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ORIGINAL RESEARCH

# Cardiac Troponin Assays With Improved Analytical Quality: A Trade-Off Between Enhanced Diagnostic Performance and Reduced Long-Term Prognostic Value

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**BACKGROUND:** Cardiac troponin (cTn) permits early rule-out/rule-in of patients admitted with possible non–ST-segment–elevation myocardial infarction. In this study, we developed an admission and a 0/1 hour rule-out/rule-in algorithm for a troponin assay with measurable results in >99% of healthy individuals. We then compared its diagnostic and long-term prognostic properties with other protocols.

**METHODS AND RESULTS:** Blood samples were collected at 0, 1, 3, and 8 to 12 hours from patients admitted with possible non–ST-segment–elevation myocardial infarction. cTnT (Roche Diagnostics), $cTnI_{(Abbott)}$ (Abbott Diagnostics), and $cTnI_{(sgx)}$ (Singulex Clarity System) were measured in 971 admission and 465 1-hour samples. An admission and a 0/1 hour rule-out/rule-in algorithm were developed for the $cTnI_{(sgx)}$ assay and its diagnostic properties were compared with $cTnT_{ESC}$ (European Society of Cardiology), $cTnI_{(Abbott)ESC}$, and 2 earlier $cTnI_{(sgx)}$ algorithms. The prognostic composite end point was all-cause mortality and future nonfatal myocardial infarction during a median follow-up of 723 days. non–ST-segment–elevation myocardial infarction prevalence was 13%. The novel $cTnI_{(sgx)}$ algorithms showed similar performance regardless of time from symptom onset, and area under the curve was significantly better than comparators. The $cTnI_{(sgx)0/1\ hour}$ algorithm classified 92% of patients to rule-in or rule-out compared with ≤78% of comparators. Patients allocated to rule-out by the prior published 0/1 hour algorithms had significantly fewer long-term events compared with the rule-in and observation groups. The novel $cTnI_{(sgx)0/1\ hour}$ algorithm used a higher troponin baseline concentration for rule-out and did not allow for prognostication.

**CONCLUSIONS:** Increasingly sensitive troponin assays may improve identification of non–ST-segment–elevation myocardial infarction but could rule-out patients with subclinical chronic myocardial injury. Separate protocols for diagnosis and risk prediction seem appropriate.

**Key Words:** chest pain ■ chronic myocardial injury ■ myocardial infarction ■ 0/1 hour algorithm

Clinical suspicion of non–ST-segment–elevation myocardial infarction (NSTEMI) is a frequent cause of hospital admission,[1] and cardiac troponin (cTn, T, or I) measurement is a cornerstone in evaluation of these patients.[2] Approximately 40% of patients are "early presenters,"[3,4] and accurate detection of low cTn

See Editorial by McCarthy and Januzzi

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## CLINICAL PERSPECTIVE

### What Is New

- Troponin assays with analytical sensitivity and precision beyond the current high sensitivity troponin assays are likely to show improved diagnostic performance for non–ST-segment–elevation myocardial infarction (NSTEMI).
- Diagnostic algorithms that are very precise for identification of NSTEMI will to a lesser extent identify patients with subclinical or overt chronic myocardial injury and consequently show lower long-term prognostic power compared with less precise algorithms.

### What Are the Clinical Implications?

- Troponin assays with improved analytical sensitivity have a high ability for early identification of NSTEMI, making early presenters with low admission troponin concentrations eligible for rule-out.
- Development of efficient diagnostic follow-up schemes allocating >90% of patients presenting with chest pain to rule-in or rule-out for NSTEMI should improve the logistics in the emergency room.
- Long-term cardiovascular risk should be considered even in patients who are ruled out for NSTEMI.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **CMI** | chronic myocardial injury |
| **ESC** | European Society of Cardiology |
| **LOD** | limit of detection |
| **Sgx** | Singulex Clarity System |

concentrations for immediate rule-out of NSTEMI may therefore have clinical utility. The European Society of Cardiology (ESC) recommends that patients with a detectable baseline cTn concentration undergo serial testing. Based on the baseline and delta concentrations obtained, such cases can be classified as rule-out, observation, or rule-in for NSTEMI.[2] A few limitations apply when troponin-based algorithms are used for rule-out of NSTEMI, for example, the suboptimal analytical sensitivity (unavailability to provide measurable concentrations in all healthy individuals) and large analytical imprecision (reproducibility of sequential measurements) at low troponin concentrations, which could produce a false low delta value leading to an inappropriate rule-out. Improved analytical sensitivity and precision of these assays might facilitate algorithms with higher specificity allowing for admission rule-out in all individuals with low concentrations regardless of time from symptom onset and greater reliability of deltas allowing for rule-out in subjects with high normal or increased baseline concentrations.

Stable troponin concentrations above the 99th percentile is considered to indicate chronic myocardial injury (CMI) and are associated with poor long-term outcomes.[5–7] Studies have shown that risk rises continuously with troponin concentrations below the 99th percentile.[8] Some studies have identified this association even at concentrations lying between the limit of detection (LOD) and limit of blank of current assays.[9] Thus, identifying subclinical myocardial injury (ie, myocardial injury with stable troponin concentration <99th percentile) may be of clinical relevance because it could indicate increased cardiovascular risk in patients with acute chest pain.[10–13] Whether assays with improved analytical performance could allow for further improvement in long-term risk prediction is not known.

Two previous reports[14,15] described findings with a cTn assay (Singulex Clarity System) ($cTnI_{(sgx)}$) with improved analytical sensitivity, providing measurable results in >99% of healthy individuals (versus corresponding values of 72% for Roche Diagnostics and 85% for Abbott Diagnostics[16]). This assay also provided 10% analytical variation at concentrations below 1 ng/L.[16] Neither study, however, directly addressed whether this increased analytical quality translated into improved clinical utility. Although this assay is currently unavailable because the company stopped trading in 2019, the data derived from it are highly relevant to understand the possible benefits and drawbacks of improved analytical quality with troponin or other cardiac injury biomarker assays.

In this cross-sectional observational study, we hypothesized that compared with currently used or suggested cTnT and cTnI algorithms, $cTnI_{(sgx)}$ could offer better performance, with a greater rate of correct rule-out and rule-in of patients presenting with possible NSTEMI. For its development, the admission sample algorithm should include all patients regardless of time between sampling and symptom onset, and the 0/1 hour algorithm should allow rule-out in patients with increased baseline concentration, given low delta values. Because algorithms using high baseline troponin concentration can rule out patients with both subclinical myocardial injury and CMI, we also analyzed data from a prospective follow-up period to evaluate the relative long-term risk-prediction ability of these algorithms.

## METHODS

### Study Design

The data that support the current findings are available from the corresponding author upon reasonable

request. The WESTCOR (Aiming Towards Evidence Based Interpretation of Cardiac Biomarkers in Patients Presenting With Chest Pain) study (Clinical Trials number NCT02620202) is a two-center, cross-sectional, prospective observational study described in detail earlier.[17] The cross-sectional study design was used to investigate the accuracy of different algorithms. Patients were then prospectively followed to determine if the different algorithms could predict future cardiovascular outcomes.[18]

The current article reports data from the WESTCOR derivation cohort (WESTCOR-D) including 985 patients admitted to Haukeland University Hospital, Norway, with suspected non–ST-segment elevation acute coronary syndrome (ACS). The inclusion period lasted from September 2015 to February 2017. 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 age >18 years admitted with chest pain or symptoms suggesting non–ST-segment elevation-ACS and who did not have a short life expectancy (eg, advanced cancer) and could provide informed consent were eligible for inclusion.[17] Patients had serum samples drawn on arrival to the emergency department and after 3 and 8 to 12 hours. Samples were centrifuged after 30 minutes, and material for the biobank was aliquoted and frozen. High-sensitivity cTnT was measured in fresh samples, and the results were reported to the attending clinician. After an initial period of fine-tuning of the study, an additional biobank sample was drawn 1 hour after admission, and the results were not reported to the attending clinician. This adjustment was planned a priori as a part of the study.[17] Biobank admission samples were available from 971 patients, and a 1-hour sample was available for 465 patients.

## Biochemical Analyses

Routine and 1-hour samples were measured for cTnT (Roche Diagnostics) with limit of blank 3 ng/L, LOD 5 ng/L, 99th percentile 14 ng/L, and analytical within-series coefficient of variation 10% at 4.5 ng/L. For cTnI (biobanked samples), measured using the Abbott Diagnostics assay ($cTnI_{(Abbott)}$), these values were limit of blank 0.9 ng/L, LOD 1.7 ng/L, 99th percentile 26 ng/L, and 10% coefficient of variation 4.6 ng/L. For the cTnI measured using the Singulex Clarity System, these values were limit of blank 0.02 ng/L, LOD 0.08 ng/L, 99th percentile 8.67 ng/L, and 10% coefficient of variation 0.53 ng/L.[16] All other clinical chemistry tests were measured using Cobas e602 or Cobas 8000 from Roche Diagnostics. The glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula and an enzymatic isotope dilution mass spectrometry traceable creatinine assay (Roche Diagnostics).

