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Keywords:	Troponin, Acute Coronary Syndrome, Clinical Investigation, Laboratory Methods and Tools

Diagnostic performance of novel troponin algorithms for the rule-out of non-ST-elevation acute coronary syndrome

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Troponin algorithms for rule-out of NSTE-ACS

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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: Non-coronary chest pain

ED: Emergency Department

MACE: Major cardiovascular events

RCV: Reference change value

CV_A: Coefficient of variation

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration

ECG: Electrocardiogram

CI: Confidence interval

Abstract

Background: The European Society of Cardiology (ESC) rule-out algorithms use cut-offs optimised 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: 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 d) 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) 0-1 and 0-3 h algorithms.

Results: The prevalence of NSTE-ACS was 24.8%, 60.0% had non-cardiac 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% versus 63%, and hs-cTnI: 87% versus 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.

Keywords: Troponin, Acute Coronary Syndrome, Clinical Investigation, Laboratory Methods and Tools

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 work-load 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 two important characteristics found in healthy individuals: 1) normal baseline troponin concentrations a few hours after symptom onset, and 2) low delta values after 1 h 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 imaging for identification of UAP *(6)*, and the diagnostic work-flow of this group is debated *(11, 12)*.

The cut offs 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 non-necrotic ischemia and are in a clinically unstable situation, show larger variation in hs-cTn concentrations compared to patients with non-cardiac 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 if 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 if 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 earlier *(15, 20)*. The current article reports data from the WESTCOR derivation and validation cohorts (as pre-specified 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. STEMI patients were excluded. Included patients were ≥18 y, 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 full blood drawn into serum tubes (Greiner Bio-one, Austria) on arrival and after 3 and 8–12 h as part of routine clinical care. Samples coagulated for 30-60 minutes and were centrifuged at 2200 G for 10 minutes. 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 degrees Celsius. 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 online Supplemental Methods file. 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 d after hospitalization or urgent (within 24 h after admission) revascularization. The adjudicating process *(15, 20)* was undertaken by two independent cardiologists (definitions provided in the Supplemental Methods file) 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 cut offs for admission samples *(24)*, and are associated with low long-term risk of MACE *(15, 25-27)*. 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 *(28-30)*. 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, 31)*. 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, 32)*.

Furthermore, from a clinical point of view the optimal novel rule-out algorithms should have: 1) clinical sensitivity for NSTE-ACS of ≥ 95.0% and ≥ 99% for the secondary endpoint *(33)*, and the maximum possible specificity. The cut off 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 pre-specified 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 non-parametric 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 cut offs 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 two 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 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 d 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 shown for patients with UAP who were rule-out by the ESC or the novel 0-3 h algorithm, differences were tested using McNemar test.

We used SPSS Statistics 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 like pneumonia or cholecystitis and other cardiac diseases like atrial fibrillation or heart-failure. Median age was 63 y, and 60% were males. 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 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, one and three hour absolute delta values

Table 2 shows troponin concentrations at baseline, and the absolute delta values at 1 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 latter lot returning lower concentrations.

The patients with UAP had significantly (p-value < 0.001) higher baseline hs-cTnT and hs-cTnI concentrations (see **Table 2)** and delta values compared to the patients with NCCP, see **Figure 1 and Supplemental Table 3**.

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 hour and 0-3 hour algorithms had clinical sensitivities of 95.4% and 97.5%, respectively, compared to the significantly lower 62.8% for the ESC 0-1 hour algorithm (p-value <0.001). This was at the expense of significantly lower clinical specificity (p-value <0.001), the algorithms showed up to 4.2 times reduction in rule-out rate compared to the ESC 0-1 hour 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 hour algorithm) and 87.6% (0-3 hour 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 hour hs-TnI algorithm had a significantly lower clinical sensitivity of 63.9% (p-value <0.001). Also here, 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 d 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 4A**), and the novel 0-3 hour 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 hour algorithms showed a 2-6 times reduction in rule-out rate compared to the ESC 0-1 hour algorithms. The novel algorithms showed no benefit regarding the secondary high-risk endpoint (**Supplemental Table 4B**).

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, see **Supplemental Table 5**. All NSTE-ACS patients who were ruled-out were UAP patients. A detailed description of patients missed for the secondary endpoint is given in Supplemental Results file.

