Novel criteria for the Observe-Zone of the ESC 0/1h-hs-cTnT Algorithm

SUPPLEMENTAL MATERIAL

Trial Registration: **ClinicalTrials.gov number, NCT00470587**

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Abbreviations

ED – Emergency department

AMI – Acute Myocardial Infarction

NSTEMI – Non-ST elevation Myocardial Infarction

ECG – Electrocardiography

cTn – Cardiac troponin

hs-cTn – High-sensitivity cardiac troponin

cMyC – cardiac Myosin-binding Protein C

eGFR – Estimated glomerular filtration rate

NPV – Negative predictive value

PPV – Positive predictive value

IQR – Interquartile range

AUC – Area under the curve

ROC – receiver operating characteristic

Supplemental Methods

Main cohort (APACE)

Adjudication of the final diagnosis

NSTEMI was defined and cTn levels interpreted as recommended in current guidelines.[1] In brief, NSTEMI was diagnosed when there was evidence of myocardial necrosis diagnosed by at least 1 cTn value above the uniform 99th percentile with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Patients with AMI were further subdivided into type 1 AMI (primary coronary events) and type 2 AMI (ischemia due to increased demand or decreased supply, for example tachyarrhythmia or hypertensive crisis)[14,37]. The adjudication of the final diagnoses (the cause of acute chest discomfort in patients presenting to the ED) was performed centrally in the core lab (University Hospital Basel) for all patients incorporating levels of hs-cTnT. More specifically, two independent cardiologists not directly involved in patient care reviewed all available medical records (including patient history, physical examination, results of laboratory testing including (hs-)cTnT levels, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, discharge summary) pertaining to the patient from the time of ED presentation to 90-day follow-up.

Late samples were available for adjudication of final diagnosis in a relevant proportion of patients (e.g., 1320 patients [63%] had hs-cTnT values ≥6h after ED presentation and 1445 [70%] above 3h, **Supplemental Table IIA, IIB and IIC**). In general, serial sampling was performed until at least 3 to 6 h after presentation to the ED or onset of chest pain. The most common reasons for missing samples after 1 h or later were early transfer to the catheter laboratory or coronary care unit and diagnostic procedures around the time window that precluded blood draws. In situations of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third cardiologist. While discharge diagnoses were most often correct and in agreement with the final adjudicated diagnosis, there were also cases in which the discharge diagnosis needed to be revised, usually because more information became available from medical testing during early follow-up, and more rarely, because the discharge diagnosis was not in agreement with the Universal Definition of MI (UDMI).

The 99th percentile (hs-cTnT: 14 ng/l) was used as the cutoff for myocardial necrosis. Absolute cTn changes were used to determine significant changes based on the diagnostic superiority of absolute over relative changes [38-43]. Based on studies of the

biological variation of cTn,[44, 45] as well as on data from previous chest pain cohort studies,[38, 46] a significant absolute change was defined as a rise or fall of at least 10 ng/l within 6 h, or, in an assumption of linearity, as an absolute change of 6 ng/L within 3 hours. If previous (e.g., 2 days prior) or later clinical samples (e.g., at 24, 48, or 72 hours) revealed a lower hs-cTnT level than that measured during the period of sampling in the ED, the previous/later level was considered the true baseline level for the calculation of the change criteria. Therefore, according to the 4th UDMI the criterion of at least one hs-cTn above the 99th percentile was absolute, the criterion for the magnitude of absolute change to be present was not mandatory for the short period in the ED, but for the acute event in general. E.g., the "rise and/or fall" criterion could also be documented using baseline concentrations obtained prior to ED or after ED presentation. This allowed to also appropriately identify patients as NSTEMI who had their plateau of hs-cTnT/I concentration during the short blood sampling period in the ED (e.g., 0h 100ng/L, 1h 101ng/L, baseline obtained 2 days prior 6ng/L). Predefined alternative diagnoses included 'unstable angina' (UA), 'Cardiac symptoms of origin other than coronary artery disease' and 'non-cardiac chest pain'. Those patients, in whom the final diagnosis remained unclear despite central adjudication by two independent cardiologists and had at least one elevated hs-cTnT concentration, received a final adjudicated diagnosis of unknown (hs-cTnT >14ng/L and no clear diagnosis). Cardiac work-up and particularly cardiac imaging, including coronary angiography, in these patients was not sufficient or provided equivocal results to allow reliable diagnosis and or exclusion of NSTEMI, reflecting clinical reality.

Clinical Care: The (hs-)cTn assays and cut-off levels used for local clinical care

Routine clinical care comprised five different cTn assays at the different hospitals and at the different recruitment periods. The cTn assays used clinically in most of the participating institutions changed during the study from a contemporary cTn assay to the hs-cTnT assay. In order to take advantage of the higher sensitivity and higher overall diagnostic accuracy offered by the hs-cTnT assay, patients were adjudicated using the hs-cTnT values in all patients. In patients in whom clinically a contemporary cTn assay was used, the concentrations of both, contemporary cTn and hs-cTnT, were available for the adjudication. Where hs-cTnT was the only clinically available biomarker, only hs-cTnT results were available for adjudication. The following conventional cTn assays were used:

For Elecsys hs-cTnT measured clinically, the same change criteria were applied as for hs-cTnT measured from the study blood samples.

For the Siemens Dimension Vista s-cTnI, the 10% CV level is 40 ng/L. The limit of detection is 15 ng/L and the 99th percentile is 45 ng/L. An absolute change of 20 ng/L or more within 3-6h was considered significant.

For the Beckmann Coulter Accu cTnI, the 10% CV level is 0.06 µg/L. A patient having 0.06 µg/L at presentation would qualify if the second is ≥0.08 µg/L. A patient having 0.05 µg/L at presentation would qualify if the second is 0.07 µg/L, but not 0.06 µg/L. A patient with an undetectable cTnI concentration (cTnI <0.01 µg/L) at presentation would qualify if the second is ≥0.06 µg/L.

For the Abbott Axsym cTnI ADV, the 10% CV level is 0.16 µg/L. A patient having 0.16 µg/L at presentation would meet the criteria for significant change if the second was ≥0.21 µg/L. A patient having <0.12 µg/L at presentation (limit of detection) would qualify if the second is >0.16 µg/L.

For the Roche cTnT 4th generation assay, the 10% CV level is 0.035 µg/L. The laboratories of the participating sites reported only two decimals; therefore 0.04 µg/L was used as a cut-off for myocardial necrosis. In order to fulfil the criteria of a significant change (30% of 99th percentile or 10% CV level), a patient would e.g., need to have a level of <0.01 µg/L at presentation and 0.04 µg/L at 6h. A patient would also qualify if the first level is 0.02 µg/L and the second 0.04 µg/L. A patient would not fulfil the criteria if the first level is 0.03 µg/L and the second is 0.04 µg/L. If the first level is 0.04 µg/L, the second level needs to be at least 0.06 µg/L.

Bootstrap internal validation

The bootstrap validation approach is a resampling technique and is favored over the split-sample technique.[13] The bootstrap validation allows to use the whole sample for developing the model/algorithm and provides a mechanism to account for model overfitting, therefore quantifying optimism in the final model/algorithm. The bootstrap validation used for validating the novel derived observe-zone 0/3h-criteria can be visualized in 6 steps:

1. Develop the algorithm (multiple cut-off criteria) using the entire original sample (n) and determine the apparent performance of your final algorithm (e.g., sensitivity and NPV for rule-out and specificity and PPV for rule-in). In this case

the tested cut-off combinations for rule-out ranged from 13 to 18 ng/L for the 3h single hs-cTnT measurement and from 1 to 5 ng/L for hs-cTnT 0/3h absolute change.

2. Generate a bootstrap sample by sampling individuals with replacement from the original sample (n).
3. Develop a new algorithm (applying the same modelling and cut-offs selection methods, as in step 1: from 13 to 18 ng/L for single measurement and from 1 to 5 ng/L for absolute change strategy) using the bootstrap sample
    a. Determine the apparent performance (e.g., sensitivity) of the cut-off combination newly derived on the bootstrap sample (bootstrap apparent performance)
    b. Determine the performance of the bootstrap cut-off combination in the original sample (test performance)
4. Calculate the optimism as the difference between the bootstrap performance and the test performance for the performance measure of interest (e.g. sensitivity)
5. Repeat 1000 times steps 2 to 4.
6. Average the estimates of optimism in step 5 and subtract the value from the apparent performance calculated in step 1. The final result will be the optimism-corrected estimate of performance (internal validation).

For calculating the 95% confidence intervals of the point estimates obtained in the internal validation, 500 repetitions were used (at least 200 are recommended). This is in fact 500 repetitions of the bootstrap internal validation, leading to 500,000 repetitions, providing robust enough confidence intervals.

The 3h single hs-cTnT rule-out cut-offs ranged from 13 ng/L to 18 ng/L. The lower boundary of 13 ng/L was chosen because 12 ng/L is already a rule-out cut-off in the hs-cTnT ESC 0/1h-Algorithm. The upper boundary was decided to reach 18 ng/L to reduce overfitting (a large number of cut-off combinations might lead to overfitting). The hs-cTnT 0/3h absolute change measurement for rule-out ranged from 1 to 5 ng/L once again allowing for a sufficient number of combinations while controlling for overfitting. For rule-in, a hs-cTnT 0/3h absolute change from 5 to 7 ng/L was chosen to see if it was possible to improve the already good performance obtained with the suggested ≥7 ng/L.[8]

Statistical analysis

Continuous variables are described as medians with interquartile range (IQR); categorical variables as numbers and percentages. Differences in baseline characteristics were assessed using the Mann-Whitney-U test or Kruskal-Wallis Test for continuous variables when appropriate, and the Pearson chi-square test for categorical variables.

Four ECG features (ST-segment depression, T-wave inversion, ST-segment depression or T wave inversion and ST-segment depression and T wave inversion) were evaluated for aiding further triage to rule-in among those patients still remaining in the observe-zone after applying the novel derived observe-zone 0/3h-criteria. Predefined performance targets for ECG-based rule-in were specificity >95% and PPV >75%.

Prognostic verification of our central diagnostic adjudication was assessed by means of Kaplan-Meier curves. A comparison between NSTEMI patients ruled-in (true positives) and NSTEMI patients ruled-out (false negatives) at 3 hours; and between NSTEMI patients ruled-out (false negatives) and non-NSTEMI patients ruled-out (true negatives) at 3 hours when applying the suggested 0/3h-hs-cTnT-change of <7ng/L was performed to see if the Kaplan-Meier curves split as expected between NSTEMI/no NSTEMI patients. The primary prognostic composite endpoint was non-index AMI or CV death during thirty-days and two-years of follow-up. The secondary prognostic endpoint was non-index AMI during thirty-days and two-years of follow-up, CV death during thirty-days and two-years of follow-up, as well as the composite endpoint non-index AMI or all-cause death during thirty-days and two-years of follow-up. Cumulative rates of the primary and secondary composite endpoints were plotted in Kaplan-Meier curves. The log-rank test was used to assess differences in survival between groups.

There are no generally accepted approaches to estimate the sample size requirements for external validation studies of prediction models.[13] Some have suggested having a minimum of 100 events (NSTEMI in this case) for external validation of clinical prediction rules.[23] Our sample and the number of events fulfills all those requirements for the sample size and therefore is expected to provide robust performance estimates. Nonetheless, a power calculation for the available sample size was conducted (**Supplemental Table III**).

Power calculation was performed for comparing the sensitivity of Parkland's pathway in the validation cohort with a reference sensitivity of 100% (chosen to be equal to the sensitivity estimate for Rule-Out in the original derivation study). Therefore, a one sample-proportion test (Z-score test) was used. Due to the optimistic apparent

performance in the derivation study (very low NSTEMI numbers [11/536 = 2.1%], no internal validation performed and single center], we expected at least a 10% drop in the sensitivity. A sensitivity of 90% in the validation cohort yields a power of 99.9% to reject that the Parkland algorithm yields a sensitivity of 100% for rule-out (**Supplemental Table III**). In addition, a post-hoc power calculation using a two-sample proportion test (chi-squared test), comparing sensitivity differences between the original derivation cohort (100%) and the sensitivity obtained in our validation cohort (33.3%), yielded a power of 1.0 for the available sample size.

Validation Cohort (TRAPID-AMI)

Patient population

TRAPID-AMI (High sensitivity cardiac Troponin T assay for RAPID rule-out of Acute Myocardial Infarction) was a prospective multicenter international diagnostic study conducted between August 2011 to June 2013 including 12 sites on 3 continents worldwide (USA, Australia, Great Britain, Sweden, Belgium, Germany, Switzerland and Spain).[16]

Adult patients presenting to the ED with symptoms suggestive of acute myocardial infarction, with a chest pain onset or maximum within the previous 6 hours were identified by study personnel, qualified for the study and recruited. A threshold of less than 6 hours was chosen in order to enrich the study population with the particularly challenging early presenters. Exclusion criteria were: A) patients with renal failure requiring chronic hemodialysis, B) patients with cardioversion, defibrillation, thrombolytic therapy or trauma prior to inclusion, C) patients receiving coronary artery bypass grafting within the last month or hospitalized for AMI within the last 3 weeks, and D) pregnant or breastfeeding mothers. In order to allow the study blood draw to be performed as quickly as possible, definite interpretation of the initial ECG was not required prior to inclusion. Accordingly, STEMI patients were not excluded by the protocol. For this external validation, STEMI patients as well as patients missing 0h, 1h, 2h or 5/6h measurements of hs-cTnT were excluded (**Supplemental Figure XV**).

The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethic committees. Written informed consent was obtained from all patients.

Routine clinical assessment

Patients underwent an initial clinical assessment that included clinical history, physical examination, 12-lead ECG, pulse oximetry, standard blood tests (including local cTn assays), and chest radiograph in accordance with local protocols. Treatment of patients was left at discretion of the attending physician. Standard data were collected on study-specific case report forms.

Adjudication of the final diagnosis

To determine the final diagnosis for each patient, adjudication of final diagnoses was performed by a dedicated group of cardiologists selected for the Clinical Event

Committee of this study according to the universal definition of AMI.[47] Each patient was adjudicated by two independent cardiologists. Adjudicators reviewed all available medical records, including patient history, physical examination, results of laboratory testing including s-cTnI-ultra levels, as well as local cTn levels obtained before the first or after the last blood draw for the study if available, creatinine, cystatic C, free hemoglobin (to quantify hemolysis), NT-proBNP levels, radiologic imaging, ECG, echocardiography, cardiac stress test, and lesion severity and morphology in coronary angiography pertaining to the patient from the time of ED presentation to 30-day follow up. A third cardiologist solved any discrepancies. The s-cTnI-ultra assay was chosen for the adjudication to achieve complete blinding to hs-cTnT levels during the study period in the ED. This assay was the best-validated sensitivite cTn assay available at the study start with similar early diagnostic accuracy to the hs-cTnT assay.[38,48,49]

AMI was defined and s-cTnI-ultra levels interpreted as recommended in guidelines.[5,14-16] In brief, AMI was diagnosed when there was evidence of myocardial necrosis with a significant rise and/or fall of s-cTnI in a clinical setting consistent with myocardial ischemia. The 99th percentil (40ng/L) was used as cut-off for myocardial necrosis. An absolute s-cTnI-ultra change of at least 20ng/L during the study period was used to define a significant rise or fall.[38] Other predefined diagnostic groups included unstable angina, other cardiac disease including myocarditis, Tako-Tsubo cardiomyopathy, acute heart failure, or tachyarrhythmias, non-cardiac disease, and symptoms of unknown origin in which AMI was excluded, but the work-up was considered insufficient for a clear alternative diagnosis.

Blood sampling and laboratory methods

Blood samples for central measurement of hs-cTnT (Roche Diagnostics) and sensitive(s)-cardiac Troponin I (cTnI)-ultra (Siemens Healthcare) were collected in EDTA plasma tubes at presentation to the ED after written informed consent was obtained. To ensure that the first study blood draw was performed within a short time window from ED presentation, this period was required to be either within 45 minutes of presentation to the ED or less than 45 minutes after the first routine blood draw. Additional samples were collected after 1, 2, and 5 to 6 hours. For the ADVIA Centaur (Siemenes Healthcare) s-cTnI-ultra assay, the 99th percentile is 40 ng/L, with a coefficient of variation <10% at 30 ng/L and a limit of detection at 6 ng/L. None of the study blood

results were available to the treating physician. The results of s-cTnI-ultra, but not those of hs-cTnT were available to the adjudicating cardiologists.

Statistical Analysis

The primary diagnostic endpoint was NSTEMI (type 1 and 2) at presentation to the ED. To study the performance of the novel alternative 3-hour algorithm in the TRAPID-AMI validation cohort, safety was assessed as the sensitivity and NPV of ruling-out index NSTEMI, accuracy as the specificity and PPV of ruling-in index NSTEMI, and efficacy was quantified by the proportion of patients triaged toward rule-out or rule-in of NSTEMI at 3 hours. Differences in independent proportions were assessed using a 2-sample test for equality of proportions Confidence intervals were calculated using Wilsons method.

Three-hour blood samples were not taken in the TRAPID-AMI study. Taking into account the near linear release of hs-cTn,[50-52] and that only patients with a chest pain onset or maximum within the previous 6 hours were recruited in this study, 3-hour hs-cTnT values were estimated by calculating the mean between the 2-hour and 5/6-hour hs-cTnT measurements.

Two sensitivity analysis were performed to assess the robustness of the external validation. In the first sensitivity analysis we assessed the diagnostic performance of the novel derived observe-zone 0/3h-criteria by using 2-hour hs-cTnT values as if they were 3-hour values. In a second sensitivity analysis more weight was given to the 2-hour values ($2/3^{rd}$) than to the 5/6-hour values ($1/3^{rd}$), which corresponds to a linear interpolation at 3-hour timepoint. (Equation: estimated TnT3h = 2/3*TnT2h + 1/3*TnT5_6h)

Supplemental Results

Main cohort (APACE)

Comparison of eligible patients versus excluded patients due to missing 3h sample

Comparison of the baseline characteristics and final adjudicated diagnosis in the population eligible for the main analysis versus those patients in whom no 3-hour hs-cTnT sample was available is presented in **Supplemental Table IV**.

Patient characteristics, management and Outcome in true detected NSTEMIs (True Positives) vs missed NSTEMIs (False negatives) according to the suggested 0/3h-hs-cTnT-change of <7ng/L

After applying the suggested 0/3h-hs-cTnT-change of <7ng/L to patients triaged to the observe-zone, a total of 80 NSTEMI patients were triaged to the rule-out group (false negatives) and 40 NSTEMI patients were triaged to the rule-in group (true positives). Kaplan-Meier curves showed no difference in 30- and 730-days cumulative incidence between true positives and false negatives NSTEMI patients for all-cause death or AMI, cardiovascular death or AMI and AMI (Log-rank test non-significant for all endpoints, **Supplemental Figure III and IV**). Furthermore, no difference in patient characteristics, ECG characteristics, hs-cTnT/I concentrations or management was observed between false negatives and true positives, strengthening the central adjudication of NSTEMI (**Supplemental Table VIII-X)**.