## Diagnosis

The diagnostic end point was NSTEMI during the index hospitalization. The adjudicating process has been described earlier,[17] but briefly, 2 independent cardiologists adjudicated the final diagnosis based on all available clinical, routine laboratory (including cTnT at admission and at 3 and 8–12 hours from admission), electrocardiogram, ultrasound, and imaging findings, including cardiac computed tomographic angiography and conventional angiography. A third adjudicator resolved disagreements. Specific diagnostic criteria were predefined for 22 different medical conditions based on guidelines that were available during planning of the study (see Data S1). NSTEMI was defined according to the third universal definition for myocardial infarction (MI), including a significant rise and fall of cTn with at least 1 value above the 99th percentile combined with symptoms of ischemia, electrocardiogram changes, and image evidence of loss of viable myocardium or intracoronary thrombus.[19] Delta values of 20% (baseline cTnT concentration >14 ng/L) or 50% (baseline cTnT concentration ≤14 ng/L) in serial cTnT measures were regarded as significant, as suggested by the ESC in 2012.[17,19] Since 2012, several studies have found a significantly lower 99th percentile concentration of cTn for women compared with men,[20–22] but knowledge of sex-specific cutoffs for women regarding diagnosing NSTEMI is partial because of a lack of data on pathophysiology.[23] Consequently, we chose to apply a common cutoff for all patients.

## Follow-Up and Prognostic End Points

Follow-up data were collected through the Norwegian Patient Register and Norwegian Cause of Death Registry. The prognostic end point was a composite of all-cause mortality and subsequent nonfatal MI (all MIs after the index NSTEMI). Patients were followed until an end point occurred or until a median follow-up time of 723 days after inclusion (ranging from 4 to 900 days).

## Comparator Algorithms

According to current recommendations, patients are eligible for early discharge or further investigations for ACS based on the troponin results.[2] The algorithms encompass an initial review of the admission sample in patients who present more than 3 hours after symptom onset. If the concentration is below

the LOD of the troponin assay, the patient is eligible for "rule-out" and may be discharged if the electrocardiogram and/or the clinical symptoms suggest a lower likelihood of ACS. The remaining patients undergo serial sampling at 1-hour intervals. Based on the baseline and delta values obtained, patient status is established as "rule-out," "observation," or "rule-in" for NSTEMI. Again, ruled-out patients may be discharged if the clinical suspicion of ACS is low, those who are ruled in may go directly to cardiac angiography and eventually invasive treatment, and those in the observation group undergo diagnostic follow-up. We compared the recommended ESC (cTnT and $cTnI_{(Abbott)}$) algorithms[2] and those suggested by Body et al[14] and Neumann et al[15] to the novel algorithms described in Tables 1 and 2.

## Development of Novel Algorithms

The novel $cTnI_{(sgx)}$ rule-out algorithms were defined based on the following hierarchy of criteria: diagnostic sensitivity for NSTEMI ≥99.0%, as previously described,[24] and the maximum possible specificity. Sensitivity was preferred over negative predictive value as the criterion because sensitivity is independent of disease prevalence and applicable in chest pain cohorts with higher and lower prevalences compared with our cohort. Applicable concentrations for the rule-in algorithms were based on the following criteria: diagnostic specificity for NSTEMI ≥95% (<5% rule-in of patients with non-NSTEMI) and a simultaneously maximized sensitivity for NSTEMI. Specificity was considered preferable to positive predictive value because specificity is independent of prevalence.

We chose the preferred algorithms based on the number of ruled-out and ruled-in patients who would give a sensitivity and specificity corresponding to the prespecified criteria. "Direct rule-out" was defined as rule-out regardless of time since symptom onset. Diagnostic performance was calculated with and without early presenters, defined as patients with <3 hours since symptom onset.

## 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 test for continuous variables and the chi-square and Fisher's exact test for categorical variables, as appropriate. Statistical analyses included calculation of sensitivity, specificity, negative predictive value, positive predictive value, and likelihood ratios, receiver operating characteristic curve analysis, and calculation of area under the curve (AUC) for all algorithms. Significant differences in AUC were evaluated using the Delong test, and efficiency

**Table 1. Single Sample Rule-Out of NSTEMI**

| | Sensitivity | Negative Predictive Value | Negative Likelihood Ratio | Specificity | Positive Predictive Value | Positive Likelihood Ratio | Area Under the Curve |
|---|---|---|---|---|---|---|---|
| Direct rule-out (N=971) | | | | | | | |
| $cTnT_{ESC}$ <5 ng/L | 98.4 (94.4–99.8) | 99.3 (97.4–99.8) | 0.04 (0.01–0.18) | 35.1 (31.8–38.4) | 15.5 (17.7–19.3) | 1.52 (1.44–1.60) | 0.67 (0.64–0.70) |
| $cTnI_{(Abbott)ESC}$ <2 ng/L | 97.6 (93.3–99.5) | 98.7 (96.0–99.6) | 0.09 (0.03–0.28) | 26.0 (23.0–29.1) | 16.6 (15.9–17.2) | 1.32 (1.26–1.38) | 0.62 (0.59–0.65) |
| $cTnI_{(sgx)Neumann}$ <1.0 ng/L | 100 (97.1–100) | 100 (NC) | 0.0 (NC) | 10.3 (8.3–12.6) | 14.4 (14.1–14.7) | 1.11 (1.09–1.14) | 0.55 (0.52–0.58) |
| $cTnI_{(sgx)Body}$ <1.5 ng/L ng/L | 99.2 (95.7–99.9) | 99.6 (97.1–99.9) | 0.03 (0.00–0.20) | 28.0 (25.0–31.1) | 17.2 (16.5–17.8) | 1.38 (1.32–1.44) | 0.64 (0.60–0.67) |
| $cTnI_{(sgx)}$ <2 ng/L* | 99.2 (95.7–100.0)* | 99.7 (98.1–100.0)* | 0.02 (0–0.13)* | 42.5 (39.2–46.0)* | 20.6 (20.0–21.6)* | 1.73 (1.63–1.83)* | 0.71 (0.68–0.74)* |
| Admission sample rule-out and history ≥3 h (N=772) | | | | | | | |
| $cTnT_{ESC}$ <5 ng/L | 98.9 (94.2–100.0) | 99.5 (96.8–99.9) | 0.03 (0–0.22) | 34.5 (30.9–38.2) | 18.4 (17.5–19.3) | 1.51 (1.42–1.60) | 0.67 (0.63–0.70) |
| $cTnI_{(Abbott)ESC}$ <2 ng/L | 97.9 (92.5–99.7) | 98.8 (95.2–99.7) | 0.08 (0.02–0.34) | 25.4 (22.2–28.9) | 16.4 (15.7–17.1) | 1.31 (1.24–1.38) | 0.62 (0.58–0.65) |
| $cTnI_{(sgx)}$ <2 ng/L* | 98.9 (94.2–100.0)* | 99.6 (97.4–100.0)* | 0.03 (0–0.18)* | 42.0 (38.2–45.8)* | 20.3 (19.2–21.4)* | 1.70 (1.59–1.82)* | 0.70 (0.67–0.74)* |

Values are n (%) with 95% CIs. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; and NSTEMI, non–ST-segment elevation myocardial infarction.

*ESC and earlier published Singulex Clarity System (sgx) algorithms and the novel algorithm are referenced in the table. A minor nonsignificant change in the diagnostic performance, for example, sensitivity, is seen when early presenters are excluded because of a change in the nominator.

**Table 2. Serial 0/1 Hour Algorithms for Rule-Out and Rule-In of NSTEMI**

| | Sensitivity | Negative Predictive Value | Negative Likelihood Ratio | Specificity | Positive Predictive Value | Positive Likelihood Ratio | Area Under the Curve |
|---|---|---|---|---|---|---|---|
| 0/1 h rule-out (N=465) | | | | | | | |
| $cTnT_{ESC}$ <12 ng/L and $\Delta_{0-1}$ <3 ng/L | 100.0 (94.1–100.0) | 100 (NC) | 0 (NC) | 74.3 (69.7–78.5) | 37.0 (33.2–40.9) | 3.88 (3.29–4.58) | 0.87 (0.84–0.90) |
| $cTnI_{(Abbott)ESC}$ <5 ng/L and $\Delta_{0-1}$ <2 ng/L | 100.0 (94.1–100.0) | 100 (NC) | 0 (NC) | 65.4 (60.5–70.0) | 30.4 (27.6–33.3) | 2.89 (2.52–3.30) | 0.83 (0.79–0.86) |
| $cTnI_{(sgx)Neumann}$ <2 ng/L and $\Delta_{0-1}$ <1 ng/L | 100 (94.1–100.0) | 100 | 0 | 46.0 (41.1–51.0) | 21.9 (20.4–23.4) | 1.85 (1.69–2.03) | 0.73 (0.69–0.77) |
| $cTnI_{(sgx)}$ <8.67 ng/L and $\Delta_{0-1}$ <3 ng/L | 100 (94.1–100.0) | 100 (NC) | 0 (NC) | 87.1 (83.5–90.2) | 53.7 (47.4–59.9) | 7.77 (6.03–10.01) | 0.94 (0.91–0.96) |
| $cTnI_{(sgx)}$ <10 ng/L and $\Delta_{0-1}$ <3 ng/L* | 100.0 (94.1–100.0)* | 100 (NC)* | 0 (NC)* | 88.6 (85.1–91.5)* | 57.0 (50.3–63.5)* | 8.78 (6.69–11.53)* | 0.94 (0.92–0.96)* |
| 0/1 h rule-in (N=465) | | | | | | | |
| $cTnT_{ESC}$ ≥52 ng/L or $\Delta_{0-1}$ ≥5 ng/L | 78.7 (66.3–88.1) | 96.8 (94.9–98.0) | 0.22 (0.14–0.36) | 96.5 (94.3–98.1) | 77.4 (66.8–85.4) | 22.7 (13.4–38.6) | 0.88 (0.84–0.90) |
| $cTnI_{(Abbott)ESC}$ ≥52 ng/L or $\Delta_{0-1}$ ≥6 ng/L | 91.8 (81.9–97.3) | 98.7 (97.1–99.4) | 0.09 (0.04–0.20) | 94.6 (91.9–96.6) | 71.8 (62.7–79.4) | 16.9 (11.2–25.5) | *0.93 (0.90–0.95) |
| $cTnI_{(sgx)Neumann}$ ≥25 ng/L or $\Delta_{0-1}$ ≥6 ng/L | 90.2 (79.8–96.3) | 98.5 (96.8–99.3) | 0.10 (0.05–0.22) | 95.3 (92.8–97.2) | 74.3 (64.9–81.9) | 19.2 (12.3–30.0) | 0.93 (0.90–0.95) |
| $cTnI_{(sgx)0}$ ≥70 ng/L or $\Delta_{0-1}$ ≥5 ng/L* | 90.5 (80.4–96.4)* | 98.7 (97.2–99.4)* | 0.10 (0.05–0.21)* | 97.0 (94.9–98.5)* | 80.6 (70.3–87.9)* | 30.5 (17.4–53.5)* | 0.94 (0.91–0.96)* |
| $cTnI_{(sgx)0}$ ≥30 ng/L or $\Delta_{0-1}$ ≥5 ng/L* | 93.4 (84.1–98.2)* | 99.1 (97.7–99.6)* | 0.07 (0.03–0.18)* | 96.3 (94.0–97.9)* | 77.4 (67.5–85.0)* | 25.2 (15.3–41.5)* | 0.95 (0.92–0.97)* |

Values are n (%) with 95% CIs. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac Troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; NC, not calculated; and NSTEMI, non–ST-elevation myocardial infarction.

