Diagnostic Performance of Novel Troponin Algorithms for the Rule-Out of Non-ST-Elevation Acute Coronary Syndrome

Hilde L. Tjora ,[a] Ole-Thomas Steiro,[b] Jørund Langørgen,[b] Rune O. Bjørneklett,[a,c] Øyvind Skadberg,[d] Vernon V.S. Bonarjee,[e] Øistein R. Mjelva,[e] Paul Collinson ,[f] Torbjørn Omland,[g,h] Kjell Vikenes,[b,i] and Kristin M. Aakre [b,i,j,*]

BACKGROUND: The European Society of Cardiology (ESC) rule-out algorithms use cutoffs optimized for exclusion of non-ST elevation myocardial infarction (NSTEMI). We investigated these and several novel algorithms for the rule-out of non-ST elevation acute coronary syndrome (NSTE-ACS) including less urgent coronary ischemia.

METHOD: A total of 1504 unselected patients with suspected NSTE-ACS were included and divided into a derivation cohort ($n = 988$) and validation cohort ($n = 516$). The primary endpoint was the diagnostic performance to rule-out NSTEMI and unstable angina pectoris during index hospitalization. The secondary endpoint was combined MI, all-cause mortality (within 30 days) and urgent (24 h) revascularization. The ESC algorithms for high-sensitivity cardiac troponin T (hs-cTnT) and I (hs-cTnI) were compared to different novel low-baseline (limit of detection), low-delta (based on the assay analytical and biological variation), and 0–1-h and 0–3-h algorithms.

RESULTS: The prevalence of NSTE-ACS was 24.8%, 60.0% had noncardiac chest pain, and 15.2% other diseases. The 0–1/0–3-h algorithms had superior clinical sensitivity for the primary endpoint compared to the ESC algorithm (validation cohort); hs-cTnT: 95% vs 63%, and hs-cTnI: 87% vs 64%, respectively. Regarding the secondary endpoint, the algorithms had similar clinical sensitivity (100% vs 94%–96%) but lower clinical specificity (41%–19%) compared to the ESC algorithms (77%–74%). The rule-out rates decreased by a factor of 2–4.

CONCLUSION: Low concentration/low-delta troponin algorithms improve the clinical sensitivity for a combined endpoint of NSTEMI and unstable angina pectoris, with the cost of a substantial reduction in total rule-out rate. There was no clear benefit compared to ESC for diagnosing high-risk events.

Introduction

Atherosclerotic cardiovascular disease is an important health challenge and a common cause of death worldwide (1). Patients with symptoms suggestive of acute coronary syndrome are frequently referred to the emergency department (ED) and impose a high workload on hospitals (2, 3). Since 2009, high-sensitivity troponin (hs-cTn) assays have become a crucial ED tool for differentiating between patients with and without non-ST-elevation myocardial infarction (NSTEMI) (4, 5).

Accordingly, the European Society of Cardiology (ESC) recommends 0–1-h algorithms that use hs-cTn for rule-out and rule-in of NSTEMI (6). The algorithms for hs-cTnT from Roche Diagnostics and hs-cTnI from Abbott Diagnostics are fairly well validated, shown to be safe, and of high efficiency (7–10).

The ESC algorithms are based on 2 important characteristics found in healthy individuals: (*a*) normal baseline troponin concentrations a few hours after symptom onset, and (*b*) low-delta values after 1 h of observation. A drawback with these algorithms is that they were not developed to identify patients with unstable angina pectoris (UAP) (6). Accordingly, the 2020 ESC guidelines recommend the use of clinical judgment and

[a]Emergency Care Clinic, Haukeland University Hospital, Bergen, Norway; [b]Department of Heart Disease, Haukeland University Hospital, Bergen, Norway; [c]Department of Clinical Medicine, University of Bergen, Bergen, Norway; [d]Laboratory of Medical Biochemistry, Stavanger University Hospital, Stavanger, Norway; [e]Cardiology Department, Stavanger University Hospital, Stavanger, Norway; [f]Cardiovascular Clinical Academic Group St Georges University Hospitals NHS Foundation Trust and St George's University of London, London, UK; [g]Department of Cardiology, Akershus University Hospital, Oslo, Norway; [h]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [i]Department of Clinical Science, University of Bergen, Bergen, Norway; [j]Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway.
*Address correspondence to this author at: Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, 5021 Bergen, Norway.
E-mail kristin.moberg.aakre@helse-bergen.no.
Received April 26, 2021; accepted September 17, 2021.
https://doi.org/10.1093/clinchem/hvab225

imaging for identification of UAP (6), and the diagnostic workflow of this group is debated (11, 12).

The cutoffs in the ESC algorithms are pragmatically selected from research datasets. Earlier studies indicate that lower baseline concentrations than those used by the ESC 0–1-h algorithms may predict short- and long-term risk of major adverse cardiovascular events (MACE) in patients with chest pain (13–16). Furthermore, all consecutive biomarker measurements are subjected to uncertainty, due to biological variation (i.e., biomarkers measured in clinically stable individuals show homeostatic variation around a set point) and analytical variation. The combination of these variances is the reference change value (RCV) (17). The currently used ESC delta values exceed those calculated from RCV's (18). It is possible that patients with UAP, who have nonnecrotic ischemia and are in a clinically unstable situation, show larger variation in hs-cTn concentrations compared to patients with noncardiac chest pain (NCCP), who have a healthy myocardium and therefore should show troponin variation similar to or lower than the RCV (11, 19). Currently, it is unknown whether the use of delta values based on RCV could differentiate between patients with UAP and NCCP.

In this study we tested the hypothesis that the use of algorithms that combine very low baseline concentrations (similar to the limit of detection of the assay) with delta values derived from RCVs might improve the diagnostic performance for NSTE-ACS in the ED and also identify patients with UAP who have less urgent disease, and whether such algorithms could provide an improved segregation between patients with UAP and NCCP.

Methods

STUDY DESIGN

The WESTCOR study (Clinical Trials number NCT02620202) is a two-center cross-sectional prospective observational study that has been described in detail previously (15, 20). The current article reports data from the WESTCOR derivation and internal validation cohorts (as prespecified in the study protocol) including 988 and 516 patients from Haukeland University Hospital. The inclusion period lasted from September 2015 to May 2019. All patients in the validation cohort were offered computed tomographic coronary angiography unless contraindicated. The study and biobank were approved by the Regional Committees for Medical and Health Research Ethics (2014/1365 REK West and 2014/1905 REK West).