Management and Outcome in missed NSTEMIs patients (False negatives) vs non-NSTEMI patients (true negatives) according to the suggested 0/3h-hs-cTnT-change of <7ng/L

After applying the suggested 0/3h-hs-cTnT-change of <7ng/L to patients triaged to the observe-zone, a total of 80 NSTEMI and 437 non-NSTEMI patients were triaged to the rule-out group (false negative and true negative, respectively). 30-, and 730-day Kaplan-Meier curves showed NSTEMI ruled-out patients (false negative) had a higher cumulative incidence for all-cause death or AMI, CV death or AMI and AMI at 30 days (log-rank test 0.0005, 0.0002 and 0.0019, respectively (**Supplemental Figure V**) and for CV death or AMI and AMI at 730-days (log-rank test 0.013 and 0.027, respectively (**Supplemental Figure VI**) compared to non-NSTEMI ruled-out patients (true negatives). Similarly, management also differed, receiving more antiplatelet therapy and

beta-blockers NSTEMI patients triaged to rule-out than non-NSTEMI patients triaged to rul-out (**Supplemental Table XI**). These results strengthen the fact that "true NSTEMIs" missed by the Parkland approach (false negatives), because they are correctly adjudicated "true NSTEMIs", received different treatment compared to those patients correctly ruled-out and having other diagnosis (true negatives).

Sensitivity Analysis

In a first sensitivity analysis, we evaluated the diagnostic performance of the Parkland approach as well as the novel derived observe-zone 0/3h-criteria restricted to an endpoint of type 1 MI. The performance of both strategies was comparable to the main analysis (outcome not restricted to type 1 myocardial infarction), with a sensitivity of 36.5% (95% CI, 26.4-47.9) and an NPV of 90.9% (95% CI, 88.1-93.1) for rule-out in the Parkland approach and a Sensitivity of 98.6% (95% CI, 92.7-99.8) and an NPV of 99.3% (95% CI, 96.0-99.9) for rule-out in the novel derived observe-zone 0/3h-criteria. For rule-in, the Parkland approach had a specificity of 95.9% (95% CI, 93.8-97.3) and a PPV of 57.4% (95% CI, 43.3-70.5), while the novel derived observe-zone 0/3h-criteria had a specificity of 94.5% (95% CI, 92.1-96.2) and a PPV of 57.1% (95% CI, 44.9-68.6).

In a second sensitivity analysis we stratified patients with and without myocardial ischemia on the ECG (defined as ST segment depression or T wave inversion) for the Parkland approach. Performance was comparable to the main analysis for each stratified group (**Supplemental Figure XIIA**). When considering patients without ischemic ECG changes as the only candidates for rule-out, and therefore all other patients triaged to rule-in, the sensitivity moderately improved to 63.3% (95% CI, 54.4-71.4) and NPV to 89.2% (95% CI, 85.9-91.9), missing 44 NSTEMIs. With this strategy, rule-in performance, previously very high, now notably decreased to a specificity of 82.2% (95% CI: 78.4-85.5) and a PPV of 49.0% (95% CI: 41.3-56.8), **Supplemental Figure XIIB**.

In a third sensitivity analysis, the diagnostic performance of the novel derived observe-zone 0/3h-criterion was evaluated only among patients with further measurements 6-hours after ED presentation in the observe-zone (n=413/564 [73.2%]). Performance of the novel derived observe-zone 0/3h-criteria was comparable in this sensitivity analysis, with a sensitivity of 99.0% (94.6-99.8%) and a NPV of 98.9% (93.9-99.8).

In a fourth sensitivity analysis, patients with elevated cardiac troponin concentrations in whom the final adjudicated diagnosis was unclear (n=45) were assumed

to have a type 1 NSTEMI (**Supplemental Table** XVA **and B**). Again, performance of the Parkland approach, the ESC 0/1h-hs-cTnT-algorithm and the novel derived observe-zone 0/3h-hs-cTnT-criteria was comparable to the main analysis. The suggested 7ng/L approach missed 109 patients (29 patients more). The clinical discharge diagnosis was unknown in 18 patients, unstable angina in 3 patients, arrythmia in 3 patients, hypertension in 2 patients, cardiac decompensation in 1 patient, NSTEMI in 1 patient and musculoskeletal in 1 patient. The resulting sensitivity and NPV for rule-out was 28.8% (95% CI, 22.2 – 36.4) and 80.0% (95% CI, 76.5 – 83.2), respectively. The ESC 0/1h-hs-cTnT algorithm missed 5 patients (one patient more). The clinical discharge diagnosis was unknown. The resulting sensitivity and NPV for rule-out was 98.9% (95% CI, 97.6-99.6) and 99.6% (95% CI, 99.0-99.8), respectively. The novel derived observe-zone 0/3h-hs-cTnT criteria missed 3 patients (two patients more). The clinical discharge diagnosis was musculoskeletal chest pain and unknown. The resulting sensitivity and NPV for rule-out was 98.0% (95% CI 94.4-99.3) and 97.9% (95% CI 93.9-99.3), respectively.

Validation Cohort (TRAPID-AMI)

Patients characteristics

In the TRAPID-AMI cohort, 1010 patients were eligible for externally validating the novel derived observe-zone 0/3h-hs-cTnT-criteria. NSTEMI was the adjudicated final diagnosis in 168 (16.6%) patients. Overall, patient characteristics were comparable between APACE and TRAPID-AMI, although in TRAPID-AMI there was a larger proportion of early presenters (**Supplemental Table XVI**). The distribution of the adjudicated final diagnoses of myocardial injury, type 1 NSTEMI and type 2 NSTEMI among patients with baseline hs-cTnT values ≥14 ng/L or any hs-cTnT value ≥14 ng/L were comparable to those of the APACE study (**Supplemental Table XVIIA and XVIIB, respectively).** The difference regarding T2MI was due to differences in the definition of T2MI. While the 4th UDMI was used in APACE, in TRAPID-AMI, using the concept commonly used at the time of conduct of that study, the presence of coronary artery disease was requested as an additional criterion for the presence of T2MI.

After application of the ESC 0/1h-hs-cTnT-algorithm, 243 (24.1%) patients remained in the observe-zone. Of these, 58 (23.9%) had NSTEMI (46 type 1 NSTEMI, 12 type 2 NSTEMI**.** Among the observe-zone patients (n=243) median age was 74 years (interquartile range [IQR] 65-81) and 27.6% were women (**Supplemental Table XVIII**). NSTEMI and non-NSTEMI patients were comparable in many baseline characteristics. However, NSTEMI patients were younger (68 vs 75 years; p value = 0.018) or more likely to present with ischemic ECG changes (ST segment depression, 39.2% vs 22.0%; p value = 0.014).

Baseline hs-cTnT concentration in patients remaining in the observe zone after application of the ESC 0/1h-hs-cTnT-algorithm was also comparable between the APACE and TRAPID-AMI studies (18 [14-25] ng/L vs 19 [14-26] ng/L, respectively).

External validation of the Parkland's criteria (7ng/L cutoff)

After applying the suggested 0/3h-hs-cTnT-change of <7ng/L for rule-out of NSTEMI in the TRAPID-AMI cohort, 215 of the 243 patients in the observe-zone (88.5%) were triaged towards rule-out, resulting in a sensitivity of 41.4% (95%CI 29.6-54.2) and a NPV of 84.2% (95%CI 78.7-88.5), missing 34 patients with NSTEMI (58.6% of all NSTEMIs in the observe-zone). Using a 0/3h-hs-cTnT-change of ≥7ng/L, 28 patients in the observe-zone were triaged towards rule-in, of which 24 had an NSTEMI, resulting in a specificity of 97.8% (95% CI 94.6-99.2) and a PPV of 85.7% (95% CI, 68.5-94.3, **Supplemental**

Figure VII). Sensitivity analysis showed comparable results (**Supplemental Figure VIII and IX**).

Comparison between derivation and external validation performance measures

The difference in sensitivity and NPV between the apparent performance (APACE derivation cohort) and external validation performance (TRAPID-AMI cohort) for the rule-out pathway in the novel derived observe-zone 0/3-hour-hs-cTnT-criteria was comparable (difference in sensitivity 0.9%, 95% CI: -2.8 to 4.6%, p=0.59; difference in NPV 1.0%, 95% CI: -2.6 to 4.6%, p=0.52).

Sensitivity analysis for the external validation of the novel derived observe-zone 0/3h-hs-cTnT-criteria

In a first sensitivity analysis we assessed the diagnostic performance of the novel derived observe-zone 0/3h-hs-cTnT-criteria by using 2-hour hs-cTnT values as if they were 3-hour values. (**Supplemental Figure XVI**). Sensitivity and NPV for rule-out were 96.6% (95% CI, 88.3-99.0) and 96.9% (95% CI, 89.5-99.2), respectively, missing 2 NSTEMI patients. Specificity and PPV for rule-in were 96.2% (95% CI, 92.4-98.2) and 68.2% (95% CI, 47.3-83.6), respectively. After applying the novel derived 0/3h-criteria, 156 patients (15.4%) remained in the observe zone, of which 41 had a final adjudicated NSTEMI.

In a second sensitivity analysis we gave more weight to the 2-hour values (2/3rd) than to the 5/6-hour values (1/3rd), **Supplemental Figure XVII**. Sensitivity and NPV for rule-out were 100% (95% CI, 93.8-100) and 100% (95% CI, 94.1-100), respectively. Specificity and PPV for rule-in were 95.7% (95% CI, 91.7-97.8) and 75.8% (95% CI, 59.0-87.2), respectively. After applying the novel derived 0/3h-criteria, 149 patients (14.8%) remained in the observe zone, of which 33 had a final adjudicated NSTEMI.

In both sensitivity analysis, the difference in sensitivity and NPV between the apparent performance (APACE derivation cohort) and external validation performance (TRAPID-AMI cohort) for the rule-out pathway of the novel derived 0/3h-criteria was comparable (difference in sensitivity 2.6%, 95% CI: -2.3 to 7.5%, p=0.20; difference in NPV 2.4%, 95% CI: -2.0 to 6.8%, p=0.18 for sensitivity analysis 1; and difference in sensitivity -0.8%, 95% CI: -2.4 to 0.8, p=0.5; difference in NPV -0.7%, 95% CI: -2.0% to 0.7%, p=0.51, for sensitivity analysis 2).

Supplemental Tables

Supplemental Table I. TRIPOD Checklist

Section/Topic			Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	3-4
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	7-8
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	7-8
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	9,13,14
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	9,S11 Online Figure 1
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	9,13,14,S11
	5b	D;V	Describe eligibility criteria for participants.	9,S11
	5c	D;V	Give details of treatments received, if relevant.	n/a
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	10-12
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	10-11
Predictors	7a	D;V	Clearly define all predictors used in developing the multivariable prediction model, including how and when they were measured.	10-11
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	n/a
Sample size	8	D;V	Explain how the study size was arrived at.	15 S10-11 Online Table 3
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	S4
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	12-13
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	12-13 S8-9
	10c	V	For validation, describe how the predictions were calculated.	12 Online Figure 2
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	14
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	n/a
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	12-13
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	7,9,,20,21,23 S18
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	16, S12 Online Fig 1 & 15
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	16,20,S18 Table 1 Online Table 4-6, 16 and 18
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	23, S18-19 Online Table 17
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	16,20
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	n/a
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	n/a
	15b	D	Explain how to use the prediction model.	18-19
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	18-19 Table 3 Figure 3
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	n/a
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as non-representative sample, few events per predictor, missing data).	25-26
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	23-24
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	23-26
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	24,25
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	30
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	27-29

D stands for derivation; V for validation; and S for supplemental

Supplemental Table II. Distribution of serial hs-cTn measurements over time with respect to time since chest pain onset (IIA), time of presentation to the ED (IIB) and time of presentation to the ED in patients remaining in the observe zone (IIC)

IIA

Latest hs-cTnT value available x hours after symptom onset	All patients (n=2076)		NSTEMI (n=431)		Non-NSTEMI (n=1645)	
	N	%	n	%	n	%
≥0h after onset	2076	100%	431	100%	1645	100%
≥1h after onset	2076	100%	431	100%	1645	100%
≥2h after onset	2076	100%	431	100%	1645	100%
≥3h after onset	2076	100%	431	100%	1645	100%
≥4h after onset	2033	98%	431	100%	1602	97%
≥5h after onset	1914	92%	425	99%	1489	91%
≥6h after onset	1820	88%	419	97%	1401	85%

IIB

Latest hs-cTnT value available x hours after presentation	All patients (n=2076)		NSTEMI (n=431)		Non-NSTEMI (n=1645)	
	N	%	n	%	n	%
≥0h after presentation	2076	100%	431	100%	1645	100%
≥1h after presentation	2076	100%	431	100%	1645	100%
≥2h after presentation	2076	100%	431	100%	1645	100%
≥3h after presentation	2076	100%	431	100%	1645	100%
≥4h after presentation	1445	70%	407	94%	1038	63%
≥5h after presentation	1359	65%	404	94%	955	58%
≥6h after presentation	1320	64%	402	93%	918	56%

IIC

Latest hs-cTnT value available x hours after presentation	OZ patients (n=564)		NSTEMI (n=120)		Non-NSTEMI (n=444)	
	N	%	n	%	n	%
≥0h after presentation	564	100%	120	100%	444	100%
≥1h after presentation	564	100%	120	100%	444	100%
≥2h after presentation	564	100%	120	100%	444	100%
≥3h after presentation	564	100%	120	100%	444	100%
≥4h after presentation	435	77%	104	87%	331	75%
≥5h after presentation	422	75%	103	86%	319	72%
≥6h after presentation	413	73%	101	84%	312	70%

NSTEMI indicates non-ST segment elevation myocardial infarction; and hs-cTnT, high-sensitivity cardiac troponin T.

Supplemental Table III. Power calculation

N	p0	pa	delta	power
120	1	0.99	-0.01	0.7963
120	1	0.98	-0.02	0.9205
120	1	0.97	-0.03	0.9651
120	1	0.96	-0.04	0.984
120	1	0.95	-0.05	0.9935
120	1	0.94	-0.06	0.9965
120	1	0.93	-0.07	0.9983
120	1	0.92	-0.08	0.9992
120	1	0.91	-0.09	0.9996
120	1	0.90	-0.1	0.9998

Power calculation for a test of H0: = 1 versus Ha: != 1 with null proportion p0 = 1 (100% sensitivity), alternative proportion ranging from 0.9 (90% sensitivity) to 0.99 (99% sensitivity), a sample size ultimately resulting in 120 NSTEMIs remaining in the observe-zone, and a default significance level alpha of 0.05.

N represents number of NSTEMI in the observe-zone; P0 represents reference sensitivity of 100%; and Pa represents sensitivity in the external validation cohort.

Supplemental Table IV. Comparison of patient characteristics (A) and final adjudicated diagnoses (B) between the population eligible for analysis and those excluded because a 3-hour sample was not available

Table IVA	Patients with 3h hs-cTnT values (n=2076)	Patients without 3h hs-cTnT values (n=2728)	P Value
Age, y	63.0 (52.0, 76.0)	58.0 (46.0, 72.0)	<0.001
Female	658 (31.7%)	914 (33.5%)	0.19
Primary symtpom, n (%)			
Pain location mid chest	1324 (65.7%)	1629 (60.8%)	<0.001
Radiation	1209 (58.2%)	1628 (59.7%)	0.31
Dyspnea	989 (48.4%)	1297 (48.2%)	0.86
Nausea	358 (18.3%)	428 (17.5%)	0.47
Time since pain started	4.5 (2.0, 11.0)	5.0 (2.0, 12.0)	0.001
Early presenters (≤2h)	611 (29.4%)	710 (26.0%)	0.009
History, n (%)			
Coronary artery disease	737 (35.5%)	815 (29.9%)	<0.001
Previous MI	538 (25.9%)	575 (21.1%)	<0.001
Previous revascularisation	621 (29.9%)	685 (25.1%)	<0.001
Peripheral Artery Disease	117 (5.6%)	132 (4.8%)	0.22
Previous Stroke	108 (5.2%)	149 (5.5%)	0.69
Cardiovascular risk factors, n (%)			
Hypertension	1358 (65.4%)	1496 (54.8%)	<0.001
Hypercholesterolemia	1082 (52.1%)	1190 (43.6%)	<0.001
Diabetes mellitus	422 (20.3%)	417 (15.3%)	<0.001
Current smoking	470 (22.6%)	727 (26.6%)	0.001
ECG findings, n (%)			
Left Bundle-Branch Block	92 (4.4%)	91 (3.3%)	0.049
Right Bundle-Branch Block	66 (3.2%)	65 (2.4%)	0.093
ST-segment depression	174 (8.4%)	199 (7.3%)	0.16
T-wave inversion	232 (11.2%)	294 (10.8%)	0.66
Laboratory findings			
eGFR	82.0 (67.1, 97.6)	85.2 (69.5, 101.1)	<0.001
Chronic medication on admission, n(%)			
Antiplatelet therapy	898 (43.3%)	933 (34.2%)	<0.001
Oral anticoagulation	239 (11.5%)	255 (9.3%)	0.014
Beta-blocker	787 (37.9%)	831 (30.5%)	<0.001
Statin	818 (39.4%)	879 (32.2%)	<0.001
ACEIs/ARBs	956 (46.1%)	943 (34.6%)	<0.001
Calcium antagonists	394 (19.0%)	325 (11.9%)	<0.001
Nitrates	215 (10.4%)	243 (8.9%)	0.090

Table IVB	Patients with 3h hs-cTnT values (n=2076)	Patients without 3h hs-cTnT values (n=2728)	P Value
Final Adjudicated Diagnosis			
NSTEMI	431 (20.8%)	461 (16.9%)	<0.001
UA	250 (12.0%)	167 (6.1%)	
cardiac, not coronary	312 (15.0%)	332 (12.2%)	
non-cardiac	1002 (48.3%)	1701 (62.4%)	
unknown	81 (3.9%)	67 (2.5%)	

ECG indicates electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ACEIs, Angiotensin-converting enzyme 2; ARBs, Angiotensin II receptor blockers; and NSTEMI, non-ST elevation myocardial infarction.

