2
Negative bias[a]	−bias$_{min95\%CI}$ (−1.3 ng/L)	97.6 (93.1–99.5)	67.8 (64.9–70.6)	99.6 (98.8–99.9)	26.4 (22.4–30.7)	60.9
	−bias$_{mean}$ (−1.5 ng/L)	96.8 (92.0–99.1)	69.2 (66.3–72.0)	99.5 (98.6–99.9)	27.1 (23.1–31.5)	62.2
	−bias$_{max95\%CI}$ (−1.6 ng/L)	96.8 (92.0–99.1)	69.7 (66.8–72.5)	99.5 (98.6–99.9)	27.4 (23.3–31.9)	62.7

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Table 4. (continued)

Algorithm	Total error applied	Sensitivity, % (95% CI)	Specificity, % (95% CI)	NPV, % (95% CI)	PPV, % (95% CI)	Rule-out, %
hs-cTnI$_0$ <5 ng/L, Δ < 2 ng/L	**Original data**	**100.0 (96.5–100.0)**	**68.0 (64.8–71.1)**	**100.0 (99.4–100.0)**	**27.2 (22.8–31.9)**	**60.7**
Positive bias[a]	+bias$_{min95\%CI}$ (+1.3 ng/L)	100.0 (96.5–100.0)	56.7 (53.4–60.0)	100.0 (99.3–100.0)	21.6 (18.0–25.6)	50.7
	+bias$_{mean}$ (+1.5 ng/L)	100.0 (96.5–100.0)	54.1 (50.7–57.4)	100.0 (96.5–100.0)	20.6 (17.2–24.4)	48.3
	+bias$_{max95\%CI}$ (+1.6 ng/L)	100.0 (96.5–100.0)	53.3 (49.9–56.6)	100.0 (99.2–100.0)	20.4 (16.9–24.1)	47.6
Negative bias[a]	−bias$_{min95\%CI}$ (−1.3 ng/L)	100.0 (96.5–100.0)	73.7 (70.7–76.6)	100.0 (99.4–100.0)	31.2 (26.3–36.5)	65.8
	−bias$_{mean}$ (−1.5 ng/L)	100.0 (96.5–100.0)	74.4 (71.4–77.3)	100.0 (99.4–100.0)	31.8 (26.8–37.2)	66.5
	−bias$_{max95\%CI}$ (−1.6 ng/L)	100.0 (96.5–100.0)	74.5 (71.5–77.4)	100.0 (99.4–100.0)	31.9 (26.9–37.3)	66.6
hs-cTnI (Siemens Healthineers)						
hs-cTnI$_0$ <3 ng/L	**Original data**	**99.2 (95.6–100.0)**	**19.8(17.5–22.3)**	**99.5 (97.4–100.0)**	**12.7 (10.7–15.0)**	**17.8**
Positive bias[a]	+bias$_{min95\%CI}$ (+1.1 ng/L)	100.0 (97.1–100.0)	6.4 (5.0–8.1)	100.0 (94.7–100.0)	11.2 (9.4–13.2)	5.7
	+bias$_{mean}$ (+1.3 ng/L)	100.0 (97.1–100.0)	4.7 (3.5–6.2)	100.0 (92.9–100.0)	11.0 (9.3–13.0)	4.2
	+bias$_{max95\%CI}$ (+1.4 ng/L)	100.0 (97.1–100.0)	4.0 (2.9–5.3)	100.0 (91.6–100.0)	10.9 (9.2–12.9)	3.5
Negative bias[a]	−bias$_{min95\%CI}$ (−1.1 ng/L)	99.2 (95.6–100.0)	36.1 (33.2–39.1)	99.7 (98.6–100.0)	15.5 (13.0–18.2)	32.4
	−bias$_{mean}$ (−1.3 ng/L)	99.2 (95.6–100.0)	38.6 (35.6–41.6)	99.8 (98.6–100.0)	16.0 (13.5–18.8)	34.6
	−bias$_{max95\%CI}$ (−1.4 ng/L)	99.2 (95.6–100.0)	39.6 (36.7–42.6)	99.8 (98.7–100.0)	16.2 (13.7–19.0)	35.5
hs-cTnI$_0$ <6 ng/L, Δ < 3 ng/L	**Original data**	**100.0 (96.5–100.0)**	**58.8 (55.4–62.1)**	**100.0 (99.3–100.0)**	**22.6 (18.9–26.7)**	**52.5**
Positive bias[a]	+bias$_{min95\%CI}$ (+1.1 ng/L)	100.0 (96.5–100.0)	49.1 (45.8–52.5)	100.0 (99.1–100.0)	19.1 (15.9–22.7)	43.9
	+bias$_{mean}$ (+1.3 ng/L)	100.0 (96.5–100.0)	46.6 (43.3–50.0)	100.0 (99.1–100.0)	18.4 (15.3–21.8)	41.6
	+bias$_{max95\%CI}$ (+1.4 ng/L)	100.0 (96.5–100.0)	45.0 (41.7–48.4)	100.0 (99.1–100.0)	17.9 (14.9–21.3)	40.2
Negative bias[a]	−bias$_{min95\%CI}$ (−1.1 ng/L)	100.0 (96.5–100.0)	65.2 (61.9–68.3)	100.0 (99.4–100.0)	25.7 (21.5–30.2)	58.2
	−bias$_{mean}$ (−1.3 ng/L)	100.0 (96.5–100.0)	66.2 (63.0–69.3)	100.0 (94.4–100.0)	26.2 (22.0–30.9)	59.1
	−bias$_{max95\%CI}$ (−1.4 ng/L)	100.0 (96.5–100.0)	66.8 (63.5–69.9)	100.0 (99.4–100.0)	26.6 (22.3–31.2)	59.6