*ESC and earlier published Singulex Clarity System (sgx) algorithms and the novel algorithm are referenced in the table.

(defined as percentage of patients ruled out plus percentage of patients ruled in) was calculated for all algorithms. Kaplan–Meier curves were drawn for the composite end point stratified according to categories, and the number of events was calculated. Cox proportional hazards regression analysis was used to calculate the unadjusted hazard ratio for the composite end point, and adjusted analysis was undertaken using age, sex, current or previous smoking, chronic kidney disease, hypertension, hyperlipidemia, diabetes mellitus, and previous MI as covariates. Definitions of the different risk factors are given in Data S1. We used SPSS Statistics 24 and MedCalc for the statistical analyses.

# RESULTS

## Baseline Characteristics and Troponin Concentrations

The characteristics of patients according to diagnostic category are presented in Table S1 (total cohort, n=971) and Table S2 (0/1 hour cohort, n=465). The prevalence of NSTEMI was 13%, and the prevalence of unstable angina pectoris was 11%. Figure 1 shows the median (25 and 75 percentiles) troponin concentrations at admission and 1 hour for the three different assays. $cTnI_{(sgx)}$ was measurable in 99.9%, cTnT in 74%, and $cTnI_{(Abbott)}$ in 87% of samples obtained at admission.

## Derivation of a Direct Rule-Out and a 0/1 Hour Rule-Out and Rule-In Algorithm

The number of NSTEMIs that would be ruled out at different admission sample $cTnI_{(Sgx)}$ concentrations was calculated (see Table S3). A direct rule-out algorithm using <2 ng/L as the cutoff showed a diagnostic sensitivity >99%, in accordance with the prespecified criteria (Table 1). The 0/1 hour algorithm was developed in a similar way by calculating the number of NSTEMIs ruled out at different admission and delta value concentrations combined (see Tables S4 and S5). The optimal rule-out algorithm was a baseline $cTnI_{(Sgx)}$ concentration <10 ng/L and a delta value of <3 ng/L (Table 2). This algorithm did not rule out any patients with NSTEMI and consequently had a sensitivity of 100%, with a corresponding specificity of 89%. Of note, this decision threshold is higher than the 99th percentile of the assay.

If 8.67 ng/L was used as the baseline concentration in the algorithm, the resulting sensitivity was 100% and specificity was 87%. Regarding rule-in, the optimal algorithm showed a specificity of 97% using a baseline concentration of ≥70 ng/L or a delta value of ≥5 ng/L (Table S5 and Table 2) as cutoffs. An alternative

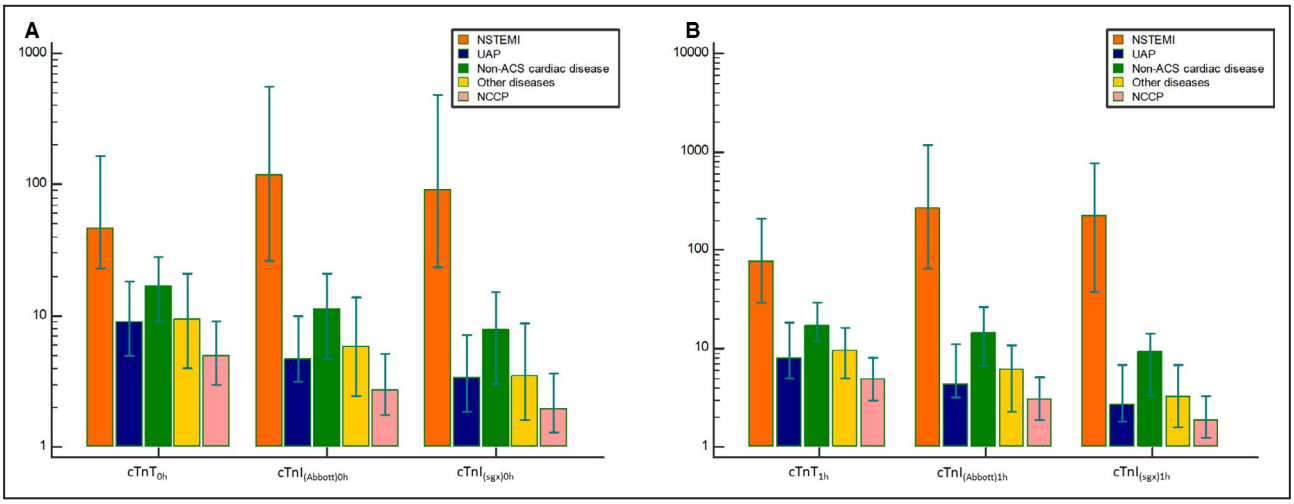


**Figure 1. Cardiac troponin concentrations; median, (interquartile range) stratified according to diagnosis.**
**A**, Cardiac troponin concentrations at admission. **B**, Cardiac troponin concentrations after 1 hour. ACS indicates acute coronary syndrome; $cTnI_{(Abbott)}$, cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; NCCP, non-cardiac chest pain; NSTEMI, non–ST-segment–elevation myocardial infarction; and UAP, unstable angina.

algorithm using a lower baseline concentration of ≥30 ng/L or a delta value of ≥5 ng/L showed similar (within 95% CIs) specificity and sensitivity.

## Comparing Diagnostic Performance of Direct Rule-Out Algorithms

Baseline concentrations analyzed as a continuous variable showed a higher AUC for $cTnI_{(Sgx)}$ compared with the other assays (Figure 2; Delong test, $P \leq 0.004$). Direct rule-out by $cTnI_{(Sgx)}$ was the only direct rule-out algorithm that fulfilled the criterion of sensitivity >99% (Figure 3 and Table 1), and only 1 patient was falsely ruled out. A similar sensitivity was achieved for $cTnT_{ESC}$ when a time lag of 3 hours between testing and symptom onset was applied.

This patient with NSTEMI was inappropriately ruled out by all admission algorithms. She was a 65-year-old woman admitted with chest pain lasting more than 3 days. A few years earlier, she had been treated with percutaneous coronary intervention in all three coronary vessels because of unstable angina pectoris. Upon admission, her electrocardiogram showed nonspecific T changes, and she had a high clinical risk for ACS (eg, a HEART (History, Electrocardiogram, Age, Risk factors, Troponin) score of 7). At 72 hours after symptom onset, the baseline troponin samples were cTnT 4 ng/L, $cTnI_{(Abbott)}$ 1.5 ng/L, and $cTnI_{(sgx)}$ 1.3 ng/L, which increased significantly to cTnT 71 ng/L/, $cTnI_{(Abbott)}$ 80 ng/L, and $cTnI_{(sgx)}$ 56 ng/L after 3 hours. The coronary angiogram revealed a thrombus in a small vessel that was not available for percutaneous coronary intervention.

The $cTnI_{(Abbott)ESC}$ algorithm had the lowest sensitivity, missing a second patient, a 73-year-old woman with one previous MI. She was admitted to the hospital with a history of chest pain related to defecation and baseline blood samples taken 3.5 hours after symptom onset showing cTnT 11 ng/L, $cTnI_{(Abbott)}$ 2 ng/L, and $cTnI_{(sgx)}$ 9.25 ng/L. After 3 hours, values for $cTnI_{(Abbott)}$ increased to 5 ng/L, but cTnT and $cTnI_{(sgx)}$ showed stable values. At 18 hours after admission, the cTnT increased to 52 ng/L and the cTnI to 29 ng/L. Unfortunately, the coronary angiography failed because of difficult arterial access.

## Comparing Diagnostic Performance Between 0/1 Hour Rule-Out and Rule-In Algorithms

None of the algorithms ruled out any patient with NSTEMI, so that the sensitivity was 100% (Figure 3 and Table 2). The novel $cTnI_{(sgx)}$ 0/1 hour rule-out algorithm had a higher rule-out rate of patients without NSTEMI (higher specificity) and an overall higher AUC (Delong test, $P<0.001$) than comparators.

Concerning the rule-in algorithms, results were quite similar for all algorithms: a few patients without NSTEMI were ruled in (false positive), for a specificity of 95% to 97%. However, the $cTnT_{ESC}$ 0/1 hour algorithm showed a lower AUC (Delong test, $P<0.05$) compared with the cTnI algorithms because of a slightly higher number of patients with NSTEMI allocated to the observation group (lower sensitivity). More than 90% of NSTEMIs were ruled in using the cTnI algorithms, whereas 78% were ruled in by $cTnT_{ESC}$ (Table S6).