The sub-group analysis undertaken in patients with UAP and NCCP (combining both cohorts), indicated better identification of UAP by the 0-3 hour compared to the 0-1 hour algorithms (**Figure 2**). Overall, 6% of patients with UAP would be ruled-out if the low delta 0-3 hour 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 hour algorithm. Corresponding rates for the 0-1 hour 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 d MIs, all-cause mortality and revascularizations for the patients with NSTE-ACS and stratified as NSTEMI and UAP are

shown in **Supplemental Table 7**. **Supplemental Table 8 s**hows 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 d (Supplemental Results file), although a significantly higher proportion of patients who needed revascularization within 30 d were rule-out by the ESC algorithms (p-value< 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 hour algorithms performed overall better compared to 0-1 hour algorithms. Third, reagent or calibrator lots that return lower concentrations may change the overall diagnostic performance of algorithms utilizing 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. Lastly, all evaluated algorithms showed similar good prognosis for a combined endpoint of 30 d 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 cut offs 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 *(34)*. 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 hour cTnT) compared to 70% for the cTnT ESC algorithm *(35)*. 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 needs a high "safety margins" and hospitalize patients with less urgent NSTE-ACS, e.g., UAP.

Future studies, including long-term outcomes, are needed to conclude if the low concentration/low delta algorithms identify a sub-population 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, whom 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 subjects who have a completely stable myocardial perfusion *(11, 19, 36)*. Indeed, a recent publication demonstrated that hs-cTn concentrations increases (time dependent) when reversible myocardial ischemia is induced by a 30-90 sec balloon occlusion of the left anterior descending coronary artery *(37)*. Patients with UAP had higher baseline concentrations, indication a situation of low- grade chronic or acute myocardial injury, combined with larger delta values, consistent with intermittent myocardial leakage of troponins *(37)*. The observation that 3-hour deltas separated better between UAP and NCCP compared to 1-hour 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 ≤ 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 a 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 *(30)*. We used two different lots of the hs-cTnI assay, one in the derivation and one 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 observation highlight the need of robust validation of algorithms, using several different clinical cohorts and reagent and calibrator lots, before implementation into clinical practice, it calls for laboratories to monitor lot variations closely and manufacturers to strive for reducing 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 two 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 performance 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 above.

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 logistic problems in the ED, a common problem in this kind of studies. Even so, the NSTE-ACS incidence was similar across cohorts and the patient characteristic were also similar to other comparable studies *(38, 39)*. It should be noted that the adjudication was based on the 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 3rd definition of MI, since this is very similar to the 4th 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.

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Disclosures

All disclosures are stated in the individual Conflict of interest forms.

Table 1. Patient characteristics. Values are n (%) or median (25 and 75 percentile).

Derivation cohort					
	Total N=988	**NSTE-ACS N=242**	**Other diseases N=156**	**NCCP N=590**	**P-value**
Age, y	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/m2 for 461patients	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					
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/m2 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, non-coronary chest pain; PCI, Percutaneous coronary intervention; CABG, Coronary artery bypass graft.

Table 2. Troponin concentrations (ng/L), median and 25 and 75 percentile. 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 hour 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 hour 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, non-coronary chest pain.

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 grey background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1 hour 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 hour 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.

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 hour) revascularization, for the different algorithms. ESC algorithms are shown on a grey background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 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 $\Delta_{0\text{-}1h}$ < 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 $\Delta_{0\text{-}1h}$ < 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 $\Delta_{0\text{-}1h}$ < 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-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 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 $\Delta_{0\text{-}3h}$ < 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.

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

Figure 2. Percentage rule-out for patients with unstable angina pectoris (UAP) and non-cardiac chest pain (NCCP) in the total cohort.

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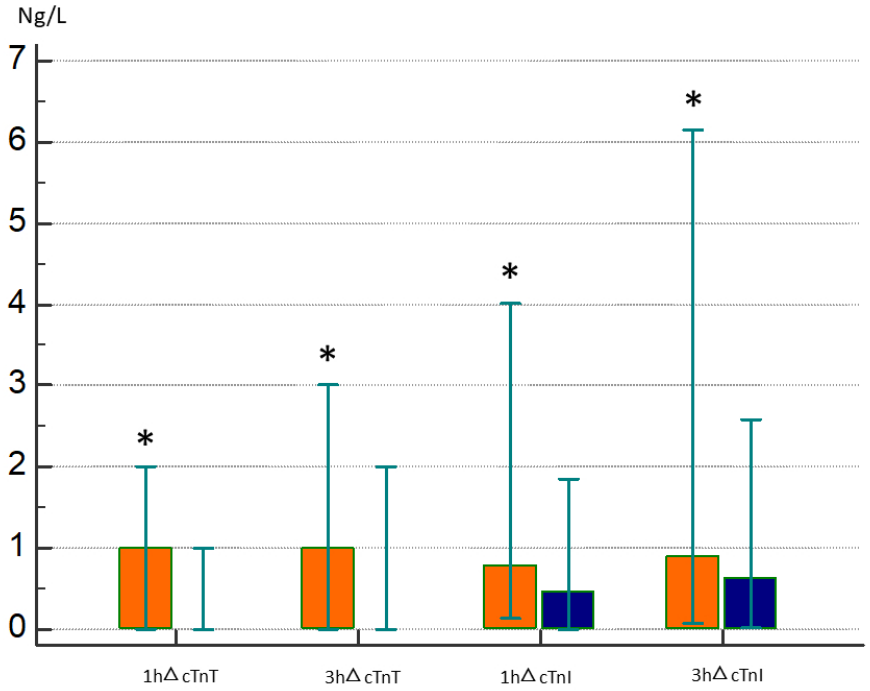

