**APPENDIX I – Supplemental Methods**

***High-Sensitivity Troponin in the Evaluation of Patients With Acute Coronary Syndrome (High-STEACS)***

**Adjudication according to the Fourth Universal Definition of Myocardial Infarction**

All patients with high-sensitivity cardiac troponin I (hs-cTnI) concentrations above the sex-specific 99th centile were classified according to the Third Universal Definition of Myocardial Infarction in use at the time of the trial. For a pre-specified secondary analysis, we updated this classification in accordance with the Fourth Universal Definition of Myocardial Infarction (ref). Two physicians independently reviewed all clinical information, blinded to study phase, with discordant diagnoses resolved by a third reviewer. Clinical information included the dates and times of presentation and final discharge, the initial emergency department assessment and final discharge letter as documented in the electronic care record, with summaries of all investigations undertaken during the index presentation including the electrocardiogram. The adjudication panel had access to raw clinical information including haemoglobin, creatinine and high-sensitivity cardiac troponin I concentrations, and the reports from invasive coronary angiography. Type 1 myocardial infarction was defined as myocardial necrosis (any hs-cTnI concentration above the 99th centile with a rise and/or fall in hs-cTnI concentration where serial testing was performed) in the context of a presentation with suspected acute coronary syndrome with symptoms or signs of myocardial ischemia on the electrocardiogram. Patients with symptoms or signs of myocardial ischemia and evidence of increased oxygen demand or decreased supply (for example, tachyarrhythmia, hypotension, or anaemia) secondary to an alternative pathology and myocardial necrosis were defined as type 2 myocardial infarction. The classification of type 2 myocardial infarction also includes patients with coronary vasospasm, embolism or spontaneous dissection without evidence of atherothrombosis related to coronary artery disease. Type 4a myocardial infarction was defined in patients with symptoms or signs of myocardial ischemia following percutaneous coronary intervention where hs-cTnI concentrations were 5-fold greater than the 99th centile, or increased further if elevated prior to the procedure. Type 4b myocardial infarction was defined where myocardial ischemia and myocardial necrosis were associated with stent thrombosis documented at angiography. Myocardial injury was defined if hs-cTnI concentrations were above the 99th centile in the absence of any clinical features of myocardial ischemia. All non-ischemic myocardial injury was classified as acute, unless a change of <20% was observed on serial testing or the final adjudicated diagnosis was chronic heart failure or chronic renal failure, where the classification was chronic myocardial injury.

***Advantageous Predictors of Acute Coronary Syndromes Evaluation (APACE)***

**Study design and population**

Advantageous Predictors of Acute Coronary Syndromes Evaluation (APACE) was a prospective international multicentre study with 12 centres in 5 countries aiming to advance the early diagnosis of myocardial infarction (ClinicalTrials.gov registry, number NCT00470587). Adult patients (≥ 18 years) presenting to the Emergency Department with symptoms suggestive of myocardial infarction were recruited. While enrolment was independent of renal function, we excluded patients with terminal kidney failure on chronic dialysis. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Written informed consent was obtained from all patients.

**Cardiac troponin measurement**

Cardiac troponin testing was performed at presentation and repeated at 1, 2, 3 and 6 hours from presentation. Serial sampling was discontinued when a patient was discharged or transferred to the catheterization laboratory. Samples were collected in plasma or serum tubes, underwent centrifugation before being frozen at −80°C until assayed in a blinded fashion in a dedicated core laboratory.

**Cardiac troponin assays**

The Abbott ARCHITECT*STAT* high-sensitivity troponin I assay (Abbott Laboratories, Illinois, USA) is a chemiluminescent microparticle immunoassay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 4.7 ng/L, a limit of detection of 1.9 ng/L and a 99th centile upper reference limit of 34 ng/L in men and 16 ng/L in women.

The Beckman Coulter Access high-sensitivity troponin I assay (Beckman Coulter, California, USA) is a sequential two–step immunoenzymatic assay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 5.6 ng/L, a limit of detection of 2.3 ng/L, a universal 99th centile upper reference limit of 17.5 ng/L with a 99th centile upper reference limit of 19.8 ng/L in men and 11.6 ng/L in women.

The LSI Medience PATHFAST high-sensitivity troponin I assay (LSI Medience Corporation, Tokyo, Japan) is a point-of-care chemiluminescent enzyme immunoassay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 15 ng/L, a limit of detection of 2.3 ng/L, and a universal 99th centile upper reference limit of 29.0 ng/L.

The Ortho Clinical Diagnostics VITROS high-sensitivity troponin I assay (Ortho Clinical Diagnostics, New Jersey, USA) is a chemiluminescent enzyme immunoassay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 1.99 ng/L, a limit of detection of 0.86 ng/L, a universal 99th centile upper reference limit of 11.0 ng/L with a 99th centile upper reference limit of 12.0 ng/L in men and 9.0 ng/L in women.

The Siemens ADVIA Centaur high-sensitivity troponin I assay (Siemens Healthiners, Erlangen, Germany) is a chemiluminescent enzyme immunoassay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 6.0 ng/L, a limit of detection of 1.6 ng/L, a universal 99th centile upper reference limit of 47.34 ng/L with a 99th centile upper reference limit of 57.3 ng/L in men and 37 ng/L in women.

The Siemens Dimension EXL high-sensitivity troponin I assay (Siemens Healthiners, Erlangen, Germany) is a chemiluminescent enzyme immunoassay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 12.0 ng/L, a limit of detection of 2.7 ng/L, a universal 99th centile upper reference limit of 60.4 ng/L with a 99th centile upper reference limit of 76.2 ng/L in men and 51.4 ng/L in women.

The Siemens Dimension Vista high-sensitivity troponin I assay (Siemens Healthiners, Erlangen, Germany) is a chemiluminescent enzyme immunoassay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 10.0 ng/L, a limit of detection of 2.0 ng/L, a universal 99th centile upper reference limit of 58.9 ng/L with a 99th centile upper reference limit of 78.5 ng/L in men and 53.7 ng/L in women.

The Singulex Clarity high-sensitivity troponin I assay (Singulex Clarity, Alameda, United States) is a microparticle-based immunoassay with a manufacturer reported inter-assay coefficient of variation of less than 10% at 0.53ng/L, a limit of detection of 0.08ng/L, and a universal 99th centile upper reference limit of 8.67 ng/L.

**Adjudicated final diagnosis**

Myocardial infarction was defined and cardiac troponin levels interpreted as recommended in current guidelines.1–3 In brief, myocardial infarction was diagnosed when there was evidence of myocardial injury with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Patients with myocardial infarction were further subdivided into type 1 myocardial infarction (primary coronary events) and type 2 myocardial infarction (ischemia due to increased demand or decreased supply, for example tachyarrhythmia or hypertensive urgency).1,4 All other patients were classified in the categories of unstable angina, non-cardiac chest pain, cardiac but non-coronary disease (e.g., tachyarrhythmia, myopericarditis), and symptoms of unknown origin with normal high-sensitivity cardiac tropoini concentrations.

The adjudication of final diagnoses was performed centrally in the core lab (University Hospital Basel) for all patients incorporating sex-specific high-sensitivity cardiac troponin I concentrations. 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 high-sensitivity cardiac troponin I levels, radiologic testing, electrocardiography, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, discharge summary) pertaining to the patient from the time of Emergency Department presentation to 90-day follow-up. In situations of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third cardiologist.

Sex-specific 99th centile upper reference limits of ARCHITECTSTAT high-sensitivity troponin I assay (women: 16 ng/L, men 34 ng/L) were used to identify those with myocardial injury. Absolute changes in cardiac tropoinin were used to determine significant changes based on the diagnostic superiority of absolute over relative changes.5–10 Based on studies of the biological variation of cTn11,12 as well as on data from previous chest pain cohort studies,5,13 a significant absolute change was defined as a rise or fall of at least 10 ng/L within six hours, or, in an assumption of linearity, as an absolute change of 6 ng/L within three hours.

**Routine clinical assessment**

Patients underwent clinical assessment that included medical history, physical examination, standard blood test including serial measurements of local cardiac troponin, 12-lead ECG, chest radiography, continuous ECG-rhythm monitoring and pulse oximetry. Management of patients was left to discretion of the attending physician.

**Follow-up and clinical endpoints**

Patients were contacted 3, 12 and 24 months after discharge by telephone calls or in written form. Information regarding death during follow up was furthermore obtained from the patient’s hospital notes, the family physician’s records and the national registry on mortality.