STUDY ENROLLMENT AND BIOBANKING

Patients were eligible for inclusion if they had chest pain or symptoms suspicious of NSTE-ACS. Patients with STEMI were excluded. Included patients were ≥18 years, did not have a coexisting clinical condition that would affect life expectancy, and were able to provide informed consent. The inclusion was performed in the ED (20) where the patients had 12 mL of full blood drawn into serum tubes (Greiner Bio-One, Austria) on arrival and after 3 h and 8–12 h as part of routine clinical care. Samples coagulated for 30–60 min and were centrifuged at 2200*g* for 10 min. Serum was used for measurement of hs-cTnT (fresh samples) with results reported to the attending clinician. Additional serum was aliquoted (1 mL) into cryotubes from Sarstedt (Sarstedt, Norway) and stored in a biobank at −80°C. After an implementation period, an additional biobank sample was drawn 1 h after admission without results being reported to the attending clinicians (20).

BIOCHEMICAL ANALYSIS

Details of the biochemical analyses are provided in the Methods in the online Data Supplement. Briefly, samples were measured for hs-cTnT (Roche Diagnostics) in fresh material using 9 different reagents and calibrator lots. Hs-cTnI were measured (biobanked samples) using the Abbott Diagnostics hs-cTnI assay using reagent lot 71164V100 and calibrator lot 65294V100 for the derivation cohort, and reagent lot 11151UI00 and calibrator lot 09906 UI00 for the validation cohort.

ENDPOINTS AND ADJUDICATION

The primary endpoint was a diagnosis of NSTEMI or UAP during index hospitalization. The secondary endpoints were MACE defined as combined myocardial infarction or all-cause mortality during the first 30 days after hospitalization or urgent (within 24 h after admission) revascularization. The adjudicating process (15, 20) was undertaken by 2 independent cardiologists (definitions provided in the Supplemental Methods) based on all available clinical, routine laboratory results (hs-cTnT), electrocardiogram (ECG), ultrasound, and imaging findings. A third adjudicator resolved disagreements. NSTE-ACS was defined as NSTEMI and UAP (21). NSTEMI and UAP was defined according to the third universal definition for MI (22). Delta values of 20% (baseline hs-cTnT concentration >14 ng/L) or 50% (baseline hs-cTnT concentration ≤14 ng/L) in serial hs-cTnT measures were regarded as clinically significant, as suggested by the ESC (23). UAP was defined as myocardial ischemia at rest or on minimal exertion, in the absence of acute myocardial injury/necrosis (21); a baseline concentration of hs-cTn above the 99th percentile of the assay did not exclude the patient from an UAP diagnosis if clinical assessment or imaging findings confirmed myocardial ischemia (11).

DEVELOPMENT OF NOVEL ALGORITHMS

As baseline concentration we chose the limit of detection of the assays (Supplemental Table 1), because these concentrations have been validated as rule-out cutoffs for admission samples (21), and are associated with low long-term risk of MACE (15, 24–26). The delta values were based on approximate RCV values for the hs-cTnT and hs-cTnI assays at low concentrations. Current assays have an analytical variation at low concentrations of approximately ±1 ng/L (27–29). Biological variation studies have shown that the short time biological variation at low concentrations is negligible in clinically stable individuals, as compared to the analytical variation (18, 30). Accordingly, an absolute delta value of ±1 ng/L or larger should be clinically sensitive for identification of minor but clinically significant variations in troponin concentrations, as could be evident in patients with UAP (18, 31).

Furthermore, from a clinical point of view the optimal novel rule-out algorithms should have: (*a*) clinical sensitivity for NSTE-ACS of ≥95.0% and ≥99% for the secondary endpoint (32), and (*b*) the maximum possible specificity. The cutoff for the primary endpoint was chosen a priori as there was no literature reporting cardiologists view on an acceptable rule-out rate for patients with UAP.

COMPARATOR ALGORITHMS

The novel algorithms were compared to the recently updated 0–1-h algorithms for rule-out of NSTEMI from the ESC. Accordingly, patients were eligible for early discharge if the baseline concentration (cTnT < 12 ng/L or cTnI < 5 ng/L) and the 1-h delta value (cTnT < ±3 ng/L and cTnI < ±2 ng/L) was below the prespecified concentration specific for the applicable troponin assay (Supplemental Table 1).

STATISTICAL ANALYSIS

The baseline characteristics are reported as medians with interquartile ranges for continuous data and percentages for categorical data. The data were analyzed using the nonparametric Kruskal–Wallis and Mann–Whitney *U*-test for continuous variables, and the Chi-square and Fisher's exact test for categorical variables, as appropriate. Statistical analyses included calculation of clinical sensitivity, specificity, negative predictive value, and positive predictive value for the cutoffs used in the different algorithms. Differences in sensitivity and specificity between algorithms were compared using McNemar test. Efficiency (defined as percentage of patients ruled out) was calculated for all algorithms. Prognosis regarding MACE (secondary endpoint) were estimated using Kaplan–Meier curves. We performed one subgroup analysis calculating the diagnostic performance of the 2 endpoints in early presenters (defined as ≤3 h since onset of symptoms). A second subgroup analysis compared the baseline and delta values, and calculated the rule-out rate in the two patient groups that are of large clinical interest to separate, i.e., the patients with UAP and NCCP. Investigations during index hospitalization, and 30-day all-cause mortality, myocardial infarction, or revascularization were calculated for all patients with NSTE-ACS and after stratifying as NSTEMI and UAP (index diagnosis), and furthermore, as shown for patients with UAP who were ruled out by the ESC or the novel 0–3-h algorithm, differences were tested using the McNemar test.

We used SPSS Statistics v.24/26 and MedCalc for the statistical analyses.

Results

Biobank admission samples were available from 1504 patients, and a 1-h sample was available from 984 patients ($n = 479$ in the derivation and $n = 505$ in the validation cohort).

Patient characteristics for the derivation and validation cohort are shown in Table 1. The prevalence of NSTE-ACS in the derivation cohort ($n = 988$) was 24.8%, while 60.0% were diagnosed with NCCP, and 15.2% had other diseases. Other diseases included non-cardiac diseases such as pneumonia or cholecystitis, and other cardiac diseases such as atrial fibrillation or heart failure. Median age was 63 years, and 60% were male. The validation group ($n = 516$) had a prevalence of NSTE-ACS of 25.8%, NCCP was diagnosed in 62.9% and 11.4% had other diseases and similar median age and percentage of males. The prevalence of NSTEMI was lower (13.2% vs 8.7%) (Table 1). Less than 7% of NSTEMIs were type 2 NSTEMI.