Figure 1. Absolute delta values (ng/L) for hs-cTnT and hs-cTnI in patients with unstable angina pectoris (orange) and non-cardiac chest pain (no colour / blue) in the total cohort. The bars show median values, poles show 10 and 90 percentile. Note that the median value for hs-cTnT deltas in non-cardiac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

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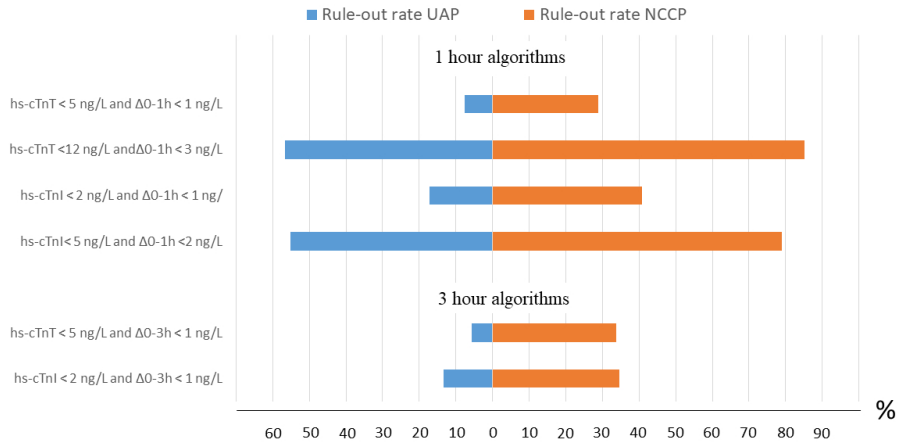

Figure 2. Percentage rule-out for patients with unstable angina pectoris (UAP) and non-cardiac chest pain (NCCP) in the total cohort.

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Supplemental Methods

Biochemical analysis

All samples were centrifuged after 30 min, and material for the biobank was aliquoted and frozen at -80°C. Routine and 1-h samples were measured for hs-cTnT (Roche Diagnostics) with limit of blank of 3 ng/L, limit of detection of 5 ng/L, 99th percentile of 14 ng/L and measurement range of 4 – 10 000 ng/L *(1)*. The 10% analytical within-series coefficient of variation (CV_A) was at 4.5 ng/L, with CV_A <5% for concentrations 10 ng/L or higher. The analysis was done continuously on fresh material using 9 different reagents and calibrator lots. For hs-cTnI, biobanked samples were measured using the Abbott Diagnostics hs-cTnI assay. The assay has a limit of blank of 0.9 ng/L, limit of detection of 1.7 ng/L, and 99th percentile of 26 ng/L *(1)*. The measurement range was 2-50 000 ng/L and the 10% CV_A was 4.6 ng/L. The CV_A was <4% for concentrations above 15 ng/L. The analysis was done 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. The glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula using an enzymatic isotope dilution-mass spectrometry traceable creatinine assay (Roche Diagnostics) with a CV_A <3% for concentration above 60 µmol/L.

Diagnostic definitions

Myocardial infarction was defined according to the third universal definition of myocardial infarction *(2)*.

Detection of a rise and/or fall of cardiac biomarker values (preferably cardiac troponins cTn) with at least one value above the 99th percentile upper reference limit and with at least one of the following:

•Symptoms of ischemia

•Development of pathologic Q waves in the electrocardiogram (ECG)

•New or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block

•Identification of an intracoronary thrombus by angiography or autopsy

•Imaging evidence of new loss of viable myocardium or a new regional wall motion abnormality

Prior myocardial infarction was defined by Q waves or QS complexes in the absence of QRS confounders in patients with ischemic heart disease regardless of symptoms *(2)*

Unstable angina pectoris (UAP) was defined as symptoms suggestive of an ACS without elevation in biomarkers with or without ECG changes indicative of ischemia *(3)*.

Stable angina was defined as typical angina symptoms lasting >1 month without an increase in magnitude, duration or frequency of the pain and a known history of coronary artery disease *(4)*.

Pericarditis was diagnosed if at least two of four diagnostic criteria were present, as defined in several studies: typical pleuritic chest pain, detection of a pericardial rub on auscultation, typical ECG changes, new or increased amount of pericardial effusion on echocardiography *(5)*.

Myocarditis was diagnosed according to the ESC's 2013 position statement *(6)*.

Takotsubo cardiomyopathy was diagnosed with the modified criteria suggested by The Mayo Clinic in 2008 *(7)*.

Heart failure was defined according to the 2016 ESC diagnostic criteria *(8)*.