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**Appendix II – Supplemental Figures & Tables**

1. **Supplementary Table 1:** Baseline characteristics of patients presenting early after symptom onset.
2. **Supplementary Table 2:** Diagnostic performance of rule out thresholds by time from symptom onset in patients presenting very early after symptom onset.
3. **Supplemental Table 3.** Diagnostic performance of rule out thresholds for type 1 or 4b myocardial infarction or cardiovascular death within 30 days of index presentation by time from symptom onset
4. **Supplementary Table 4:** Baseline characteristics of the external validation cohort stratified by time from maximal symptom severity to troponin sampling.
5. **Supplementary Table 5:** External validation of performance of rule out thresholds for a diagnosis of type 1 or 4 b myocardial infarction by time from maximal symptom onset.
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7. **Supplementary Table 7:** External validation of performance of rule out thresholds for a diagnosis of type 1 or 4 b myocardial infarction or cardiovascular death within 30 days of index presentation by time from maximal symptom onset.
8. **Supplementary Table 8:** Baseline characteristics of the external validation cohort stratified by time from maximal symptom severity to troponin sampling.
9. **Supplementary Table 9:** Validation of the performance of rule out thresholds for a diagnosis of type 1 or 4 b myocardial infarction by time from initial symptom onset.
10. **Supplementary Table 10:** Validation of the performance of rule out thresholds by time from initial symptom onset in patients presenting within 3 hours of initial symptom onset.
11. **Supplementary Table 11:** Baseline characteristics of patients in the external validation cohort who underwent additional cardiac assay troponin sampling.
12. **Supplemental Table 12:** Assessment of the limit of detection of additional cardiac troponin I assays to rule out type 1 or 4b myocardial infarction by time from maximal symptom severity to troponin sampling in the external validation cohort.
13. **Supplementary Table 13:** Diagnostic performance of rule out thresholds for a diagnosis of type 1 or 4b myocardial infarction by time from symptom onset in patients without evidence of ischaemia on 12-lead electrocardiogram.

#### **Supplementary Table 14:** Rule-out performance by time from symptom onset to troponin sampling restricted to patients with serial cardiac troponin measurements at 6-12 hours from presentation (n=9,362)

#### **Supplementary Table 15:** Diagnostic performance of rule out thresholds for a diagnosis of type 1 or 4b myocardial infarction by time from symptom onset excluding patients with a GRACE score >140.

#### **Supplementary Table 16:** Diagnostic performance of a 2ng/L threshold for the rule-out of type 1 or 4b myocardial infarction by time from symptom onset and GRACE risk category.

1. **Supplementary Table 17:** Diagnostic performance of rule out thresholds for a diagnosis of type 1 or 4b myocardial infarction by time from symptom onset stratified by age.

#### **Supplementary Table 18:** Diagnostic performance of rule out thresholds for a diagnosis of type 1, type 2 or type 4b myocardial infarction by time from symptom onset.

**Supplementary Table 1:** Baseline characteristics of patients with suspected myocardial infarction presenting early stratified by the number of hours since symptom onset (n=12,595)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Time from symptom onset to troponin testing | | |  |
|  | 1 hour | 2 hours | 3 hours | p-value |
| *Number of participants* | 2,469 | 5,303 | 4,823 |  |
| *Age, years* | 62 (17) | 62 (17) | 62 (17) | <0.001 |
| *Sex* |  |  |  | <0.001 |
| Women | 965 (39%) | 2,294 (43%) | 2,221 (46%) |  |
| Men | 1,504 (61%) | 3,009 (57%) | 2,602 (54%) |  |
| *Presenting complaint* |  |  |  |  |
| Chest pain | 1,679 (68%) | 4,154 (78%) | 3,914 (81%) | <0.001 |
| *Previous medical conditions* |  |  |  |  |
| Ischaemic heart disease | 630 (26%) | 1,385 (26%) | 1,368 (28%) | 0.010 |
| Myocardial infarction | 232 (9.4%) | 508 (9.6%) | 502 (10%) | 0.26 |
| Heart Failure | 254 (10%) | 517 (9.7%) | 423 (8.8%) | 0.076 |
| Cerebrovascular disease | 165 (6.7%) | 328 (6.2%) | 324 (6.7%) | 0.50 |
| Diabetes mellitus | 187 (7.6%) | 410 (7.7%) | 376 (7.8%) | 0.94 |
| Chronic renal disease | 562 (23%) | 993 (19%) | 1,013 (21%) | <0.001 |
| *Previous revascularisation* |  |  |  |  |
| Percutaneous coronary intervention | 198 (8.0%) | 455 (8.6%) | 445 (9.2%) | 0.20 |
| Coronary artery bypass grafting | 50 (2.0%) | 79 (1.5%) | 101 (2.1%) | 0.054 |
| *Medications at presentation* |  |  |  |  |
| Aspirin | 737 (30%) | 1,523 (29%) | 1,453 (30%) | 0.27 |
| P2Y12 inhibitor | 252 (10%) | 530 (10.0%) | 531 (11%) | 0.23 |
| Dual anti-platelet therapy† | 79 (3.2%) | 188 (3.5%) | 189 (3.9%) | 0.28 |
| Statin | 1,092 (44%) | 2,230 (42%) | 2,085 (43%) | 0.17 |
| ACE inhibitor or ARB | 909 (37%) | 1,824 (34%) | 1,658 (34%) | 0.076 |
| Beta-blocker | 752 (30%) | 1,518 (29%) | 1,440 (30%) | 0.19 |
| Oral anticoagulant‡ | 180 (7.3%) | 360 (6.8%) | 319 (6.6%) | 0.55 |
| *Physiological parameters on presentation*§ |  |  |  |  |
| Heart rate, beats per minute | 90 (30) | 86 (29) | 85 (27) | 0.003 |
| Systolic blood pressure, mmHg | 136 (34) | 136 (29) | 140 (29) | 0.025 |
| GRACE score | 152 (42) | 145 (39) | 141 (36) | 0.002 |
| Ischaemia on ECG | 141 (27%) | 311 (32%) | 234 (27%) | 0.038 |
| *Haematology and clinical chemistry measurements* |  |  |  |  |
| Haemoglobin, g/L | 136 (25) | 137 (21) | 135 (23) | <0.001 |
| Estimated glomerular filtration mL/min | 77 (26) | 80 (24) | 81 (24) | <0.001 |
| Presentation high-sensitivity cardiac troponin I, ng/L | 4 [2-16] | 4 [1-12] | 4 [2-12] | <0.001 |
| Serial troponin¶ measurement | 1,354 (55%) | 3,288 (62%) | 3,062 (63%) | <0.001 |
| *Adjudicated diagnosis* |  |  |  |  |
| Type 1 myocardial infarction | 269 (11%) | 589 (11%) | 560 (12%) | 0.59 |
| Type 2 myocardial infarction | 98 (4.0%) | 175 (3.3%) | 135 (2.8%) | 0.027 |
| Type 4b myocardial infarction | <5 (<0.1%) | 9 (0.2%) | 10 (0.2%) | 0.51 |
| Acute myocardial injury | 204 (8.3%) | 196 (3.7%) | 145 (3.0%) | <0.001 |
| Chronic myocardial injury | 67 (2.7%) | 133 (2.5%) | 138 (2.9%) | 0.54 |
| No myocardial injury | 1,829 (74%) | 4,199 (79%) | 3,835 (80%) | <0.001 |
| *Outcomes at 30 days* |  |  |  |  |
| Type 1 or 4b myocardial infarction | 17 (0.7%) | 38 (0.7%) | 29 (0.6%) | 0.8 |
| Cardiovascular death | 107 (4.3%) | 117 (2.2%) | 75 (1.6%) | <0.001 |
| Values are mean (SD) and median [25th – 75th centile]; n (%).  1Wilcoxon rank sum test; Pearson’s Chi-squared test  †Two medications from aspirin, clopidogrel, prasugrel, or ticagrelor.  ‡Includes warfarin or novel oral anticoagulants.  ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers  ¶Serial testing defined as two or more tests within 24 hours of presentation. | | | | |

#### **Supplementary Table 2:** Diagnostic performance of rule out thresholds by time from symptom onset in patients presenting very early after symptom onset

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| 1 hour | 567 | 1 | 99.6%  (99.3-99.8%) | 99.8%  (99.6-99.9%) | 23% |
| 2 hours | 1,323 | 4 | 99.3%  (99.1-99.5%) | 99.7%  (99.5-99.8%) | 25% |
| 3 hours | 1,130 | 4 | 99.3%  (99.0-99.5%) | 99.6%  (99.4-99.8%) | 24% |
|  | 5 ng/L | | | | |
| 1 hour | 1,222 | 20 | 92.6%  (91.5-93.6%) | 98.4%  (97.8-98.8%) | 50% |
| 2 hours | 2,866 | 18 | 97.0%  (96.5-97.4%) | 99.4%  (99.1-99.6%) | 54% |
| 3 hours | 2,580 | 12 | 97.9%  (97.4-98.3%) | 99.5%  (99.3-99.7%) | 54% |
|  | Sex-specific 99th centile | | | | |
| 1 hour | 1,906 | 102 | 62.4%  (60.4-64.3%) | 94.9%  (94.0-95.7%) | 81% |
| 2 hours | 4,305 | 181 | 69.7%  (68.5-71.0%) | 96.0%  (95.4-96.5%) | 85% |
| 3 hours | 3,896 | 128 | 77.5%  (76.3-78.7%) | 96.8%  (96.3-97.3%) | 83% |
| Presented as number or % (95% confidence intervals) as appropriate. High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: 1 hour = 2,469; 2 hours = 5,299; 3 hours = 4,913  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

#### **Supplementary Table 3:** Diagnostic performance of rule out thresholds for type 1 or 4b myocardial infarction or cardiovascular death within 30 days of index presentation by time from symptom onset