BASELINE CONCENTRATIONS, AND 1- AND 3-HOUR ABSOLUTE DELTA VALUES

Table 2 shows troponin concentrations at baseline, and the absolute delta values at 1 h and 3 h stratified according to the adjudicated diagnosis. The baseline concentrations were similar across cohorts for hs-cTnT (samples were analyzed continuously using 9 different reagent and calibrator lots), while the hs-cTnI baseline concentrations were significantly lower in the validation compared to the derivation cohort for all diagnoses except NSTEMI (Supplemental Table 2). This was due to samples being analyzed in batches, using one reagent/calibrator lot for each cohort, with the last lot returning lower concentrations.

The patients with UAP had significantly higher ($P < 0.001$) baseline hs-cTnT and hs-cTnI concentrations (Table 2) and delta values compared to the patients with NCCP (Fig. 1 and Supplemental Table 3).

Table 1. Patient characteristics. Values are *N* (%) or median (25th and 75th percentiles).

Derivation cohort					
	Total ***N*** **= 988**	**NSTE-ACS** ***N*** **= 242**	**Other diseases** ***N*** **= 156**	**NCCP** ***N*** **= 590**	***P*** **value**
Age, years	63.0 (52.0–74.0)	69.5 (59.0–78.0)	70.0 (58.0–80.0)	59.0 (49.0–70.0)	<0.001
Male, %	600 (60.7)	172 (71.1)	94 (60.3)	334 (56.6)	0.001
Symptom to arrival time, hours	8.0 (2.9–47.8)	8.2 (2.8–48.8)	8.6 (3.5–53.8)	7.4 (2.9–46.2)	0.539
Hospital stay, hours	29.0 (21.0–69.0)	73.5 (49.8–117.3)	43.5 (24.0–86.5)	24.0 (19.0–35.0)	<0.001
Risk factors					
Hypertension, %	413 (41.8)	124 (51.2)	66 (42.3)	223 (37.8)	0.002
Hypercholesterolemia* %	394 (39.9)	121 (50.0)	63 (40.4)	210 (35.6)	0.001
Diabetes mellitus, %	121 (12.4)	51 (21.1)	16 (10.3)	54 (9.2)	<0.001
Family history, %	195 (19.7)	45 (18.6)	25 (16.0)	125 (21.2)	0.468
Unknown	121 (12.1)	35 (14.1)	17 (10.7)	69 (11.6)	0.507
Ever smoker, %	628 (63.6)	145 (59.9)	102 (65.4)	381 (64.6)	0.392
Medical history					
Prior MI, %	211 (21.4)	77 (31.8)	34 (21.8)	100 (16.9)	<0.001
Prior PCI, %	209 (21.2)	82 (33.9)	27 (17.3)	100 (16.9)	<0.001
Prior CABG, %	83 (8.4)	45 (18.6)	12 (7.7)	26 (4.4)	<0.001
Heart failure, %	47 (4.7)	15 (6.0)	14 (8.8)	18 (3.0)	0.005
Stroke, %	30 (3.0)	9 (3.7)	7 (4.5)	14 (2.4)	0.254
Peripheral vascular disease, %	22 (2.2)	11 (4.5)	2 (1.3)	9 (1.5)	0.027
Vital parameters at admission					
Systolic BP, mmHg	142.5 (129.0–158.0)	147.0 (133.0–160.0)	133.0 (122.3–154.8)	142.0 (129.0–158.0)	<0.001
Diastolic BP, mmHg	81.0 (73.0–91.0)	81.0 (74.0–90.8.0)	80.0 (72.3–91.0)	82.0 (74.5–90.0)	0.326
Heart rate, bpm	72.0 (64.0–83.0)	72.0 (64.0–84.0)	82.0 (66.3–100.0)	70.0 (63.8–80.0.0)	<0.001
BMI, kg/m^2 for 461 patients	26.4 (24.2–29.5)	25.9 (24.2–29.1)	27.2 (25.5–29.1)	26.3 (24.1–29.7)	0.259
Electrocardiography					
ST segment depression, %	34 (3.4)	21 (8.7)	7 (4.5)	6 (1.0)	<0.001
T-wave inversion, %	31 (3.1)	16 (6.6)	5 (3.2)	10 (1.7)	0.002
Validation cohort					
	Total ***N*** **= 516**	**NSTE-ACS** ***N*** **= 133**	**Other diseases** ***N*** **= 58**	**NCCP** ***N*** **= 325**	***P*** **value**
Age, years	60.0 (51.0–70.0)	66.0 (57.0–74)	65.0 (56.0–72.5)	56.0 (47.0–67.0)	<0.001
Male, %	308 (59.7)	91 (68.4)	33 (56.9)	184 (56.4)	0.048
Symptom to arrival time, hours	11.4 (3.5–71.8)	9.9 (3.1–81.5)	15.0 (4.7–77.5)	11.5 (3.8–71.4)	0.588
Hospital stay, hours	27.0 (22.0–69.0)	73.0 (48–143.0)	33.5 (22.0–70.8)	24.0 (21.0–30.0)	<0.001
Risk factors					

Continued

Table 1. (continued)

Derivation cohort					
Hypertension, %	202 (39.1)	70 (52.2)	23 (41.8)	109.0 (34.0)	<0.001
Hypercholesterolemia*, %	191 (37.0)	66 (49.6)	21 (36.2)	104 (32.0)	0.002
Diabetes mellitus, %	60 (11.6)	26 (19.5)	8 (13.8)	26 (8.0)	0.002
Family history, %	80 (15.5)	21 (15.8)	8 (13.8)	51 (15.7)	0.469
Unknown	21 (4.1)	9 (6.3)	2 (3.4)	10 (3.1)	0.469
Ever smoker, %	312 (60.5)	87 (64.9)	31 (54.4)	196 (60.1)	0.368
Medical history					
Prior MI, %	78 (15.1)	30 (22.6)	8 (13.8)	40 (12.3)	0.020
Prior PCI, %	84 (16.3)	37 (27.6)	6 (10.3)	41 (12.6)	<0.001
Prior CABG, %	28 (5.4)	17 (12.7)	4 (6.9)	7 (2.2)	<0.001
Heart failure, %	5 (1.0)	1 (0.8)	0	4 (1.2)	0.649
Stroke, %	12 (2.3)	6 (4.5)	1 (1.7)	5 (1.5)	0.151
Peripheral vascular disease, %	7 (1.4)	5 (3.7)	0	2 (0.6)	0.020
Vital parameters at admission					
Systolic BP, mmHg	147.0 (134.0–161.0)	148.0 (136.0–161.5)	149.0 (128.5–167.3)	147.0 (133.0–161.0)	0.666
Diastolic BP, mmHg	86.0 (78.0–95.0)	85.0 (77.5–96.0)	90.0 (82.0–98.3)	85.0 (78.0–94.0)	0.113
Heart rate, bpm	71.0 (63.0–81.0)	72.0 (63.5–81.0)	74.0 (61.0–87.3)	70.0 (63.0–80.0)	0.361
BMI, kg/m^2 for 281 patients	27.7 (25.0–31.1)	27.7(24.8–30.9)	29.1 (25.2–31.4)	27.5 (25.1–31.2)	0.797
Electrocardiography					
ST segment depression, %	13 (2.5)	8 (6.0)	0	5 (1.5)	0.019
T-wave inversion, %	16 (3.1)	11 (8.3)	3 (5.2)	2 (0.6)	<0.001

*Hypercholesterolemia is defined as treatment with lipid lowering drugs.
NSTE-ACS, non-ST-elevation acute coronary syndrome; NCCP, noncoronary chest pain; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.