Atrial fibrillation, atrial flutter and other supraventricular arrhythmias were diagnosed by ECG findings and the lack of symptoms and biochemical results supporting another disease.

Aortic stenosis and other valve diseases were diagnosed in accordance with echocardiographic results and a history supporting the valve disease as cause of the symptoms *(9)*.

Myalgia was defined as chest pain provoked by palpation in lack of cardiac disease.

Gastroesophageal reflux disease was based on gastroscopic findings, also in the lack of cardiac disease.

Cholecystitis was defined by the Tokyo Guidelines of 2006 while other abdominal diseases where defined according to operative, endoscopic or radiological findings *(10)*.

Pneumonia acquired typical symptoms and a chest X-ray supporting the disease, whereas the diagnosis of both pulmonary embolism and pneumothorax was based on radiologic results and the lack of concurrent cardiac disease.

Chronic obstructive pulmonary disease was defined in accordance with the 2008 criteria of Stephens et al *(11)*, while chest pain without any specific clinical, radiologic or biochemical findings where defined as non-specific chest pain.

Definition of risk factors

Diabetes was defined by the use of insulin, oral antidiabetic, or diet to lower the concentration of blood glucose.

Hypertension was based on the use of antihypertensive medication.

Hypercholesterolemia was defined by the use of statin or other lipid-lowering drugs.

Chronic kidney disease was defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2.

Family history of cardiovascular disease was defined as cardiovascular disease in first-degree relatives, before 55 y of age in men and 65 y of age in women.

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Diagnostic performance of novel troponin algorithms for the rule-out of NSTE-ACS

Supplemental results

Supplemental Tables

Supplemental Figure

Review of "missed patients"

Supplemental Table 1. Overview of the different rule-out algorithms that were evaluated.

	Novel algorithms	ESC algorithms
hs-TnT Roche		
1 hour	hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < ±1 ng/L	hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < ±3 ng/L
3 hour	hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < ±1 ng/	
hs-TnI Abbott		
1 hour	hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < ±1 ng/L	hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h}$ < ±2 ng/L
3 hour	hs-cTnI < 2 ng/L and $\Delta_{0\text{-}3h}$ < ±1 ng/L	

ESC, European Society of Cardiology; hs-cTnT, high-sensitivity troponin T; hs-cTnI, high-sensitivity troponin I.

Supplemental Table 2. Comparison of baseline troponin concentrations (ng/L, median, 25 and 75 percentile) in the two cohorts after stratification according to diagnosis adjudicated during hospitalization. A significant calibrator shift was identified for the hs-cTnI measurements (p-value for difference were ≤ 0.01 for all groups except NSTEMI).

	hs-cTnT			hs-cTnI		
Baseline	Derivation cohort	Validation cohort	p-value	Derivation cohort	Validation cohort	p-value
Total	7.0 (3.0-18.0)	7.0 (4.0-13.0)	0.07	4.0 (2.1-11.6)	2.2 (1.0-5.2)	<0.001
NSTEMI	48.0 (22.8-172.0)	56.5 (23.0-161.5)	0.73	118.9 (26.5-560.1)	102.2 (28.2-578.3)	0.58
UAP	9.0 (5.0-18.0)	9.0 (6.0-17.0)	0.57	4.7 (3.1-9.9)	3.3 (1.7-9.3)	0.01
Other diseases	13.0 (5.8-24.0)	10.5 (5.8-16.3)	0.08	8.1 (3.2-17.7)	3.6 (1.4-10.6)	<0.001
NCCP	5.0 (3.0-9.0)	5.0 (3.0-8.0)	0.81	2.7 (1.7-5.2)	1.5 (0.8-3.1)	<0.001

Supplemental Table 3. Median, 10, 90 percentile and significance level for the 1 h and 3 h absolute delta concentrations in UAP and NCCP patients.

	hs-cTnT			**hs-cTnI**		
	UAP	NCCP	p-value	UAP	NCCP	p-value
1 h delta $_D$	1.0 (0-2.0)	0 (0-1)	0.002	0.6 (01.-3.6)	0.4 (0-1.5)	0.008
1 h delta $_V$	0.7 (0-2.3)	0 (0-1.1)	0.008	0.9 (0.2-5.6)	0.5 (0.1-2.1)	<0.001
3 h delta $_D$	1.0 (0-3.0)	0 (0-2.0)	<0.001	0.8 (0.1-4.7)	0.6 (0-2.5)	0.001
3 h delta $_V$	1.0 (0-2.9)	0 (0-2.0)	<0.001	0.9 (0.1-7.2)	0.8 (0.1-2.7)	0.19

UAP, unstable angina pectoris; NCCP, none cardiac chest pain; D, deviation cohort; V, validation cohort.