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 3,016 | 13 | 99.2%  (99.0 - 99.3%) | 99.6%  (99.4 - 99.7%) | 24% |
| 4-12 hours | 4,761 | 9 | 99.5%  (99.3 - 99.6%) | 99.8%  (99.7 - 99.9%) | 27% |
| >12 hours | 3,551 | 2 | 99.8%  (99.6 - 99.8%) | 99.9%  (99.9 - 100.0%) | 32% |
| Overall | 11,328 | 24 | 99.4%  (99.3 - 99.5%) | 99.8%  (99.7 - 99.8%) | 28% |
|  | 5 ng/L | | | | |
| ≤3 hours | 6,653 | 65 | 95.9%  (95.6 - 96.3%) | 99.0%  (98.8 - 99.2%) | 53% |
| 4-12 hours | 10,138 | 30 | 98.2%  (98.0 - 98.4%) | 99.7%  (99.6 - 99.8%) | 58% |
| >12 hours | 6,900 | 13 | 98.4%  (98.1 - 98.6%) | 99.8%  (99.7 - 99.9%) | 63% |
| Overall | 23,691 | 108 | 97.3%  (97.2 - 97.5%) | 99.5%  (99.5 - 99.6%) | 58% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 10,105 | 503 | 68.6%  (67.7 - 69.4%) | 95.2%  (94.8 - 95.6%) | 84% |
| 4-12 hours | 14,555 | 293 | 82.3%  (81.8 - 82.9%) | 98.0%  (97.8 - 98.2%) | 85% |
| >12 hours | 9,386 | 85 | 89.4%  (88.8 - 89.9%) | 99.1%  (98.9 - 99.3%) | 86% |
| Overall | 33,956 | 881 | 78.3%  (77.9 - 78.7%) | 97.5%  (97.3 - 97.6%) | 85% |
| Presented as number or % (95% confidence intervals) as appropriate. High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 12,595; 4-12 hours = 17,468;  >12 hours = 11,040; Overall = 41,103  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 4:** Baseline characteristics of the external validation cohort stratified by time from maximal symptom severity to troponin sampling (n=7,088).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Time from symptom onset to troponin testing | | | | | |  |
| **Overall** | | **≤3 hours** | **4-12 hours** | **>12 hours** | | ***p-value*** | |
| *Number of participants* | 7,088 | 2,927 | | 3,203 | 958 | |  | |
| *Age, years* | 60 (17) | 58 (16) | | 62 (16) | | 60 (17) | <0.001 | |
| *Sex* |  |  | |  | |  |  | |
| Women | 2,420 (34%) | 968  (33%) | | 1,145 (36%) | | 307 (32%) | 0.073 | |
| Men | 4,668 (66%) | 1,959 (67%) | | 2,058 (64%) | | 651 (68%) |  | |
| *Time from symptom onset to troponin sampling (hours)* | 9 (±15) | 2 (±1) | | 7 (±2) | | 38 (±25) | <0.001 | |
| *Previous medical conditions* |  |  | |  | |  |  | |
| Ischaemic heart disease | 2,262 (32%) | 903 (31%) | | 1,076 (34%) | | 283 (30%) | 0.017 | |
| Myocardial infarction | 1,607 (23%) | 667 (23%) | | 746 (23%) | | 194 (20%) | 0.14 | |
| Cerebrovascular disease | 365 (5.1%) | 145 (5.0%) | | 173 (5.4%) | | 47 (4.9%) | 0.68 | |
| Diabetes mellitus | 1,238 (17%) | 497 (17%) | | 583 (18%) | | 158 (16%) | 0.30 | |
| Chronic renal disease | 665 (9.4%) | 235 (8.0%) | | 333 (10%) | | 97 (10%) | 0.005 | |
| *Previous revascularisation* |  |  | |  | |  |  | |
| Percutaneous coronary intervention | 1,677 (24%) | 699 (24%) | | 764 (24%) | | 214 (22%) | 0.59 | |
| Coronary artery bypass grafting | 546 (7.7%) | 190 (6.5%) | | 288 (9.0%) | | 68 (7.1%) | <0.001 | |
| *Medications at presentation* |  |  | |  | |  |  | |
| Aspirin | 2,503 (35%) | 994 (34%) | | 1,177 (37%) | | 332 (35%) | 0.067 | |
| P2Y12 inhibitor | 748 (11%) | 331 (11%) | | 327 (10%) | | 90 (9.4%) | 0.17 | |
| Dual anti-platelet therapy† | 583 (8.2%) | 258 (8.8%) | | 257 (8.0%) | | 68 (7.1%) | 0.21 | |
| Statin | 2,453 (35%) | 996 (34%) | | 1,162 (36%) | | 295 (31%) | 0.005 | |
| ACE inhibitor or ARB | 2,774 (39%) | 1,121 (38%) | | 1,305 (41%) | | 348 (36%) | 0.023 | |
| Beta-blocker | 2,360 (33%) | 935 (32%) | | 1,124 (35%) | | 301 (31%) | 0.014 | |
| Oral anticoagulant‡ | 716 (10%) | 266 (9.1%) | | 369 (12%) | | 81 (8.5%) | 0.001 | |
| *Physiological parameters on presentation*§ |  |  | |  | |  |  | |
| Heart rate, beats per minute | 79 (19) | 79 (20) | | 79 (19) | | 79 (20) | 0.75 | |
| Systolic blood pressure, mmHg | 141 (23) | 140 (24) | | 142 (24) | | 141 (22) | 0.005 | |
| GRACE score | 98 [75-126] | 94 [72-121] | | 103 [80-130] | | 98 [75-125] | <0.001 | |
| Ischaemia on ECG | 1,314 (19%) | 461 (16%) | | 670 (21%) | | 183 (19%) | <0.001 | |
| *Haematology and clinical chemistry measurements* |  |  | |  | |  |  | |
| Haemoglobin, g/L | 141 (17) | 142 (17) | | 140 (17) | | 142 (17) | 0.001 | |
| Estimated glomerular filtration mL/min | 85 (23) | 86 (23) | | 83 (23) | | 84 (24) | <0.001 | |
| Presentation high-sensitivity cardiac troponin I, ng/L | 4 [2-14] | 4 [2-11] | | 5 [2-21] | | 4 [2-14] | <0.001 | |
| *Adjudicated diagnosis* |  |  | |  | |  |  | |
| Type 1 myocardial infarction | 975 (14%) | 360 (12%) | | 486 (15%) | | 129 (13%) | 0.005 | |
| *Outcome at 30 days* |  |  | |  | |  |  | |
| Type 1 or 4b myocardial infarction | 40 (0.6%) | 15 (0.5%) | | 22 (0.7%) | | 3 (0.3%) | 0.35 | |
| Cardiovascular death | 39 (0.6%) | 7 (0.2%) | | 26 (0.8%) | | 6 (0.6%) | 0.010 | |
| Values are mean (SD) and median [25th – 75th centile]; n (%).  1Wilcoxon rank sum test; Pearson's Chi-squared test  †Two medications from aspirin, clopidogrel, prasugrel, or ticagrelor.  ‡Includes warfarin or novel oral anticoagulants.  §Physiological data missing in the following number of patients: heart rate = 12, blood pressure = 10, presentation ECG = 81, GRACE score = 669  ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; ECG, electrocardiogram | | | | | | | | |