Supplemental Table V. Patient Characteristics stratified by the ESC 0/1h-hs-cTnT-algorithm

	All Patients (n=2076)	Rule-Out (n=1142)	Observe-zone (n=564)	Rule-In (n=370)	*P* Value
Age, y	63.0 (52.0, 76.0)	56.0 (47.0, 66.0)	74.0 (65.0, 81.0)	70.0 (60.0, 80.0)	<0.001
Female	658 (31.7%)	403 (35.3%)	143 (25.4%)	112 (30.3%)	<0.001
Primary symptom, n (%)					
Radiation	1209 (58.2%)	694 (60.8%)	301 (53.4%)	214 (57.8%)	0.014
Pain location mid chest	1324 (65.7%)	671 (60.6%)	382 (69.5%)	271 (75.9%)	<0.001
Dyspnea	989 (48.4%)	493 (43.9%)	294 (52.9%)	202 (55.5%)	<0.001
Nausea	358 (18.3%)	207 (19.3%)	82 (15.6%)	69 (19.5%)	0.16
Time since pain started	4.5 (2.0, 11.0)	4.0 (2.0, 12.0)	5.0 (2.5, 11.0)	5.0 (2.0, 9.5)	0.002
Early presenters (≤2h)	611 (29.4%)	386 (33.8%)	126 (22.3%)	99 (26.8%)	<0.001
History, n (%)					
Coronary artery disease	737 (35.5%)	285 (25.0%)	311 (55.1%)	141 (38.1%)	<0.001
Previous MI	538 (25.9%)	206 (18.0%)	227 (40.2%)	105 (28.4%)	<0.001
Previous revascularization	621 (29.9%)	251 (22.0%)	262 (46.5%)	108 (29.2%)	<0.001
Peripheral Artery Disease	117 (5.6%)	29 (2.5%)	50 (8.9%)	38 (10.3%)	<0.001
Previous Stroke	108 (5.2%)	31 (2.7%)	42 (7.4%)	35 (9.5%)	<0.001
Cardiovascular RF, n (%)					
Hypertension	1358 (65.4%)	609 (53.3%)	473 (83.9%)	276 (74.6%)	<0.001
Hypercholesterolemia	1082 (52.1%)	485 (42.5%)	378 (67.0%)	219 (59.2%)	<0.001
Diabetes mellitus	422 (20.3%)	155 (13.6%)	164 (29.1%)	103 (27.8%)	<0.001
Current smoking	470 (22.6%)	319 (27.9%)	71 (12.6%)	80 (21.6%)	<0.001
ECG findings, n (%)					
Left Bundle-Branch Block	92 (4.4%)	13 (1.1%)	45 (8.0%)	34 (9.2%)	<0.001
Right Bundle-Branch Block	66 (3.2%)	22 (1.9%)	26 (4.6%)	18 (4.9%)	0.002
ST-segment depression	174 (8.4%)	42 (3.7%)	56 (9.9%)	76 (20.5%)	<0.001
T-wave inversion	232 (11.2%)	69 (6.0%)	87 (15.4%)	76 (20.5%)	<0.001
Laboratory findings					
eGFR	82.0 (67.1, 97.6)	88.0 (75.2, 102.6)	71.7 (56.4, 88.2)	74.7 (56.5, 93.3)	<0.001
Chronic medication on admission, n (%)					
Antiplatelet therapy	898 (43.3%)	382 (33.5%)	334 (59.2%)	182 (49.2%)	<0.001
Oral anticoagulation	239 (11.5%)	72 (6.3%)	123 (21.8%)	44 (11.9%)	<0.001
Beta-blocker	787 (37.9%)	327 (28.6%)	314 (55.7%)	146 (39.5%)	<0.001
Statin	818 (39.4%)	343 (30.0%)	320 (56.7%)	155 (41.9%)	<0.001
ACEIs/ARBs	956 (46.1%)	401 (35.1%)	361 (64.0%)	194 (52.4%)	<0.001
Calcium antagonists	394 (19.0%)	168 (14.7%)	152 (27.0%)	74 (20.0%)	<0.001
Nitrates	215 (10.4%)	74 (6.5%)	93 (16.5%)	48 (13.0%)	<0.001

ECG indicates electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ACEIs, Angiotensin-converting enzyme 2; and ARBs, Angiotensin II receptor blockers.

Supplemental Table VI. Differences between early presenters and non-early presenters in the observe-zone group

	All Patients (n=564)	Non Early Presenter (n=438)	Early Presenter (n=126)	*P* Value
Age, y	74.0 (65.0, 81.0)	75.0 (66.0, 81.0)	72.0 (64.0, 79.0)	0.075
Female	143 (25.4%)	112 (25.6%)	31 (24.6%)	0.83
Primary symptom, n (%)				
Pain location mid chest	382 (69.5%)	301 (70.5%)	81 (65.9%)	0.33
Radiation	301 (53.4%)	226 (51.6%)	75 (59.5%)	0.12
Dyspnea	294 (52.9%)	230 (53.0%)	64 (52.5%)	0.92
Nausea	82 (15.6%)	55 (13.4%)	27 (23.3%)	0.009
History, n (%)				
Coronary artery disease	311 (55.1%)	239 (54.6%)	72 (57.1%)	0.61
Previous MI	227 (40.2%)	173 (39.5%)	54 (42.9%)	0.50
Previous revascularization	262 (46.5%)	201 (45.9%)	61 (48.4%)	0.62
Peripheral Artery Disease	50 (8.9%)	40 (9.1%)	10 (7.9%)	0.68
Previous Stroke	42 (7.4%)	35 (8.0%)	7 (5.6%)	0.36
Cardiovascular risk factors, n (%)				
Hypertension	473 (83.9%)	370 (84.5%)	103 (81.7%)	0.46
Hypercholesterolemia	378 (67.0%)	291 (66.4%)	87 (69.0%)	0.58
Diabetes mellitus	164 (29.1%)	138 (31.5%)	26 (20.6%)	0.018
Current smoking	71 (12.6%)	52 (11.9%)	19 (15.1%)	0.34
ECG findings, n (%)				
Left Bundle-Branch Block	45 (8.0%)	38 (8.7%)	7 (5.6%)	0.25
Right Bundle-Branch Block	26 (4.6%)	22 (5.0%)	4 (3.2%)	0.38
ST-segment depression	56 (9.9%)	42 (9.6%)	14 (11.1%)	0.61
T-wave inversion	87 (15.4%)	71 (16.2%)	16 (12.7%)	0.34
Laboratory findings				
eGFR	71.7 (56.4, 88.2)	72.0 (54.8, 88.5)	70.8 (59.4, 84.1)	0.79
Chronic medication on admission, n (%)				
Antiplatelet therapy	334 (59.2%)	262 (59.8%)	72 (57.1%)	0.59
Oral anticoagulation	123 (21.8%)	95 (21.7%)	28 (22.2%)	0.90
Beta-blocker	314 (55.7%)	244 (55.7%)	70 (55.6%)	0.98
Statin	320 (56.7%)	247 (56.4%)	73 (57.9%)	0.76
ACEIs/ARBs	361 (64.0%)	283 (64.6%)	78 (61.9%)	0.58
Calcium antagonists	152 (27.0%)	115 (26.3%)	37 (29.4%)	0.49
Nitrates	93 (16.5%)	75 (17.1%)	18 (14.3%)	0.45

ECG indicates electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ACEIs, Angiotensin-converting enzyme 2; and ARBs, Angiotensin II receptor blockers.

Supplemental Table VII. Baseline and 3-hour hs-cTnT, age and eGFR in patients remaining in the observe-zone after applying the ESC 0/1h Algo stratified by Sex

Variable	Male n=421	Female N=143	p value
0h hs-cTnT	18.57 (14, 25.64)	17.78 (13.53, 25)	0.25
3h hs-cTnT	19 (14,26)	18 (14, 25)	0.39
Age	73 (64, 80)	77 (70, 82)	<0.001
eGFR	73.6 (57.9, 90.3)	67.3 (54, 80)	0.011

Hs-cTnT indicates high-sensitivity cardiac troponin T; eGFR, estimated Glomerular Filtration Rate; and ESC, European Society of Cardiology

Supplemental Table VIII. Patient characteristics and ECG features of NSTEMI patients correctly triaged to rule-in (true positive) vs NSTEMI patients triaged to rule-out (false negative) using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone.

Clinical and ECG Characteristics	True positive (NSTEMI RI 3h)	False negative (Missed NSTEMI RO 3h)	p-value
	40	80	
Age, y	66.0 (56.5, 78.0)	72.0 (60.0, 80.0)	0.23
Female	6 (15%)	19 (24%)	0.27
Pain radiation	29 (73%)	51(64%)	0.34
Pain location mid-chest	29 (76%)	62 (81%)	0.60
Hypertension	34 (85%)	59 (74%)	0.16
Hypercholesterolemia	26 (65%)	49 (61%)	0.69
Diabetes mellitus	11 (28%)	28 (35%)	0.41
Current smoker	7 (18%)	16 (20%)	0.74
Coronary artery disease	24 (60%)	40 (50%)	0.30
Previous MI	19 (48%)	33 (41%)	0.51
Revascularization	20 (50%)	34 (42%)	0.44
Peripheral Artery Disease	3 (8%)	13 (16%)	0.18
Previous Stroke	1 (3%)	5 (6%)	0.37
ST segment depression	8 (20%)	22 (28%)	0.37
T wave inversion	7 (18%)	23 (29%)	0.18
Left Bundle-branch block	2 (5%)	5 (6%)	0.78
Right Bundle-branch block	1 (2%)	4 (5%)	0.52
eGFR	75.4 (63.5, 95.8)	73.4 (61.9, 92.8)	0.51

ECG indicates electrocardiogram; eGFR, estimated Glomerular Filtration Rate; NSTEMI, non-ST elevation myocardial infarction.

Supplemental Table IX. Time since chest pain onset and hs-cTnT/I concentrations in NSTEMI patients correctly triaged to rule-in (true positives) vs NSTEMI patients triaged to rule-out (false negative) (A), and in non-NSTEMI patients correctly triaged to rule-out (true negative) vs NSTEMI patients triaged to rule-out (false negative) (B), using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone.

Table IXA

Variable	True positive (NSTEMI RI 3h)	False negative (Missed NSTEMI RO 3h)	p-value
	40	80	
Chest Pain Onset ≤3h	20 (50%)	23 (29%)	0.024
Chest Pain Onset ≤2h	17 (43%)	14 (18%)	0.003
Time since chest pain onset	3.0 (1.8, 5.5)	6.5 (3.0, 11.8)	0.006
0h - Roche hs-cTnT	18.9 (14.0, 37.0)	22.0 (17.9, 34.0)	0.17
1h - Roche hs-cTnT	20.8 (17.2, 36.0)	24.0 (17.0, 34.0)	0.51
2h - Roche hs-cTnT	28.0 (20.0, 35.0)	25.1 (18.0, 34.8)	0.35
3h - Roche hs-cTnT	27.5 (24.6, 36.5)	25.6 (18.0, 34.0)	0.041
0/3h delta hs-cTnT	10.0 (7.5, 14.0)	3.0 (2.0, 4.6)	<0.001
Peak Roche hs-cTnT	37.0 (27.0, 49.0)	26.0 (19.7, 36.0)	<0.001
0h - Abbott-hs-cTnI	14.9 (10.7, 42.0)	22.6 (10.9, 55.7)	0.27
1h -Abbott- hs-cTnI	22.0 (13.0, 45.0)	24.4 (10.8, 60.6)	0.77
2h - Abbott-hs-cTnI	40.8 (21.7, 67.2)	33.6 (15.6, 77.0)	0.44
3h - Abbott-hs-cTnI	38.6 (27.8, 71.5)	36.9 (14.0, 96.4)	0.41
0/3h delta hs-cTnI	25.2 (9.0, 54.5)	8.6 (2.8, 22.0)	0.024
Peak Abbott hs-cTnI	45.5 (32.5, 113.1)	36.5 (15.0, 98.2)	0.088

For 0h-Abbott-hs-cTnI 40 and 80 patients had available TnI values. For 1h-Abbott-hs-cTnI 40 and 76 patients had available TnI values. For 2h-Abbott-hs-cTnI 30 and 73 patients had available TnI values. For 3h-Abbott-hs-cTnI 33 and 65 patients had available TnI values. hs-cTnT/I indicates high-sensitivity cardiac Troponin T or I; NSTEMI, non-ST elevation myocardial infarction.

Table IXB

Variable	True negative (non-NSTEMI RO 3h)	False negative (Missed NSTEMI RO 3h)	p-value
	437	80	
Chest Pain Onset ≤3h	145 (33%)	23 (29%)	0.44
Chest Pain Onset ≤2h	93 (21%)	14 (18%)	0.44
Time since chest pain onset	5.0 (2.5,11.3)	6.5 (3.0, 11.8)	0.39
0h - Roche hs-cTnT	17.5 (14.0, 24.0)	22.0 (17.9, 34.0)	<0.001
1h - Roche hs-cTnT	17.2 (14.0, 24.0)	24.0 (17.0, 34.0)	<0.001
2h - Roche hs-cTnT	17.6 (13.5, 23.4)	25.1 (18.0, 34.8)	<0.001
3h - Roche hs-cTnT	17.0 (13.0, 23.0)	25.6 (18.0, 34.0)	<0.001
0/3h delta hs-cTnT	1.0 (1.0, 2.3)	3.0 (2.0, 4.6)	<0.001
Peak Roche hs-cTnT	19.0 (14.9, 29.0)	26.0 (19.7, 36.0)	<0.001
0h - Abbott-hs-cTnI	9.0 (5.0, 17.0)	22.6 (10.9, 55.7)	<0.001
1h -Abbott- hs-cTnI	9.4 (5.0, 18.0)	24.4 (10.8, 60.6)	<0.001
2h - Abbott-hs-cTnI	9.7 (5.8, 18.1)	33.6 (15.6, 77.0)	<0.001
3h - Abbott-hs-cTnI	10.0 (6.0, 20.0)	36.9 (14.0, 96.4)	<0.001
0/3h delta hs-cTnI	1.0 (0.4, 3.0)	8.6 (2.8, 22.0)	<0.001
Peak Abbott hs-cTnI	11.0 (6.5, 21.5)	36.5 (15.0, 98.2)	<0.001

For 0h-Abbott-hs-cTnI 433 and 80 patients had available TnI values. For 1h-Abbott-hs-cTnI 422 and 76 patients had available TnI values. For 2h-Abbott-hs-cTnI 399 and 73 patients had available TnI values. For 3h-Abbott-hs-cTnI 377 and 65 patients had available TnI values. hs-cTnT/I indicates high-sensitivity cardiac Troponin T or I; NSTEMI, non-ST elevation myocardial infarction.

Supplemental Table X. Management (medication on discharge, invasive therapy and diagnostic workup) in NSTEMI patients correctly triaged to rule-in (true positives) vs NSTEMI patients triaged to rule-out (false negative) using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone.

Medication on discharge	True positive (NSTEMI RI 3h)	False negative (Missed NSTEMI RO 3h)	p-value
	40	80	
Antiplatelet therapy	33 (83%)	63 (79%)	0.63
Oral anticoagulants	8 (20%)	17 (21%)	0.87
Beta-blocker	32 (80%)	62 (78%)	0.75
Statin	34 (85%)	55 (69%)	0.06
ACEIs/ARBs	33 (83%)	59 (74%)	0.29
Calcium antagonists	15 (38%)	23 (29%)	0.33
Nitrates	10 (25%)	19 (24%)	0.88

Diagnosis and invasive therapy	True positive (NSTEMI RI 3h)	False negative (Missed NSTEMI RO 3h)	p-value
	40	80	
Coronary angiography	21 (53%)	43 (54%)	0.90
PCI performed	19 (48%)	30 (38%)	0.29
- **Drug eluting Stent**	16 (40%)	25 (31%)	0.34
- **Metallic stent**	2 (5%)	3 (4%)	0.75
Ergometry	13 (33%)	19 (24%)	0.31
- **Clinical positive**	5 (13%)	4 (5%)	0.14
- **Electrical positive**	6 (15%)	6 (8%)	0.20

NSTEMI indicates non-ST elevation myocardial infarction; ACEIs, Angiotensin-converting enzyme 2; and ARBs, Angiotensin II receptor blockers.

Supplemental Table XI. Management (medication on discharge, invasive therapy and diagnostic workup) differences between non-NSTEMI patients correctly triaged to rule-out (true negative) vs NSTEMI patients triaged to rule-out (false negative) using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone.

Medication on discharge	**True negative (non NSTEMI ruled-out)**	**False negative (NSTEMI ruled-out)**	**p-value**
	437	80	
Antiplatelet therapy	284 (65%)	63 (79%)	**0.02**
Oral anticoagulants	123 (28%)	17 (21%)	0.20
Beta-blockers	284 (65%)	62 (78%)	**0.03**
Statin	300 (69%)	55 (69%)	0.99
ACEIs/ARBs	300 (69%)	59 (74%)	0.36
Calcium antagonists	134 (31%)	23 (29%)	0.73
Nitrates	87 (20%)	19 (24%)	0.43

Diagnosis and invasive therapy	**True negative (non NSTEMI ruled-out)**	**False negative (NSTEMI ruled-out)**	**p-value**
	437	80	
Coronary angiography	114 (26%)	43 (54%)	**<0.001**
PCI performed	57 (13%)	30 (38%)	**<0.001**
- **Drug eluting Stent**	47 (11%)	25 (31%)	**<0.001**
- **Metallic Stent**	7 (2%)	3 (4%)	0.20
Ergometry	115 (26%)	19 (24%)	0.63
- **Clinical positive**	23 (5%)	4 (5%)	0.92
- **Electrical positive**	36 (8%)	6 (8%)	0.82

NSTEMI indicates non-ST elevation myocardial infarction; ACEIs, Angiotensin-converting enzyme 2; and ARBs, Angiotensin II receptor blockers.

Supplemental Table XII. NPV and PPV for different hypothetical prevalence's of the Outcome (index NSTEMI) in the observe-zone group.

Hypothetical prevalence	Rule-out NPV	Rule-in PPV
2	98.6%	29.8%
4	97.3%	46.4%
6	95.9%	57.1%
8	94.4%	64.4%
10	93.0%	69.8%
12	91.5%	73.9%
14	90.1%	77.2%
16	88.6%	79.9%
18	87.0%	82.0%
20	85.5%	83.9%
22	84.0%	85.4%

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

Supplemental Table XIII. Apparent performance for the tested cut-off combinations for rule-out.

Combined cut-off strategy		Rule-out Diagnostic performance			
3h hs-cTnT value (ng/L)	0/3h delta (ng/L)	Sensitivity	NPV	Number of patients Ruled out	Number of NSTEMI missed
13	1	100	100	14	0
	2	100	100	40	0
	3	100	100	60	0
	4	100	100	75	0
	5	99.2	98.8	81	1
14	1	100	100	29	0
	2	100	100	68	0
	3	100	100	93	0
	4	100	100	108	0
	5	97.5	97.4	116	3
15	1	100	100	35	0
	2	100	100	93	0
	3	99.2	99.2	123	1
	4	99.2	99.3	138	1
	5	95.8	96.6	147	5
16	1	100	100	44	0
	2	98.3	98.3	115	2
	3	97.5	98.0	153	3
	4	96.7	97.6	170	4
	5	93.3	95.5	179	8
17	1	99.2	98.1	52	1
	2	97.5	97.8	134	3
	3	95.8	97.2	178	5
	4	93.3	96.0	199	8
	5	90.0	94.3	210	12
18	1	99.2	98.3	59	1
	2	95.8	96.7	150	5
	3	94.2	96.5	202	7
	4	90.8	95.2	228	11
	5	86.7	93.4	241	16

NPV indicates negative predictive value; NSTEMI, non-ST elevation myocardial infarction; and hs-cTnT, high-sensitivity cardiac Troponin T.

Supplemental Table XIV. Diagnostic performance of four different ischemic ECG criteria evaluated in patients still remaining in the observe-zone after applying the novel derived observe-zone 0/3h-criteria (3h hs-cTnT measurement in combination with a 0/3h-hs-cTnT absolute change)

ECG Criteria	Apparent Diagnostic Performance			
	Specificity	**PPV**	**Total Rule in**	**NSTEMI Rule in**
ST segment depression	93.6% (90.3-95.9)	50.0% (34.8-65.2)	38	19
T wave inversion	88.9% (84.9-92.0)	37.7% (25.9-51.2)	53	20
ST depression **or** T wave inversion	84.6% (80.0-88.2)	41.0% (30.8-52.1)	78	32
ST depression **and** T wave inversion	98.0% (95.7-99.1)	53.8% (29.1-76.8)	13	7

PPV indicates positive predictive value; NSTEMI, non-ST elevation myocardial infarction.

Supplemental Table XV. Patient characteristics (A) and clinical discharge diagnoses (B) among patients with an adjudicated unknown diagnosis and available baseline, 1-, and 3-hour hs-cTn values.