Supplemental Table 4A. 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 in early presenters (≤ 3 hour since symptom onset). ESC algorithms are shown on a grey background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	91.7 (73.0-99.0)	91.3 (72.6-97.7)	28.8 (18.8-40.6)	29.7 (25.9-33.8)	23 (23.7)
Validation cohort N=94	92.3 (74.9-99.1)	88.2 (64.6-96.8)	22.1 (12.9-33.8)	31.2 (27.7-34.9)	17 (18.1)
hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < 3 ng/L					
Derivation cohort N= 97	83.3 (62.6-95.3)	92.3 (82.9-96.8)	65.8 (53.7-76.5)	44.4 (35.7-53.5)	52 (53.6)
Validation cohort N= 94	61.5 (40.6-79.8)	85.5 (78.2-90.6)	86.8 (76.4-93.8)	64.0 (47.4-77.8)	69 (73.4)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	91.7 (73.0-99.0)	90.5 (70.5-97.4)	26.3 (16.5-37.6)	29.0 (25.4-32.8)	21 (21.6)
Validation cohort N=94	80.8 (60.7-93.5)	87.2 (74.9-93.9)	50.0 (37.6-62.4)	38.2 (31.3-45.5)	39 (41.5)
hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h}$ < 2 ng/L					
Derivation cohort N=97	83.3 (62.6-95.3)	92.1 (82.5-96.7)	64.4 (52.3-75.3)	43.5 (35.0-52.4)	51 (52.6)
Validation cohort N =94	57.7 (36.9-76.7)	84.3 (77.2-89.5)	86.8 (76.4-93.8)	62.5 (45.5-76.9)	70 (74.5)
3-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=214	98.2 (90.3-99.9)	98.0 (87.4-99.7)	30.8 (23.8-38.6)	32.9 (30.6-35.4)	50 (23.4)
Validation cohort N=90	95.7 (78.1-99.9)	96.0 (77.5-99.4)	35.8 (24.5-48.5)	35.9 (29.5-38.4)	25 (27.8)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=206	96.2 (87.0-99.5)	95.5 (84.0-98.8)	27.5 (20.6-35.2)	31.9 (29.1-33.9)	44 (21.4)
Validation cohort N=90	87.0 (66.4-97.2)	90.6 (76.5-96.6)	43.3 (31.2-56.0)	34.5 (28.8-40.6)	32 (35.6)

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

Supplemental Table 4B. Diagnostic performance (95% confidence intervals) and efficacy (total rule-out, percentages in brackets) for the secondary endpoint combining 30 days MI and all-cause mortality and urgent (24 hour) revascularization for the different algorithms in early presenters (≤ 3 hour since symptom onset). European Society of Cardiology algorithms are shown on a grey background.

	Sensitivity	NPV	Specificity	PPV	Rule-out rate
1-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	100 (85.2-100.0)	100	21.6 (12.9-32.7)	28.4 (26.0-30.9)	16 (16.5)
Validation cohort N=94	100 (80.5-100.0)	100	15.6 (8.3-25.6)	20.7 (19.2-22.4)	12 (12.8)
hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < 3 ng/L					
Derivation cohort N=97	100 (79.4-100.0)	100	64.2 (52.3-74.6)	33.6 (29.2-42.5)	52 (53.6)
Validation cohort N=94	100 (73.5-100.0)	100	82.2 (74.4-91.3)	48.0 (35.9-60.3)	69 (73.4)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L					
Derivation cohort N=97	100 (79.4-100.0)	100	25.9 (16.8-36.9)	21.1 (19.0-23.8)	21 (21.6)
Validation cohort N=94	100 (73.5-100.0)	100	47.6 (36.4-58.9)	21.8 (18.5-25.5)	39 (41.5)
hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h}$ < 2 ng/L					
Derivation cohort N=97	100 (79.4-100.0)	100	63.0 (51.5-73.4)	34.8 (28.7-41.5)	51 (52.6)
Validation cohort N =94	100 (73.5-100.0)	100	85.4 (75.8-92.2)	50.0 (37.2-62.8)	70 (74.5)
3-hour algorithms					
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=214	100 (91.2-100.0)	100	28.7 (22.1-36.1)	24.4 (22.7-26.2)	50 (23.4)
Validation cohort N=90	100 (71.5-100.0)	100	31.6 (21.6-43.1)	16.9 (14.9-19.1)	25 (27.8)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L					
Derivation cohort N=206	100 (90.8-100.0)	100	26.2 (19.7-33.5)	23.5 (21.9-25.1)	44 (21.4)
Validation cohort N=90	100 (71.5-100.0)	100	40.5 (29.6-52.2)	19.0 (16.3-21.9)	32 (35.6)

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

Supplemental Table 5. Absolute rule-out numbers (percentages in brackets) for the different algorithms, patients are stratified according to the diagnosis adjudicated during index hospitalization. European Society of Cardiology algorithms are shown on a grey background.