**Supplementary Table 5:** External validation of performance of rule out thresholds for a diagnosis of type 1 or 4 b myocardial infarction by time from maximal symptom onset (n=7,088)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 697 | 0 | 100%  (99.9-100%) | 100%  (99.9-100%) | 24% |
| 4-12 hours | 610 | 0 | 100%  (99.9-100%) | 100%  (99.9-100%) | 19% |
| >12 hours | 243 | 0 | 100%  (99.6-100%) | 100%  (99.6-100%) | 25% |
| Overall | 1,550 | 0 | 100%  (99.9-100%) | 100%  (99.9-100%) | 22% |
|  | 5 ng/L | | | | |
| ≤3 hours | 1,713 | 5 | 98.6%  (98.1-99.0%) | 99.7%  (99.4-99.8%) | 59% |
| 4-12 hours | 1,571 | 0 | 100%  (99.9-100%) | 100%  (99.9-100%) | 49% |
| >12 hours | 534 | 1 | 99.2%  (98.4-99.6%) | 99.8%  (99.3-100%) | 56% |
| Overall | 3,818 | 6 | 99.4%  (99.2-99.5%) | 99.8%  (99.7-99.9%) | 54% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 2,386 | 128 | 64.4%  (62.7-66.2%) | 94.9%  (94.1-95.6%) | 86% |
| 4-12 hours | 2,430 | 55 | 88.7%  (87.5-89.7%) | 97.8%  (97.2-98.2%) | 78% |
| >12 hours | 754 | 6 | 95.3%  (93.8-96.5%) | 99.2%  (98.4-99.6%) | 79% |
| Overall | 5,570 | 189 | 80.6%  (79.7-81.5%) | 96.7%  (96.3-97.1%) | 81% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 2,927; 4-12 hours = 3,203;  >12 hours = 958; Overall = 7,088  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 6:** External validation of performance of rule out thresholds by time from maximal symptom onset in patients presenting very early after symptom onset (n=2,927)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| 1 hour | 153 | 0 | 100%  (99.6 – 100%) | 100%  (99.4 - 100%) | 24% |
| 2 hours | 313 | 0 | 100%  (99.7 – 100%) | 100%  (99.7 - 100%) | 25% |
| 3 hours | 231 | 0 | 100%  (99.6 – 100%) | 100%  (99.6 - 100%) | 22% |
|  | 5 ng/L | | | | |
| 1 hour | 377 | 1 | 98.6%  (97.4-99.3%) | 99.7%  (98.9-99.9%) | 60% |
| 2 hours | 750 | 1 | 99.3%  (98.6-99.6%) | 99.9%  (99.5-100%) | 61% |
| 3 hours | 586 | 3 | 98.0%  (96.9-98.7) | 99.5%  (98.8-99.8%) | 56% |
|  | Sex-specific 99th centile | | | | |
| 1 hour | 526 | 33 | 54.8%  (50.9-58.6%) | 94.1%  (92.0-95.7%) | 88% |
| 2 hours | 1031 | 51 | 63.3%  (60.6-65.9%) | 95.3%  (94.0-96.3%) | 87% |
| 3 hours | 829 | 44 | 70.3%  (67.4-73.0%) | 95.0%  (93.5-96.1%) | 83% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: 1 hour = 634; 2 hours = 1,240; 3 hours = 1,053  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 7:** External validation of performance of rule out thresholds for a diagnosis of type 1 or 4 b myocardial infarction or cardiovascular death within 30 days of index presentation by time from maximal symptom onset (n=7,088)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 696 | 1 | 99.7%  (99.5-99.9%) | 99.9%  (99.6-99.9%) | 24% |
| 4-12 hours | 608 | 2 | 99.6%  (99.3-99.8%) | 99.7%  (99.4-99.8%) | 19% |
| >12 hours | 243 | 0 | 100%  (99.6-100%) | 100%  (99.6-100%) | 26% |
| Overall | 1,547 | 3 | 99.7%  (99.5-99.8%) | 99.8%  (99.7-99.9%) | 22% |
|  | 5 ng/L | | | | |
| ≤3 hours | 1,711 | 7 | 98.1%  (97.5-98.5%) | 99.6%  (99.3-99.8%) | 59% |
| 4-12 hours | 1,566 | 5 | 99.0%  (98.6-99 3%) | 99.7 %  (99.4-99.8%) | 49% |
| >12 hours | 533 | 2 | 98.5%  (97.5-99.1%) | 99.6%  (99.0-99 9%) | 56% |
| Overall | 3,810 | 14 | 98.6%  (98.3-98.9%) | 99.6%  (99.5-99.8%) | 54% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 2,378 | 136 | 63.1%  (61.4-64.9%) | 94.6%  (93.7-95.4%) | 86% |
| 4-12 hours | 2,416 | 69 | 86.3%  (85.1-87.5%) | 97.2%  (96.6-97.7%) | 78% |
| >12 hours | 751 | 9 | 93.2%  (91.4-94.6%) | 98.8%  (97.9-99.3%) | 79% |
| Overall | 5,545 | 214 | 78.7%  (77.8-79.7) | 96.3%  (95.8-96.7%) | 81% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 2,927; 4-12 hours = 3,203;  >12 hours = 958; Overall = 7,088  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 8:** Baseline characteristics of the external validation cohort stratified by time from maximal symptom severity to troponin sampling (n=7,088).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Time from symptom onset to troponin testing | | |  |
|  | **Overall** | **≤3 hours** | **4-12 hours** | **>12 hours** | ***p-value*** |
| *Number of participants* | 7,088 | 1,880 | 2,964 | 2,244 | <0.01 |
| *Age, years* | 60 (17) | 58 (16) | 63 (16) | 59 (17) | <0.001 |
| *Sex* |  |  |  |  | 0.036 |
| Women | 4,668 (66%) | 1,280 (68%) | 1,945 (66%) | 1,443 (64%) |  |
| Men | 2,420 (34%) | 600 (32%) | 1,019 (34%) | 801 (36%) |  |
| *Time from symptom onset to troponin sampling (hours)* | 19 (27) | 2 (1) | 7 (2) | 48 (31) | <0.001 |
| *Previous medical conditions* |  |  |  |  |  |
| Ischaemic heart disease | 2,262 (32%) | 566 (30%) | 1,058 (36%) | 638 (28%) | <0.001 |
| Myocardial infarction | 1,607 (23%) | 418 (22%) | 732 (25%) | 457 (20%) | <0.001 |
| Cerebrovascular disease | 365 (5.1%) | 95 (5.1%) | 164 (5.5%) | 106 (4.7%) | 0.41 |
| Diabetes mellitus | 1,238 (17%) | 322 (17%) | 557 (19%) | 359 (16%) | 0.030 |
| Chronic renal disease | 665 (9.4%) | 137 (7.3%) | 308 (10%) | 220 (9.8%) | 0.001 |
| *Previous revascularisation* |  |  |  |  |  |
| Percutaneous coronary intervention | 1,677 (24%) | 429 (23%) | 765 (26%) | 483 (22%) | <0.001 |
| Coronary artery bypass grafting | 546 (7.7%) | 114 (6.1%) | 260 (8.8%) | 172 (7.7%) | 0.003 |
| *Medications at presentation* |  |  |  |  |  |
| Aspirin | 2,503 (35%) | 639 (34%) | 1,122 (38%) | 742 (33%) | <0.001 |
| P2Y12 inhibitor | 748 (11%) | 208 (11%) | 328 (11%) | 212 (9.4%) | 0.12 |
| Dual anti-platelet therapy† | 583 (8.2%) | 160 (8.5%) | 253 (8.5%) | 170 (7.6%) | 0.40 |
| Statin | 2,453 (35%) | 639 (34%) | 1,114 (38%) | 700 (31%) | <0.001 |
| ACE inhibitor or ARB | 2,774 (39%) | 718 (38%) | 1,237 (42%) | 819 (36%) | <0.001 |
| Beta-blocker | 2,360 (33%) | 604 (32%) | 1,071 (36%) | 685 (31%) | <0.001 |
| Oral anticoagulant‡ | 716 (10%) | 166 (8.8%) | 322 (11%) | 228 (10%) | 0.072 |
| *Physiological parameters on presentation*§ |  |  |  |  |  |
| Heart rate, beats per minute | 79 (19) | 78 (20) | 79 (19) | 79 (19) | 0.048 |
| Systolic blood pressure, mmHg | 141 (23) | 139 (24) | 142 (24) | 142 (23) | <0.001 |
| GRACE score | 98 [75-126] | 94 [72-122] | 104 [80-130] | 96 [74- 124] | <0.001 |
| Ischaemia on ECG | 1,314 (19%) | 298 (16%) | 596 (20%) | 420 (19%) | <0.001 |
| *Haematology and clinical chemistry measurements* |  |  |  |  |  |
| Haemoglobin, g/L | 141 (17) | 142 (16) | 140 (17) | 142 (17) | <0.001 |
| Estimated glomerular filtration mL/min | 85 (23) | 86 (23) | 83 (23) | 85 (24) | <0.001 |
| Presentation high-sensitivity cardiac troponin I, ng/L | 4 [2-14] | 4 [2-10] | 5 [2-20] | 4 [2-14] | <0.001 |
| *Adjudicated diagnosis* |  |  |  |  |  |
| Type 1 myocardial infarction | 975 (14%) | 246 (13%) | 449 (15%) | 280 (12%) | 0.013 |
| *Outcome at 30 days* |  |  |  |  |  |
| Type 1 or 4b myocardial infarction | 40 (0.6%) | 9 (0.5%) | 16 (0.5%) | 15 (0.7%) | 0.70 |
| Cardiovascular death | 39 (0.6%) | 6 (0.3%) | 19 (0.6%) | 14 (0.6%) | 0.29 |
| Values are mean (SD) and median [25th – 75th centile]; n (%).  1Wilcoxon rank sum test; Pearson's Chi-squared test  †Two medications from aspirin, clopidogrel, prasugrel, or ticagrelor.  ‡Includes warfarin or novel oral anticoagulants.  §Physiological data missing in the following number of patients: heart rate = 12, blood pressure = 10, presentation ECG = 81, GRACE score = 669  ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; ECG, electrocardiogram | | | | | |