## Efficiency of the Algorithms

The novel $cTnI_{(sgx)}$ direct rule-out and 0/1 hour algorithms were more efficient than the comparators

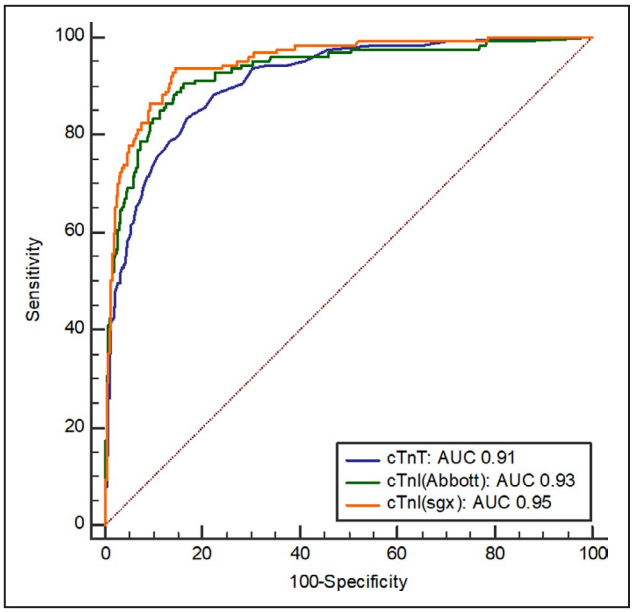


**Figure 2. AUC-ROC for admission troponin concentrations as a continuous variable in patients with NSTEMI vs patients with non-NSTEMI.**
AUC indicates area under the curve; $cTnI_{(Abbott)}$, cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; NSTEMI, non–ST-segment–elevation myocardial infarction; and ROC, receiver operating characteristic curve.

(Figure 3). The algorithm classified 37% of admitted patients as candidates for early discharge, compared with 31% with $cTnT_{ESC}$, 23% with $cTnI_{(Abbott)ESC}$, 24% with $cTnI_{(sgx)Body}$, and 9% with $cTnI_{(sgx)Neumann}$. When the early presenters were excluded for the ESC algorithms in accordance with the guideline, the direct rule-out rate dropped to 24% with $cTnT_{ESC}$ and 18% with $cTnI_{(Abbott)ESC}$ (Figure 3).

Concerning 0/1 hour serial sampling algorithms, the novel $cTnI_{(sgx)}$ rule-out algorithm would have suggested discharge for 77% of patients, compared with 64% with $cTnT_{ESC}$ and 57% with $cTnI_{(Abbott)ESC}$ (Table S4). Only 40% would be eligible for discharge if the $cTnI_{(sgx)Neumann}$ 0/1 hour algorithm were applied. Rule-in would be recommended for 13% to 17% (Table S5). Total efficiency values showed that the novel $cTnI_{(sgx)}$ 0/1 hour algorithms would allocate 92% (95% CI, 89%–94%) of the patients to either rule-out or rule-in. Corresponding numbers for the $cTnT_{ESC}$, $cTnI_{(Abbott)ESC}$, and $cTnI_{(Neumann)}$ 0/1 hour algorithms were 78% (95% CI, 74%–82%), 74% (95% CI, 70%–78%), and 56% (95% CI, 51%–60%), respectively.

## Long-Term Prognostic Value

A total of 82 events occurred among the 971 patients included in the admission sample cohort. Table S7 shows the number of end points stratified according to the different algorithms. With the exception of $cTnI_{(sgx)Neumann}$ (which allocated only 9% of patients to rule-out), the direct rule-out algorithms showed a significant ability to predict long-term end points. The discrimination power of the rule-out algorithms was confirmed in a Cox regression analysis (Table 3), after adjustment for well-established risk factors.

The 465 patients included in the 0/1 hour cohort experienced 32 events. Patients who were ruled out by the novel $cTnI_{(sgx)}$ 0/1 hour algorithm had an event rate of 5.3% (Table S7), which was not significantly different from the event rate in the observation and

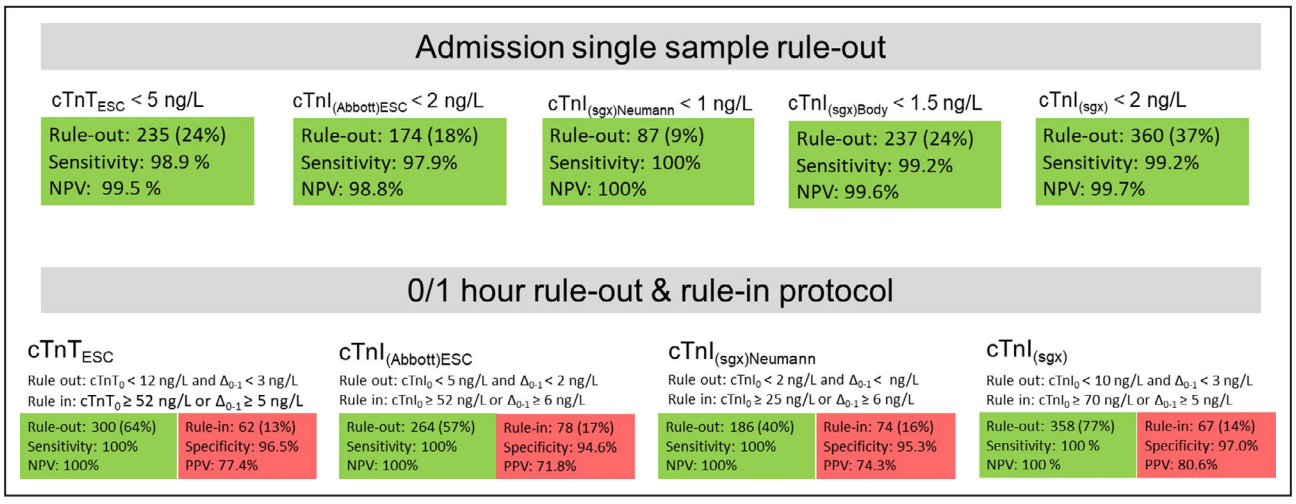


**Figure 3. Main diagnostic performance measures and efficiency of the different algorithms.**
Diagnostic performance of the admission ESC rule-out algorithm was calculated based on late presenters (n=772) because the ESC does not recommend direct rule-out until >3 hours after onset of symptoms. The cardiac troponin I from the Singulex Clarity System ($cTnI_{(sgx)}$) data are based on all participants (N=971). Efficiency was calculated as the percentage eligible for rule-out from the total cohort (all algorithms). $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; and NPV, negative predictive value.

**Table 3. Cox Regression Analysis**

| | Univariable | | Multivariable* | |
|---|---|---|---|---|
| | HR (95% CI) | *P* Value | HR (95% CI) | *P* Value |
| Direct rule-out (n=971) | | | | |
| $cTnT_{ESC}$ <5 ng/L vs observation and rule-in | 9.361 (3.423–25.583) | <0.001 | 3.421 (1.170–10.000) | 0.025 |
| $cTnI_{(Abbott)\ ESC}$ <2 ng/L vs observation and rule-in | 8.179 (2.582–25.909) | <0.001 | 3.050 (0.917–10.146) | 0.069 |
| $cTnI_{(sgx)Neumann}$ <1 ng/L vs observation and rule in | 8.296 (1.155–59.609) | 0.035 | 2.365 (0.318–17.583) | 0.400 |
| $cTnI_{(sgx)}$ <2 ng/L vs observation and rule-in | 7.769 (3.363–17.840) | <0.001 | 3.286 (1.359–7.946) | 0.008 |
| 0/1 h rule-out (n=465) | | | | |
| $cTnT_{ESC}$ <12 ng/L and $\Delta_{0-1}$ <3 ng/L vs observation and rule-in | 4.959 (2.294–10.718) | <0.001 | 3.190 (1.345–7.562) | 0.008 |
| $cTnI_{(Abbott)\ ESC}$ <5 ng/L and $\Delta_{0-1}$ <2 ng vs observation and rule-in | 3.456 (1.599–7.469) | 0.002 | 2.227 (0.972–5.105) | 0.058 |
| $cTnI_{(sgx)Neumann}$ <2 ng/L and $\Delta_{0-1}$ <1 ng/L, vs observation and rule in | 6.537 (1.991–21.461) | 0.002 | 3.671 (1.046–12.885) | 0.042 |
| $cTnI_{(sgx)}$ <10 ng/L and $\Delta_{0-1}$ <3 ng/L, vs observation and rule-in | 2.373 (1.172–4.805) | 0.016 | 1.603 (0.754–3.406) | 0.220 |

95% CI in brackets. Unadjusted and adjusted hazard ratios for the composite end point of future non-fatal myocardial infarction and all-cause mortality. Patients are dichotomized based on the admission sample algorithm, that is, rule-out (reference category) vs observation/rule-in. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; ESC, European Society of Cardiology; and HR, hazard ratio.

*Included in the multivariable model: algorithm as applicable, age, sex, current or previous smoking, estimated glomerular filtration rate above vs below 60 mL/min per 1.73 $m^2$, diabetes mellitus, hypertension, hyperlipidemia, and previous MI.

rule-in groups of 10% to 13% (Figure 4 and Table 3). In contrast, those allocated to rule-out by the ESC or the Neumann 0/1 hour algorithms had significantly lower event rates (1.6%–3.4%) compared with the corresponding observation and rule-in groups. Cox regression analysis results confirmed the discrimination power of rule-out by these algorithms (Table 3).