	NSTE-ACS	Other diseases	NCCP	Total
1-hour algorithms				
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L				
Derivation cohort	5 (4.2)	8 (11.8)	102 (34.9)	115 (24.0)
Validation cohort	6 (4.7)	4 (7.1)	75 (23.4)	85 (16.8)
hs-cTnT <12 ng/L and$\Delta_{0\text{-}1h}$ < 3 ng/L				
Derivation cohort	34 (28.6)	30 (44.1)	245 (83.9)	309 (64.5)
Validation cohort	48 (37.2)	31 (55.4)	276 (86.3)	355 (70.3)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L				
Derivation cohort	8 (6.7)	7 (10.3)	94 (32.8)	109 (23.0)
Validation cohort	17 (13.1)	17 (29.8)	153 (47.8)	187 (36.8)
hs-cTnI< 5 ng/L and Δ0-1h < 2 ng/L				
Derivation cohort	33 (27.7)	20 (29.4)	216 (75.3)	269 (56.0)
Validation cohort	47 (36.2)	33 (57.9)	263 (82.2)	343 (67.7)
3-hour algorithms				
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L				
Derivation cohort	8 (3.5)	19 (13.5)	203 (34.6)	230 (23.4)
Validation cohort	3 (2.5)	6 (11.5)	99 (32.0)	108 (22.4)
hs-cTnI< 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng				
Derivation cohort	10 (4.2)	12 (7.7)	175 (31.2)	197 (20.2)
Validation cohort	15 (12.4)	13 (24.5)	127 (41.1)	155 (32.1)

NSTE-ACS, non-ST-elevation acute coronary syndrome; NCCP, non-coronary chest pain.

Supplemental Table 6. Rule-out rate for the different algorithms in the sub-groups of patients with unstable angina pectoris (UAP) and non-cardiac chest pain (NCCP) (diagnosis adjudicated during index hospitalization). Percentages and Confidence intervals in brackets. European Society of Cardiology algorithms are shown on a grey background.

	UAP	NCCP
1-hour algorithms		
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L		
Derivation cohort	8.8 (1.5-16.2)	34.9 (25.7-44.2)
Validation cohort	6.8 (1.6-12.1)	23.4 (18.8-28.0)
hs-cTnT <12 ng/L and$\Delta_{0\text{-}1h}$ < 3 ng/L		
Derivation cohort	59.6 (46.9-72.3)	83.9 (79.7-88.1)
Validation cohort	54.5 (44.1-65.0)	86.3 (82.5- 90.1)
hs-cTnI < 2 ng/L and $\Delta_{0\text{-}1h}$ < 1 ng/L		
Derivation cohort	14.0 (5.0-23.0)	32.8 (27.4-38.2)
Validation cohort	19.5 (9.2-29.8)	47.8 (42.3-53.3)
hs-cTnI< 5 ng/L and Δ0-1h < 2 ng/L		
Derivation cohort	57.9 (45.1-70.7)	75.3 (70.3-80.3)
Validation cohort	53.4 (43.0-63.8)	82.2 (78.0-86.4)
3-hour algorithms		
hs-cTnT < 5 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng/L		
Derivation cohort	7.1 (2.3-11.9)	34.6 (30.8-38.5)
Validation cohort	3.8 (0-8.8)	32.0 (26.8-37.2)
hs-cTnI< 2 ng/L and $\Delta_{0\text{-}3h}$ < 1 ng		
Derivation cohort	9.3 (3.8-14.8)	31.2 (27.4-35.0)
Validation cohort	18.8 (10.2-27.3)	41.1 (35.6-46.6)

Supplemental Table 7. The table shows the number of investigations, revascularizations and 30 days major cardiac adverse events (MACE) in the different groups, stratified by index diagnosis. MACE was defined as death, myocardial infarction or revascularization. The increased numbers of CCTA in the validation cohort was in accordance with the study protocol (see method section).

	NSTE-ACS	**NSTEMI**	**UAP**
Derivation cohort	N=242	N=130	N=112
Investigations			
Echocardiography	180 (74.4)	109 (83.8)	71 (63.4)
CCTA*	39 (16.1)	5 (3.8)	34 (30.4)
Coronary angiography	187 (77.3)	112 (86.2)	75 (67.0)
Revascularization			
PCI‡ within 24 hours	38 (15.7)	34 (26.2)	4 (3.6)
PCI >24 hours after admission	96 (39.7)	49 (37.7)	47 (42.0)
CABG£	14 (5.8)	8 (6.2)	6 (5.3)
30 days all-cause mortality, MI or revascularization			
Total	195 (80.6)	130 (100.0)	65 (58.0)
Deaths	1 (0.4)	1 (0.8)	0
MI	133 (55.0)	130 (100)	3 (2.7)
Revascularization	157 (64..9)	93 (71.5)	64 (57.1)
Validation cohort	N=133	N=44	N=89
Investigations			
Echocardiography	110 (82.7)	38 (86.4)	72 (80.9)
CCTA	42 (31.6)	6 (13.6)	36 (40.4)
Coronary angiography	104 (78.2)	38 (86.4)	66(74.2)
Revascularization			
PCI within 24 hours	15 (11.3)	11 (25.0)	4 (4.5)
PCI >24 hours after admission	48 (36.1)	14 (31.8)	34 (38.2)
CABG	11 (8.3)	6 (13.6)	5 (5.6)
30 days all-cause mortality, MI or revascularization			
Total	98 (73.7)	44 (100.0)	54 (60.7)
Deaths	1 (0.8)	0	1 (1.1)
MI	45 (33.8)	44 (100)	1 (1.1)
Revascularization	88 (66.2)	34 (77.3)	54 (60.7)