Table XVA	Patients with an adjudicated unknown diagnosis (n=45)
Age, y	79.0 (64.0, 86.0)
Female	19 (42.2%)
Primary symptom, n (%)	
Pain location mid chest	25 (55.6%)
Radiation	25 (55.6%)
Dyspnea	19 (42.2%)
Nausea	6 (13.3%)
Time since pain started	5.0 (2.7, 10.5)
Early Presenters	8 (17.8%)
History, n (%)	
Coronary artery disease	16 (35.6%)
Previous MI	13 (28.9%)
Previous revascularisation	14 (31.1%)
Peripheral Artery Disease	5 (11.1%)
Previous Stroke	4 (8.9%)
Cardiovascular risk factors, n (%)	
Hypertension	37 (82.2%)
Hypercholesterolemia	29 (64.4%)
Diabetes mellitus	17 (37.8%)
Current smoking	10 (22.2%)
ECG findings, n (%)	
Left Bundle-Branch Block	2 (4.4%)
Right Bundle-Branch Block	3 (6.7%)
ST-segment depression	3 (6.7%)
T-wave inversion	8 (17.8%)
Laboratory findings	
eGFR	65.3 (54.2, 79.4)
Admission medication	
Antiplatelet therapy	25 (55.6%)
Oral anticoagulant	8 (17.8%)
Beta-blocker	21 (46.7%)
Statin	26 (57.8%)
ACEIs/ARBs	24 (53.3%)
Calcium antagonists	8 (17.8%)
Nitrates	9 (19.6%)

Table XVB	Clinical discharge diagnosis (n=45)
NSTEMI	3 (6.7%)
Unstable Angina	3 (6.7%)
Arrhythmia	4 (8.9%)
Hypertension	3 (6.7%)
Cardiac decompensation	1 (2.2%)
Cardiac Other	2 (4.4%)
Musuculoskeletal Chest Pain	1 (2.2%)
Pleuritis/pneumonia	1 (2.2%)
Unknown	27 (60.0%)

ECG indicates electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ACEIs, Angiotensin-converting enzyme 2; ARBs, Angiotensin II receptor blockers; and NSTEMI, non ST elevation myocardial infarction.

Supplemental Table XVI. Patient characteristics in the TRAPID-AMI cohort

	All patients (n=1010)
Age, y	62.0 (51.0, 74.0)
Female	371 (36.7%)
Primary symptom, n (%)	
Radiation	602 (59.6%)
Dyspnea	511 (50.6%)
Time since pain started	2.7 (1.5, 5.2)
Time since maximum pain started	1.7 (0.9, 2.8)
Early Presenters (<2h)	365 (36.1%)
History, n (%)	
Previous MI	272 (27.1%)
Previous revascularisation	327 (32.7%)
Previous Stroke	110 (10.9%)
Cardiovascular risk factors, n (%)	
Hypertension	657 (65.6%)
Diabetes mellitus	221 (22.1%)
Current smoking	217 (21.5%)
ECG findings, n (%)	
Left Bundle-Branch Block	30 (3.1%)
Right Bundle-Branch Block	46 (4.8%)
ST-segment depression	137 (14.6%)
T-wave inversion	147 (15.7%)
Laboratory findings	
eGFR	81.2 (64.2, 97.0)
Admission medication	
Antiplatelet therapy	576 (57.3%)
Oral anticoagulant	163 (16.4%)
Beta-blocker	400 (40.2%)
ACEIs/ARBs	479 (48.0%)
Calcium antagonists	211 (21.2%)
Nitrates	336 (33.5%)

ECG indicates electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ACEIs, Angiotensin-converting enzyme 2; and ARBs, Angiotensin II receptor blockers.

Supplemental Table XVII. Comparison between the APACE and TRAPID-AMI cohort studies showing the proportion of patients with a baseline hs-cTnT value ≥14 ng/L (A) or any hs-cTnT value ≥14 ng/L (B), classified as type 1 NSTEMI, type 2 NSTEMI and myocardial injury

Adjudicated Diagnosis	Cohort Study	
	APACE n=775	TRAPID AMI n=312
NSTEMI type 1	298 (38.5%)	124 (39.7%)
NSTEMI type 2	86 (11.1%)	24 (7.7%)
myocardial injury	391 (50.4%)	164 (52.6%)

Pearson chi2(2) = 2.8378 P value = 0.24

Adjudicated Diagnosis	Cohort Study	
	APACE n=855	TRAPID AMI n=354
NSTEMI type 1	324 (37.9%)	136 (38.4%)
NSTEMI type 2	106 (12.4%)	27 (7.6%)
myocardial injury	425 (49.7%)	191 (54.0%)

Pearson chi2(2) = 6.0834 Pr = 0.05

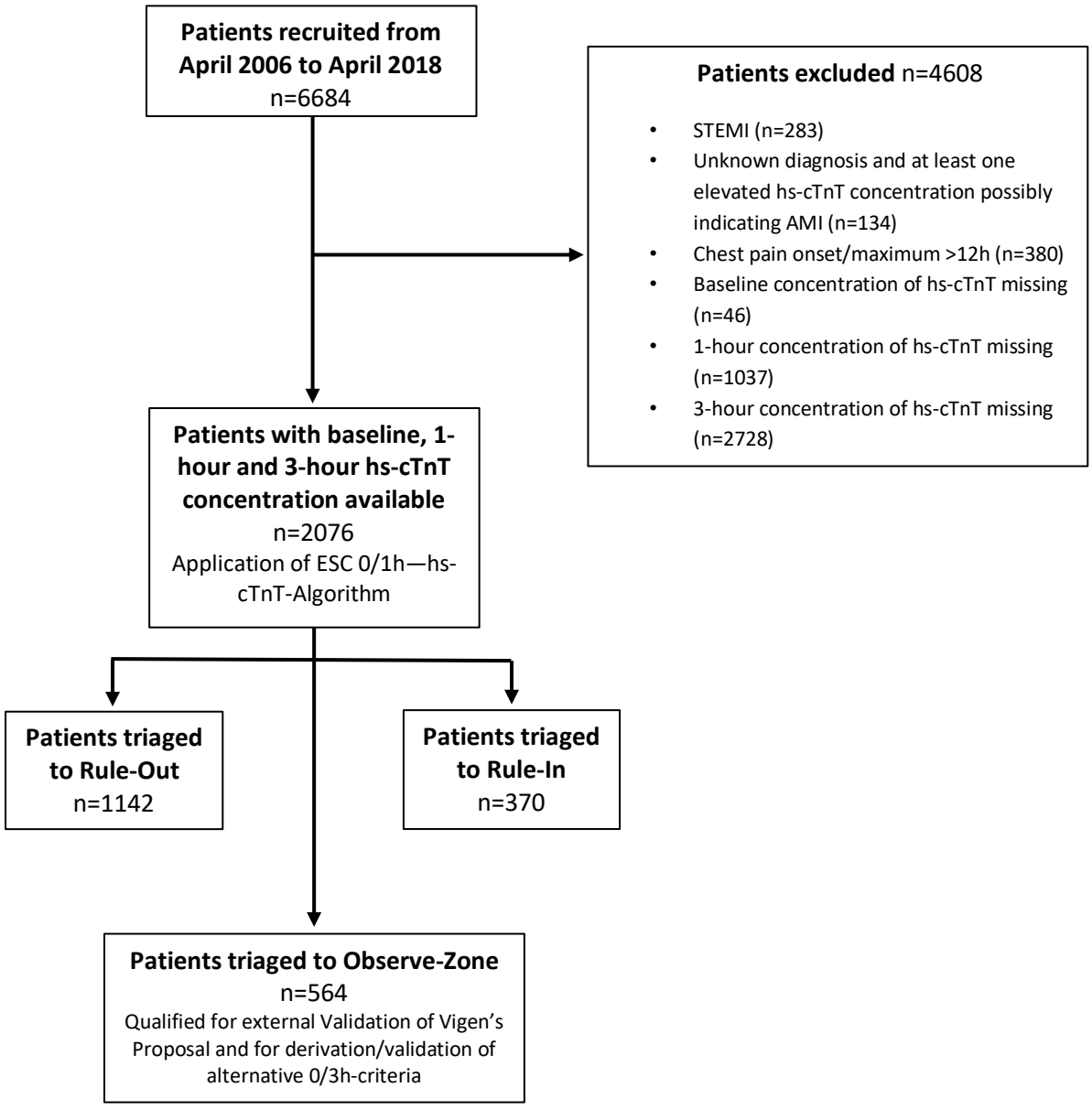
NSTEMI indicates non-ST elevation myocardial infarction.

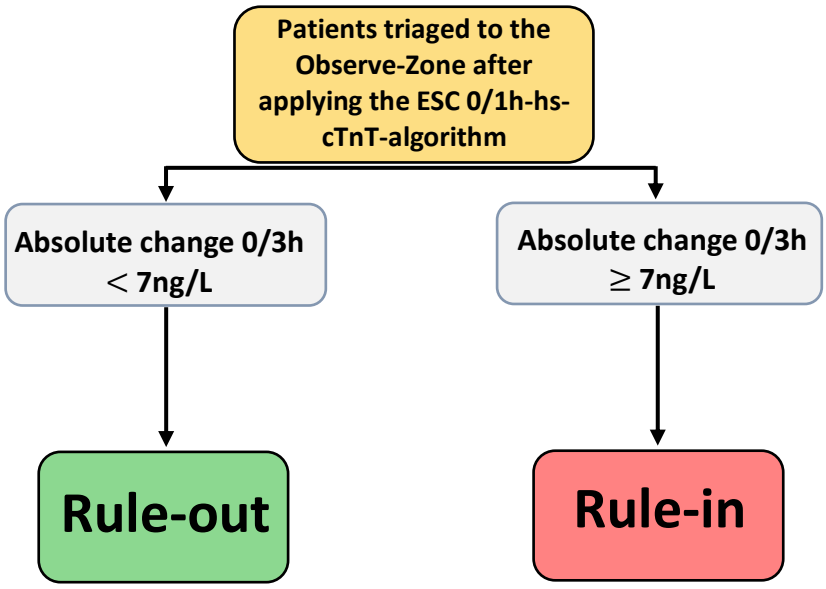
Supplemental Table XVIII. Characteristics of Patients triaged to the Observe-Zone in the TRAPID-AMI cohort

	Observe-zone patients (n=243)	No NSTEMI (n=185)	NSTEMI (n=58)	p value
Age, y	74.0 (65.0, 81.0)	75.0 (68.0, 81.0)	68.0 (60.0, 80.0)	0.018
Female	67 (27.6%)	51 (27.6%)	16 (27.6%)	1
Primary symtpom, n (%)				
Radiation	128 (52.7%)	93 (50.3%)	35 (60.3%)	0.18
Dyspnea	136 (56.0%)	107 (57.8%)	29 (50.0%)	0.29
Time since pain started	2.5 (1.6, 5.1)	2.6 (1.5, 5.2)	2.5 (1.8, 4.4)	0.77
Time since maximum pain started	1.9 (1.2, 3.0)	1.9 (1.2, 3.0)	1.9 (1.3, 3.4)	0.74
Early Presenters (<2h)	82 (33.7%)	65 (35.1%)	17 (29.3%)	0.41
History, n (%)				
Previous MI	100 (41.5%)	74 (40.4%)	26 (44.8%)	0.55
Previous revascularisation	119 (49.6%)	89 (48.6%)	30 (52.6%)	0.60
Previous Stroke	37 (15.2%)	26 (14.1%)	11 (19.0%)	0.36
Cardiovascular risk factors, n (%)				
Hypertension	201 (83.1%)	155 (84.2%)	46 (79.3%)	0.38
Diabetes mellitus	89 (36.8%)	70 (37.8%)	19 (33.3%)	0.54
Current smoking	35 (14.4%)	24 (13.0%)	11 (19.0%)	0.26
ECG findings, n (%)				
Left Bundle-Branch Block	14 (6.2%)	12 (7.0%)	2 (3.7%)	0.38
Right Bundle-Branch Block	23 (10.2%)	17 (9.9%)	6 (11.1%)	0.80
ST-segment depression	57 (26.0%)	37 (22.0%)	20 (39.2%)	0.014
T-wave inversion	63 (29.3%)	46 (28.0%)	17 (33.3%)	0.47
Laboratory findings				
eGFR	67.4 (52.1, 83.8)	66.9 (52.2, 83.1)	68.1 (49.9, 84.8)	0.56
Admission medication				
Antiplatelet therapy	174 (71.9%)	133 (71.9%)	41 (71.9%)	1.00
Oral anticoagulant	64 (26.6%)	52 (28.3%)	12 (21.1%)	0.28
Beta-blocker	135 (56.5%)	109 (59.9%)	26 (45.6%)	0.058
ACEIs/ARBs	153 (63.5%)	114 (62.0%)	39 (68.4%)	0.38
Calcium antagonists	75 (31.2%)	58 (31.7%)	17 (29.8%)	0.79
Nitrates	108 (44.6%)	79 (42.7%)	29 (50.9%)	0.28

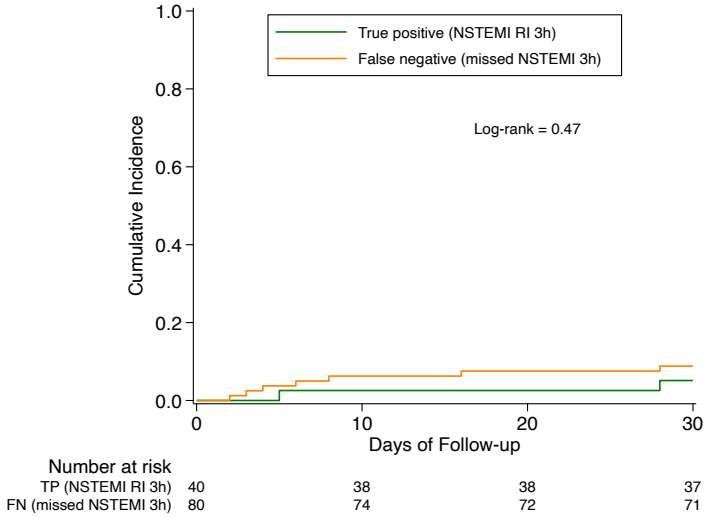
ECG indicates electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ACEIs, Angiotensin-converting enzyme 2; and ARBs, Angiotensin II receptor blockers.

Supplemental Figures



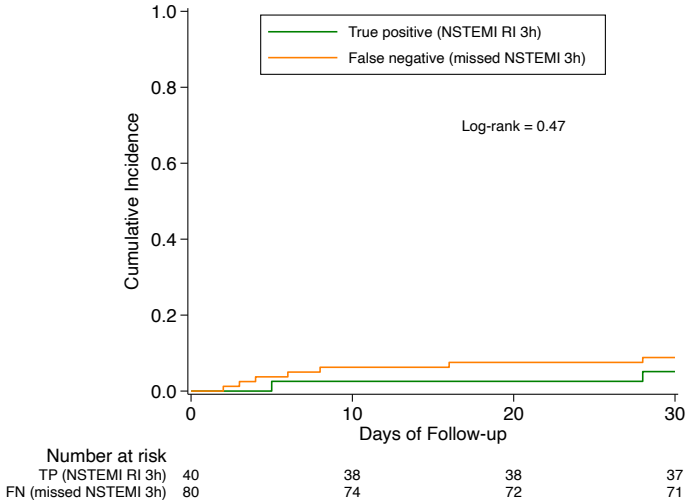

Patients triaged to the Observe-Zone after applying the ESC 0/1h-hs-cTnT-algorithm
Absolute change 0/3h < 7ng/L
Absolute change 0/3h ≥ 7ng/L
Rule-out
Rule-in