DIAGNOSTIC PERFORMANCE OF THE NOVEL AND ESC ALGORITHMS FOR NSTE-ACS AND MACE

Overall, the low concentration/low-delta value algorithms showed superior clinical sensitivity for the primary endpoint (NSTEMI or UAP) compared to the ESC algorithms (Table 3). In the validation cohort, the novel hs-cTnT 0–1-h and 0–3-h algorithms had clinical sensitivities of 95.4% and 97.5%, respectively, compared to the significantly lower 62.8% for the ESC 0–1-h algorithm ($P < 0.001$). This was at the expense of significantly lower clinical specificity ($P < 0.001$), the algorithms showed up to a 4.2× reduction in rule-out rate compared to the ESC 0–1-h algorithm (Table 3).

The findings were less clear for the novel hs-cTnI algorithms. The 95% clinical sensitivity criterion was not met in the validation cohort, with a clinical sensitivity of 86.9% (0–1-h algorithm) and 87.6% (0–3-h algorithm). This cohort was analyzed using a reagent/calibrator lot measuring overall lower hs-cTnI concentrations compared to the derivation cohort (Table 2). The ESC 0–1-h hs-TnI algorithm had a significantly lower clinical sensitivity of 63.9% ($P < 0.001$). Again, the novel algorithms showed less efficacy, and the rule-out rate was reduced by a factor of 1.8.

The low concentration/low-delta value algorithms did not show any clear advantage compared to the ESC algorithms for the secondary endpoint [MI or all-cause mortality within 30 days or urgent (24 h) revascularization] (Table 4, Supplemental Fig. 1). The clinical sensitivity of the novel algorithms was similar to the ESC (100% vs 94%–96%), but the clinical specificity was substantially lower compared to ESC, reducing overall diagnostic efficiency.

The analysis in early presenters showed similar but overall slightly lower clinical sensitivity for all algorithms (Supplemental Table 4, A), and the novel 0–3-h algorithm for cTnT was the only one fulfilling the 95% clinical sensitivity criterion. Again, this was at the expense of significantly lower specificity, where the novel 0–1-h algorithms showed a 2–6× reduction

Table 2. Troponin concentrations (ng/L), median, and 25th and 75th percentiles. D; derivation cohort. V; validation cohort.

	NSTEMI	UAP	Other diseases	NCCP	*P* value
Baseline concentrations					
hs-cTnT$_D$	48.0 (22.8–172.0)	9.0 (5.0–18.0)	13.0 (5.8–24.0)	5.0 (3.0–9.0)	<0.001
hs-cTnT$_V$	56.5 (23.0–161.5)	9.0 (6.0–17.0)	10.5 (5.8–16.3)	5.0 (3.0–8.0)	<0.001
hs-cTnI$_D$	118.9 (26.5–560.1)	4.7 (3.1–9.9)	8.1 (3.2–17.7)	2.7 (1.7–5.2)	<0.001
hs-cTnI$_V$	102.2 (28.2–578.3)	3.3 (1.7–9.3)	3.6 (1.4–10.6)	1.5 (0.8–3.1)	<0.001
Absolute 1-h delta					
hs-cTnT$_D$	12.5 (6.0–28.3)	1.0 (0–1.0)	1.0 (0–2.0)	0 (0–1.0)	<0.001
hs-cTnT$_V$	8.0 (2.4–22.5)	0.7 (0.1–1.0)	0.7 (0–1.0)	0 (0–1.0)	<0.001
hs-cTnI$_D$	72.5 (17.8–261.3)	0.6 (0.2–1.4)	0.6 (0–1.9)	0.4 (0.1–0.7)	<0.001
hs-cTnI$_V$	37.5 (10.4–132.7)	0.9 (0.3–2.3)	0.7 (0.3–1.8)	0.5 (0.2–1.2)	<0.001
Absolute 3-h delta					
hs-cTnT$_D$	47.5 (14.0–142.3)	1.0 (0–2.0)	1.0 (0–3.0)	0 (0–1.0)	<0.001
hs-cTnT$_V$	23.0 (6.0–90.0)	1.0 (0–2.0)	1.0 (0–2.0)	0 (0–1.0)	<0.001
hs-cTnI$_D$	315.8 (47.2–1360.0)	0.8 (0.4–1.8)	1.6 (0.4–4.4)	0.6 (0.2–1.2)	<0.001
hs-cTnI$_V$	59.5 (15.6–489.3)	0.9 (0.2–2.7)	1.1 (0.2–1.9)	0.8 (0.3–1.6)	<0.001

NSTEMI, non-ST elevation myocardial infarction; UAP, unstable angina pectoris; NCCP, noncoronary chest pain.

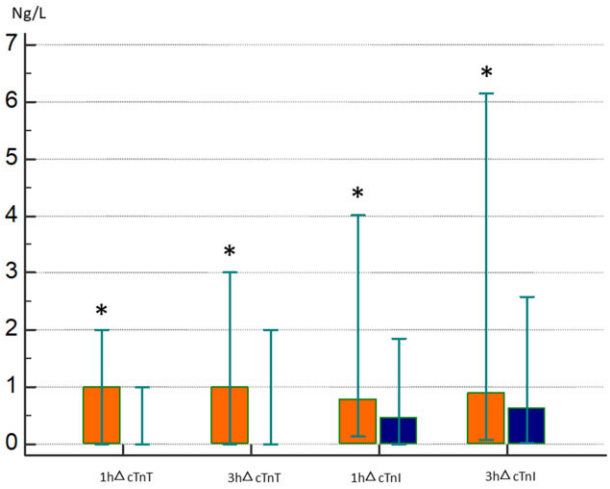

Figure 1. Absolute delta values (ng/L) for hs-cTnT and hs-cTnI in patients with unstable angina pectoris (orange) and noncardiac chest pain (no color/blue) in the total cohort. The bars show median values, poles show the 10th and 90th percentiles. Note that the median value for hs-cTnT deltas in noncardiac chest pain patients was 0 ng/L, similar to the 10th percentile and is therefore shown without color. **P* value <0.001. See color figure online at clinchem.org.

in rule-out rate compared to the ESC 0–1-h algorithms. The novel algorithms showed no benefit regarding the secondary high-risk endpoint (Supplemental Table 4, B).