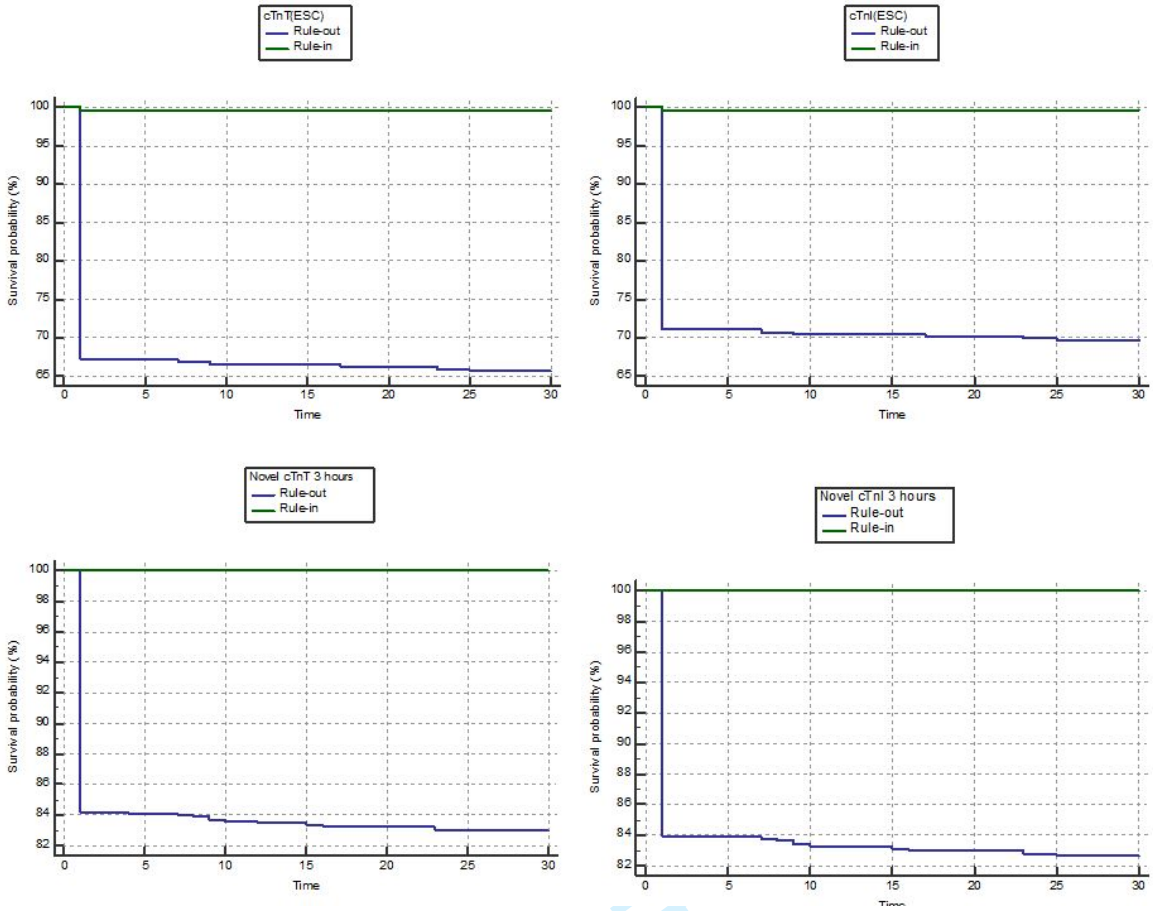
CCTA, Coronary computed tomography angiography; NSTE-ACS, non-ST-elevation acute coronary syndrome; NSTEMI, non-ST elevation myocardial infarction; UAP, unstable angina pectoris; PCI, Percutaneous coronary intervention.

Supplemental Table 8. The investigations, revascularization and 30 days major cardiac adverse events (MACE) defined as death, myocardial infarction or revascularization in the group of patients with unstable angina pectoris (UAP) who were rule-out by the European Society of Cardiology algorithms and the most favorable of the novel algorithms (0-3 hour). Percentages (in brackets) are calculated using all patients with UAP in the nominator (n=57 (in the derivation cohort only the 57/112 patients who had a 1-hour sample were included) and n=88 (validation cohort)).