A



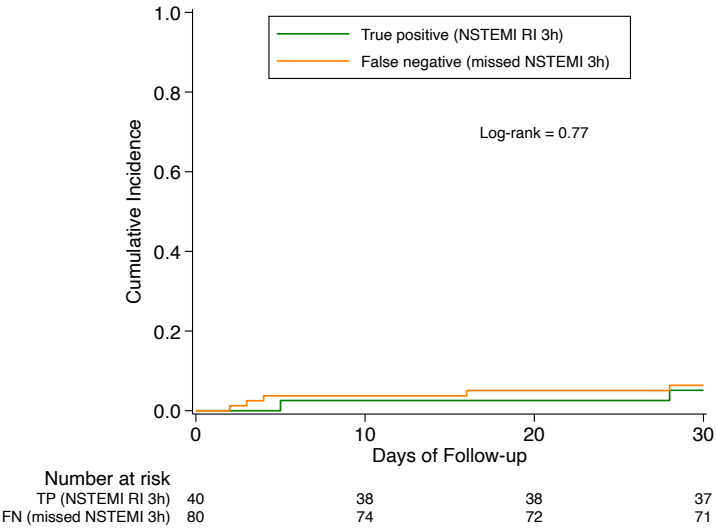
Outcome: CV death or AMI 30d

B



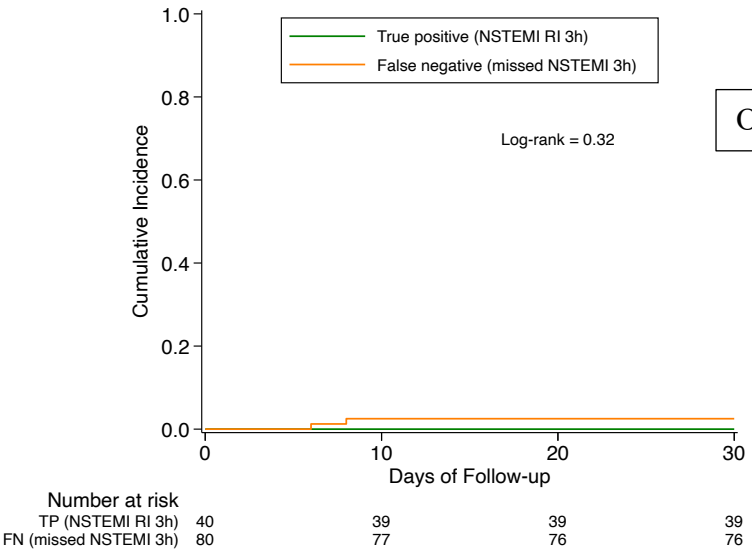
Outcome: all cause death or AMI 30d

C



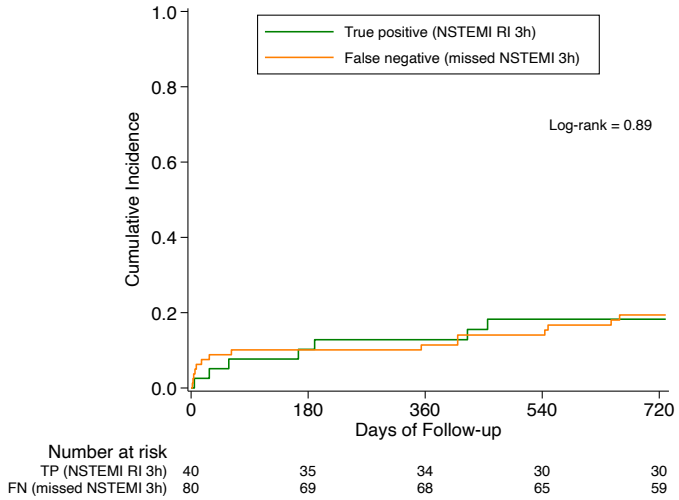
Outcome: AMI 30d

D



Outcome: CV death 30d

A

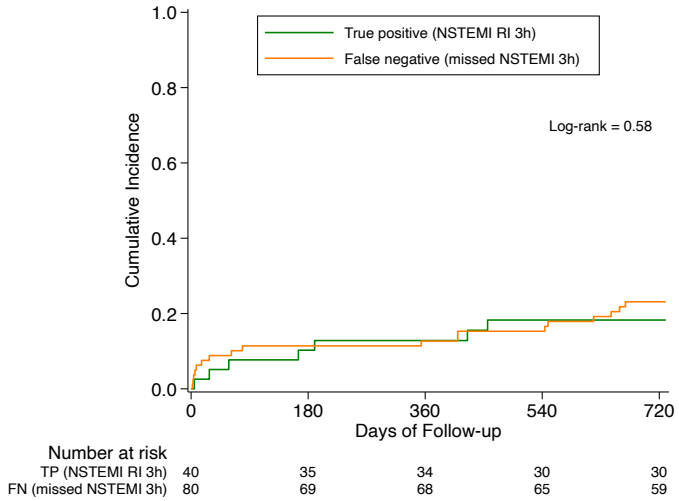


Number at risk

TP (NSTEMI RI 3h)	40	35	34	30	30
FN (missed NSTEMI 3h)	80	69	68	65	59

Outcome: CV death or AMI 730d

B

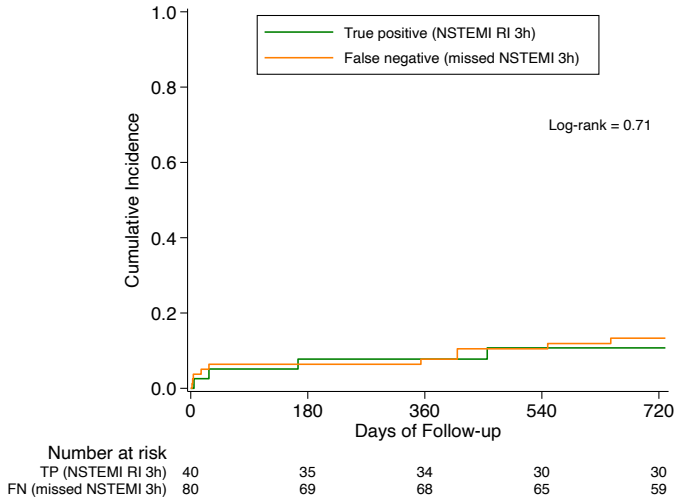


Number at risk

TP (NSTEMI RI 3h)	40	35	34	30	30
FN (missed NSTEMI 3h)	80	69	68	65	59

Outcome: all cause death or AMI 730d

C

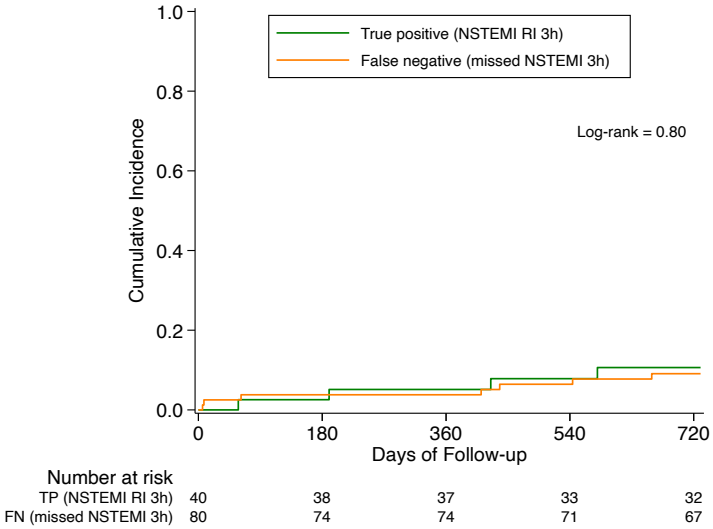


Number at risk

TP (NSTEMI RI 3h)	40	35	34	30	30
FN (missed NSTEMI 3h)	80	69	68	65	59

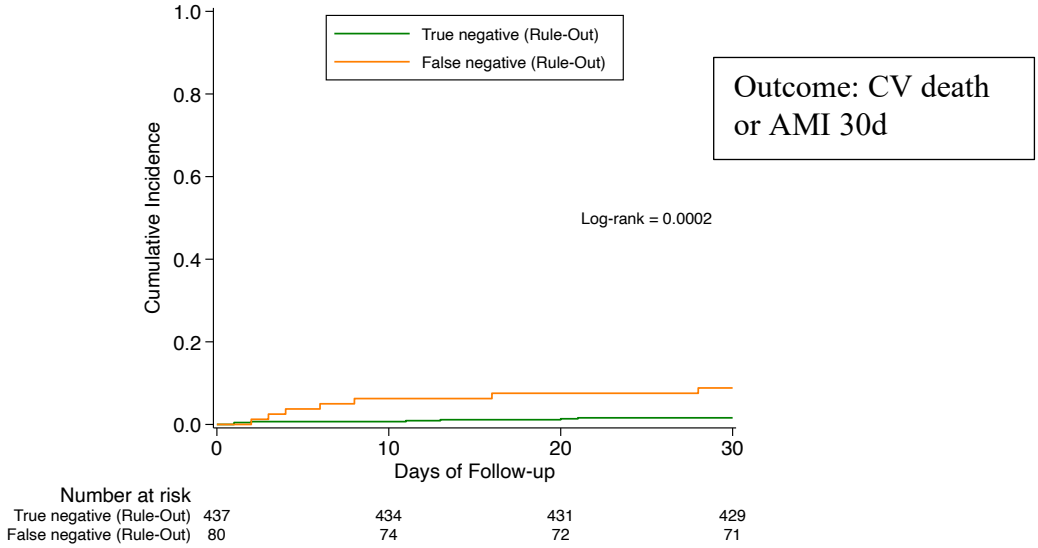
Outcome: AMI 730d

D

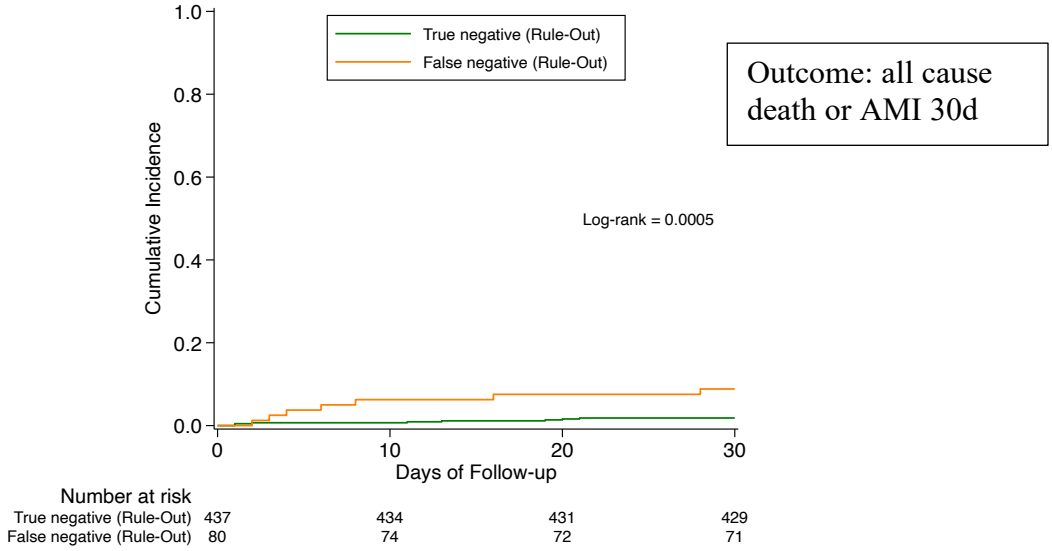


Outcome: CV death 30d

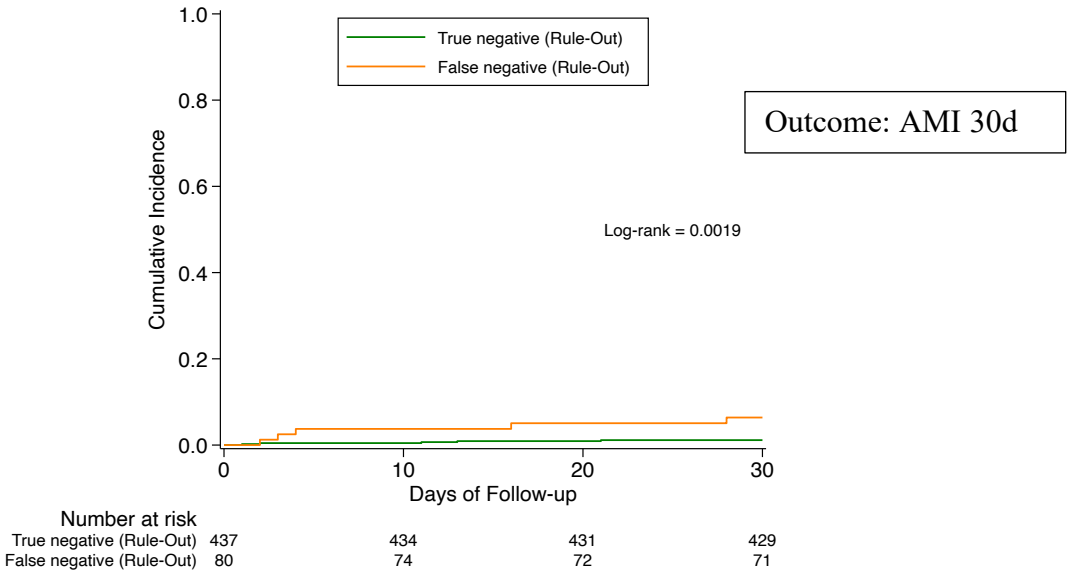
A


True negative (Rule-Out)
False negative (Rule-Out)
Outcome: CV death or AMI 30d
Log-rank = 0.0002
Cumulative Incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Days of Follow-up
Number at risk
True negative (Rule-Out) 437 434 431 429
False negative (Rule-Out) 80 74 72 71

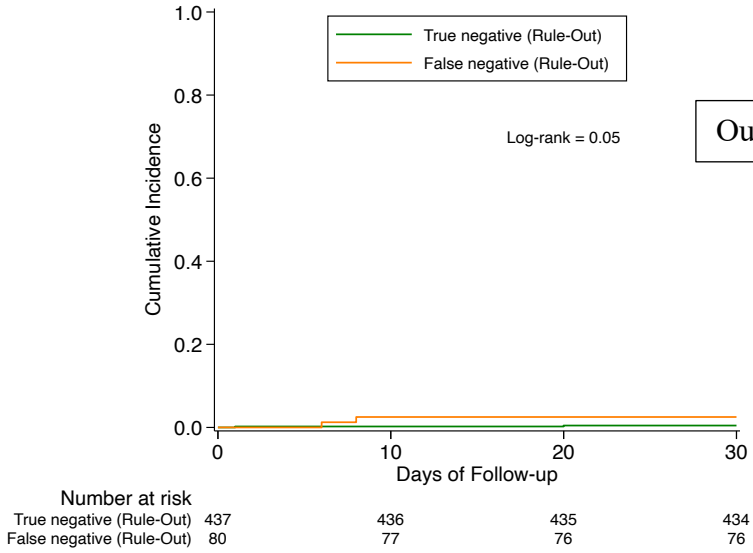
B


True negative (Rule-Out)
False negative (Rule-Out)
Outcome: all cause death or AMI 30d
Log-rank = 0.0005
Cumulative Incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Days of Follow-up
Number at risk
True negative (Rule-Out) 437 434 431 429
False negative (Rule-Out) 80 74 72 71

C

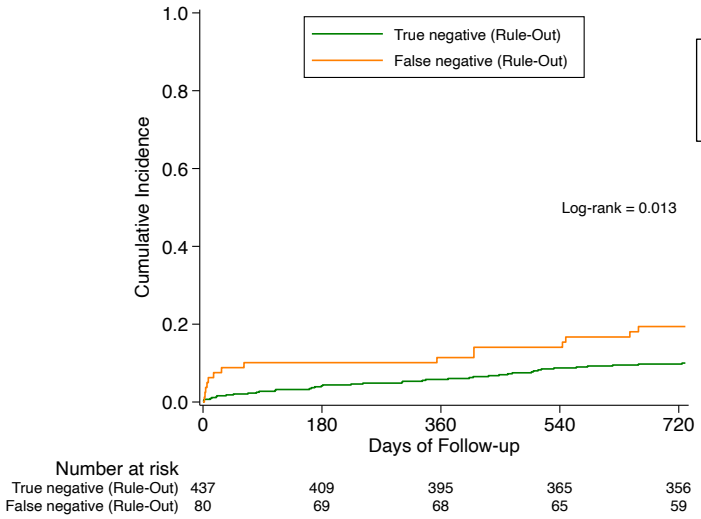

True negative (Rule-Out)
False negative (Rule-Out)
Outcome: AMI 30d
Log-rank = 0.0019
Cumulative Incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Days of Follow-up
Number at risk
True negative (Rule-Out) 437 434 431 429
False negative (Rule-Out) 80 74 72 71