RULE-OUT RATES FOR THE DIFFERENT ALGORITHMS

Patients were stratified according to index diagnosis and the number being ruled out by the different algorithms were calculated (Supplemental Table 5). All patients with

Table 3. Diagnostic performance (95% confidence intervals) and efficacy (total rule-out, percentages in brackets) for the primary endpoint combining NSTEMI and UAP during index hospitalization for the different algorithms. European Society of Cardiology algorithms are shown on a gray background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-h algorithms					
hs-cTnT <5 ng/L and $\Delta_{0\text{-}1h}$ <1 ng/L					
Derivation cohort $N = 479$	95.8 (90.5–98.6)	95.7 (90.2–98.1)	30.6 (25.8– 35.6)	31.3 (29.7–33.0)	115 (24.0)
Validation cohort $N = 505$	95.4 (90.2–98.3)	92.9 (85.5–96.7)	21.0 (17.0–25.5)	29.3 (28.0–30.6)	85 (16.8)
hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ <3 ng/L					
Derivation cohort $N = 479$	71.4 (62.7–79.7)	89.0 (85.8–91.5)	76.4 (71.7–80.7)	50.0 (44.6–55.4)	309 (64.5)
Validation cohort $N = 505$	62.8 (53.8–71.1)	86.5 (83.6–88.9)	81.7 (77.4–85.4)	54 (47.7–60.2)	355 (70.3)
hs-cTnI <2 ng/L and $\Delta_{0\text{-}1h}$ <1 ng/L					
Derivation cohort $N = 474$	93.3 (87.2– 97.1)	92.7 (86.4– 96.2)	28.5 (23.8–33.5)	30.4 (28.7–.32.2)	109 (23.0)
Validation cohort $N = 507$	86.9 (79.9–92.2)	90.9 (86.4–94.1)	45.1 (40.0–50.3)	35.3 (32.8–37.9)	187 (36.8)
hs-cTnI <5 ng/L and $\Delta_{0\text{-}1h}$ <2 ng/L					
Derivation cohort $N = 474$	72.3 (63.3–80.1)	87.7 (84.1–90.6)	66.5 (61.3–71.4)	42.0 (37.6–46.5)	269 (56.0)
Validation cohort $N = 507$	63.9 (55.0–72.1)	86.3 (83.3–88.9)	78.5 (74.0–82.6)	50.6 (44.8–56.4)	343 (67.7)
3-h algorithms					
hs-cTnT <5 ng/L and $\Delta_{0\text{-}3h}$ <1 ng/L					
Derivation cohort $N = 982$	96.7 (93.6– 98.6)	96.5 (93.3 – 98.2)	30.0 (26.7–33.4)	31.1 (30.0– 32.3)	230 (23.4)
Validation cohort $N = 482$	97.5 (92.9–99.5)	97.2 (91.9–99.1)	29.1 (24.5–34.1)	31.6 (30.0–33.1)	108 (22.4)
hs-cTnI <2 ng/L and $\Delta_{0\text{-}3h}$ <1 ng/L					
Derivation cohort $N = 936$	95.7 (92.2–97.9)	94.9 (91.0–97.2)	26.6 (23.3–30.0)	30.0 (28.9–31.2)	197 (20.2)
Validation cohort $N = 483$	87.6 (80.4–92.9)	90.3 (85.1–93.9)	38.6 (32.4–42.5)	32.3 (30.1–34.7)	155 (32.1)

NPV, negative predictive value; PPV, positive predictive value; NSTEMI, non-ST elevation myocardial infarction; UAP, unstable angina pectoris.

NSTE-ACS who were ruled out were patients with UAP. A detailed description of patients missed for the secondary endpoint is given in the Supplemental Results.

The subgroup analysis undertaken in patients with UAP and NCCP (combining both cohorts), indicated better identification of UAP by the 0–3-h compared to the 0–1-h algorithms (Fig. 2). Overall, 6% of patients with UAP would be ruled out if the low-delta 0–3-h hs-cTnT algorithm was used, with a simultaneously rule-out rate >34% in patients with NCCP. Somewhat higher rule-out rates of approximately 13% (UAP) and 35% (NCCP), respectively, were shown for the hs-cTnI 0–3-h algorithm. Corresponding rates for the 0–1-h ESC algorithms were significantly higher; 56% (cTnT) and 55% (cTnI) for UAP patients, and 85% (cTnT) and 79% (cTnI) for the patients with NCCP. Results were overall similar when analyzed separately in the derivation and validation cohort (Supplemental Table 6).

INVESTIGATIONS, REVASCULARIZATIONS, AND 30-DAYS FOLLOW UP IN THE NSTE-ACS GROUP

The number of investigations, urgent revascularizations (24 h), 30-day MIs, all-cause mortality, and revascularizations for the patients with NSTE-ACS and stratified as

Table 4. Diagnostic performance (95% confidence intervals) and efficacy (total rule-out, percentages in brackets) for the combined secondary endpoint of MACE defined as 30 days MI, 30 days all-cause mortality, or urgent (24 h) revascularization, for the different algorithms. ESC algorithms are shown on a gray background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-h algorithms					
hs-cTnT <5 ng/L and Δ_{0-1h} <1 ng/L					
Derivation cohort $N = 479$	100.0 (94.6–100.0)	100	27.9 (23.6–32.4)	18.1 (17.3–19.4)	115 (24.0)
Validation cohort $N = 505$	100.0 (92.5–100.0)	100	18.6 (15.1–22.4)	11.2 (10.8–11.6)	85 (16.8)
hs-cTnT <12 ng/L and Δ_{0-1h} <3 ng/L					
Derivation cohort $N = 479$	100.0 (94.6–100.0)	100	74.8 (70.3–78.9)	38.8 (34.9–42.9)	309 (64.5)
Validation cohort $N = 505$	93.6 (82.5–98.7)	99.2 (97.2–100.0)	77.0 (72.7–80.6)	29.3 (25.7–33.1)	355 (70.3)
hs-cTnI <2 ng/L and Δ_{0-1h} <1 ng/L					
Derivation cohort $N = 474$	100.0 (94.6–100.0)	100	26.7 (22.5–31.3)	18.1 (17.2–19.0)	109 (23.0)
Validation cohort $N = 507$	100.0 (92.6–100.0)	100	40.7 (36.2–45.4)	15.6 (14.1–16.0)	187 (36.8)
hs-cTnI <5 ng/L and Δ_{0-1h} <2 ng/L					
Derivation cohort $N = 474$	100.0 (94.6–100.0)	100	65.9 (61.0–70.5)	32.2 (29.3–35.3)	269 (56.0)
Validation cohort $N = 507$	95.8 (85.8–99.5)	99.4 (97.8–99.9)	74.3 (70.0–78.2)	28.1 (24.8–31.5)	343 (67.7)
3-h algorithms					
hs-cTnT <5 ng/L and Δ_{0-3h} <1 ng/L					
Derivation cohort $N = 982$	100.0 (97.5–100.0)	100	27.4 (24.4–30.6)	19.0 (18.4–19.7)	230 (23.4)
Validation cohort $N = 482$	100.0 (92.5–100.0)	100	24.8 (20.8–29.2)	12.6 (12.0–13.2)	108 (22.4)
hs-cTnI <2 ng/L and Δ_{0-3h} <1 ng/L					
Derivation cohort $N = 936$	100.0 (97.3–100.0)	100	24.6 (21.7–27.8)	18.4 (17.8–19.0)	197 (20.2)
Validation cohort $N = 483$	100.0 (92.5–100.0)	100	35.6 (31.1–40.2)	14.3 (13.5–15.2)	155 (32.1)