Performance as calculated for the original (raw) data is highlighted in bold.
[a]The bias equals the total error assigned based on data from Kavsak et al. (CODE-MI study) (21).
[b]Not calculable (NC) as the cutoff after adding a bias of 3.8 ng/L to original value of 1.5 ng/L (LoB/2) is too high to result in any false or true negatives, nor any rule-out proportions.

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the hs-cTnI assay from Siemens might be too low, as a sensitivity of 99% has been demonstrated even at cutoffs of 5 ng/L (24–27). The cost of using a low rule-out concentration is a corresponding reduction in rule-out rate. It is unknown how clinicians react to this, and if this leads to more emphasis on clinical gestalt and less trust in the guideline-suggested cutoffs (28). However, patients are more likely to be risk adverse and may prefer to undergo further testing rather than risk an event after discharge (29). This should be a topic of future studies focusing on the implementation of ADPs.

LABORATORY AND MANUFACTURER IMPLICATIONS OF OUR STUDY

Our data highlight the importance of stringent long-term internal quality assessment in hospitals that use troponin-based ADPs. Laboratories and EQA schemes should assess the TE at the applicable cutoffs using commutable QC material (e.g., serum or plasma pools) (30). Laboratories may also measure their internal bias by long-term monitoring the fluctuation in ED patients measured with troponin who are allocated to the low-risk category (15). Publishing such data should be encouraged, providing an estimate of the expected clinical performance of the assay over time (21). Manufacturers producing hs-cTn assays intended for use in ADPs should monitor assay shift internally and openly communicate the expected bias when in routine use.

SCIENTIFIC IMPLICATIONS OF OUR FINDINGS

Our data demonstrate the importance of taking the total analytical variation into consideration when troponin algorithms for ED ADPs are developed. Even though the cutoffs are based on large robust clinical studies, later performance of the ADPs may periodically shift when in clinical use, depending on the bias of the assay. For example, studies suggesting troponin cutoffs may estimate the effect of bias of the assay at the applicable concentration by anticipating bias from ±1 to 5 ng/L, indicating a range of performance (17). This is particularly important for studies using one reagent and calibrator lot (e.g., biobank studies) (19). It may also be useful to consider the analytical bias for studies developing and validating machine learning or artificial intelligence tools. Finally, scientific studies measuring the total TE of key laboratory assays using commutable material should be encouraged (16, 20, 21) to provide independent data on the overall performance of assays when in routine use.