	UAP ruled-out $cTnT_{ESC}$	UAP ruled-out $cTnT_{\Delta 0\text{-}3}$	P-value	UAP ruled-out $cTnI_{ESC}$	UAP ruled-out $cTnI_{\Delta 0\text{-}3}$	P-value
Derivation cohort	**N=34/57**	**N=3/57**	<0.001	**N=33/57**	**N=7/53**	<0.001
Investigations						
Echocardiography	21 (36.8)	1 (1.8)	<0.001	22 (38.6)	4 (7.0)	<0.001
CCTA*	17 (29.8)	2 (3.5)	<0.001	15 (26.3)	6 (10.5)	0.002
Coronary angiography	22 (38.6)	1 (1.8)	<0.001	23 (40.4)	6 (10.5)	<0.001
Revascularization						
PCI‡ within 24 hours	0	0	NA	0	0	NA
PCI >24 hours but during admission	15 (26.3)	1 (1.8)	<0.001	14 (24.6)	4 (7.0)	0.01
CABG£ during admission	0	0	NA	0	0	NA
30 days all-cause mortality, MI or revascularization						
Total	21 (36.8)	2 (3.5)	<0.001	19 (33.3)	4 (7.5)	<0.001
Deaths	0	0		0	0	
MI	0	0		0	0	
Revascularization	21 (36.8)	2 (3.5)	<0.001	19 (33.3)	4 (7.5)	<0.001
Validation cohort	**N=48/88**	**N=3/79**	<0.001	**N=47/88**	**N=15/79**	<0.001
Investigations						
Echocardiography	38 (43.1)	2 (2.2)	<0.001	35 (39.8)	12 (13.6)	<0.001
CCTA	23 (26.1)	1 (1.1)	<0.001	20 (22.7)	6 (6.8)	0.002
Coronary angiography	34 (38.6)	2 (2.2)	<0.001	34 (38.6)	12 (13.6)	<0.001
Revascularization						
PCI within 24 hours	3 (3.4)	0	0.5	2 (2.3)	0	1.0
PCI >24 hours but during admission	15 (17.0)	0	<0.001	15 (17.0)	3 (3.4)	0.04
CABG during admission	2 (2.3)	0	0.5	2 (2.3)	1 (1.1)	1.0
30 days all-cause mortality, MI or revascularization						
Total	28 (31.8)	1 (1.1)	<0.001	28 (31.8)	8 (10.1)	<0.001
Deaths	0	0	NA	0	0	NA
MI	0	0	NA	1 (1.1)	0	NA
Revascularization	26 (29.5)	1 (1.1)	<0.001	28 (31.8)	8 (10.1)	<0.001

*Coronary computer tomography angiography
‡ Percutaneous coronary intervention
£ Coronary artery bypass graft

Supplemental Figure 1. Kaplan-Meier curves showing 30 days all-cause mortality, 30 days MI or 24 hours revascularization for patients ruled-in and ruled-out by the European Society of Cardiology and the novel 3-hour algorithms.



Review of "missed" patients

The list include an overview of patients who were missed by the algorithms and developed an MI or died within 30 days after admission or were treated with an urgent (24 hour) revascularization.

Review of "missed" UAP patients

Patient 1 and 2 are the same patients in both groups

ESC cTnT algorithm (hs-cTnT <12 ng/L and $\Delta_{0\text{-}1h}$ < ±3 ng/L)

Validation cohort

Patient 1

60 year old male with previous STEMI, admitted with a four hour history of chest pain. Had PCI 21 hours after admittance with a stent in CX. Diagnosed with UAP.

Hs-TnT_{0h} 7 ng/l, TnT_{1h} 8 ng/l and TnT_{3h} 7 ng/L

Patient 2

70 year old female with known atherosclerotic heart disease, admitted with a 16 hour history of chest pain. PCI at 24 hours, stented in LAD. Diagnosed with UAP.

Hs-TnT_{0h} 6 ng/l, TnT_{1h} 6 ng/l and TnT_{3h} 6 ng/L

Patient 3

50 year old male, previously healthy, admitted with two weeks history of chest pain, PCI at 24 hours, stented in LAD. Diagnosed with UAP.

Hs-TnT_{0h} 8 ng/l, TnT_{1h} 8 ng/l and TnT_{3h} 8 ng/L

ESC cTnI algorithm (hs-cTnI < 5 ng/L and $\Delta_{0\text{-}1h}$ < ±2 ng/L)

Patient 1: Hs-TnI_{0h} 3 ng/L, TnI_{1h} 2 ng/L and TnI_{3h} 3 ng/L

Patient 2: Hs-TnI_{0h} 3 ng/L and TnI_{1h} 4 ng/L TnI_{3h} 3 ng/L