**Supplementary Table 9:** Validation of the performance of rule out thresholds for a diagnosis of type 1 or 4 b myocardial infarction by time from initial symptom onset (n=7,088)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 449 | 0 | 100%  (99.8-100%) | 100%  (99.8-100%) | 24% |
| 4-12 hours | 526 | 0 | 100%  (99.9-100%) | 100%  (99.9-100%) | 18% |
| >12 hours | 575 | 0 | 100%  (99.8-100%) | 100%  (99.8-100%) | 26% |
| Overall | 1550 | 0 | 100%  (99.9-100%) | 100%  (99.9-100%) | 22% |
|  | 5 ng/L | | | | |
| ≤3 hours | 1092 | 4 | 98.4%  (97.7-98.9%) | 99.6%  (99.2-99.8%) | 58% |
| 4-12 hours | 1454 | 1 | 99.8%  (99.5-99.9%) | 99.9%  (99.8-100%) | 50% |
| >12 hours | 1272 | 1 | 99.6%  (99.3-99.8%) | 99.9%  (99.7-100%) | 57% |
| Overall | 3818 | 6 | 99.4%  (99.2-99.5%) | 99.8%  (99.7-99.9%) | 54% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 1532 | 104 | 57.7%  (55.5-59.9%) | 93.6%  (92.4-94.7%) | 87% |
| 4-12 hours | 2254 | 59 | 86.9%  (85.6-88.0%) | 97.4%  (96.8-98.0%) | 78% |
| >12 hours | 1784 | 26 | 90.7%  (89.4-91.8%) | 98.6%  (98.0-99.0%) | 81% |
| Overall | 5570 | 189 | 80.6%  (79.7-81.5%) | 96.7%  (96.3-97.1%) | 81% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 1,880; 4-12 hours = 2,964;  >12 hours = 2,244; Overall = 7,088  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 10:** Validation of the performance of rule out thresholds by time from initial symptom onset in patients presenting within 3 hours of initial symptom onset (n=2,420)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| 1 hour | 153 | 0 | 100%  (99.4-100%) | 100%  (99.4-100%) | 24% |
| 2 hours | 313 | 0 | 100%  (99.7-100%) | 100%  (99.7-100%) | 25% |
| 3 hours | 231 | 0 | 100%  (99.6-100%) | 100%  (99.6-100%) | 22% |
|  | 5 ng/L | | | | |
| 1 hour | 377 | 1 | 98.6%  (97.4-99.3%) | 99.7%  (98.9-99.9%) | 60% |
| 2 hours | 750 | 1 | 99.3%  (98.6-99.6%) | 99.9%  (99.5-100%) | 61% |
| 3 hours | 586 | 3 | 98.0%  (96.9-98.7%) | 99.5%  (98.8-99.8%) | 56% |
|  | Sex-specific 99th centile | | | | |
| 1 hour | 526 | 33 | 54.8%  (50.9-58.6%) | 94.1%  (92.0-95.7%) | 88% |
| 2 hours | 1031 | 51 | 63.3%  (60.6-65.9%) | 95.3%  (94.0-96.3%) | 87% |
| 3 hours | 829 | 44 | 70.3%  (67.4-73.0%) | 95.0%  (93.5-96.1%) | 83% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: 1 hour = 687; 2 hours = 1,027; 3 hours = 706  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 11:** Baseline characteristics of patients in the external validation cohort who underwent additional cardiac assay troponin sampling.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cardiac troponin assay | | | | | | |
| **Beckman Coulter Access** | **LSI Medicine PATHFAST** | **Ortho Clinical Diagnostics VITROS** | **Siemens ADVIA Centaur** | **Siemens Dimension Vista** | **Siemens Dimension EXL** | **Singulex Clarity** |
| *Number of participants* | 1,895 | 1,534 | 3,197 | 2,908 | 2,120 | 1,438 | 2,787 |
| *Age, years* | 60 (17) | 60 (17) | 60 (17) | 61 (16) | 61 (16) | 60 (17) | 61 (16) |
| *Sex* |  |  |  |  |  |  |  |
| Women | 626 (33%) | 530 (35%) | 1,141 (36%) | 1,958 (67%) | 712 (34%) | 499 (35%) | 910 (33%) |
| Men | 1,269 (67%) | 1,004 (65%) | 2,056 (64%) | 950 (33%) | 1,408 (66%) | 939 (65%) | 1,877 (67%) |
| *Time from symptom onset to troponin sampling (hours)* | 17 (30) | 18 (32) | 18 (29) | 17 (27) | 18 (29) | 18 (32) | 17 (23) |
| *Previous medical conditions* |  |  |  |  |  |  |  |
| Ischaemic heart disease | 614 (32%) | 481 (31%) | 979 (31%) | 1,003 (34%) | 727 (34%) | 436 (30%) | 973 (35%) |
| Myocardial infarction | 440 (23%) | 367 (24%) | 717 (22%) | 689 (24%) | 503 (24%) | 326 (23%) | 662 (24%) |
| Cerebrovascular disease | 124 (6.5%) | 82 (5.3%) | 171 (5.3%) | 157 (5.4%) | 128 (6.0%) | 77 (5.4%) | 159 (5.7%) |
| Diabetes mellitus | 325 (17%) | 265 (17%) | 550 (17%) | 523 (18%) | 364 (17%) | 259 (18%) | 477 (17%) |
| Chronic renal disease | 168 (8.9%) | 132 (8.6%) | 304 (9.5%) | 277 (9.5%) | 206 (9.7%) | 123 (8.6%) | 261 (9.4%) |
| *Previous revascularisation* |  |  |  |  |  |  |  |
| Percutaneous coronary intervention | 471 (25%) | 376 (25%) | 766 (24%) | 706 (24%) | 521 (25%) | 342 (24%) | 673 (24%) |
| Coronary artery bypass grafting | 146 (7.7%) | 113 (7.4%) | 217 (6.8%) | 249 (8.6%) | 201 (9.5%) | 108 (7.5%) | 262 (9.4%) |
| *Medications at presentation* |  |  |  |  |  |  |  |
| Aspirin | 651 (34%) | 525 (34%) | 1,101 (34%) | 1,048 (36%) | 789 (37%) | 480 (33%) | 1,027 (37%) |
| P2Y12 inhibitor | 212 (11%) | 169 (11%) | 350 (11%) | 334 (11%) | 244 (12%) | 155 (11%) | 329 (12%) |
| Dual anti-platelet therapy† | 154 (8.1%) | 138 (9.0%) | 288 (9.0%) | 243 (8.4%) | 182 (8.6%) | 124 (8.6%) | 233 (8.4%) |
| Statin | 669 (35%) | 531 (35%) | 1,108 (35%) | 1,038 (36%) | 753 (36%) | 480 (33%) | 995 (36%) |
| ACE inhibitor or ARB | 726 (38%) | 624 (41%) | 1,306 (41%) | 1,121 (39%) | 828 (39%) | 572 (40%) | 1,054 (38%) |
| Beta-blocker | 619 (33%) | 503 (33%) | 1,066 (33%) | 985 (34%) | 750 (35%) | 466 (32%) | 971 (35%) |
| Oral anticoagulant‡ | 211 (11%) | 205 (13%) | 370 (12%) | 288 (9.9%) | 192 (9.1%) | 190 (13%) | 247 (8.9%) |
| *Physiological parameters on presentation*§ |  |  |  |  |  |  |  |
| Heart rate, beats per minute | 80 (20) | 79 (19) | 79 (19) | 80 (20) | 80 (20) | 79 (18) | 80 (20) |
| Systolic blood pressure, mmHg | 142 (23) | 141 (23) | 140 (23) | 143 (24) | 143 (24) | 140 (23) | 143 (24) |
| GRACE score | 97 [74-126] | 98 [75-125] | 98 [75-125] | 98  [74-127] | 98  [74-126] | 96  [73-126] | 99  [74- 127] |
| Ischaemia on ECG | 328 (18%) | 560 (18%) | 560 (18%) | 564 (20%) | 401 (19%) | 220 (16%) | 551 (20%) |
| *Haematology and clinical chemistry measurements* |  |  |  |  |  |  |  |
| Haemoglobin, g/L | 142 (17) | 141 (17) | 141 (17) | 141 (17) | 142 (17) | 141 (17) | 141 (17) |
| Estimated glomerular filtration mL/min | 86 (23) | 84 (23) | 84 (23) | 85 (24) | 85 (23) | 86 (23) | 85 (23) |
| Presentation high-sensitivity cardiac troponin I, ng/L | 4 [2-11] | 2 [1-8] | 2 [1-8] | 6 [3-20] | 4 [1-17] | 7 [4-21] | 2 [1-6] |
| *Adjudicated diagnosis* |  |  |  |  |  |  |  |
| Type 1 myocardial infarction | 245 (13%) | 423 (13%) | 423 (13%) | 414 (14%) | 309 (15%) | 165 (11%) | 408 (15%) |
| *Outcome at 30 days* |  |  |  |  |  |  |  |
| Type 1 or 4b myocardial infarction | 7 (0.4%) | 19 (0.6%) | 19 (0.6%) | 15 (0.5%) | 8 (0.4%) | 6 (0.4%) | 11 (0.4%) |
| Cardiovascular death | 8 (0.4%) | 13 (0.4%) | 13 (0.4%) | 16 (0.6%) | 13 (0.6%) | 10 (0.7%) | 17 (0.6%) |
| Values are mean (SD) and median [25th – 75th centile]; n (%).  1Wilcoxon rank sum test; Pearson's Chi-squared test  †Two medications from aspirin, clopidogrel, prasugrel, or ticagrelor.  ‡Includes warfarin or novel oral anticoagulants.  ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers | | | | | | | |

**Supplemental Table 12:** Assessment of the limit of detection of additional cardiac troponin I assays to rule out type 1 or 4b myocardial infarction by time from maximal symptom severity to troponin sampling in the external validation cohort.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Presentation high-sensitivity cardiac troponin I assay (limit of detection) | | | | | | |
| Hours from  symptom onset | **Number of**  **patients** | **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
| *Beckman Coulter Access (3 ng/L)* | | | | | | |
| ≤3 hours | 525 | 339 | 0 | 100 (99.6-100) | 100 (99.6-100) | 40 |
| 4-12 hours | 831 | 361 | 0 | 100 (99.6-100) | 100 (99.6-100) | 39 |
| >12 hours | 539 | 37 | 0 | 100 (96.7-100) | 100 (96.7-100) | 33 |
| Overall | 1,895 | 737 | 0 | 100 (99.8-100) | 100 (99.8-100) | 39 |
| *LSI Medicine PATHFAST (3 ng/L)* | | | | | | |
| ≤3 hours | 478 | 389 | 1 | 98.9 (97.9-99.4) | 99.7 (99.1-99.9) | 48 |
| 4-12 hours | 595 | 276 | 1 | 98.9 (97.8-99.5) | 99.6 (98.8-99.9) | 42 |
| >12 hours | 461 | 25 | 0 | 100 (94.3-100) | 100.0 (94.3-100) | 39 |
| Overall | 1,534 | 690 | 2 | 98.9 (98.3-99.3) | 99.7 (99.3-99.9) | 45 |
| *Ortho Clinical Diagnostics VITROS (1 ng/L)* | | | | | | |
| ≤3 hours | 947 | 469 | 0 | 100 (99.7-100) | 100.0 (99.7-100) | 31 |
| 4-12 hours | 1,271 | 353 | 1 | 99.5 (99.0-99.8) | 99.7 (99.3-99.9) | 25 |
| >12 hours | 979 | 73 | 0 | 100 (98.7-100) | 100.0 (98.7-100) | 26 |
| Overall | 3,197 | 895 | 1 | 99.8 (99.5-99.9) | 99.9 (99.7-100) | 28 |
| *Siemens ADVIA Centaur (2 ng/L)* | | | | | | |
| ≤3 hours | 250 | 222 | 0 | 100 (99.7-100) | 100 (99.7-100) | 19 |
| 4-12 hours | 1,244 | 191 | 0 | 100 (99.7-100) | 100 (99.7-100) | 13 |
| >12 hours | 814 | 35 | 0 | 100 (98.7-100) | 100 (98.7-100) | 12 |
| Overall | 2,908 | 448 | 0 | 100 (99.9-100) | 100 (99.9-100) | 15 |
| *Siemens Dimension EXL (3 ng/L)* | | | | | | |
| ≤3 hours | 460 | 81 | 0 | 100 (99.5-100) | 100 (99.5-100) | 11 |
| 4-12 hours | 550 | 67 | 0 | 100 (99.4-100) | 100 (99.4-100) | 11 |
| >12 hours | 428 | 2 | 0 | 100 (93.4-100) | 100 (93.4-100) | 4 |
| Overall | 1,438 | 150 | 0 | 100 (99.7-100) | 100 (99.7-100) | 10 |
| *Siemens Dimension Vista (2 ng/L)* | | | | | | |
| ≤3 hours | 534 | 271 | 0 | 100 (99.5-100) | 100 (99.5-100) | 33 |
| 4-12 hours | 911 | 295 | 0 | 100 (99.6-100) | 100 (99.6-100) | 31 |
| >12 hours | 675 | 97 | 0 | 100 (98.9-100) | 100 (98.9-100) | 28 |
| Overall | 2,120 | 663 | 0 | 100 (99.8-100) | 100 (99.8-100) | 31 |
| *Singulex Clarity (1ng/L)* | | | | | | |
| ≤3 hours | 668 | 396 | 2 | 98.7 (97.7-99.2) | 99.5 (98.8-99.8) | 39 |
| 4-12 hours | 1,317 | 484 | 0 | 100 (99.7-100) | 100 (99.7-100) | 35 |
| >12 hours | 802 | 127 | 0 | 100 (99.0-100) | 100 (99.0-100) | 32 |
| Overall | 2,787 | 1007 | 2 | 99.5 (99.2-99.7) | 99.8 (99.6-99.9) | 36 |
| Presented as number or % (95% confidence intervals) as appropriate.  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | | |

**Supplementary Table 13:** Diagnostic performance of rule out thresholds for a diagnosis of type 1 or 4b myocardial infarction by time from symptom onset in patients without evidence of ischaemia on 12-lead electrocardiogram (n=39,457)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 3,019 | 7 | 99.3%  (99.1 - 99.4%) | 99.8%  (99.7 - 99.8%) | 25% |
| 4-12 hours | 4,764 | 5 | 99.6%  (99.5 - 99.7%) | 99.9%  (99.8 - 99.9%) | 28% |
| >12 hours | 3,552 | 1 | 99.8%  (99.7 - 99.9%) | 100.0%  (99.9 - 100.0%) | 33% |
| Overall | 11,335 | 13 | 99.5%  (99.4 - 99.6%) | 99.9%  (99.8 - 99.9%) | 29% |
|  | 5 ng/L | | | | |
| ≤3 hours | 6,657 | 36 | 96.3%  (95.9 - 96.6) | 99.5%  (99.3 - 99.6%) | 56% |
| 4-12 hours | 10,145 | 17 | 98.5%  (98.3 - 98.7%) | 99.8%  (99.8 - 99.9%) | 60% |
| >12 hours | 6,907 | 6 | 98.8%  (98.6 - 99.0%) | 99.9%  (99.8 - 100.0%) | 65% |
| Overall | 23,709 | 59 | 97.8%  (97.6 - 97.9%) | 99.8%  (99.7 - 99.8%) | 60% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 10,046 | 305 | 68.7%  (67.8 - 69.5%) | 97.1%  (96.7 - 97.3%) | 87% |
| 4-12 hours | 14,587 | 176 | 84.6%  (84.0 - 85.1%) | 98.8%  (98.6 - 99.0%) | 88% |
| >12 hours | 9,407 | 44 | 91.3%  (90.8 - 91.9%) | 99.5%  (99.4 - 99.6%) | 88% |
| Overall | 34,040 | 525 | 80.0%  (79.6 - 80.4%) | 98.5%  (98.4 - 98.6%) | 88% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 11,909; 4-12 hours = 16,854;  >12 hours = 10,689; Overall = 39,457  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

#### **Supplementary Table 14:** Rule-out performance by time from symptom onset to troponin sampling restricted to patients with serial cardiac troponin measurements at 6-12 hours from presentation (n=9,362)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 744 | 7 | 99.1%  (98.5-99.5%) | 99.1%  (98.7-99.3%) | 18% |
| 4-12 hours | 163 | 3 | 99.6%  (99.3-99.7%) | 99.6%  (99.3-99.7%) | 17% |
| >12 hours | 163 | 1 | 99.5%  (98.9-99.8%) | 99.4%  (98.7-99.7%) | 16% |
| Overall | 744 | 7 | 99.1%  (98.5-99.5%) | 99.1%  (98.7-99.3%) | 18% |
|  | 5 ng/L | | | | |
| ≤3 hours | 1,934 | 24 | 97.0%  (96.5-97.5%) | 98.8%  (98.4-99.1%) | 46% |
| 4-12 hours | 1,871 | 11 | 98.4%  (97.9-98.7%) | 99.4%  (99.1-99.6%) | 46% |
| >12 hours | 399 | 2 | 99.0%  (98.2-99.5%) | 99.5%  (98.9-99.8%) | 39% |
| Overall | 1,934 | 24 | 97.0%  (96.5-97.5%) | 98.8%  (98.4-99.1%) | 46% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 3,082 | 253 | 68.5%  (67.1-69.9%) | 92.4%  (91.6-93.2%) | 78% |
| 4-12 hours | 2,989 | 122 | 82.1%  (80.8-83.2%) | 96.1%  (95.4-96.6%) | 77% |
| >12 hours | 640 | 30 | 85.2%  (82.9-87.2%) | 95.5%  (94.1-96.6%) | 64% |
| Overall | 3,082 | 253 | 68.5%  (67.1-69.9%) | 92.4%  (91.6-93.2%) | 78% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 12,595; 4-12 hours = 17,468;  >12 hours = 11,040; Overall = 41,103  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 15:** Diagnostic performance of rule out thresholds for a diagnosis of type 1 or 4b myocardial infarction by time from symptom onset excluding patients with a GRACE score >140 (n=39,076)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 3,019 | 7 | 99.4%  (99.2 - 99.5%) | 99.8%  (99.7 - 99.8%) | 26% |
| 4-12 hours | 4,764 | 6 | 99.5%  (99.4 - 99.6%) | 99.9%  (99.8 - 99.9%) | 29% |
| >12 hours | 3,552 | 1 | 99.8%  (99.7 - 99.9%) | 100.0%  (99.9 - 100.0%) | 34% |
| Overall | 11,335 | 14 | 99.5%  (99.5 - 99.6%) | 99.9%  (99.8 - 99.9%) | 29% |
|  | 5 ng/L | | | | |
| ≤3 hours | 6,658 | 41 | 96.3%  (95.9 - 96.6%) | 99.4%  (99.2 - 99.5%) | 57% |
| 4-12 hours | 10,145 | 20 | 98.4%  (98.2 - 98.5%) | 99.8%  (99.7 - 99.9%) | 61% |
| >12 hours | 6,907 | 5 | 99.2%  (99.0 - 99.3%) | 99.9%  (99.9 - 100.0%) | 65% |
| Overall | 23,710 | 66 | 97.8%  (97.6 - 97.9%) | 99.7%  (99.7 - 99.8%) | 88% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 10,016 | 315 | 71.4%  (70.6 - 72.2%) | 97.0%  (96.6 - 97.2%) | 88% |
| 4-12 hours | 14,556 | 190 | 84.5%  (83.9 - 85.0%) | 98.7%  (98.5 - 98.9%) | 89% |
| >12 hours | 9,408 | 44 | 92.8%  (92.3 - 93.3%) | 99.5%  (99.4 - 99.6%) | 89% |
| Overall | 33,980 | 549 | 81.3%  (80.9 - 81.7%) | 98.4%  (98.3 - 98.5%) | 88% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 11,804; 4-12 hours = 16,652;  >12 hours = 10,620; Overall = 39,076  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**Supplementary Table 16:** Diagnostic performance of a 2ng/L threshold for the rule-out of type 1 or 4b myocardial infarction by time from symptom onset and GRACE risk category.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | TN | FN | Sensitivity  (95% CI) | NPV  (95% CI) | Proportion ruled out (%) |
| *GRACE score <104* | | | | | |
| ≤3 hours | 564 | 0 | 100%  (99.8-100%) | 100%  (99.8-100%) | 33% |
| 4-12 hours | 496 | 0 | 100%  (99.8-100%) | 100% (99.8-100%) | 31% |
| >12 hours | 202 | 0 | 100%  (99.3-100%) | 100% (99.3-100%) | 38% |
| Overall | 1,262 | 0 | 100%  (99.9-100%) | 100% (99.9-100%) | 33% |
| *GRACE score 104-140* | | | | | |
| ≤3 hours | 53 | 0 | 100% (99.3-100%) | 100%  (99.3-100%) | 10% |
| 4-12 hours | 47 | 0 | 100% (99.5-100%) | 100%  (99.5-100%) | 6% |
| >12 hours | 19 | 0 | 100% (98.2-100%) | 100%  (98.2-100%) | 9% |
| Overall | 119 | 0 | 100% (99.7-100%) | 100%  (99.7-100%) | 8% |
| *GRACE score >140* | | | | | |
| ≤3 hours | 10 | 0 | 100%  (98.9-100%) | 100%  (98.9-100%) | 3% |
| 4-12 hours | 6 | 0 | 100%  (99.3-100%) | 100%  (99.3-100%) | 1% |
| >12 hours | 2 | 0 | 100%  (97.4-100%) | 100%  (97.4-100%) | 1% |
| Overall | 18 | 0 | 100%  (99.6-100%) | 100%  (99.6-100%) | 2% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  Number of patients in each group: GRACE Score <104 = 3,860, GRACE Score 104-140 = 1,518, GRACE Score >140 = 1,040, Overall = 6,418  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value, GRACE = global registry of acute coronary events | | | | | |