D



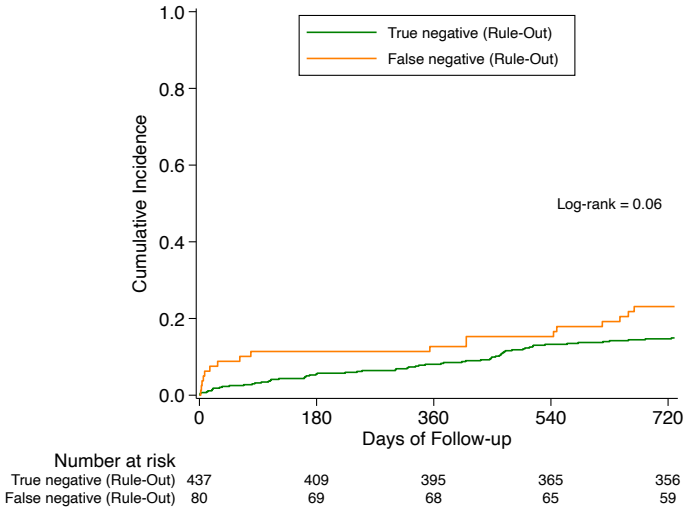
Outcome: CV death 30d

A



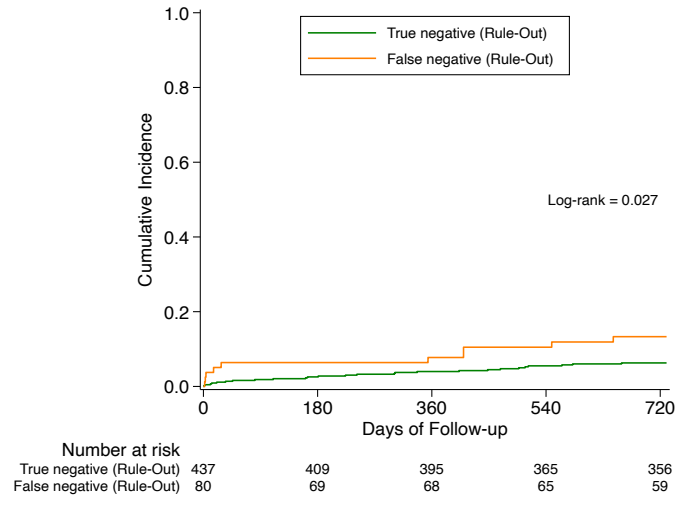
Outcome: CV death or AMI 730d

B



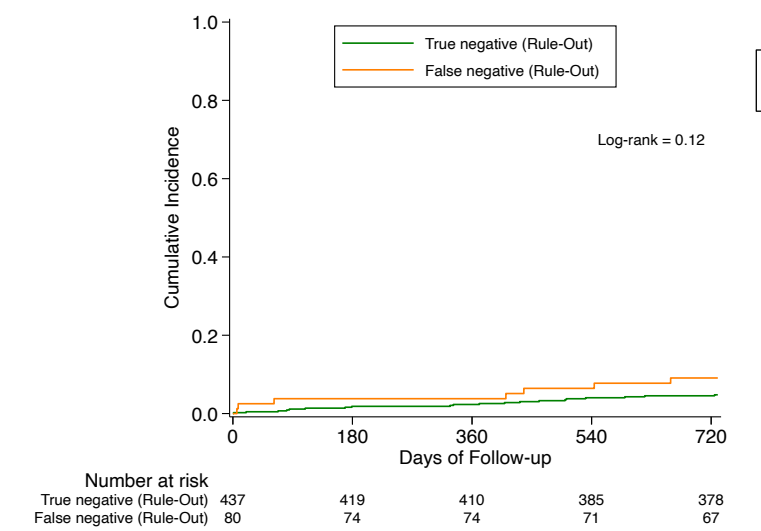
Outcome: all cause death or AMI 730d

C

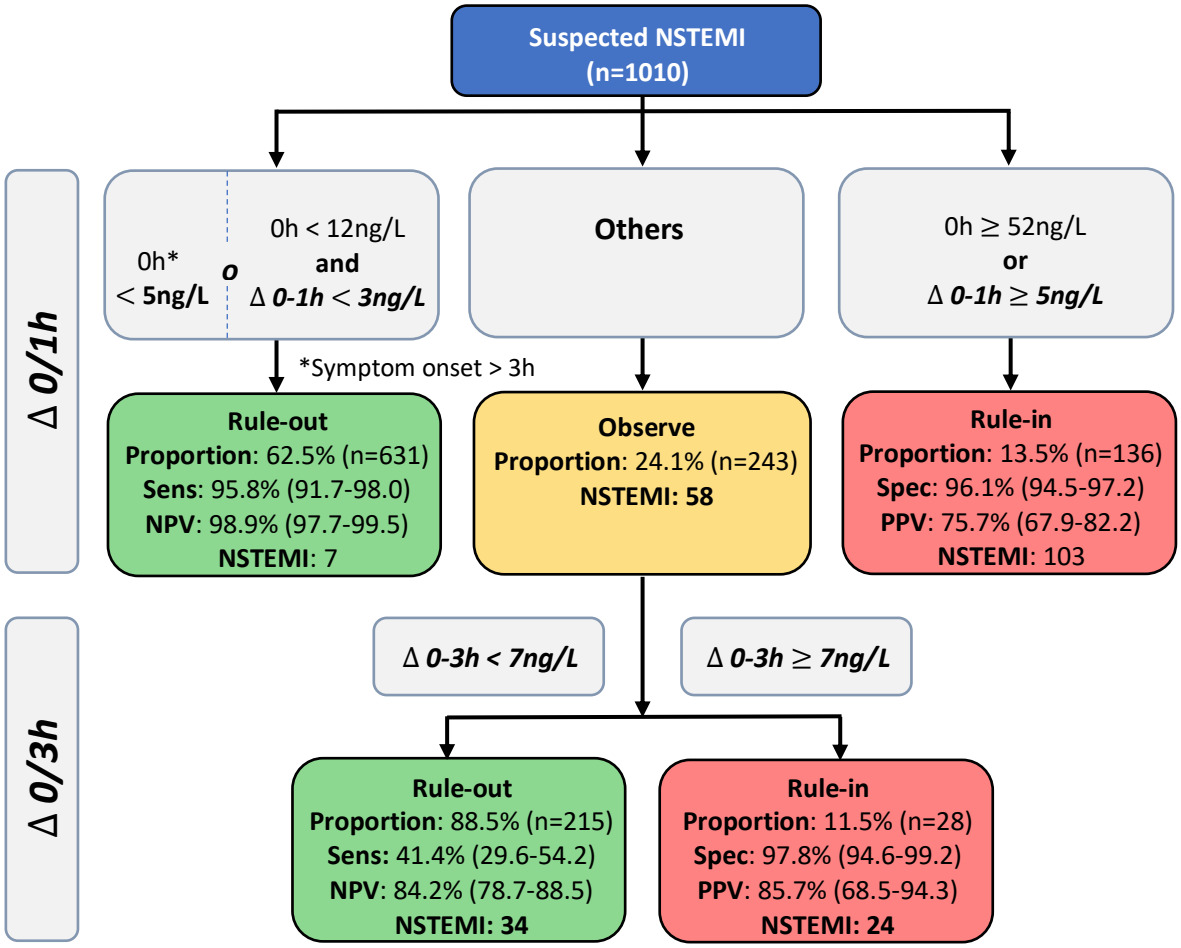


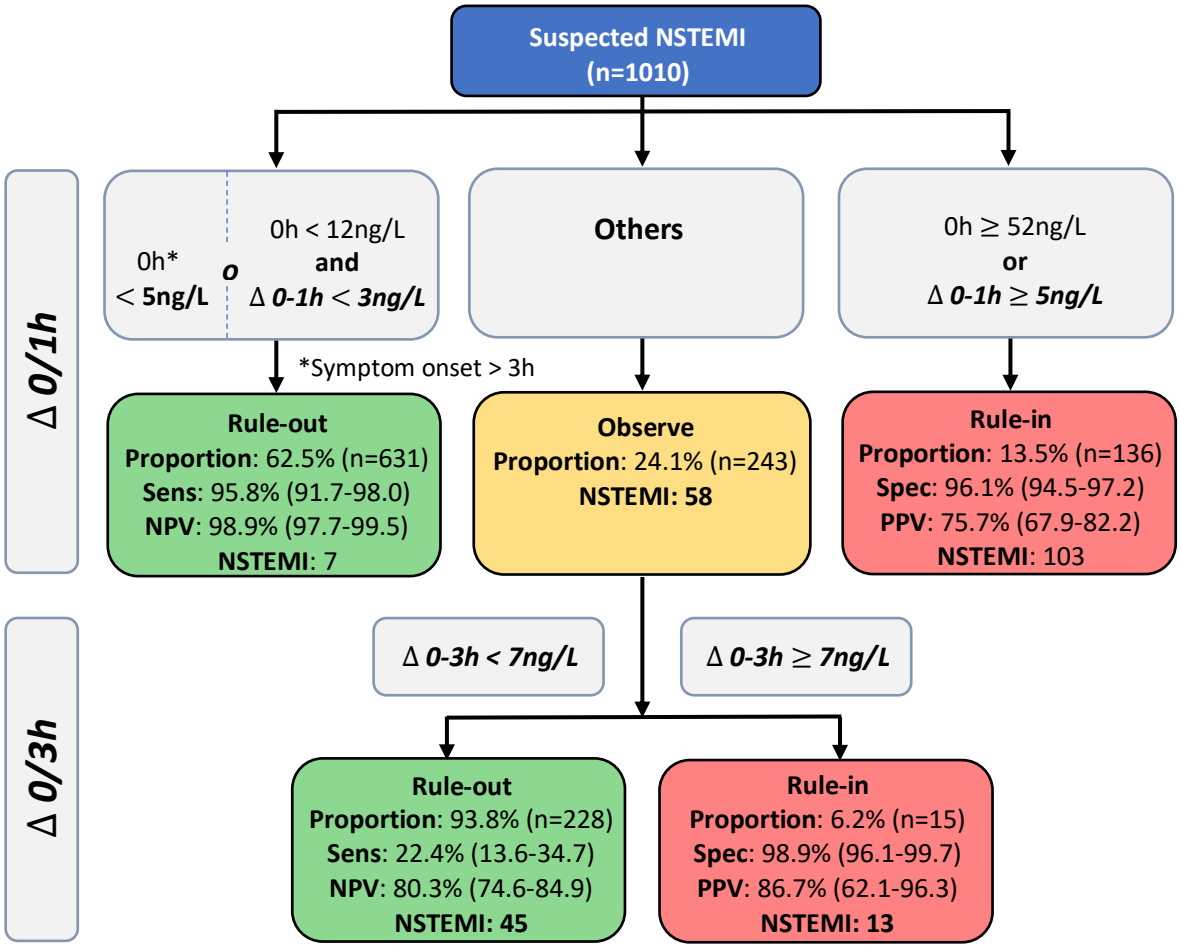
Outcome: AMI 730d

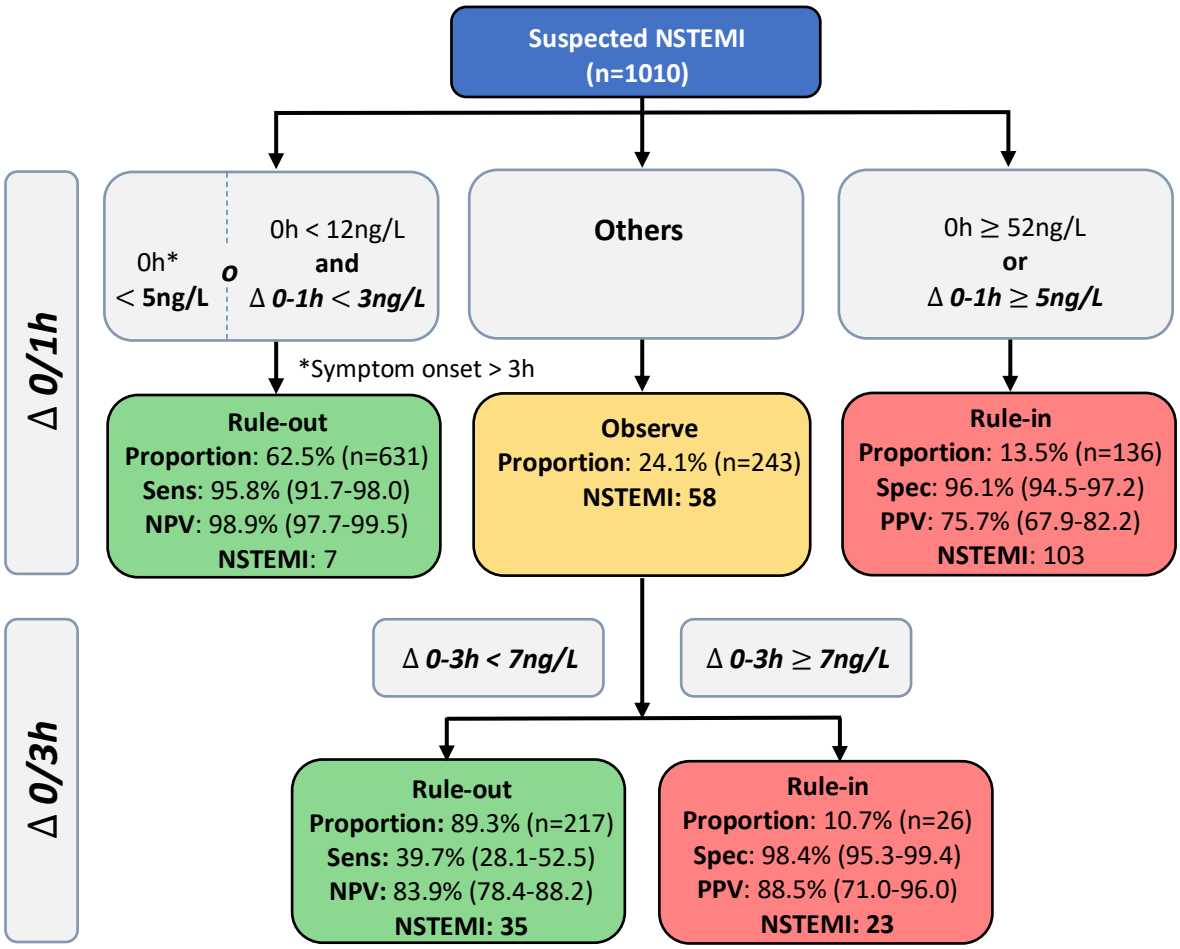
D

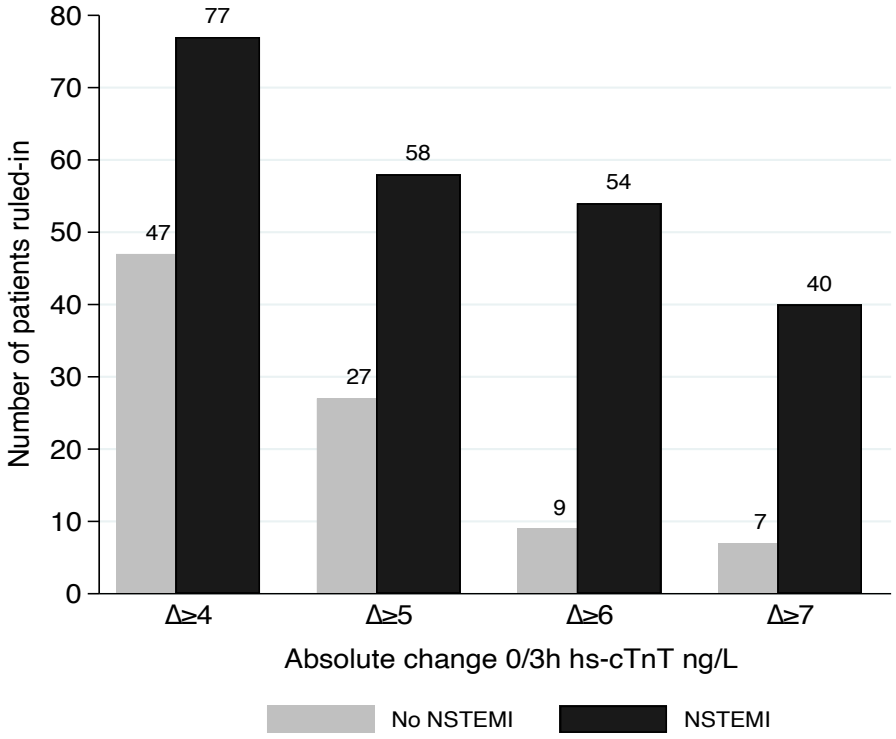


Outcome: CV death 30d

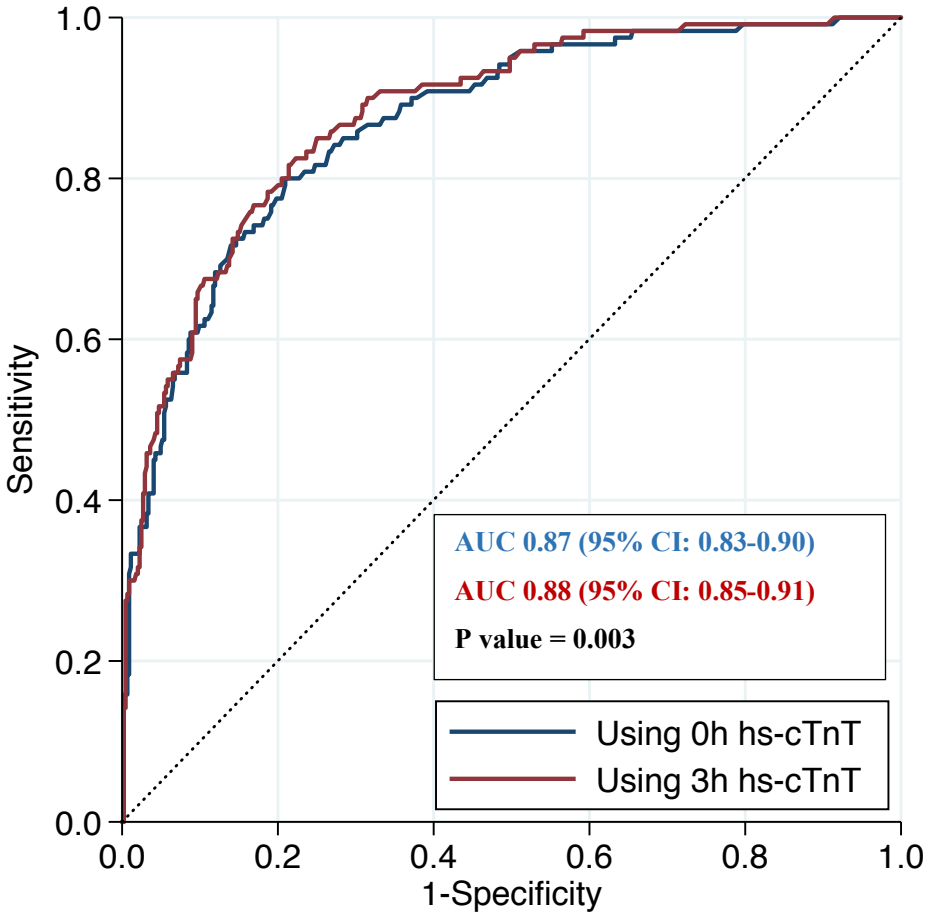

Suspected NSTEMI
(n=1010)
Δ 0/1h
0h*
< 5ng/L
o
0h < 12ng/L
and
Δ 0-1h < 3ng/L
Others
0h ≥ 52ng/L
or
Δ 0-1h ≥ 5ng/L
*Symptom onset > 3h
Rule-out
Proportion: 62.5% (n=631)
Sens: 95.8% (91.7-98.0)
NPV: 98.9% (97.7-99.5)
NSTEMI: 7
Observe
Proportion: 24.1% (n=243)
NSTEMI: 58
Rule-in
Proportion: 13.5% (n=136)
Spec: 96.1% (94.5-97.2)
PPV: 75.7% (67.9-82.2)
NSTEMI: 103
Δ 0/3h
Δ 0-3h < 7ng/L
Δ 0-3h ≥ 7ng/L
Rule-out
Proportion: 88.5% (n=215)
Sens: 41.4% (29.6-54.2)
NPV: 84.2% (78.7-88.5)
NSTEMI: 34
Rule-in
Proportion: 11.5% (n=28)
Spec: 97.8% (94.6-99.2)
PPV: 85.7% (68.5-94.3)
NSTEMI: 24

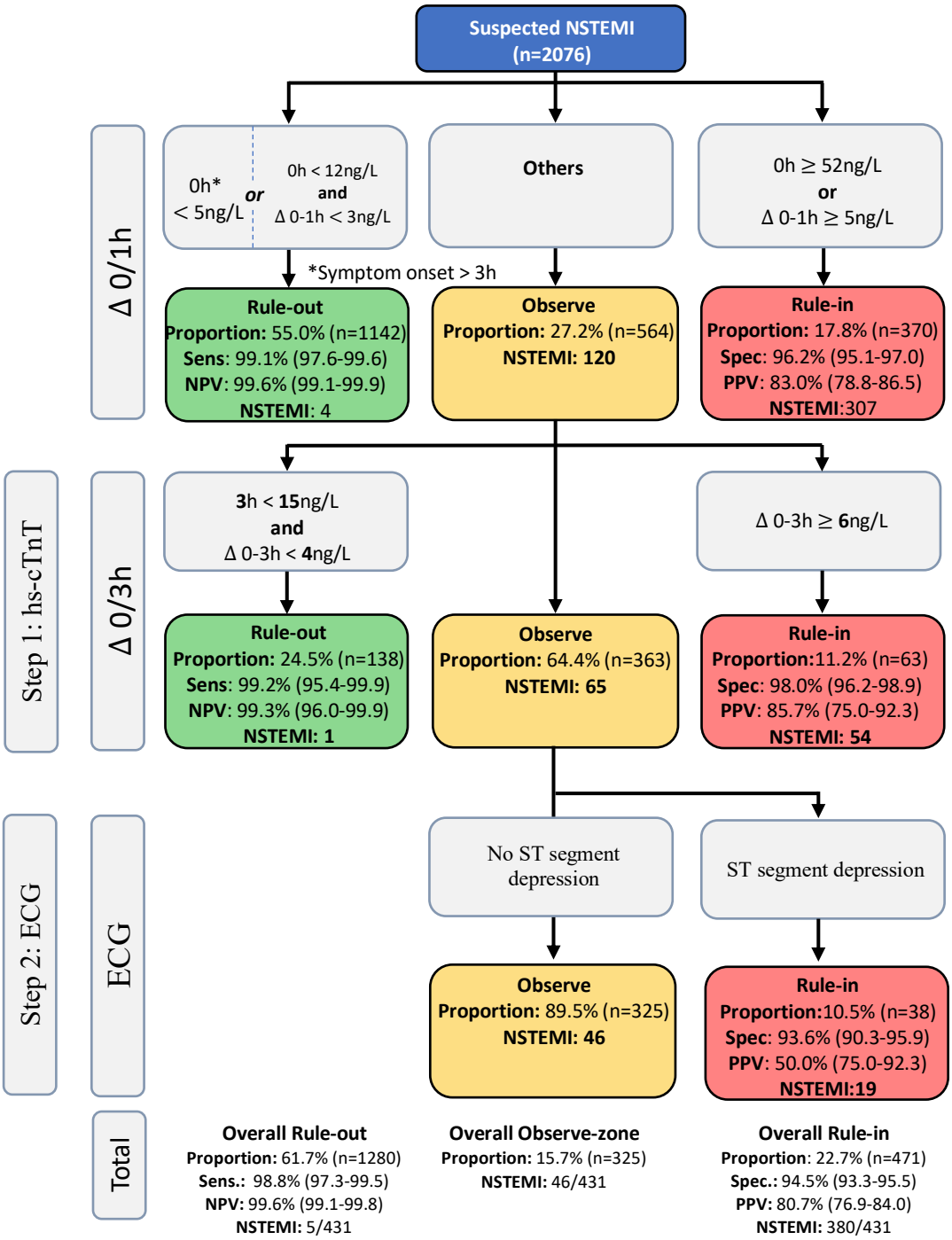

Suspected NSTEMI
(n=1010)
Δ 0/1h
0h*
< 5ng/L
o
0h < 12ng/L
and
Δ 0-1h < 3ng/L
Others
0h ≥ 52ng/L
or
Δ 0-1h ≥ 5ng/L
*Symptom onset > 3h
Rule-out
Proportion: 62.5% (n=631)
Sens: 95.8% (91.7-98.0)
NPV: 98.9% (97.7-99.5)
NSTEMI: 7
Observe
Proportion: 24.1% (n=243)
NSTEMI: 58
Rule-in
Proportion: 13.5% (n=136)
Spec: 96.1% (94.5-97.2)
PPV: 75.7% (67.9-82.2)
NSTEMI: 103
Δ 0/3h
Δ 0-3h < 7ng/L
Δ 0-3h ≥ 7ng/L
Rule-out
Proportion: 93.8% (n=228)
Sens: 22.4% (13.6-34.7)
NPV: 80.3% (74.6-84.9)
NSTEMI: 45
Rule-in
Proportion: 6.2% (n=15)
Spec: 98.9% (96.1-99.7)
PPV: 86.7% (62.1-96.3)
NSTEMI: 13