NPV, negative predictive value; PPV, positive predictive value.

NSTEMI and UAP are shown in Supplemental Tables 7 and 8, which show the same variables in the subgroup of patients with UAP who were ruled out by the ESC and the most sensitive of the novel algorithms (0–3 h). None of the ruled-out patients died or experienced an MI within 30 days (Supplemental Results), although a significantly higher proportion of patients who needed revascularization within 30 days were ruled out by the ESC algorithms ($P < 0.001$).

Discussion

Our study has several important findings. First, the use of algorithms combining a low baseline concentration with delta values derived from RCVs may improve the segregation between patients with UAP and NCCP and avoid rule-out of patients who need a recent revascularization. This was particularly clear for algorithms developed for the hs-cTnT assay. Second, the timing of the sampling seems important, as 0–3-h algorithms performed overall better compared to 0–1-h algorithms. Third, reagent or calibrator lots that return lower concentrations may change the overall diagnostic performance of algorithms using low concentrations and deltas, as was demonstrated for the hs-cTnI assay. Fourth, compared to the ESC algorithms, the novel algorithms showed a substantial reduction in patients eligible for rule-out. Last, all

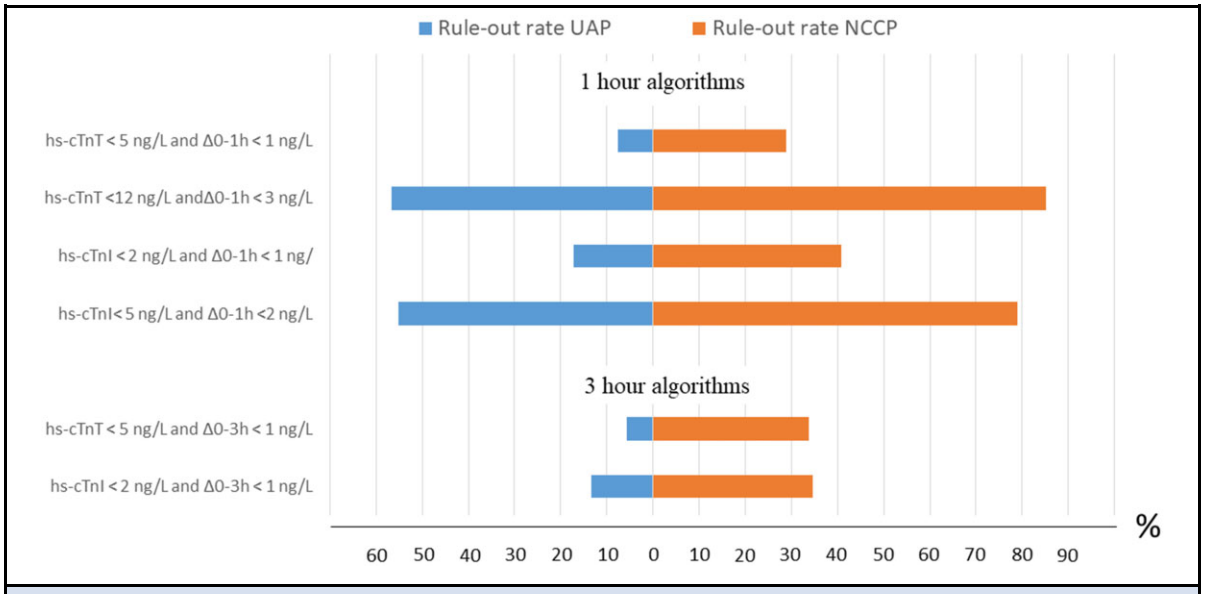

Figure 2. Percentage rule-out for patients with unstable angina pectoris (UAP) and noncardiac chest pain (NCCP) in the total cohort. See color figure online at clinchem.org.

evaluated algorithms showed similar good prognosis for a combined endpoint of 30-day all-cause mortality and MI or urgent (24 h) revascularization.

The most recent guideline from the ESC stress that even if patients are ruled out for NSTEMI, they still may have UAP and may require follow up or treatment within a recent time frame (6). Our data show that the sensitivity for less urgent NSTE-ACS could be increased from approximately 60% to 87%–95%, if the cutoffs applied are based on baseline and delta values that are derived from individuals without apparent underlying myocardial disease. Patients with UAP have increased risk of death and cardiovascular events (11, 19) and revascularization reduces symptom burden and improve quality of life (33). The prognosis is still far better compared to patients with NSTEMI and it is uncertain if rule-out of patients with UAP compromises patient safely as long as invasive treatment is offered during outpatient follow up. It should be noted that the rule-out rate for some of the novel algorithms was as low as 17% (0–1-h cTnT) compared to 60% for the cTnT ESC algorithm (10). This is an important drawback. EDs that have implemented the ESC algorithms may find the novel approach to conservative allocating too many patients to the observational zone. The rule-out rate was somewhat better in the NCCP subgroup, correctly ruling out around 30%–40% of patients with NCCP. Accordingly, the novel algorithms may be useful in EDs that aim to reduce low risk admissions but need high "safety margins" and hospitalize patients with less urgent NSTE-ACS, e.g., UAP.

Future studies, including long-term outcomes, are needed to conclude whether the low concentration/low-delta algorithms identify a subpopulation within the NCCP cohort who may be safely discharged (16).