**Supplementary Table 17:** Diagnostic performance of rule out thresholds for a diagnosis of type 1 or 4b myocardial infarction by time from symptom onset stratified by age (n=41,103).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | | | | |
| **Age group** | **TP** | **FP** | **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
| 2 ng/L | | | | | | | | |
| ≤3 hours | <75 years | 913 | 5,332 | 2,953 | 9 | 99.0% (98.8-99.2%) | 99.7% (99.6-99.8%) | 32% |
|  | 75 years | 517 | 2,804 | 67 | 0 | 100% (99.9-100%) | 100% (99.9-100%) | 2% |
| 4-12 hours | <75 years | 921 | 7,340 | 4,675 | 5 | 99.5% (99.3-99.6%) | 99.9% (99.8-99.9%) | 36% |
|  | 75 years | 626 | 3,811 | 89 | 1 | 99.8% (99.7-99.9%) | 98.9% (98.5-99.2) | 2% |
| >12 hours | <75 years | 492 | 4,796 | 3,499 | 1 | 99.8% (99.7-99.9%) | 100% (99.9-99.8%) | 40% |
|  | 75 years | 243 | 1,956 | 53 | 0 | 100% (99.8-100%) | 100% (99.8-100%) | 2% |
| Overall | <75 years | 2,326 | 17,468 | 11,127 | 15 | 99.4% (99.3-99.4%) | 99.9% (99.8-99.9%) | 36% |
|  | 75 years | 1,386 | 8,571 | 209 | 1 | 99.9% (99.9-100%) | 99.5% (99.4-99.6%) | 2% |
| 5 ng/L | | | | | | | | |
| ≤3 hours | <75 years | 876 | 2,336 | 5,949 | 46 | 95.0% (94.5-95.4%) | 99.2% (99.0-99.4%) | 65% |
|  | 75 years | 513 | 2,152 | 719 | 4 | 99.2% (98.9-99.5%) | 99.4% (99.1-99.6%) | 21% |
| 4-12 hours | <75 years | 910 | 2,896 | 9,119 | 16 | 98.3 (98.0-98.5%) | 99.8% (99.7-99.9%) | 71% |
|  | 75 years | 623 | 2,871 | 1,029 | 4 | 99.4% (99.1-99.6%) | 99.6% (99.2-99.8%) | 23% |
| >12 hours | <75 years | 489 | 1,890 | 6,405 | 4 | 99.2% (99.0-99.4%) | 99.9% (99.9-100%) | 73% |
|  | 75 years | 241 | 1,507 | 502 | 2 | 99.2% (98.7-99.5%) | 99.6% (99.4-99.7%) | 22% |
| Overall | <75 years | 2,275 | 7,122 | 21,473 | 66 | 97.2% (97.0-97.4%) | 99.7% (99.6-99.7%) | 70% |
|  | 75 years | 1,377 | 6,530 | 2,250 | 10 | 99.3% (99.1-99.4%) | 99.6% (99.4-99.7%) | 22% |
| Sex-specific 99th centile | | | | | | | | |
| ≤3 hours | <75 years | 793 | 517 | 7,844 | 283 | 69.3% (68.4-70.2%) | 96.5% (96.1-96.9%) | 88% |
|  | 75 years | 389 | 608 | 2,263 | 128 | 75.2% (73.8-76.7%) | 94.6% (96.4-97.4%) | 71% |
| 4-12 hours | <75 years | 793 | 517 | 11,498 | 133 | 85.6% (85.0-86.2%) | 98.9% (98.7-99.0%) | 90% |
|  | 75 years | 528 | 782 | 3,118 | 99 | 84.2% (83.1-85.2%) | 96.9% (96.4-97.4%) | 71% |
| >12 hours | <75 years | 459 | 344 | 7,951 | 34 | 93.1% (92.6-93.6%) | 99.6% (99.4-99.7%) | 91% |
|  | 75 years | 222 | 544 | 1,465 | 21 | 91.4% (90.1-92.4%) | 98.6% (98.0-99.0%) | 66% |
| Overall | <75 years | 1,891 | 1,302 | 27,293 | 450 | 80.8% (80.3-81.2%) | 98.4% (98.2-98.5%) | 90% |
|  | 75 years | 1,139 | 1,934 | 6,846 | 248 | 82.1% (81.4-82.9%) | 96.5% (96.1-96.8%) | 70% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group:   * Age <75 years: ≤3 hours = 9,207; 4-12 hours = 12,941; >12 hours = 8,788; Overall = 30,936 * Age ≥75 years: ≤3 hours = 3,388; 4-12 hours = 4,527; >12 hours = 2,252; Overall = 10,167   Abbreviations: TP = true positive, FP = false positive, TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | | | | |

#### **Supplementary Table 18:** Diagnostic performance of rule out thresholds for a diagnosis of type 1, type 2 or type 4b myocardial infarction by time from symptom onset (n=41,103)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hours from  symptom onset | Presentation high-sensitivity cardiac troponin I | | | | |
| **TN** | **FN** | **Sensitivity**  **(95% CI)** | **NPV**  **(95% CI)** | Proportion ruled out (%) |
|  | 2 ng/L | | | | |
| ≤3 hours | 3,019 | 10 | 99.5%  (99.3 - 99.6%) | 99.6%  (99.4 – 99.7%) | 24% |
| 4-12 hours | 4,764 | 6 | 99.7%  (99.6 - 99.8%) | 99.8%  (99.7 - 99.9%) | 27% |
| >12 hours | 3,552 | 1 | 99.9%  (99.8 - 99.9%) | 99.9%  (99.9 – 100%) | 32% |
| Overall | 11,335 | 17 | 99.6%  (99.6 - 99.7%) | 99.8%  (99.7 – 99.8%) | 28% |
|  | 5 ng/L | | | | |
| ≤3 hours | 6,653 | 65 | 96.5%  (96.1 - 96.8%) | 99.0%  (98.8 - 99.2%) | 53% |
| 4-12 hours | 10,145 | 23 | 98.8%  (98.7 - 99.0%) | 99.8%  (99.7 - 99.8%) | 58% |
| >12 hours | 6,907 | 6 | 99.4%  (99.2 - 99.5%) | 99.9%  (99.8 - 100.0%) | 63% |
| Overall | 23,705 | 94 | 98.0%  (97.9 - 98.2%) | 99.6%  (99.5 - 99.7%) | 58% |
|  | Sex-specific 99th centile | | | | |
| ≤3 hours | 9,979 | 539 | 70.8%  (70.0 - 71.6%) | 99.7%  (99.6 - 99.8%) | 84% |
| 4-12 hours | 14,538 | 310 | 84.3%  (83.7 - 84.8%) | 99.9%  (99.8 - 99.9%) | 85% |
| >12 hours | 9,404 | 67 | 92.8%  (92.4 - 93.3%) | 100.0% (99.9 - 100.0%) | 86% |
| Overall | 33,921 | 916 | 80.7%  (80.3 - 81.1%) | 99.9%  (99.8 - 99.9%) | 85% |
| Presented as number or % (95% confidence intervals) as appropriate.  High-sensitivity cardiac troponin I assay = ARCHITECTSTAT  \*Sex-specific 99th centile = 34 ng/L men, 16 ng/L women.  Number of patients in each time group: ≤3 hours = 12,595; 4-12 hours = 17,468;  >12 hours = 11,040; Overall = 41,103  Abbreviations: TN = true negatives, FN = false negatives, NPV = negative predictive value | | | | | |

**FIGURE LEGENDS**

**Supplementary Figure 1:** Distribution of time from symptom onset to troponin sampling across cohorts and definitions of symptom onset.