STRENGTHS AND LIMITATIONS

One strength of this study is the inclusion of a large clinical dataset measured with 3 different troponin assays. Our study did not evaluate cutoffs used in the 0-/2-h ADPs but our principal findings (sensitivity is less affected compared to rule-out rate) should be valid for all ADPs that use absolute cTn concentrations to allocate patients to a low-risk category. The TE used as basis for the bias simulations is based on a study calculating TE based on long-term data from many routine laboratories (21). It should be noted as a limitation, however, that real-life observations of analytical error are influenced by number of laboratories reporting data, especially if the between-laboratory analytical variation is a large part of the TE. The larger TE reported for hs-cTnT by Kavsak et al. (21). might be attributed to the higher participation rate of sites with Roche platforms. If more observations had been available for other assays, the estimates for TE might have been different. Another limitation is that the estimates present maximum effects, as all concentrations are skewed in accordance with mean and maximum bias (95% CI) estimates, implying that a systematic deviation (bias) corresponding to the TE occurred in all samples. This is an inherited limitation as TE consist of both bias and analytical impression. Compared to real life the analytical error in our simulation is overestimated, as TE will never consist of bias alone and imprecision and bias may also show opposite effects. This assumption might explain the higher false negative rate estimated in our dataset compared to a recent simulation in the High-STEACS study for which it was assumed that the TE was attributed to analytical impression alone (17). The main limitation in the simulation from Li et al. (17) was that it did not account for how between-laboratory bias and differences in institution size affected patient classifications. A large bias at a large hospital will affect more patients compared to the same bias at a smaller hospital. We therefore chose to simulate that the TE consisted exclusively of bias to estimate a worst-case-scenario, addressing the questions related to safety sometimes raised for the ADPs. We acknowledge that the current estimates are based on simulated data and should be interpreted with care as it assumed to represent worst-case scenarios. Per contra, it is reassuring that a very high sensitivity and NPV for the outcome prevailed, even under a worst-case approach.

A further limitation is that we assigned the study concentration as the original value even though some of the measurements were obtained using biobank samples measured with one reagent lot (hs-cTnI-S assay). Therefore, the original concentration might not represent the true level of that assay. Even so, the true value should be within the limits of the reported TE, indicating that large skewness of data is unlikely. The hs-cTnT measurements were undertaken as routine measurements and within-laboratory bias was therefore embedded in the original data. Earlier studies (15) have shown the within-laboratory bias for hs-cTnT is

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approximately ±1–2 ng/L. The bias effect seen for the $\pm bias_{max95\%CI}$ in our simulation should therefore be interpreted with care, as it might be too large compared to a real-life setting. The findings should be viewed as examples of how TE affects clinical performance of hs-cTn assays used in ADPs. A final limitation is that all patients were clinically adjudicated based on the hs-cTnT assay and we cannot rule out that our data would have been slightly different if adjudication had been based on one of the hs-cTnI assays. However, since 96% of the index NSTEMIs were type 1 MI we do not think this has caused a major effect since the concordance between troponin assays is better for diagnosing type 1 MI compared to type 2 MI (31).

Conclusions

Long-term analytical bias of hs-cTn assays influences the clinical performance of troponin-based ADPs, particularly the rule-out rate, and should be acknowledged by the scientific, commercial, routine laboratory, and clinical milieus working with these assays. Further analytical improvements in hs-cTn assays should aim to reduce assay bias at the clinical thresholds used for ruling out MI.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: ADPs, accelerated diagnostic protocols; hs-cTn, high-sensitivity cardiac troponin; hs-cTnT, hs-cTn from Roche Diagnostics; hs-cTnI, hs-cTn from Abbott Diagnostics (hs-cTnI-A); hs-cTnI-S, hs-cTn from Siemens Healthineers; ED, Emergency Department; NSTEMI, non-ST elevation myocardial infarction; MI, myocardial infarction; EQA, External Quality Assessment; TE, total analytical error; ACS, acute coronary syndrome; ESC, European Society of Cardiology; ROC-AUC, receiver operating characteristic area under the curve; NPV, negative predictive value; PPV, positive predictive values.

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and McMaster University has filed patent: "Quality Control Materials For Cardiac Troponin Testing" with P.A. Kavsak as a listed inventor. A.S. Jaffe has consulted for Abbott Diagnostics, Siemens, Roche Diagnostics, Radiometer, Beckman-Coulter, Ortho Diagnostics, ET Healthcare, Sphingotec, SpinChip, LumiraDx, Mindray, Hytest, and Moderna, and has stock options in RCE Technologies. P.O. Collinson has served on advisory boards for Siemens Healthineers and Radiometer; has received honoraria from QuidelOrtho and Psyros; and is a consultant to the IFCC Clinical Applications of Cardiac Biomarkers Committee (unpaid).