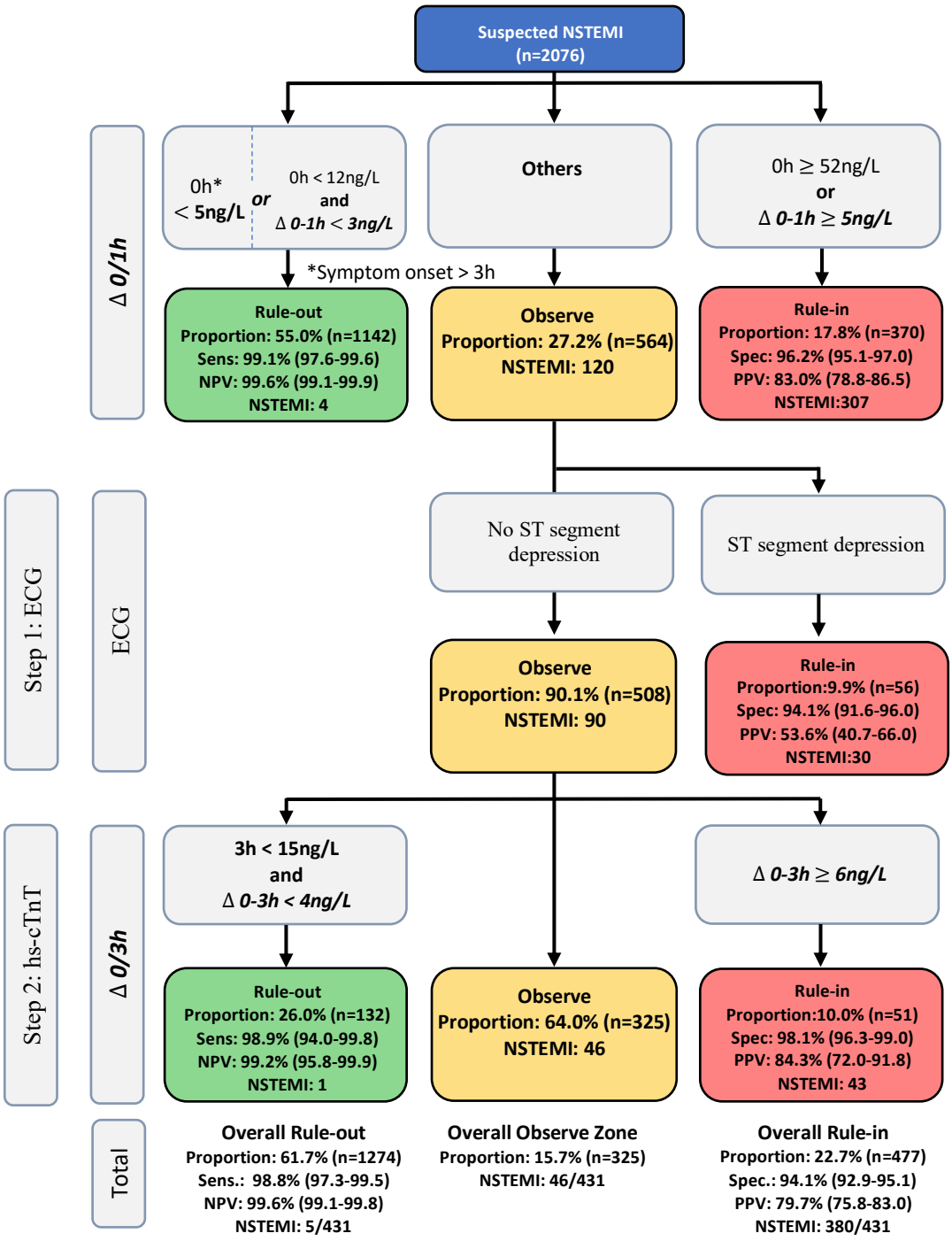

Suspected NSTEMI
(n=1010)
Δ 0/1h
0h*
< 5ng/L
o
0h < 12ng/L
and
Δ 0-1h < 3ng/L
Others
0h ≥ 52ng/L
or
Δ 0-1h ≥ 5ng/L
*Symptom onset > 3h
Rule-out
Proportion: 62.5% (n=631)
Sens: 95.8% (91.7-98.0)
NPV: 98.9% (97.7-99.5)
NSTEMI: 7
Observe
Proportion: 24.1% (n=243)
NSTEMI: 58
Rule-in
Proportion: 13.5% (n=136)
Spec: 96.1% (94.5-97.2)
PPV: 75.7% (67.9-82.2)
NSTEMI: 103
Δ 0/3h
Δ 0-3h < 7ng/L
Δ 0-3h ≥ 7ng/L
Rule-out
Proportion: 89.3% (n=217)
Sens: 39.7% (28.1-52.5)
NPV: 83.9% (78.4-88.2)
NSTEMI: 35
Rule-in
Proportion: 10.7% (n=26)
Spec: 98.4% (95.3-99.4)
PPV: 88.5% (71.0-96.0)
NSTEMI: 23

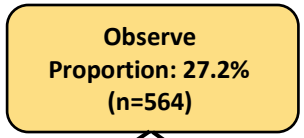


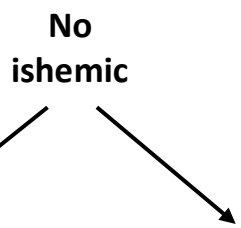
Absolute change 0/3h hs-cTnT ng/L	**≥4**	**≥5**	**≥6**	**≥7**
Specificity	89.4 (86.2-91.9)	93.9 (91.3-95.8)	98.0 (96.2-98.9)	98.4 (96.8-99.2)
PPV	62.1 (53.3-70.2)	68.2 (57.7-77.2)	85.7 (75.0-92.3)	85.1 (72.3-92.6)

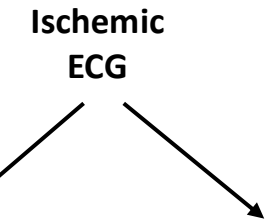

1.0
0.8
0.6
0.4
0.2
0.0
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1-Specificity
AUC 0.87 (95% CI: 0.83-0.90)
AUC 0.88 (95% CI: 0.85-0.91)
P value = 0.003
Using 0h hs-cTnT
Using 3h hs-cTnT


Suspected NSTEMI
(n=2076)
Step 1: hs-cTnT
Δ 0/1h
0h* < 5ng/L or 0h < 12ng/L and Δ 0-1h < 3ng/L
Others
0h ≥ 52ng/L or Δ 0-1h ≥ 5ng/L
*Symptom onset > 3h
Rule-out
Proportion: 55.0% (n=1142)
Sens: 99.1% (97.6-99.6)
NPV: 99.6% (99.1-99.9)
NSTEMI: 4
Observe
Proportion: 27.2% (n=564)
NSTEMI: 120
Rule-in
Proportion: 17.8% (n=370)
Spec: 96.2% (95.1-97.0)
PPV: 83.0% (78.8-86.5)
NSTEMI:307
Δ 0/3h
3h < 15ng/L and Δ 0-3h < 4ng/L
Δ 0-3h ≥ 6ng/L
Rule-out
Proportion: 24.5% (n=138)
Sens: 99.2% (95.4-99.9)
NPV: 99.3% (96.0-99.9)
NSTEMI: 1
Observe
Proportion: 64.4% (n=363)
NSTEMI: 65
Rule-in
Proportion:11.2% (n=63)
Spec: 98.0% (96.2-98.9)
PPV: 85.7% (75.0-92.3)
NSTEMI: 54
Step 2: ECG
ECG
No ST segment depression
ST segment depression
Observe
Proportion: 89.5% (n=325)
NSTEMI: 46
Rule-in
Proportion:10.5% (n=38)
Spec: 93.6% (90.3-95.9)
PPV: 50.0% (75.0-92.3)
NSTEMI:19
Total
Overall Rule-out
Proportion: 61.7% (n=1280)
Sens.: 98.8% (97.3-99.5)
NPV: 99.6% (99.1-99.8)
NSTEMI: 5/431
Overall Observe-zone
Proportion: 15.7% (n=325)
NSTEMI: 46/431
Overall Rule-in
Proportion: 22.7% (n=471)
Spec.: 94.5% (93.3-95.5)
PPV: 80.7% (76.9-84.0)
NSTEMI: 380/431

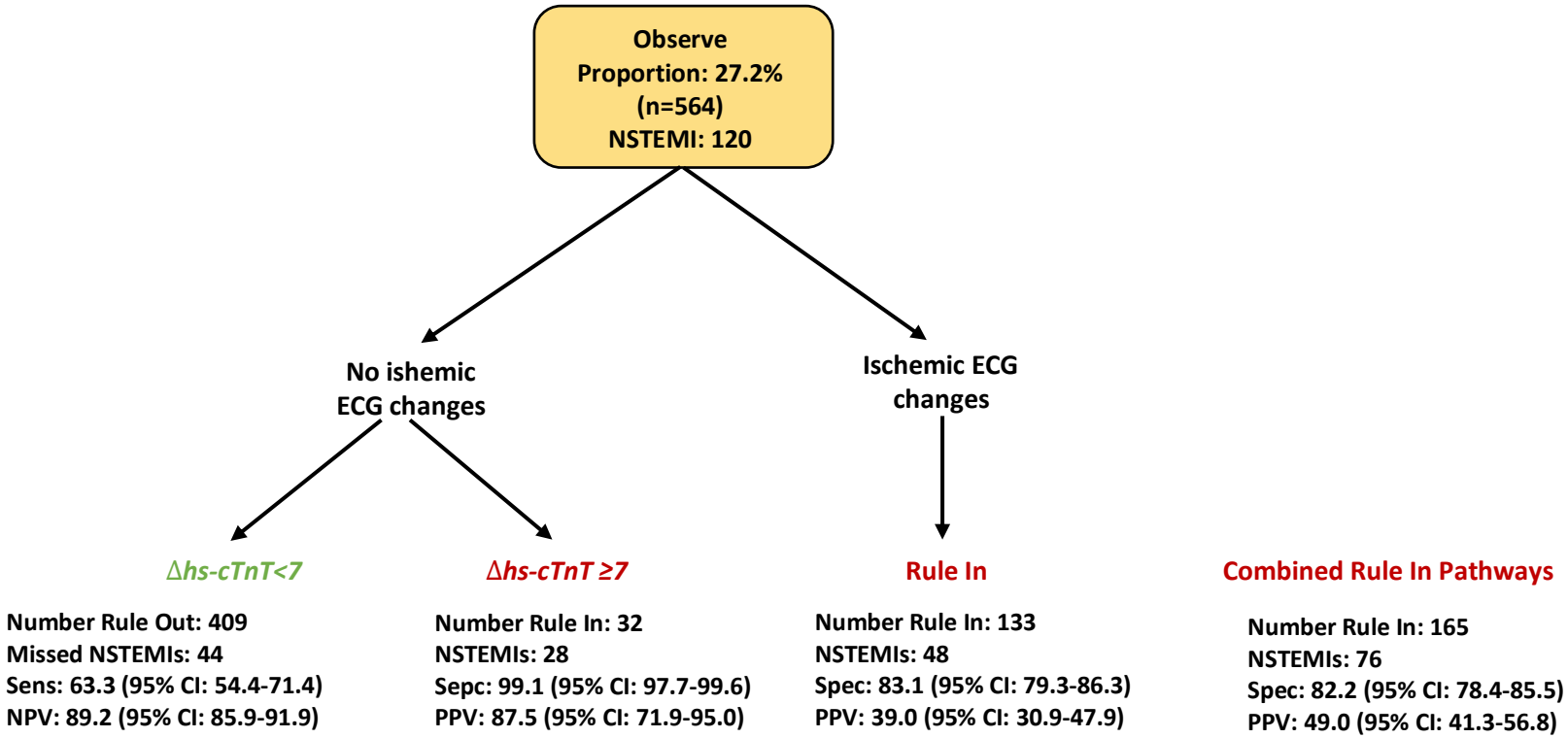

Suspected NSTEMI
(n=2076)
Δ 0/1h
0h*
< 5ng/L
or
0h < 12ng/L
and
Δ 0-1h < 3ng/L
Others
0h ≥ 52ng/L
or
Δ 0-1h ≥ 5ng/L
*Symptom onset > 3h
Rule-out
Proportion: 55.0% (n=1142)
Sens: 99.1% (97.6-99.6)
NPV: 99.6% (99.1-99.9)
NSTEMI: 4
Observe
Proportion: 27.2% (n=564)
NSTEMI: 120
Rule-in
Proportion: 17.8% (n=370)
Spec: 96.2% (95.1-97.0)
PPV: 83.0% (78.8-86.5)
NSTEMI:307
Step 1: ECG
ECG
No ST segment depression
ST segment depression
Observe
Proportion: 90.1% (n=508)
NSTEMI: 90
Rule-in
Proportion:9.9% (n=56)
Spec: 94.1% (91.6-96.0)
PPV: 53.6% (40.7-66.0)
NSTEMI:30
Step 2: hs-cTnT
Δ 0/3h
3h < 15ng/L
and
Δ 0-3h < 4ng/L
Δ 0-3h ≥ 6ng/L
Rule-out
Proportion: 26.0% (n=132)
Sens: 98.9% (94.0-99.8)
NPV: 99.2% (95.8-99.9)
NSTEMI: 1
Observe
Proportion: 64.0% (n=325)
NSTEMI: 46
Rule-in
Proportion:10.0% (n=51)
Spec: 98.1% (96.3-99.0)
PPV: 84.3% (72.0-91.8)
NSTEMI: 43
Total
Overall Rule-out
Proportion: 61.7% (n=1274)
Sens.: 98.8% (97.3-99.5)
NPV: 99.6% (99.1-99.8)
NSTEMI: 5/431
Overall Observe Zone
Proportion: 15.7% (n=325)
NSTEMI: 46/431
Overall Rule-in
Proportion: 22.7% (n=477)
Spec.: 94.1% (92.9-95.1)
PPV: 79.7% (75.8-83.0)
NSTEMI: 380/431

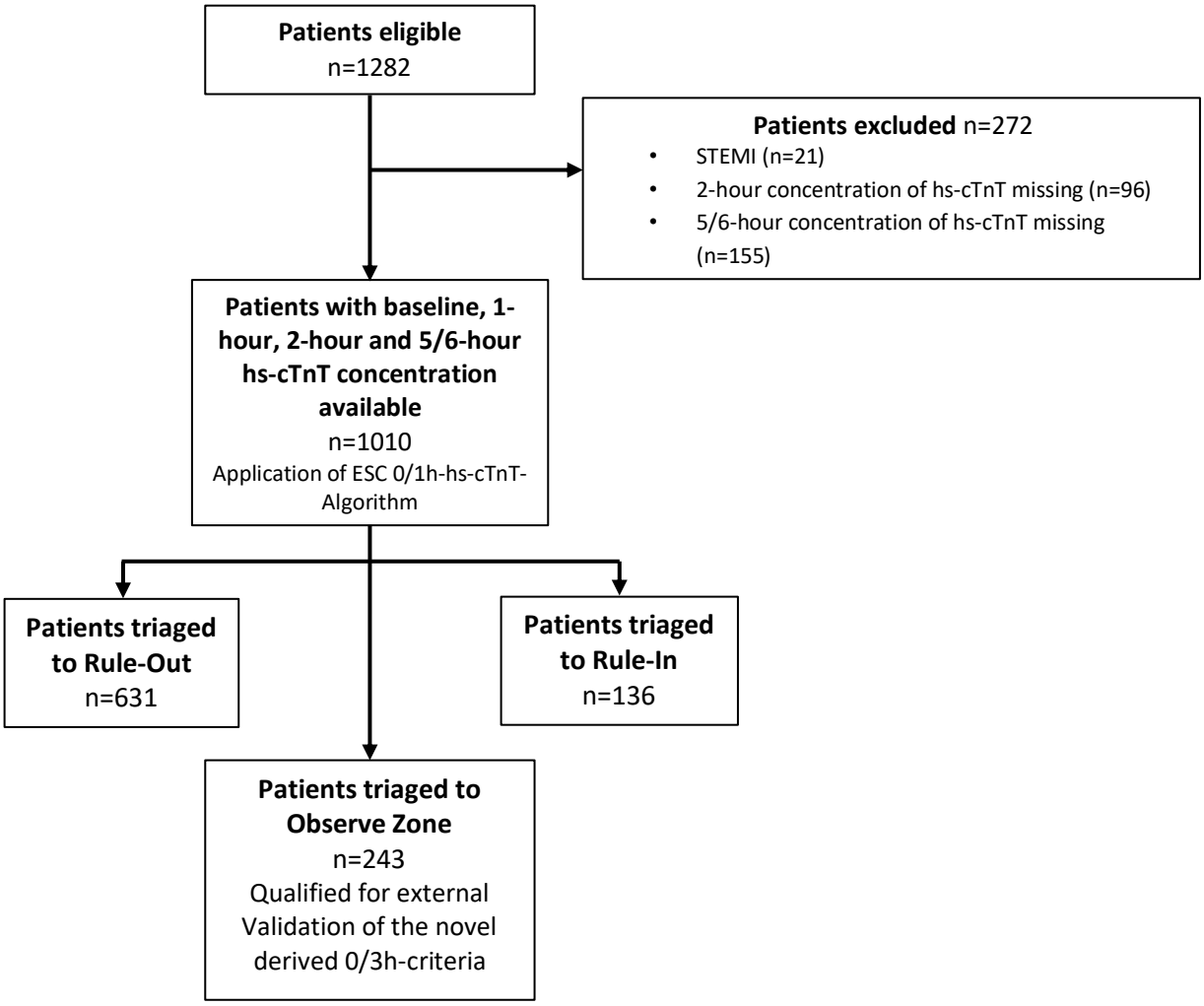


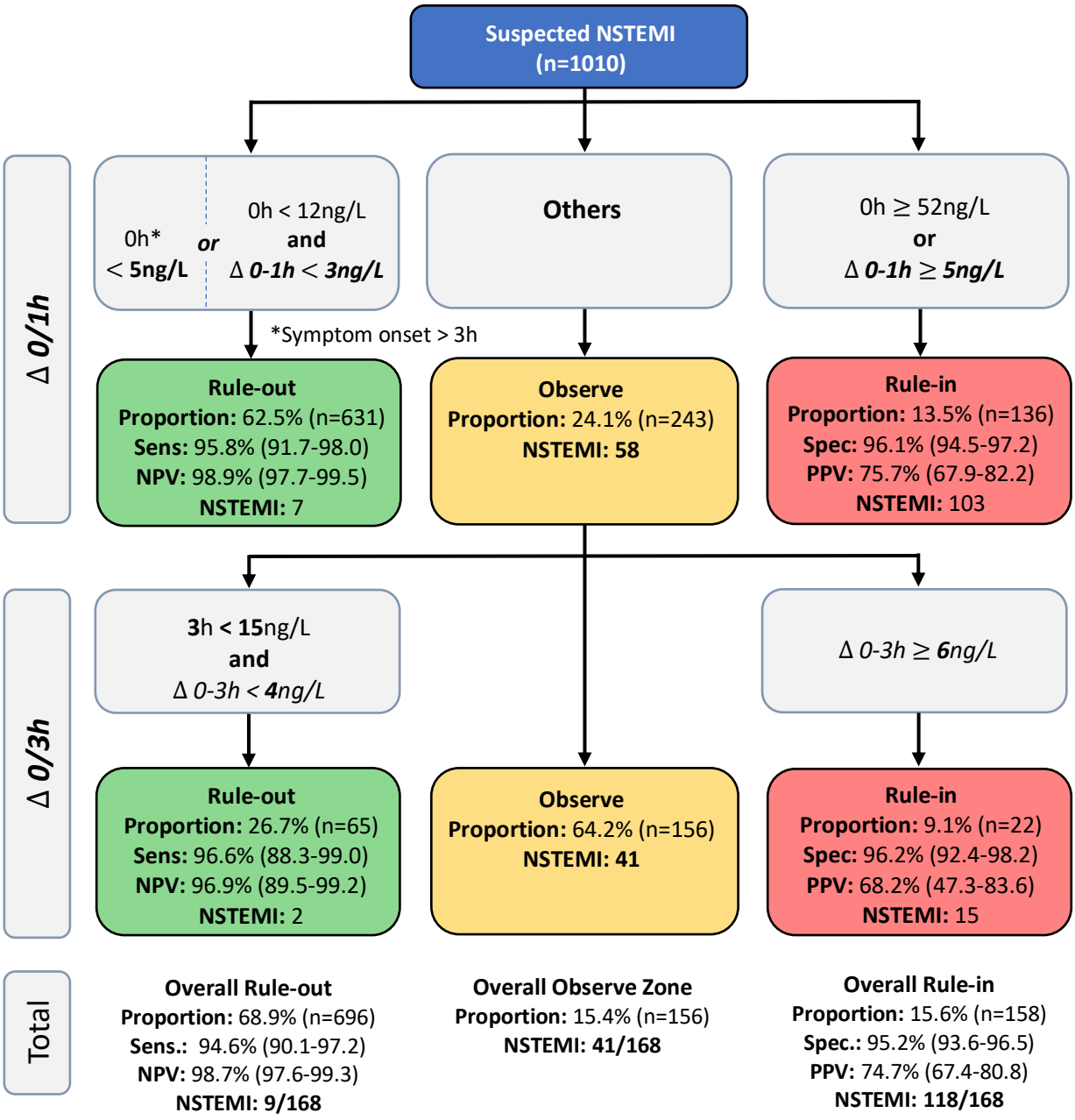




In the ECG stratified group	*Δhs-cTnT<7*	*Δhs-cTnT ≥7*	*Δhs-cTnT<7*	*Δhs-cTnT*
	Number Rule Out: 409 Missed NSTEMIs: 44 Sens: 38.9 (95% CI: 28.5-50.4) NPV: 89.2 (95% CI: 85.9-91.9)	Number Rule In: 32 NSTEMIs: 28 Spec: 98.9 (95% CI: 97.2-99.6) PPV: 87.5 (95% CI: 71.9-95.0)	Number Rule Out: 108 Missed NSTEMIs: 36 Sens: 25.0 (95% CI: 14.9-38.8) NPV: 66.7 (95% CI: 57.3-74.8)	Number Rule In: 15 NSTEMIs: 12 Sepc: 96.0 (95% CI: 88.9-98.6) PPV: 80.0 (95% CI: 54.8-93.0)