## DISCUSSION

This study yielded three main findings. First a cardiac injury marker with improved analytical sensitivity and precision beyond the current high-sensitivity assays could improve logistics and categorizing of patients investigated for NSTEMI. Second, the cost of this screening could be higher rates of rule-out of patients with increased long-term risk because of subclinical myocardial injury or CMI. Third, our data are in agreement with the proposed ESC algorithms but only partly correspond to 2 earlier reports for the $cTnI_{(sgx)}$ assay. This pattern highlights a need for robust validation before rule-in and rule-out algorithms are implemented for any particular assay.

Our data suggest that the improved analytical sensitivity of the $cTnI_{(sxg)}$ assay translates into a better "signal-to-noise ratio" compared with the other high sensitivity assays and reduces the time window required for reliably detecting myocardial injury. Body et al[14] reported similar outcomes using a slightly lower cutoff than ours (1.5 ng/L). The very low direct rule-out cutoff suggested by Neumann et al[15] was 100% sensitive but proved clinically unsuitable in our cohort because of the low number of patients eligible for rule-out (9%). Using a $cTnI_{(sgx)}$ cutoff of <2 ng/L would lead to allocation of more patients to direct rule-out than the comparator algorithms suggested by ESC and Body et al.[4,14,25,26]

We used a 0/1 rule-out algorithm that depended more on delta values and allowed for rule-out at high baseline concentrations (>99th percentile of the assay), without compromising sensitivity. This approach resulted in a highly specific algorithm, ruling out large numbers of patients without NSTEMI. It is noteworthy that the novel $cTnI_{(sgx)}$ 0/1 hour algorithm could allocate more than 90% of patients to either rule-out or rule-in, with a similar or higher diagnostic accuracy compared with the other algorithms.

The second important finding in our study is that the ability to predict long-term MI and all-cause mortality seems to depend on the algorithm used for rule-out. The direct rule-out algorithms that used low troponin concentrations as cutoffs showed an excellent prognostic ability. This finding was robust for the $cTnI_{(sgx)}$ and $cTnT_{ESC}$ algorithms after adjusting for well-known risk factors and borderline significant for $cTnI_{(Abbott)ESC}$.[11,27,28] That the direct rule-out according to Neumann et al did not predict long-term prognosis may be explained by the low rule-out frequency of 9%.

Concerning the 0/1 hour algorithms, our data suggest that the $cTnI_{(sgx)}$ algorithm could not predict long-term risk. This algorithm had twice as many end points compared with the others. The higher baseline concentration used for rule-out included more patients with subclinical myocardial injury (high-normal troponin concentrations) and even CMI,[5] which could explain this observation because these patients have increased long-term risk.[11,27,28] For all algorithms, we observed a similar event rate in patients allocated to observation and to rule-in. The prevalence of NSTEMI and CMI was

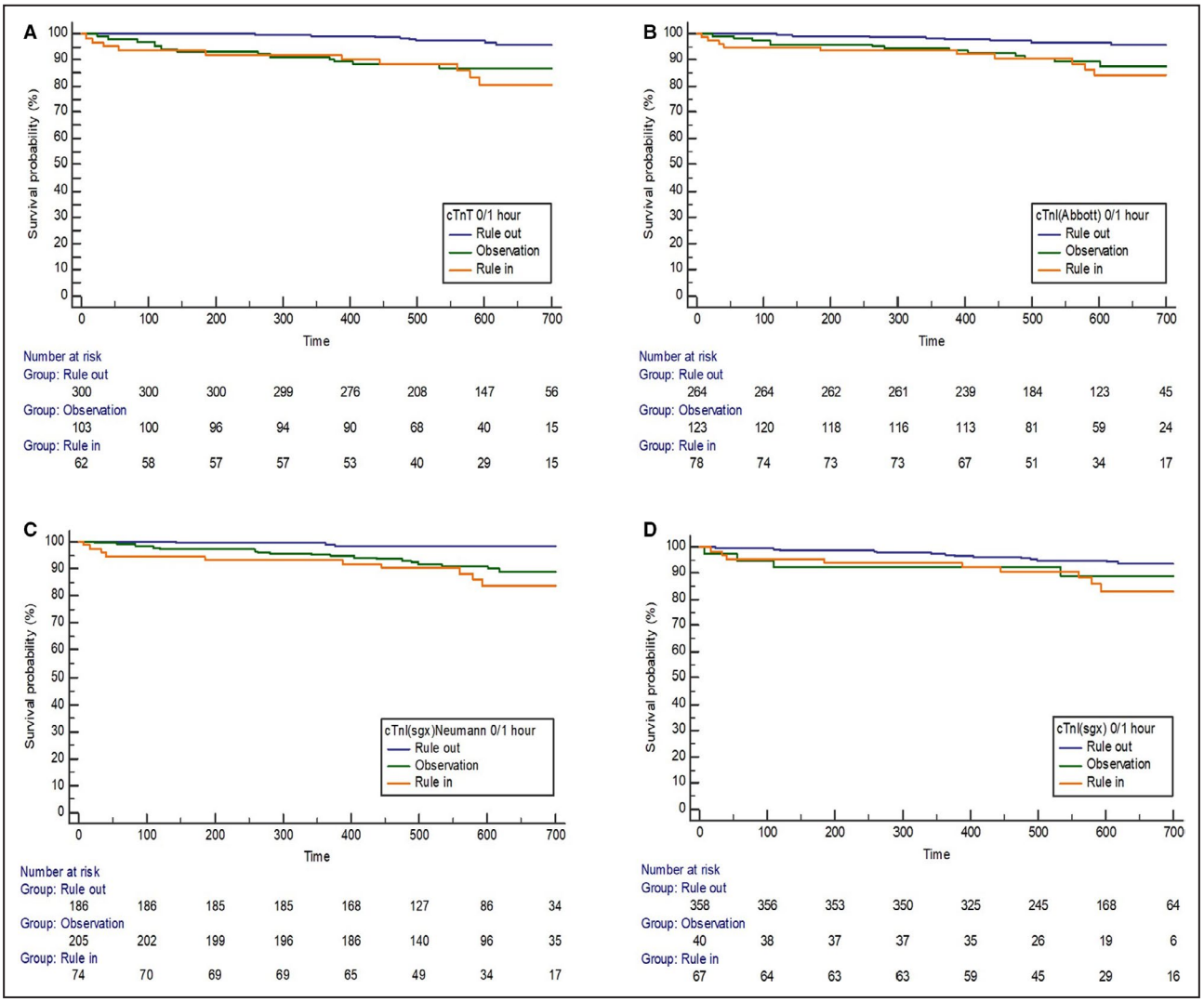


**Figure 4. Kaplan–Meier curves for the prognostic composite end point of future myocardial infarction (MI) and all-cause mortality stratified according to the algorithms.**
**A**, 0/1 hour $cTnT_{ESC}$. **B**, 0/1 hour $cTnI_{(Abbott)ESC}$. **C**, 0/1 hour $cTnT_{(sgx)Neumann}$. **D**, 0/1 hour $cTnI_{(sgx)}$. $cTnI_{(Abbott)}$ indicates cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$, cardiac troponin I (Singulex Clarity System); cTnT, cardiac troponin T; and ESC, European Society of Cardiology.

reciprocal in the two groups, with high NSTEMI frequency in the rule-in group and high CMI frequency in the observation group (Tables S6 and S7).[27,29] These findings highlight that NSTEMI and CMI are both serious conditions with increased long-term risk. Future studies should target identifying more accurate diagnostic and treatment options for patients with CMI.

The last important observation is the discrepancy between our findings and the data reported by Neumann et al.[15] The reason could be related to coincidence, as the cutoffs in both studies were based on the few patients with NSTEMI with low baseline concentration. Also, both studies were single center, and differences in health care systems could have affected the patient cohort that was recruited. The fact that Neumann et al also ruled out fewer patients for the $cTnI_{(ESC)}$ algorithm suggests that the cohorts likely were different. Furthermore, analytical issues such as reagent and calibrator lot variations are highly likely to influence the performance of cutoffs in the low range of an assay.[30] This assumption is strengthened by the observation that the diagnostic performance for the different rule-in algorithms showed better alignment, given that lot-to-lot differences are usually less prominent at higher cTn concentrations. Our data demonstrate the need for large sample sets, validation in several different patient cohorts, and knowledge about long-term analytical performance before rule-in and rule-out algorithms are implemented into practice.

An obvious strength of our study is the comparison of the $cTnI_{(sgx)}$ algorithm to well-validated algorithms

from ESC, including cTnT and another cTnI assay. Other strengths are a long observation time during the index hospitalization, during which the patients were observed for at least 8 hours, ensuring the validity of the adjudicated diagnosis. The study closely mirrored clinical practice by not excluding patients with end-stage renal disease or with more than a 12-hour history of symptoms suggestive of ACS. The last strength is a long follow-up period registering end points after the index NSTEMI/hospitalization, allowing for prognostication.

## Study Limitations

The main limitation of the study is that the $cTnI_{(sgx)}$ assay currently is no longer available on the market because of bankruptcy. The baseline concentrations used in the rule-out and rule-in algorithms were chosen based on sensitivity, specificity, and efficiency for diagnosing NSTEMI, and more studies are necessary to confirm those concentrations that can indicate subclinical myocardial injury and increased long-term risk. The suggested algorithms should therefore be taken as an example of possibilities and limitations that might be expected from high-precision cardiac injury markers with measurable concentrations in almost all healthy participants. Other limitations are the relatively low number of patients used for development of the 0/1 hour algorithm, the single-center inclusion, the lack of a validation cohort, and the relatively low number of early presenters. As we have noted, our data should be seen as hypothesis-generating and as offering examples, and all new high-sensitivity biomarkers and algorithms need extensive validation in multiple cohorts before they can be ready for clinical use. Another limitation is that cTnT was used as part of the adjudication process. This use could have introduced a positive bias for the cTnT algorithms and underestimation of the performance of cTnI algorithms. Finally, this study involved a long inclusion period, which is a common problem in similar studies; however, the broad inclusion criteria should ensure a broad and representative inclusion. In addition, the NSTEMI rate and patient characteristics in this cohort are similar to those from comparable studies.[31,32]

# CONCLUSIONS

In this study, we show that cardiac injury markers with improved analytical performance may improve emergency department efficiency by ruling out and ultimately categorizing more patients compared with current recommendations and algorithms. Our data indicate that patients with increased baseline troponin concentrations who might suffer from subclinical myocardial injury should not be deemed low risk even if they are ruled out for NSTEMI. Future studies should aim at simultaneous development of dedicated algorithms identifying both patients with NSTEMI and those with increased long-term risk. Our final observation is that the intercohort variability in algorithm performance should not be underestimated, and validation including several different cohorts and clinical settings is necessary for all suggested emergency department algorithms.