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References

1. Jones S, Moulton C, Swift S, Molyneux P, Black S, Mason N, et al. Association between delays to patient admission from the emergency department and all-cause 30-day mortality. Emerg Med J 2022;39:168–73.
2. Sun BC, Hsia RY, Weiss RE, Zingmond D, Liang L-J, Han W, et al. Effect of emergency department crowding on outcomes of admitted patients. Ann Emerg Med 2013;61:605–11.e6.
3. Neumann JT, Twerenbold R, Ojeda F, Sorensen NA, Chapman AR, Shah ASV, et al. Application of high-sensitivity troponin in suspected myocardial infarction. N Engl J Med 2019;380:2529–40.
4. Tjora HL, Steiro O-T, Langorgen J, Bjorneklett RO, Skadberg O, Bonarjee VVS, et al. Diagnostic performance of novel troponin algorithms for the rule-out of non-ST-elevation acute coronary syndrome. Clin Chem 2022;68:291–302.
5. Steiro O-T, Tjora HL, Langorgen J, Bjorneklett R, Nygard OK, Skadberg O, et al. Clinical risk scores identify more patients at risk for cardiovascular events within 30 days as compared to standard ACS risk criteria: the WESTCOR study. Eur Heart J Acute Cardiovasc Care 2021;10:287–301.
6. Sandoval Y, Lewis BR, Mehta RA, Ola O, Knott JD, De Michieli L, et al. Rapid exclusion of acute myocardial injury and infarction with a single high-sensitivity cardiac troponin T in the emergency department: a multicenter United States evaluation. Circulation 2022;145:1708–19.
7. Shah ASV, Anand A, Sandoval Y, Lee KK, Smith SW, Adamson PD, et al. High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study. Lancet 2015;386:2481–8.
8. Twerenbold R, Neumann JT, Sorensen NA, Ojeda F, Karakas M, Boeddinghaus J, et al. Prospective validation of the 0/1-h algorithm for early diagnosis of myocardial infarction. J Am Coll Cardiol 2018;72:620–32.
9. Reichlin T, Schindler C, Drexler B, Twerenbold R, Reiter M, Zellweger C, et al. One-hour rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. Arch Intern Med 2012;172:1211–8.
10. Gulati M, Levy PD, Mukherjee D, Amsterdam E, Bhatt DL, Birtcher KK, et al. 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR guideline for the evaluation and diagnosis of chest pain: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation 2021;144:e368–454.
11. Byrne RA, Rossello X, Coughlan JJ, Barbato E, Berry C, Chieffo A, et al. 2023 ESC guidelines for the management of acute coronary syndromes. Eur Heart J 2023;44:3720–826.
12. National Institute for Health and Care Excellence. High-sensitivity tests for the early rule-out of NSTEMI; 2020. https://www.nice.org.uk/guidance/dg40 (Accessed September 2025).
13. Kavsak PA, Clark L, Jaffe AS. Effect of repeat measurements of high-sensitivity cardiac troponin on the same sample using the European Society of Cardiology 0-hour/1-hour or 2-hour algorithms for early rule-out and rule-in for myocardial infarction. Clin Chem 2017;63:1163–5.
14. Chiang C-H, Chiang C-H, Pickering JW, Stoyanov KM, Chew DP, Neumann JT, et al. Performance of the European Society of Cardiology 0/1-hour, 0/2-hour, and 0/3-hour algorithms for rapid triage of acute myocardial infarction: an international collaborative meta-analysis. Ann Intern Med 2022;175:101–13.
15. Haagensen K, Collinson P, Åsberg A, Aakre KM. How does the analytical quality of the high-sensitivity cardiac troponin T assay affect the ESC rule out algorithm for NSTEMI? Clin Chem 2019;65:494–6.
16. Kavsak PA, Mills NL, Clark L, Ko DT, Sharif S, Chen-Tournoux A, et al. Assay precision and risk of misclassification at rule-out cutoffs for high-sensitivity cardiac troponin. Can J Cardiol 2024;40:2102–5.