Our study used hs-cTn delta values that were based on RCV values to identify patients with UAP, who by definition have "stable" troponin concentrations (6). It is biologically plausible that troponin concentrations are slightly increased and/or show larger variations in this group compared to participants who have a completely stable myocardial perfusion (11, 19, 34). Indeed, a recent publication demonstrated that hs-cTn concentrations increased (time dependent) when reversible myocardial ischemia was induced by a 30–90 s balloon occlusion of the left anterior descending coronary artery (35). Patients with UAP had higher baseline concentrations, indicative of a situation of low-grade chronic or acute myocardial injury, combined with larger delta values, consistent with intermittent myocardial leakage of troponins (35). The observation that 3-h deltas separated better between UAP and NCCP, compared to 1-h deltas, strengthens this assumption. It should be noted that our NSTE-ACS cohort had an overall time from symptom onset to first sampling of 8–10 h. The subgroup analysis showed lower sensitivity in patients with NSTE-ACS with $\leq$3 h since onset of symptoms, and usability in this group is uncertain. Overall, if confirmed

in other studies, our data could have consequences for the logistics in the ED, including duration of observation. Future assays with lower analytical variation could have potential for even further improved diagnostic differentiation between patients with UAP and NCCP.

Finally, our data demonstrate how the analytical performance of the assays may influence the diagnostic performance of rule-out algorithms (29). We used 2 different lots of the hs-cTnI assay, 1 in the derivation and 1 in the validation cohort. The lot used in the validation cohort returned lower troponin results (Supplemental Table 2). Consequently, more patients with NSTE-ACS showed concentrations below the limit of detection, resulting in higher rule-out of patients with UAP in this cohort (Supplemental Table 6). The patients with NCCP in the validation cohort also experienced larger delta values, similar to those observed in patients with UAP (Table 2 and Supplemental Table 3), likewise due to more measurements being done at the lowest concentrations (higher analytical variability). In sum, this led to an overall lower diagnostic performance for the cTnI algorithms in the validation cohort (Table 3). Similar systematic evaluation of lot variations could not be done for hs-cTnT because measurements were done on fresh samples during the whole inclusion period, using a larger number of reagent and calibrator lots in both cohorts. The current observations highlight the need of robust validation of algorithms, using several different clinical cohorts and reagent and calibrator lots, before implementation into clinical practice; this calls for laboratories to monitor lot variations closely, and for manufacturers to strive to reduce such variations and develop assays with incremental analytical performance.

STRENGTH AND LIMITATIONS

The study has several strengths. The inclusion criteria are broad, mimicking real-life practice. The study encompassed a derivation and a validation cohort, and evaluated 2 different high-sensitivity troponin assays. The derivation and validation cohort were slightly divergent. This should not affect the clinical sensitivity and specificity of algorithms and the diagnostic performances for hs-cTnT were similar across cohorts, in line with this assumption. The difference observed between cohorts for hs-cTnI is explained by lot variations, as outlined previously.

Our data lack validation in an external cohort; this is a limitation and our findings should therefore be seen as hypothesis generating. Another important limitation in our study is that not all eligible patients with chest pain were included, an important reason for the NSTEMI incidence being lower in the validation compared to the derivation cohort. This was due to logistical problems in the ED, a common problem in this kind of study. Even so, the NSTE-ACS incidence was similar across cohorts and the patient characteristics were also similar to other comparable studies (36, 37). It should be noted that the adjudication was based on routine hs-cTnT measurements, which could positively bias the results for the hs-cTnT algorithms. The use of all-cause mortality instead of cardiovascular mortality as an endpoint may underestimate the performance of the algorithms. Our NSTEMI adjudication was based on the third definition of MI, since this is very similar to the fourth definition it is unlikely to affect results. Finally, the clinical sensitivity was lower in early presenters, questioning the applicability in this group. The cohort of early presenters is quite small and further validation is necessary.

Conclusion

The current study shows that troponin algorithms using low baseline concentrations and delta values show improved clinical sensitivity for NSTE-ACS by improved differentiation between patients with UAP and NCCP. A major drawback was that the overall rule-out rate of patients investigated for NSTE-ACS was reduced with a factor of 2–4 compared to the ESC algorithms, which is substantial and may result in a less efficient patient flow through the ED. Our study demonstrates that timing of samples, lot variations, and analytical variability may substantially influence the diagnostic performance of rule-out algorithms that encompass low hs-cTn concentrations and deltas. This study demonstrates that high-sensitivity assays could play a role in identifying patients with UAP and NCCP in the ED, and that even further improvement of the analytical performance of troponin assays may have a clear clinical benefit.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: ESC, European Society of Cardiology; NSTEMI, non-ST-elevation myocardial infarction; NSTE-ACS, non-ST-elevation acute coronary syndrome; hs-cTnT, high-sensitivity cardiac troponin T; hs-cTnI, high-sensitivity cardiac troponin I; UAP, unstable angina pectoris; NCCP, noncoronary chest pain; ED, emergency department; MACE, major cardiovascular events; RCV, reference change value; CVA, coefficient of variation; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; ECG, electrocardiogram.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design,*

acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: T. Omland, K.M Aakre, Paul Collinson, IFCC Committee on Cardiovascular Biomarkers; K.M. Aakre, Paul Collinson, EFLM Working Group on Cardiac Markers; P. Collinson, *The Journal of Applied Laboratory Medicine*, AACC.
Consultant or Advisory Role: T. Omland, Roche Diagnostics, Abbott Diagnostics, CardiNor; K.M. Aakre, Roche Diagnostics.
Stock Ownership: None declared.
Honoraria: T. Omland, Siemens Healthineers, Roche Diagnostics, Abbott Diagnostics; K.M. Aakre, Siemens Healthineers, and Roche Diagnostics.
Research Funding: The study was financed by a grant from the Western Norway Regional Health Authority (grant number: 912265). H.L. Tjora has a Ph.D. grant from the Western Norway Regional Health Authority (grant number: 912208). T. Omland, nonfinancial support to institution from Novartis, Abbott Diagnostics, Roche Diagnostics, and SomaLogic.
Expert Testimony: None declared.
Patents: T. Omland, Granin proteins as markers of heart disease (PCT/GB0818650), SgII as a prognostic marker in conditions that require critical care (CT/GB0919901.9).