# ARTICLE INFORMATION

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## Disclosures

Aakre has served on one advisory board for Roche Diagnostics and received speaker's honoraria from Siemens Healthineers. Torbjørn Omland has served on advisory boards for Abbott Diagnostics, Roche Diagnostics, and Novartis and has received research support from AstraZeneca, Abbott Diagnostics, Roche Diagnostics, ThermoFisher, Singulex and Biomedica via Akershus University Hospital, and speaker's honoraria from Roche Diagnostics and Novartis. Skadberg has received lecture fees from Abbott Diagnostics. The remaining authors have no disclosures to report.

## Supplementary Material

**Data S1**
**Tables S1–S7**
**References 33–40**

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# SUPPLEMENTAL MATERIAL

**Table S1. Baseline characteristics for the total cohort.**

| **Baseline characteristics** | **Total N=971** | **NSTEMI 127 (13.1)** | **UAP 111 (11.4)** | **Other diseases 153 (15.7)** | **NCCP 580 (59.7)** | **p-value** |
|---|---|---|---|---|---|---|
| Age, years | 63 (52.0-74.0) | 70 (57.0-79.0) | 69 (62.0-77.0) | 70 (58.0-80.0) | 59.0 (49.0-70.0) | <0.001 |
| Male | 588 (60.6) | 87 (68.5) | 83 (74.8) | 91 (59.5) | 327 (56.4) | <0.001 |
| Hours from symptom onset to first troponin sample | 8.0 (3.4-46.2) | 5.2 (2.8-25.4) | 14.6 (5.5-81.6) | 8.5 (3.5-47.7) | 8.0 (3.3-45.7) | <0.001 |
| Early presenters < 3 hours | N=199 (20.5) | N=34 (27) | N=17(15.3) | 29 (18.9) | 119 (20.5) | 0.163 |
| Percentage of patients observed > 8 hours | N=941 (96.8) | N=127 (100) | N=110(99.1) | 141(92.2) | 563 (97.1) | <0.001 |
| **Risk factors*** | | | | | | |
| Hypertension | 403 (41.5) | 62 (48.8) | 60 (54.1) | 64 (41.8) | 217 (37.4) | 0.003 |
| Diabetes mellitus | 120 (12.4) | 22 (17.3) | 28 (25.2) | 16 (10.5) | 54 (9.3) | <0.001 |
| Current smoker | 202 (20.8) | 23 (18.1) | 20 (18.0) | 33 (21.6) | 124 (21.4) | 0.89 |
| Previous smoking | 410 (42.2) | 69 (54.3) | 59 (53.2) | 59 (38.6) | 219 (37.8) | 0.001 |
| **History** | | | | | | |
| Previous MI | 203 (20.9) | 33 (26) | 43 (38.7) | 28 (24.3) | 96 (16.6) | <0.001 |
| Previous PCI | 204 (21.0) | 29 (22.8) | 52 (46.8) | 26 (17.0) | 97 (16.7) | <0.001 |
| Previous CABG | 81 (8.3) | 17 (13.4) | 28 (25.2) | 11 (7.2) | 25 (4.3) | <0.001 |
| Previous heart failure | 46 (4.7) | 8 (6.3) | 6 (5.4) | 14 (9.2) | 18 (3.1) | 0.013 |
| **Medication** | | | | | | |
| Statins/other lipidlowering | 385 (39.6) | 48 (37.8) | 71 (64.0) | 61 (39.9) | 205 (35.3) | <0.001 |
| Diuretics | 176 (18.1) | 23 (18.1) | 30 (27.0) | 38 (24.8) | 85 (14.7) | 0.002 |
| ACE inhibitor/ARB | 326 (33.6) | 46 (36.2) | 50 (45.0) | 56 (36.6) | 174 (30.0) | 0.012 |
| Beta-blocker | 336 (34.6) | 45 (35.4) | 59 (53.2) | 68 (44.4) | 164 (28.3) | <0.001 |
| Aspirin | 340 (35.0) | 54 (42.5) | 70 (63.1) | 106 (47) | 169 (29.1) | <0.001 |
| Oral Anticoagulant | 118 (12.2) | 12 (9.4) | 13 (11.7) | 39 (25.5) | 54 (9.3) | <0.001 |
| Antithrombotic agents | 71 (7.3) | 7 (5.5) | 22 (19.8) | 9 (5.9) | 33 (5.7) | <0.001 |
| **Baseline measurements** | | | | | | |
| BMI, kg/m$^2$ (n=454) | 26.4 (24.2-29.7) | 25.9 (24.1-28.6) | 25.8 (24.5-29.6) | 27.2 (25.7-29.3) | 26.3 (24.1-29.8) | 0.337 |
| HEART score | 4.0 (3.0-5.0) | 6.0 (5.0-7.0) | 5.0 (4.0-6.0) | 4.0 (2.5-6.0) | 3.0 (2.0-5.0) | <0.001 |
| **Baseline biomarkers** | | | | | | |
| Glucose, mmol/L | 5.8 (5.3-6.7) | 6.5 (5.8-8.0) | 5.9 (5.4-6.7) | 6.1 (5.5-7.3) | 5.6 (5.2-6.4) | <0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| eGFR, ml/min/1.73m$^2$ | 85.2 (70.2-97.1) | 79.6 (62.8-92.3) | 77.7 (64.8-91.4) | 74.3 (58.0-91.6) | 88.4 (75.9-100.1) | <0.001 |
| cTnT, ng/L | 7.0 (3.0-18.0) | 47 (23.0-168.0) | 9 (5.0-18.0) | 13 (5.5-24.0) | 5 (3.0-9.0) | <0.001 |
| cTnI, ng/L | 4.0 (2.1-11.2) | 117.7 (26.1-570.9) | 4.7 (3.1-10.0) | 8.0 (3.2-18.0) | 2.7 (1.7-5.2) | <0.001 |
| $cTnI_{(sgx)}$, ng/L | 2.8 (1.5-7.7) | 91.0 (23.1-487.7) | 3.4 (1.9-7.3) | 4.8 (2.1-12.2) | 2.0 (1.3-3.6) | <0.001 |
| **ECG findings** | | | | | | |
| ST depression | 33 (3.4) | 17 (13.4) | 3(2.7) | 7 (4.6) | 6 (1.0) | <0.001 |
| ST elevation | 15 (1.5) | 2( 1.6) | 0 | 7 (4.6) | 6 (1.0) | <0.001 |
| T-wave inversion | 30 (3.1) | 10 (7.9) | 6 (5.4) | 4 (2.6) | 10 (1.7) | <0.001 |

Values are n (%) or median (interquartile range).

ACE=angiotensin-converting enzyme; ARB=angiotensin receptor blocker; BMI=body mass index; cTnI=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; CABG=coronary artery bypass graft; eGFR=estimated glomerular filtration rate; HEART score=acronym for History, ECG=electrocardiogram, A=age, R=risk factors, T=troponin; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; MI=myocardial infarction; PCI=percutaneous coronary intervention; UAP=unstable angina pectoris.

*Hypercholesterolemia is defined as treatment with lipid-lowering drugs.