17. Li Z, Tew YY, Kavsak PA, Aakre KM, Jaffe AS, Apple FS, et al. Impact of high-sensitivity cardiac troponin I assay imprecision on the safety of a single-sample rule-out approach for myocardial infarction. Clin Chem Lab Med 2024;63:e59–62.
18. Hatherley JD, Miller G, Collinson P, Shantsila E, Fearon H, Lambert A, et al. Why is single sample rule out of non-ST elevation myocardial infarction using high-sensitivity cardiac troponin T safe when analytical imprecision is so high? A joint statistical and clinical demonstration. Clin Chem Lab Med 2025;63:e63–6.
19. Aakre KM, Apple FS, Mills NL, Meex SJR, Collinson PO; International Federation of Clinical Chemistry Committee on Clinical Applications of Cardiac Biomarkers (IFCC C-CB). Lower limits for reporting high-sensitivity cardiac troponin assays and impact of analytical performance on patient misclassification. Clin Chem 2024;70:497–505.
20. Kavsak PA, Clark L, Arnoldo S, Lou A, Shea JL, Eintracht S, et al. Imprecision of high-sensitivity cardiac troponin assays at the female 99th-percentile. Clin Biochem 2024;125:110731.
21. Kavsak PA, Clark L, Arnoldo S, Lou A, Shea JL, Eintracht S, et al. Analytic result variation for high-sensitivity cardiac troponin: interpretation and consequences. Can J Cardiol 2023;39:947–51.
22. International Federation of Clinical Chemistry and Laboratory Medicine. Biomarkers Reference Tables; 2025; v072025. https://ifcc.org/ifcc-education-division/emd-committees/committee-on-clinical-applications-of-cardiac-biomarkers-c-cb/biomarkers-reference-tables/ (Accessed July 2025).
23. Tjora HL, Steiro O-T, Langorgen J, Bjorneklett R, Nygard OK, Renstrom R, et al. Aiming toWards Evidence baSed inTerpretation of Cardiac biOmarkers in patients pResenting with chest pain-the WESTCOR study: study design. Scand Cardiovasc J 2019;53:280–5.
24. Chapman AR, Fujisawa T, Lee KK, Andrews JP, Anand A, Sandeman D, et al. Novel high-sensitivity cardiac troponin I assay in patients with suspected acute coronary syndrome. Heart 2019;105:616–22.
25. Sandoval Y, Nowak R, deFilippi CR, Christenson RH, Peacock WF, McCord J, et al. Myocardial infarction risk stratification with a single measurement of high-sensitivity troponin I. J Am Coll Cardiol 2019;74:271–82.
26. Sörensen NA, Goßling A, Neumann JT, Hartikainen TS, Haller PM, Scharlemann L, et al. Diagnostic validation of a high-sensitivity cardiac troponin I assay. Clin Chem 2021;67:1230–9.
27. Restan IZ, Steiro O-T, Pickering JW, TjoraHL, Langørgen J, Omland T, et al.

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Clinical derivation and data simulated validation of rule-out and rule-in algorithms for the Siemens Atellica IM high-sensitivity cardiac troponin I assay. Eur Heart J Acute Cardiovasc Care 2025;14:155–68.
28. McDermott M, Kimenai DM, Anand A, Huang Z, Houston A, Williams S, et al. Adoption of high-sensitivity cardiac troponin for risk stratification of patients with suspected myocardial infarction: a multicentre cohort study. Lancet Reg Health Eur 2024;43:100960.
29. Greenslade JH, Wilkinson S, Parsonage W, Cullen L. What is an acceptable risk of major adverse cardiac event soon after discharge from emergency? The patient's perspective. Emerg Med J 2022;39:519–20.
30. Kavsak PA, Kumaritakis A, Wong-Fung M, Badrick T, Knauer M. Validation of analytical performance limits for accuracy with high-sensitivity cardiac troponin assays. Clin Chem 2024;71:332–4.
31. Saeed N, Steiro O-T, Langørgen J, TjoraHL, Bjørneklett RO, Skadberg Ø, et al. Diagnosing myocardial injury in an acute chest pain cohort; long-term prognostic implications of cardiac troponin T and I. Clin Chem 2024;70:1241–55.

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