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

References

1. World Health Organization. Cardiovascular diseases. http://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (Accessed August 2021). Geneva, Switzerland; WHO.
2. Goodacre S, Cross E, Arnold J, Angelini K, Capewell S, Nicholl J. The health care burden of acute chest pain. Heart 2005;91:229–30.
3. Langlo NM, Orvik AB, Dale J, Uleberg O, Bjornsen LP. The acute sick and injured patients: an overview of the emergency department patient population at a Norwegian university hospital emergency department. Eur J Emerg Med 2014;21:175–80.
4. Garg P, Morris P, Fazlanie AL, Vijayan S, Dancso B, Dastidar AG, et al. Cardiac biomarkers of acute coronary syndrome: from history to high-sensitivity cardiac troponin. Intern Emerg Med 2017;12:147–55.
5. Jarolim P. High sensitivity cardiac troponin assays in the clinical laboratories. Clin Chem Lab Med 2015;53:635–52.
6. Collet JP, Thiele H, Barbato E, Barthelemy O, Bauersachs J, Bhatt DL, et al.; ESC Scientific Document Group. 2020 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2021;42:1289–367.
7. Anand A, Lee KK, Chapman AR, Ferry AV, Adamson PD, Strachan FE, et al. High-sensitivity cardiac troponin on presentation to rule out myocardial infarction: a stepped-wedge cluster randomized controlled trial. Circulation 2021;143:2214–24.
8. Chapman AR, Anand A, Boeddinghaus J, Ferry AV, Sandeman D, Adamson PD, et al. Comparison of the efficacy and safety of early rule-out pathways for acute myocardial infarction. Circulation 2017;135:1586–96.
9. Shah AS, 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.
10. 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.
11. Eggers KM, Jernberg T, Lindahl B. Unstable angina in the era of cardiac troponin assays with improved sensitivity-a clinical dilemma. Am J Med 2017;130:1423–30.e5.
12. Yang S, Bhatia N, Xu M, McPherson JA. Incidence and predictors of obstructive coronary artery disease and the role of cardiac troponin assays in patients with unstable angina. Tex Heart Inst J 2019;46:161–6.
13. Andruchow JE, Boyne T, Innes G, Vatanpour S, Seiden-Long I, Wang D, et al. Low high-sensitivity troponin thresholds identify low-risk patients with chest pain unlikely to benefit from further risk stratification. CJC Open 2019;1:289–96.
14. Pickering JW, Than MP, Cullen L, Aldous S, Ter Avest E, Body R, et al. Rapid rule-out of acute myocardial infarction with a single high-sensitivity cardiac troponin T measurement below the limit of detection: a collaborative meta-analysis. Ann Intern Med 2017;166:715–24.
15. Tjora HL, Steiro OT, Langørgen J, Bjørneklett R, Nygård OK, Skadberg Ø, et al. Cardiac troponin assays with improved analytical quality: a trade-off between enhanced diagnostic performance and reduced long-term prognostic value. J Am Heart Assoc 2020;9:e017465.
16. Sandoval Y, Smith SW, Sexter A, Gunsolus IL, Schulz K, Apple FS. Clinical features and outcomes of emergency department patients with high-sensitivity cardiac troponin I concentrations within sex-specific reference intervals. Circulation 2019;139:1753–5.
17. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci. 1989;27:409–37.
18. Aakre KM, Saeed N, Wu AHB, Kavsak PA. Analytical performance of cardiac troponin assays–current status and future needs. Clin Chim Acta 2020;509:149–55.
19. Giannitsis E, Biener M, Hund H, Mueller-Hennessen M, Vafaie M, Gandowitz J, et al. Management and outcomes of patients with unstable angina with undetectable, normal, or intermediate hsTnT levels. Clin Res Cardiol 2020;109:476–87.
20. Tjora HL, Steiro OT, 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.
21. Roffi M, Patrono C, Collet J-P, Mueller C, Valgimigli M, Andreotti F, et al.; ESC Scientific Document Group. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2016;37:267–315.
22. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al.; ESC Committee for Practice Guidelines (CPG). Third universal definition of myocardial infarction. Eur Heart J 2012;33:2551–67.
23. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al.; Study Group on Biomarkers in Cardiology of ESC Working Group on Acute Cardiac Care. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J 2012;33:2252–7.
24. Everett BM. Cardiac troponin as a novel tool for cardiovascular risk prediction in ambulatory populations. Trends Cardiovasc Med 2017;27:41–7.
25. Kontos MC, Turlington JS. High-sensitivity troponins in cardiovascular disease. Curr Cardiol Rep 2020;22:30.
26. deFilippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, Seliger SL. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. Jama 2010;304:2494–502.
27. Lan NSR, Bell DA. Revisiting the biological variability of cardiac troponin: implications for clinical practice. Clin Biochem Rev 2019;40:201–16.
28. Kavsak PA, Jaffe AS, Greene DN, Christenson RH, Apple FS, Wu AHB. Total analytic error for low cardiac troponin concentrations (</=10 ng/l) by use of a high-sensitivity cardiac troponin assay. Clin Chem 2017;63:1043–5.
29. Haagensen K, Collinson P, Asberg 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.
30. Aakre KM, Røraas T, Petersen PH, Svarstad E, Sellevoll H, Skadberg Ø, et al. Weekly and 90-minute biological variations in cardiac troponin T and cardiac troponin I in hemodialysis patients and healthy controls. Clin Chem 2014;60:838–47.
31. Sandoval Y, Apple FS, Smith SW. High-sensitivity cardiac troponin assays and unstable angina. Eur Heart J Acute Cardiovasc Care 2018;7:120–8.
32. Than M, Herbert M, Flaws D, Cullen L, Hess E, Hollander JE, et al. What is an acceptable risk of major adverse cardiac event in chest pain patients soon after discharge from the emergency department? A clinical survey. Int J Cardiol 2013;166:752–4.
33. Boden WE, O'Rourke RA, Teo KK, Hartigan PM, Maron DJ, Kostuk WJ, et al.; COURAGE Trial Research Group. Optimal medical therapy with or without PCI for stable coronary disease. N Engl J Med 2007;356:1503–16.

34. Januzzi JL Jr, Suchindran S, Hoffmann U, Patel MR, Ferencik M, Coles A, et al. Single-molecule hsTnI and short-term risk in stable patients with chest pain. J Am Coll Cardiol 2019;73:251–60.
35. Árnadóttir Á, Pedersen S, Bo Hasselbalch R, Goetze JP, Friis-Hansen LJ, Bloch-Münster AM, et al. Temporal release of high-sensitivity cardiac troponin T and I and copeptin after brief induced coronary artery balloon occlusion in humans. Circulation 2021; 143:1095–104.
36. Chapman AR, Hesse K, Andrews J, Ken Lee K, Anand A, Shah ASV, et al. High-sensitivity cardiac troponin I and clinical risk scores in patients with suspected acute coronary syndrome. Circulation 2018;138:1654–65.
37. Reichlin T, Twerenbold R, Reiter M, Steuer S, Bassetti S, Balmelli C, et al. Introduction of high-sensitivity troponin assays: impact on myocardial infarction incidence and prognosis. Am J Med 2012;125:1205–13.e1.