**Table S2. Baseline characteristics for the 0/1 hour cohort (n=465).**

| | **Total N=465** | **NSTEMI 61 (13.1)** | **UAP 56 (12.0)** | **Other diseases 66 (14.2)** | **NCCP 282 (60.6)** | **p-value** |
|---|---|---|---|---|---|---|
| Age, years | 61 (51.0-71.0) | 67 (56.5.0-78.0) | 68 (62.0-72.8) | 70 (58.8-80.0) | 57.0 (49.0-67.00) | <0.001 |
| Male | 278 (59.8) | 43 (70.5) | 47 (83.9) | 33 (50.0) | 278 (59.8) | <0.001 |
| Hours from symptom onset to first troponin sample | 8.8 (3.5-49.1) | 5.2 (2.8-27.6) | 25.4 (7.2-173.4) | 5.8 (3.3-26.5) | 8.8 (3.7-47.9) | <0.001 |
| **Risk factors*** | | | | | | |
| Hypertension | 199 (43.1.5) | 28 (45.9) | 28 (50.0) | 33 (51.6) | 110 (39.1) | 0.173 |
| Diabetes mellitus | 51 (11.0) | 7 (11.5) | 16 (28.6) | 9 (13.6) | 19 (6.7) | <0.001 |
| Current smoker | 110 (23.7) | 8 (13.1) | 13 (23.2) | 15 (22.7) | 74 (26.3) | 0.61 |
| Previous smoking | 189 (40.7) | 34 (55.7) | 28 (50.0) | 31 (47.0) | 96 (34.2) | 0.018 |
| Previous PCI | 83 (17.8) | 12 (19.7) | 25 (44.6) | 8 (12.1) | 38(13.5) | <0.001 |
| Previous CABG | 32 (6.9) | 9 (14.8) | 10 (17.9) | 5 (7.6) | 8 (2.8) | <0.001 |
| Previous heart failure | 14 (3.0) | 2 (3.3) | 3 (5.4) | 4 (6.1) | 5 (1.8) | 0.170 |
| **Medication** | | | | | | |
| Statins/other lipidlowering | 171 (36.8) | 25 (41.0) | 33 (58.9) | 29 (43.9) | 84 (29.8) | <0.001 |
| Diuretics | 81 (17.4) | 9 (14.8) | 15 (26.8) | 15 (22.7) | 42 (14.9) | 0.100 |
| ACE inhibitor/ARB | 165 (35.5) | 19 (31.1) | 24 (42.9) | 29 (43.9) | 93 (33.0) | 0.198 |
| Beta-blocker | 147 (31.6) | 21 (34.4) | 25 (44.6) | 33 (50.0) | 68 (24.1) | <0.001 |
| Aspirin | 154 (33.1) | 28 (45.9) | 37 (66.1) | 22 (33.3) | 67 (23.8) | <0.001 |
| Oral Anticoagulant | 41 (8.8) | 4 (6.6) | 3 (5.4) | 13 (19.7) | 21 (7.4) | <0.022 |
| Antithrombotic agents | 31 (6.7) | 3 (5.0) | 9 (16.1) | 4 (6.2) | 15 (5.4) | <0.057 |
| **Baseline measurements** | | | | | | |
| BMI, kg/m$^2$ (n=231) | 26.4 (24.2-29.7) | 25.9 (24.1-28.6) | 25.8 (24.5-29.6) | 26.3 (24.1-29.8) | 27.2 (25.7-29.3) | 0.337 |
| HEART score | 4.0 (2.0-5.0) | 6.0 (5.0-7.0) | 5.0 (4.0-6.0) | 4.0 (3.0-5.0) | 3.0 (2.0-4.0) | <0.001 |
| Glucose, mmol/L | 5.8 (5.3-6.6) | 6.3 (5.7-7.6) | 6.1 (5.4-7.6) | 6.2 (5.7.5) | 5.6 (5.2-6.2) | <0.001 |
| eGFR, ml/min/1.73m$^2$ | 87.9 (72.4-98.6) | 86.3 (71.6-97.0) | 83.1 (69.9-94.8) | 74.7 (58.0-93.9) | 90.5 (76.1-101.2) | <0.001 |
| cTnT, ng/L | 7.0 (3.0-16.0) | 49.0 (21.5-185.0) | 8.5 (5.0-19.5) | 12 (7.0-20.5) | 5 (3.0-8.0) | <0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| $cTnI_{(Abbott)}$, ng/L | 3.8 (2.0-10.1) | 144.5 (27.1-549.2) | 3.9 (2.7-9.9) | 8.1 (3.3-14.9) | 2.7 (1.7-4.6) | <0.001 |
| $cTnI_{(sgx)}$, ng/L | 2.6 (1.4-6.8) | 105.9 (28.4-501.7) | 2.6(1.8-7.0) | 4.6 (2.0-10.8) | 1.8 (1.2-3.1) | <0.001 |
| **ECG findings** | | | | | | |
| ST depression | 10 (2.2) | 6 (9.8) | 1 (1.8) | 1 (1.5) | 2 (0.7) | <0.001 |
| ST elevation | 3 (0.6) | 2 (3.3) | 0 (0.0) | 1 (1.5) | 0 (0.0) | <0.001 |
| T-wave inversion | 13 (2.8) | 5 (8.2) | 4 (7.1) | 0 | 4 (1.4) | <0.001 |

Values are n (%) or median (interquartile range).

ACE=angiotensin-converting enzyme; ARB=angiotensin receptor blockers; BMI=body mass index; cTnI=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; CABG=coronary artery bypass graft; eGFR=estimated glomerular filtration rate; HEART score=acronym for History, ECG=electrocardiogram, A=age, R=risk factors, T=troponin; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; MI=myocardial infarction; PCI=percutaneous coronary intervention; UAP=unstable angina pectoris.

*Hypercholesterolemia is defined as treatment with lipid-lowering drugs.

**Table S3. Number of patients ruled out using different protocols.**

| | NSTEMI | UAP | Non-ACS cardiac disease | Non-cardiac chest pain | Other diseases | Total |
|---|---|---|---|---|---|---|
| **Rule-out, total cohort** | **N=127** | **N=111** | **N=73** | **N=580** | **N=80** | **N=971** |
| $cTnT_{ESC}$ < 5 ng/L | 2 (2) | 20 (18) | 6 (8) | 247 (43) | 23 (29) | 298 (31) |
| $cTnI_{(Abbott)ESC}$ < 2 ng/L | 3 (2) | 11 (10) | 3 (4) | 192 (33) | 13 (16) | 222 (23) |
| $cTnI_{(sgx)Neumann}$ < 1.0 ng/L | 0 | 5 (4.5) | 0 | 76 (13.1) | 6 (7.5) | 87 (9.0) |
| $cTnI_{(sgx)Body}$ < 1.5 ng/L | 1 (1) | 15 (14) | 2 (3.0) | 203 (35) | 16 (20) | 237 (24) |
| $cTnI_{(sgx)}$ < 2 ng/L | 1 (1) | 31 (28) | 6 (8) | 293 (51) | 29 (36) | 360 (37) |
| **Rule-out, total cohort (all early presenters were automatically ruled-in)** | **N=127** | **N=111** | **N=73** | **N=580** | **N=80** | **N=971** |
| $cTnT_{ESC}$ < 5 ng/L | 1 (1) | 16 (14) | 3 (4) | 194 (33) | 21 (26) | 235 (24) |
| $cTnI_{(Abbott)ESC}$ < 2 ng/L | 2 (1.5) | 9 (8) | 2 (3) | 149 (26) | 12 (15) | 174 (18) |
| $cTnI_{(sgx)Neumann}$ < 1.0 ng/L | 0 | 3 (3) | 0 | 54 (12) | 5 (6) | 62 (6) |
| $cTnI_{(sgx)Body}$ < 1.5 ng/L | 1 (1) | 12 (11) | 1 (1) | 159 (27) | 15 (19) | 188 (19) |
| $cTnI_{(sgx)}$ < 2 ng/L | 1 (1) | 24 (22) | 4 (5) | 231 (40) | 26 (33) | 286 (29) |
| **Rule-out, late presenters only ( ≥ 3 hours)** | **N=93** | **N=94** | **N=55** | **N=461** | **N=69** | **N=772** |
| $cTnT_{ESC}$ < 5 ng/L | 1 (1) | 16 (17) | 3 (6) | 194 (42) | 21 (30) | 235 (30) |
| $cTnI_{(Abbott)ESC}$ < 2 ng/L | 2 (2) | 9 (10) | 2 (4) | 149 (32) | 12 (17) | 174 (23) |
| $cTnI_{(sgx)Neumann}$ < 1.0 ng/L | 0 | 3 (3.2) | 0 | 54 (12) | 5 (7) | 62 (8) |
| $cTnI_{(sgx)Body}$ < 1.5 ng/L | 1 (1) | 12 (13) | 1 (2) | 159 (35) | 15 (22) | 188 (24) |
| $cTnI_{(sgx)}$ < 2 ng/L | 1 (1) | 24 (26) | 4 (7) | 231 (50) | 26 (38) | 286 (37) |

Percentages in brackets.

The upper panel shows the number of patients who would be ruled out if all patients (independent of time between symptom onset and testing) were included.

Middle panel shows number of rule-outs in the total cohort when all early presenters were directly transformed to serial sampling, and the last panel shows the number of rule-outs in late presenters only (used for calculation of diagnostic performance in late presenters; see Table 1, main text).

ACS=acute coronary syndrome; $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; UAP=unstable angina pectoris.

**Table S4. Number of patients ruled in using 0/1 hour protocols.**

| | NSTEMI | UAP | Non-ACS cardiac disease | Non-cardiac chest pain | Other diseases | Total |
|---|---|---|---|---|---|---|
| **0/1 hour rule-out** | **N=61** | **N=56** | **N=30** | **N=282** | **N=36** | **N=465** |
| **Evaluation of $cTn_{ESC}$ and Neumann algorithms:** | | | | | | |
| $cTnT_{ESC}$ < 12 ng/L and $\Delta_{0\text{-}1}$ < 3 ng/L | 0 | 33 (59) | 8 (31) | 237 (84) | 22 (61) | 300 (64) |
| $cTnI_{(Abbott)ESC}$ < 5 ng/L and $\Delta_{0\text{-}1}$ < 2 ng/L | 0 | 32 (57) | 6 (20) | 212 (75) | 14 (39) | 264 (57) |
| $cTnI_{(sgx)Neumann}$ < 2.0 and $\Delta_{0\text{-}1}$ < 1 ng/L | 0 | 20 (36) | 2 (7) | 152 (54) | 12 (33) | 186 (40) |
| **Evaluation of $cTnI_{(sgx)}$ baseline and delta values combined:** | | | | | | |
| $cTnI_{(sgx)}$ < 4.0 and $\Delta_{0\text{-}1}$ < 3 ng/L | 0 | 35 (64) | 8 (27) | 230 (82) | 19 (53) | 293 (63) |
| $cTnI_{(sgx)}$ < 6.0 and $\Delta_{0\text{-}1}$ < 3 ng/L | 0 | 41 (73) | 11 (37) | 256 (91) | 25 (69) | 333 (71) |
| $cTnI_{(sgx)}$ < 8.0 and $\Delta_{0\text{-}1}$ < 3 ng/L | 0 | 44 (79) | 14 (47) | 264 (94) | 28 (78) | 350 (75) |
| $cTnI_{(sgx)}$ < 8.67 and $\Delta_{0\text{-}1}$ < 3 ng/L | 0 | 44 (79) | 15 (50) | 264 (94) | 29 (81) | 352 (76) |
| **$cTnI_{(sgx)}$ < 10.0 and $\Delta_{0\text{-}1}$ < 3 ng/L** | **0** | **45 (80)** | **16 (53)** | **259 (95)** | **39 (87)** | **358 (77)** |
| $cTnI_{(sgx)}$ < 12.0 and $\Delta_{0\text{-}1}$ < 3 ng/L | 1 (2) | 46 (82) | 19 (63) | 268 (95) | 31 (86) | 365 (78) |

Percentages in brackets.