Observe
Proportion: 27.2%
(n=564)
NSTEMI: 120
No ishemic
ECG changes
Ischemic ECG
changes
Δhs-cTnT<7
Δhs-cTnT ≥7
Rule In
Combined Rule In Pathways
Number Rule Out: 409
Missed NSTEMIs: 44
Sens: 63.3 (95% CI: 54.4-71.4)
NPV: 89.2 (95% CI: 85.9-91.9)
Number Rule In: 32
NSTEMIs: 28
Sepc: 99.1 (95% CI: 97.7-99.6)
PPV: 87.5 (95% CI: 71.9-95.0)
Number Rule In: 133
NSTEMIs: 48
Spec: 83.1 (95% CI: 79.3-86.3)
PPV: 39.0 (95% CI: 30.9-47.9)
Number Rule In: 165
NSTEMIs: 76
Spec: 82.2 (95% CI: 78.4-85.5)
PPV: 49.0 (95% CI: 41.3-56.8)


Patients eligible
n=1282
Patients excluded n=272
STEMI (n=21)
2-hour concentration of hs-cTnT missing (n=96)
5/6-hour concentration of hs-cTnT missing (n=155)
Patients with baseline, 1-hour, 2-hour and 5/6-hour hs-cTnT concentration available
n=1010
Application of ESC 0/1h-hs-cTnT-Algorithm
Patients triaged to Rule-Out
n=631
Patients triaged to Rule-In
n=136
Patients triaged to Observe Zone
n=243
Qualified for external Validation of the novel derived 0/3h-criteria


Suspected NSTEMI
(n=1010)
Δ 0/1h
0h* < 5ng/L or 0h < 12ng/L and Δ 0-1h < 3ng/L
Others
0h ≥ 52ng/L or Δ 0-1h ≥ 5ng/L
*Symptom onset > 3h
Rule-out
Proportion: 62.5% (n=631)
Sens: 95.8% (91.7-98.0)
NPV: 98.9% (97.7-99.5)
NSTEMI: 7
Observe
Proportion: 24.1% (n=243)
NSTEMI: 58
Rule-in
Proportion: 13.5% (n=136)
Spec: 96.1% (94.5-97.2)
PPV: 75.7% (67.9-82.2)
NSTEMI: 103
Δ 0/3h
3h < 15ng/L and Δ 0-3h < 4ng/L
Δ 0-3h ≥ 6ng/L
Rule-out
Proportion: 26.7% (n=65)
Sens: 96.6% (88.3-99.0)
NPV: 96.9% (89.5-99.2)
NSTEMI: 2
Observe
Proportion: 64.2% (n=156)
NSTEMI: 41
Rule-in
Proportion: 9.1% (n=22)
Spec: 96.2% (92.4-98.2)
PPV: 68.2% (47.3-83.6)
NSTEMI: 15
Total
Overall Rule-out
Proportion: 68.9% (n=696)
Sens.: 94.6% (90.1-97.2)
NPV: 98.7% (97.6-99.3)
NSTEMI: 9/168
Overall Observe Zone
Proportion: 15.4% (n=156)
NSTEMI: 41/168
Overall Rule-in
Proportion: 15.6% (n=158)
Spec.: 95.2% (93.6-96.5)
PPV: 74.7% (67.4-80.8)
NSTEMI: 118/168

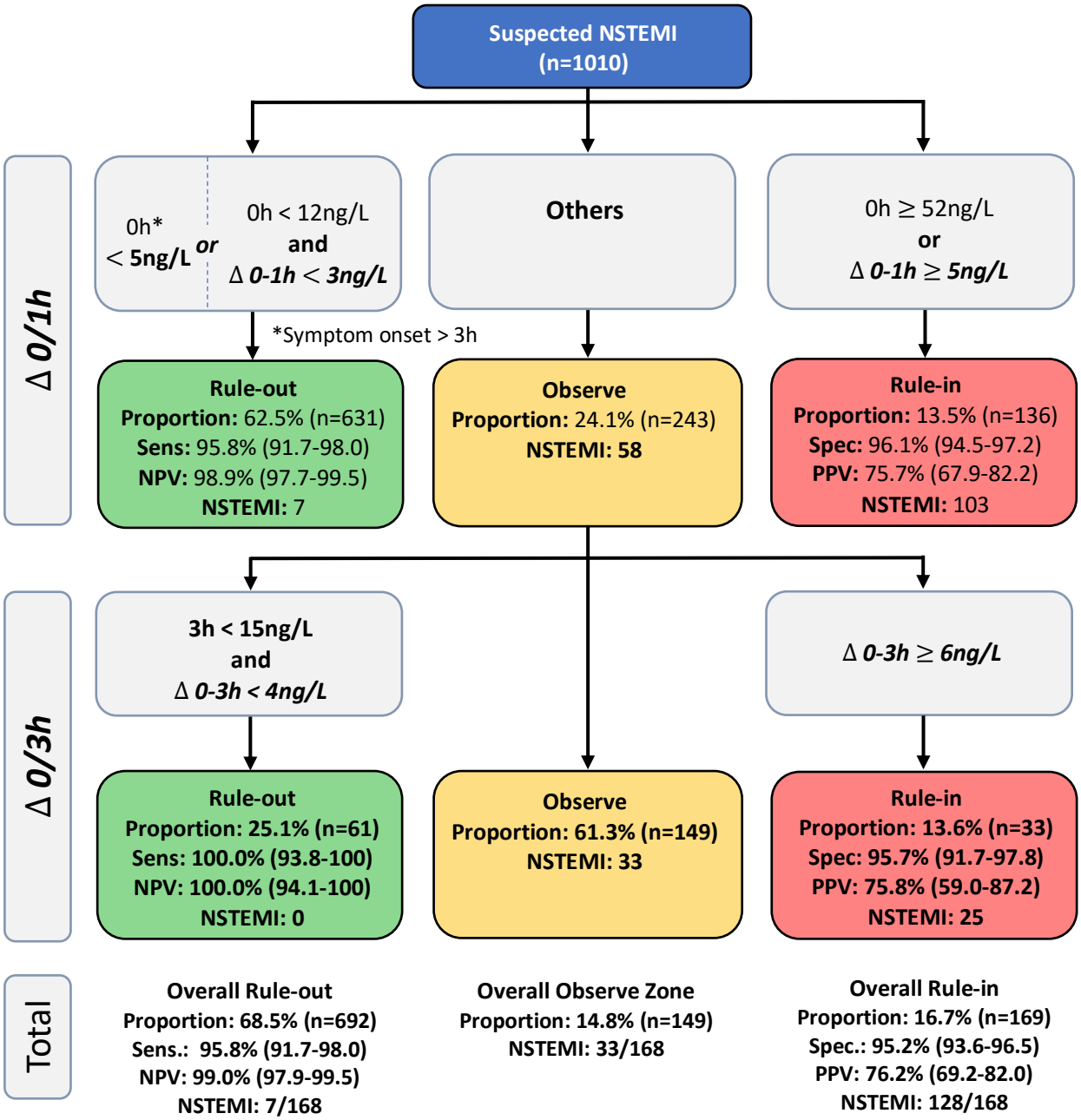

Suspected NSTEMI
(n=1010)
Δ 0/1h
0h*
< 5ng/L
or
0h < 12ng/L
and
Δ 0-1h < 3ng/L
Others
0h ≥ 52ng/L
or
Δ 0-1h ≥ 5ng/L
*Symptom onset > 3h
Rule-out
Proportion: 62.5% (n=631)
Sens: 95.8% (91.7-98.0)
NPV: 98.9% (97.7-99.5)
NSTEMI: 7
Observe
Proportion: 24.1% (n=243)
NSTEMI: 58
Rule-in
Proportion: 13.5% (n=136)
Spec: 96.1% (94.5-97.2)
PPV: 75.7% (67.9-82.2)
NSTEMI: 103
Δ 0/3h
3h < 15ng/L
and
Δ 0-3h < 4ng/L
Δ 0-3h ≥ 6ng/L
Rule-out
Proportion: 25.1% (n=61)
Sens: 100.0% (93.8-100)
NPV: 100.0% (94.1-100)
NSTEMI: 0
Observe
Proportion: 61.3% (n=149)
NSTEMI: 33
Rule-in
Proportion: 13.6% (n=33)
Spec: 95.7% (91.7-97.8)
PPV: 75.8% (59.0-87.2)
NSTEMI: 25
Total
Overall Rule-out
Proportion: 68.5% (n=692)
Sens.: 95.8% (91.7-98.0)
NPV: 99.0% (97.9-99.5)
NSTEMI: 7/168
Overall Observe Zone
Proportion: 14.8% (n=149)
NSTEMI: 33/168
Overall Rule-in
Proportion: 16.7% (n=169)
Spec.: 95.2% (93.6-96.5)
PPV: 76.2% (69.2-82.0)
NSTEMI: 128/168

Figure Legends

Supplemental Figure I. Patient Flow in the APACE study.

STEMI indicates ST elevation myocardial infarction; AMI, acute myocardial infarction; hs-cTnT, high-sensitivity cardiac troponin T; and ESC, European society of cardiology.

Supplemental Figure II. Parkland's proposal (0/3h-hs-cTnT-change of <7ng/L).

The concept of the suggested 0/3h-hs-cTnT-change criteria (7ng/L) for patients triaged to the observe-zone after applying the ESC 0/1h-hs-cTnT-algorithm. Patients are triaged towards either rule-out or rule-in, eliminating the observe-zone group. ESC indicates European society of cardiology.

Supplemental Figure III. 30-day Kaplan Meier for true positives vs false negatives.

Kaplan Meier curves depicting cumulative incidence within 30-days for NSTEMI patients ruled-in at 3 hours (true positive) vs NSTEMI patients missed at 3 hours (false negative) using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone. Different endpoints were assessed: **A**) composite endpoint of 30-days CV death or non-index AMI, **B**) composite endpoint of 30-day all-cause death or non-index AMI, **C**) 30-day non-index AMI, and **D**) 30-days CV death. NSTEMI stands for non-ST elevation myocardial infarction; AMI stands for acute myocardial infarction; and CV stand for cardiovascular.

Supplemental Figure IV. 730-day Kaplan Meier for true positives vs false negatives.

Kaplan Meier curves depicting cumulative incidence within 730-day for NSTEMI patients ruled-in at 3 hours (true positive) vs NSTEMI patients missed at 3 hours (false negative) using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone. Different endpoints were assessed: **A**) composite endpoint of 730-days CV death or non-index AMI, **B**) composite endpoint of 730-day all-cause death or non-index AMI, **C**) 730-day non-index AMI, and **D**) 730-days CV death. NSTEMI stands for non-ST elevation myocardial infarction; AMI stands for acute myocardial infarction; and CV stand for cardiovascular.

Supplemental Figure V. 30-day Kaplan Meier for false negatives vs true negatives.

Kaplan Meier curves depicting cumulative incidence within 30-days for missed NSTEMI at 3 hours (false negative) vs non-NSTEMI ruled-out at 3 hours (true negative) using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone. Different endpoints were assessed: **A**) composite endpoint of 30-days CV death or non-index AMI, **B**) composite endpoint of 30-day all-cause death or non-index AMI, **C**) 30-day non-index AMI, and **D**) 30-days CV

death. NSTEMI stands for non-ST elevation myocardial infarction; AMI stands for acute myocardial infarction; and CV stand for cardiovascular.

Supplemental Figure VI. 730-day Kaplan Meier for false negatives vs true negatives.
Kaplan Meier curves depicting cumulative incidence within 730-day for missed NSTEMI at 3 hours (false negative) vs non-NSTEMI ruled-out at 3 hours (true negative) using the suggested 0/3h hs-cTnT-change <7 ng/L as single criterion for the observe-zone. Different endpoints were assessed: **A**) composite endpoint of 730-days CV death or non-index AMI, **B**) composite endpoint of 730-day all-cause death or non-index AMI, **C**) 730-day non-index AMI, and **D**) 730-days CV death. NSTEMI stands for non-ST elevation myocardial infarction; AMI stands for acute myocardial infarction; and CV stand for cardiovascular.

Supplemental Figure VII. External Validation of the Parkland's criteria (0/3h-hs-cTnT-change criteria 7 ng/L) in the TRAPID-AMI cohort.
The algorithm displays patient flow and diagnostic performance for the ESC 0/1h-algorithm and the Parkland's criteria (7 ng/L). 3h values were estimated by calculating the mean between the 2h and 5/6h hs-cTnT measurements. Sens indicates sensitivity; Spec, specificity; NPV, negative predictive value; PPV, positive predictive value; and NSTEMI, non-ST elevation myocardial infarction; Δ, delta.
*****Patients with a chest pain onset<3h can't be directly rule-out with a 0h hs-cTnT value.

Supplemental Figure VIII. Sensitivity analysis 1 for the external validation of the Parkland's criteria (0/3h-hs-cTnT-change criteria 7 ng/L) in the TRAPID-AMI cohort.
The algorithm displays patient flow and diagnostic performance for the ESC 0/1h-algorithm and the Parkland's criteria (7 ng/L). 2h hs-cTnT values were used as 3h values. Sens indicates sensitivity; Spec, specificity; NPV, negative predictive value; PPV, positive predictive value; and NSTEMI, non-ST elevation myocardial infarction; Δ, delta.
*****Patients with a chest pain onset<3h can't be directly rule-out with a 0h hs-cTnT value.

Supplemental Figure IX. Sensitivity analysis 2 for the external validation of the Parkland's criteria (0/3h-hs-cTnT-change criteria 7 ng/L) in the TRAPID-AMI cohort.
The algorithm displays patient flow and diagnostic performance for the ESC 0/1h-algorithm and the Parkland's criteria (7 ng/L). 3h hs-cTnT values were calculated by giving more weight to 2h values (2/3rd weight) than to 5/6h values (1/3rd weight). Sens indicates sensitivity; Spec,

specificity; NPV, negative predictive value; PPV, positive predictive value; and NSTEMI, non-ST elevation myocardial infarction; Δ, delta.

*****Patients with a chest pain onset<3h can't be directly rule-out with a 0h hs-cTnT value.

Supplemental Figure X. Diagnostic performance and number of patients ruled-in for different hs-cTnT 0/3h- absolute change cut-offs.

Number of patients ruled-in stratified by presence or absence of NSTEMI according to each 0/3h-hs-cTnT absolute change cut-offs from Δ ≥4ng/L until Δ ≥7ng/L. The table shows diagnostic performance metrics for each specific 0/3h-hs-cTnT absolute change cut-off. hs-cTnT indicates high-sensitivity cardiac Troponin T; PPV, positive predictive value and NSTEMI, non-ST elevation myocardial infarction; Δ, delta.

Supplemental Figure XI. Comparison of the AUC between 2 different hs-cTnT strategies.

Single hs-cTnT measurement at 0h in combination with a 0/3h-hs-cTnT absolute change criteria (blue ROC curve) or single hs-cTnT measurement at 3h in combination with a 0/3h-hs-cTnT absolute change criteria (red ROC curve). AUC indicates area under the curve; ROC, receiver-operating characteristic curve; and hs-cTnT, high-sensitivity cardiac troponin T.

Supplemental Figure XII. Overall performance of the novel proposed hs-cTnT-based observe-zone 3h strategy including the ECG criterion.

Patient flow and diagnostic performance for the ESC 0/1h-hs-cTnT-algorithm and the novel derived observe-zone 0/3h-criteria combining a 3h hs-cTnT concentration with a 0/3h absolute change criterion (step 1) and ST-segment depression (step 2). Sens indicates sensitivity; Spec, specificity; NPV, negative predictive value; PPV, positive predictive value; NSTEMI, non-ST elevation myocardial infarction; and ECG, electrocardiogram; Δ, delta.

*****Patients with a chest pain onset<3h can't be directly rule-out with a 0h hs-cTnT value.

Supplemental Figure XIII. Application of the ECG criterion before the novel proposed observe-zone 3-hour hs-cTnT cut-offs.

Patient flow and diagnostic performance for the ESC 0/1h-hs-cTnT-algorithm and the novel derived observe-zone 0/3h-criteria combining a 3h hs-cTnT concentration with a 0/3h absolute change criterion (step 1) and ST-segment depression (step 2). Sens indicates sensitivity; Spec, specificity; NPV, negative predictive value; PPV, positive predictive value; NSTEMI, non-ST elevation myocardial infarction; and ECG, electrocardiogram; Δ, delta.

*****Patients with a chest pain onset<3h can't be directly rule-out with a 0h hs-cTnT value.

Supplemental Figure XIV. Diagnostic performance of the 7ng/L approach in observe-zone patients stratified by evidence of myocardial ischemia on the ECG.

External validation of the 7ng/L approach in patients with and without myocardial ischemia on the ECG, defined as ST segment depression or T wave inversion (A); and external validation of the 7ng/L approach in patients with and without myocardial ischemia on the ECG, defined as ST segment depression or T wave inversion, however patients with ischemic ECG changes were directly ruled-in overruling the 7ng/L approach (B).

Supplemental Figure XV. Patient flow for the TRAPID-AMI cohort

STEMI indicates ST elevation myocardial infarction; AMI, acute myocardial infarction; hs-cTnT, high-sensitivity cardiac troponin T; and ESC, European society of cardiology.

Supplemental Figure XVI. Sensitivity analysis 1 for the external validation of the novel proposed 3h strategy in the TRAPID-AMI cohort.

The algorithm displays patient flow and diagnostic performance for the ESC 0/1h-algorithm and the novel derived observe-zone 0/3h-criteria which combines a 3h hs-cTnT concentration with a 0/3h absolute change criterion. 2h hs-cTnT values were used as 3h values. Sens indicates sensitivity; Spec, specificity; NPV, negative predictive value; PPV, positive predictive value; and NSTEMI, non-ST elevation myocardial infarction; Δ, delta.

*****Patients with a chest pain onset<3h can't be directly rule-out with a 0h hs-cTnT value.

Supplemental Figure XVII. Sensitivity analysis 2 for the external validation of the novel proposed 3h strategy in the TRAPID-AMI cohort.

The algorithm displays patient flow and diagnostic performance for the ESC 0/1h-algorithm and the novel derived observe-zone 0/3h-criteria which combines a 3h hs-cTnT concentration with a 0/3h absolute change criterion. 3h hs-cTnT values were calculated by giving more weight to 2h values (2/3rd weight) than to 5/6h values (1/3rd weight). Sens indicates sensitivity; Spec, specificity; NPV, negative predictive value; PPV, positive predictive value; and NSTEMI, non-ST elevation myocardial infarction; Δ, delta.

*****Patients with a chest pain onset<3h can't be directly rule-out with a 0h hs-cTnT value.