ESC protocols for cTnT and $cTnI_{(Abbott)}$ and different protocols for $cTnI_{(sgx)}$.

ACS=acute coronary syndrome; $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; UAP=unstable angina pectoris.

**Table S5. Number of patients ruled in using 0/1 hour protocols.**

| | NSTEMI | UAP | Non-ACS cardiac disease | Non-cardiac chest pain | Other diseases | Total |
|---|---|---|---|---|---|---|
| **0/1 hour rule-in** | **N=61** | **N=56** | **N=30** | **N=282** | **N=36** | **N=465** |
| $cTnT_{ESC} \geq 52$ ng/L or $\Delta_{0\text{-}1} \geq 5$ ng/L | 48 (79) | 2 (4) | 8 (27) | 2 (1) | 2 (6) | 62 (13) |
| $cTnI_{(Abbott)ESC} \geq 52$ ng/L or $\Delta_{0\text{-}1} \geq 6$ ng/L | 56 (92) | 6 (11) | 9 (30) | 5 (2) | 2 (6) | 78 (17) |
| $cTnI_{(sgx)Neumann} \geq 25.0$ ng/L or $\Delta_{0\text{-}1} \geq 6$ ng/L | 55 (90) | 5 (9) | 7 (23) | 5 (2) | 2 (5) | 74 (16) |
| **Evaluation of $cTnI_{(sgx)}$ baseline and delta values combined:** | | | | | | |
| $cTnI_{(sgx)} \geq 8.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 61 (100) | 12 (21) | 16 (53) | 20 (7) | 8 (22) | 117 (25) |
| $cTnI_{(sgx)} \geq 10.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 61 (100) | 12 (21) | 16 (54) | 20 (7) | 8 (22) | 117 (25) |
| $cTnI_{(sgx)} \geq 12.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 60 (98) | 10 (18) | 11 (37) | 16 (6) | 5 (14) | 102 (22) |
| $cTnI_{(sgx)} \geq 14.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 58 (95) | 10 (18) | 10 (33) | 13 (5) | 4 (11) | 95 (20) |
| $cTnI_{(sgx)} \geq 18.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 58 (95) | 9 (16) | 7 (23) | 10 (4) | 3 (8) | 86 (19) |
| $cTnI_{(sgx)} \geq 20.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 58 (95) | 8 (14) | 7 (23) | 10 (4) | 3 (8) | 86 (19) |
| $cTnI_{(sgx)} \geq 30.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 58 (95) | 6 (11) | 7 (23) | 8 (3) | 2 (6) | 81 (17) |
| $cTnI_{(sgx)} \geq 40.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 6 (11) | 7 (23) | 7 (3) | 2 (6) | 79 (17) |
| $cTnI_{(sgx)} \geq 50.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 6 (11) | 7 (23) | 7 (3) | 2 (6) | 79 (17) |
| $cTnI_{(sgx)} \geq 60.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 6 (11) | 7 (23) | 7 (3) | 2 (6) | 79 (17) |
| $cTnI_{(sgx)} \geq 70.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 6 (11) | 7 (23) | 7 (3) | 2 (6) | 78 (17) |
| $cTnI_{(sgx)} \geq 80.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 4 (7) | 6 (20) | 4 (1) | 2 (6) | 73 (16) |
| $cTnI_{(sgx)} \geq 90.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 4 (7) | 6 (20) | 4 (1) | 2 (6) | 73 (16) |
| $cTnI_{(sgx)} \geq 100.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 3 (5) | 6 (20) | 4 (1) | 2 (6) | 72 (16) |
| $cTnI_{(sgx)} \geq 150.0$ or $\Delta_{0\text{-}1} \geq 3$ ng/L | 57 (93) | 2 (4) | 6 (20) | 4 (19 | 2 (6) | 71 (15) |
| | | | | | | |
| $cTnI_{(sgx)} \geq 10.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L | 60 (98) | 11 (20) | 14 (47) | 13 (5) | 5 (14) | 103 (22) |
| $cTnI_{(sgx)} \geq 20.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L | 57 (93) | 6 (11) | 7 (23) | 5 (2) | 3 (8) | 78 (17) |
| **$cTnI_{(sgx)} \geq 30.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L** | **57 (93)** | **3 (5)** | **7 (23)** | **3 (1)** | **2 (6)** | **72 (16)** |
| $cTnI_{(sgx)} \geq 40.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L | 55 (90) | 3 (5) | 7 (23) | 2 (1) | 1 (3) | 68 (15) |
| $cTnI_{(sgx)} \geq 50.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L | 55 (90) | 3 (5) | 7 (23) | 2 (1) | 1 (3) | 68 (15) |
| $cTnI_{(sgx)} \geq 60.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L | 55 (90) | 3 (5) | 7 (23) | 2 (1) | 1 (3) | 68 (15) |
| **$cTnI_{(sgx)} \geq 70.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L** | **55 (90)** | **3 (5)** | **6 (20)** | **2 (1)** | **1 (3)** | **67 (14)** |

| | | | | | | |
|---|---|---|---|---|---|---|
| $cTnI_{(sgx)} \geq 80.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 90.0$ or $\Delta_{0\text{-}1} \geq 5$ ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 100.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 110.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 120.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 130.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 140.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 150.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |
| $cTnI_{(sgx)} \geq 250.0$ or $\Delta_{0\text{-}1} \geq$ 5 ng/L | 55 (90) | 3 (5) | 6 (20) | 2 (1) | 1 (3) | 67 (14) |

Percentages in brackets.

ACS=acute coronary syndrome; $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NCCP=non-cardiac chest pain; NSTEMI=non–ST-elevation myocardial infarction; UAP=unstable angina pectoris.

ESC protocols for TnT and $cTnI_{(Abbott)}$ and different protocols for $cTnI_{(sgx)}$.

**Table S6. Allocation of NSTEMI patients.**

| Rule-out / rule-in protocol | Rule-out | Observation | Rule-in |
|---|---|---|---|
| $cTnT_{ESC}$ 0/1 hour | 0 | 13 (21.3) | 48 (78.7) |
| $cTnI_{(Abbott)ESC}$ 0/1 hour | 0 | 5 (8.2) | 56 (91.8) |
| $cTnI_{(sgx)Neumann}$ 0/1 hour | 0 | 6 (9.8) | 55 (90.2) |
| **$cTnI_{(sgx)}$ 0/1 hour** | **0** | **6 (9.8)** | **55 (90.2)** |

Percentages in brackets.

The table shows the category to which the different 0/1 hour rule-out and rule-in protocols would allocate patients who were finally diagnosed with an index NSTEMI (n=61). $cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; ESC=European Society of Cardiology; NSTEMI=non–ST-elevation myocardial infarction.

**Table S7. Prevalence of events stratified according to protocol classification.**

| | None-fatal MI and all-cause mortality |
|---|---|
| Direct rule-out | N=82 |
| Rule out | |
| cTnT < 5 ng/L | 4 (1.4) |
| $cTnI_{(Abbott)}$ < 2 ng/L | 3 (1.4) |
| $cTnI_{(sgx)Neumann}$ < 1 ng/L | 1 (1.2) |
| **$cTnI_{(sgx)}$ < 2 ng/L** | **6 (1.7)** |
| | |
| Observation/rule in | |
| cTnT | 78 (11.6) |
| $cTnI_{(Abbott)}$ | 79 (10.6) |
| $cTnI_{(sgx)Neumann}$ | 81 (9.2) |
| **$cTnI_{(sgx)}$** | **76 (12.4)** |
| 0/1 hour protocol | N=32 |
| Rule-out | |
| cTnT < 12 ng/L and $\Delta_{0-1}$ < 3 ng/L | 9 (3.0) |
| $cTnI_{(Abbott)}$ < 5 ng/L and $\Delta_{0-1}$ < 2 ng/ | 9 (3.4) |
| $cTnI_{(sgx)Neumann}$ < 2 and $\Delta_{0-1}$ < 1 ng/L | 3 (1.6) |
| **$cTnI_{(sgx)}$ < 10 and $\Delta_{0-1}$ < 3 ng/L** | **19 (5.3)** |
| | |
| Observation | |
| cTnT | 13 (12.6) |
| $cTnI_{(Abbott)}$ | 13 (10.6) |
| $cTnI_{(sgx)Neumann}$ | 19 (9.3) |
| **$cTnI_{(sgx)}$** | **4 (10.0)** |
| | |
| Rule-in | |
| cTnT ≥52 ng/L or $\Delta_{0-1} \geq$ 5 ng/L | 10 (16.1) |
| $cTnI_{(Abbott)} \geq$ 52 ng/L or $\Delta_{0-1} \geq$ 6 ng/L | 10 (12.8) |
| $cTnI_{(sgx)} \geq$ 25.0 or $\Delta_{0-1} \geq$ 6 ng/L | 10 (13.5) |
| **$cTnI_{(sgx)} \geq$ 70.0 or $\Delta_{0-1} \geq$ 5 ng/L** | **9 (13.4)** |

Percentages in brackets.

$cTnI_{(Abbott)}$=cardiac troponin I (Abbott Diagnostics); $cTnI_{(sgx)}$=cardiac troponin I (Singulex Clarity system); cTnT=cardiac troponin T; MI=myocardial infarction.