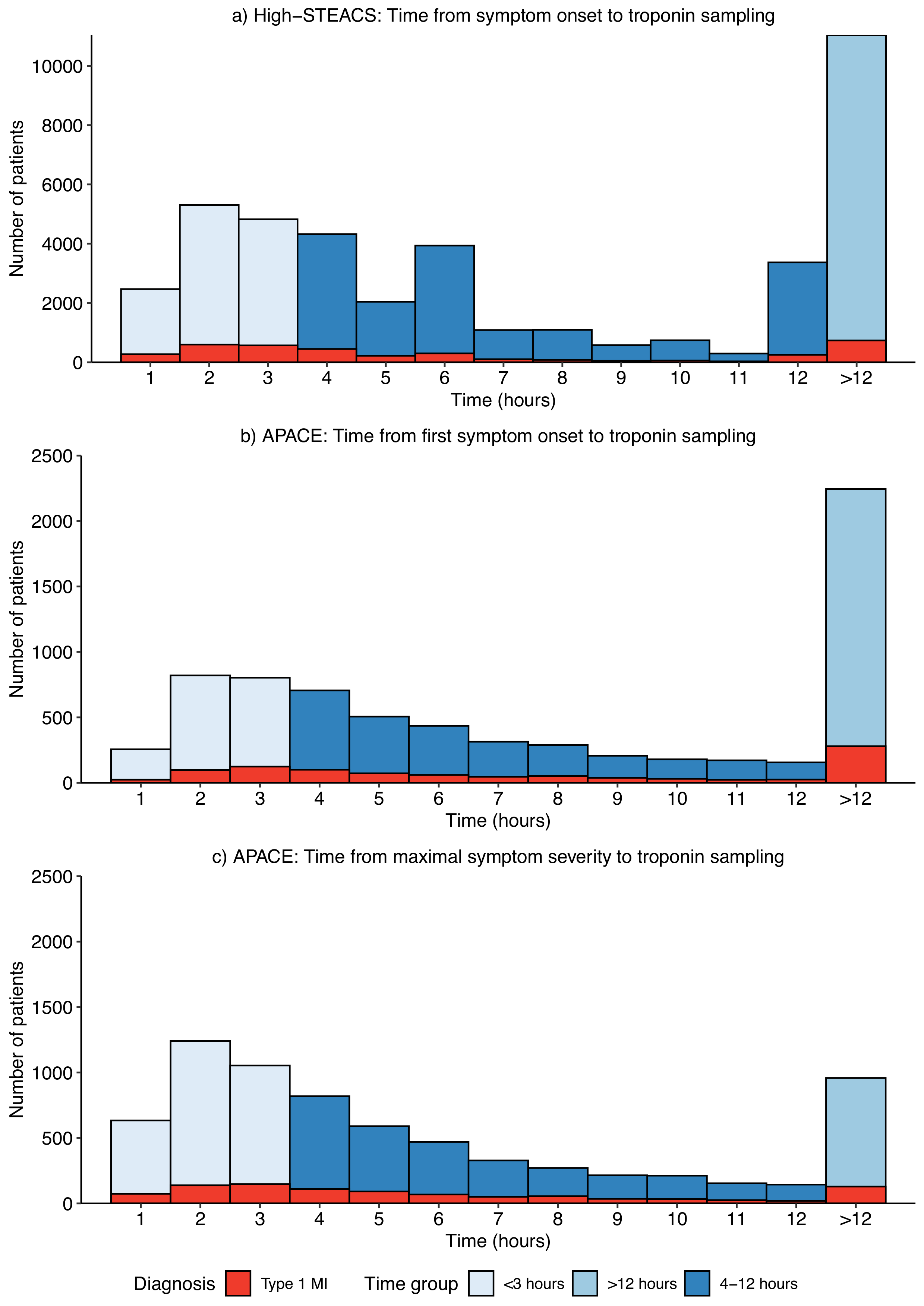
**Supplementary Figure 2:** Fourth Universal Definition of Myocardial Infarction by time of symptom onset. Abbreviations: *MI = myocardial infarction.*

**Supplementary Figure 3.** Density estimtate of troponin concentrations by time from symptom onset

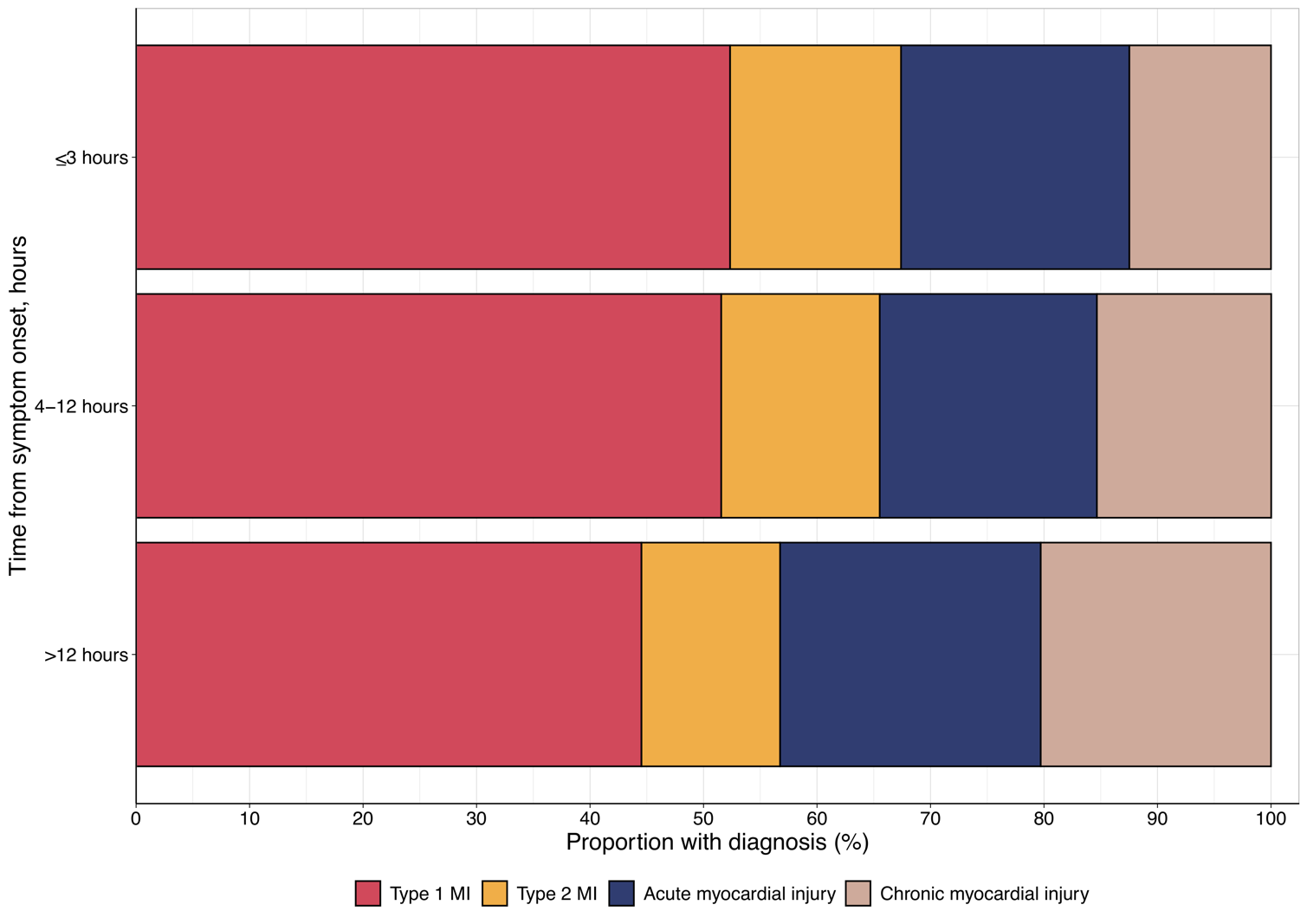
**Supplementary Figure 4.** Scatter plot with 95% confidence intervals (CI) showing the sensitivity and negative predictive value (A) and sensitivity (B) for patients with cardiac troponin concentrations below 2 ng/L (red), 5 ng/L (blue) and the sex-specific 99th centile (grey) at presentation by time from symptom onset for an outcome of type 1 or 4b myocardial infarction or cardiovascular death within 30 days from the index presentation.

**Supplemental Figure 5:** Combined scatter and bar plot showing the sensitivity, negative predictive value and proportion of patients with cardiac troponin concentrations below 2 ng/L (red), 5 ng/L (blue) and the sex-specific 99th centile (grey) at presentation in the external validation cohort stratified by time from maximal symptom severity to troponin sampling.

**Supplementary Figure 1:** Distribution of time from symptom onset the to troponin sampling and the diagnosis of myocardial infarction across cohorts and definitions of symptom onset.

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**Supplementary Figure 2:** Fourth Universal Definition of Myocardial infarction by time of symptom onset.

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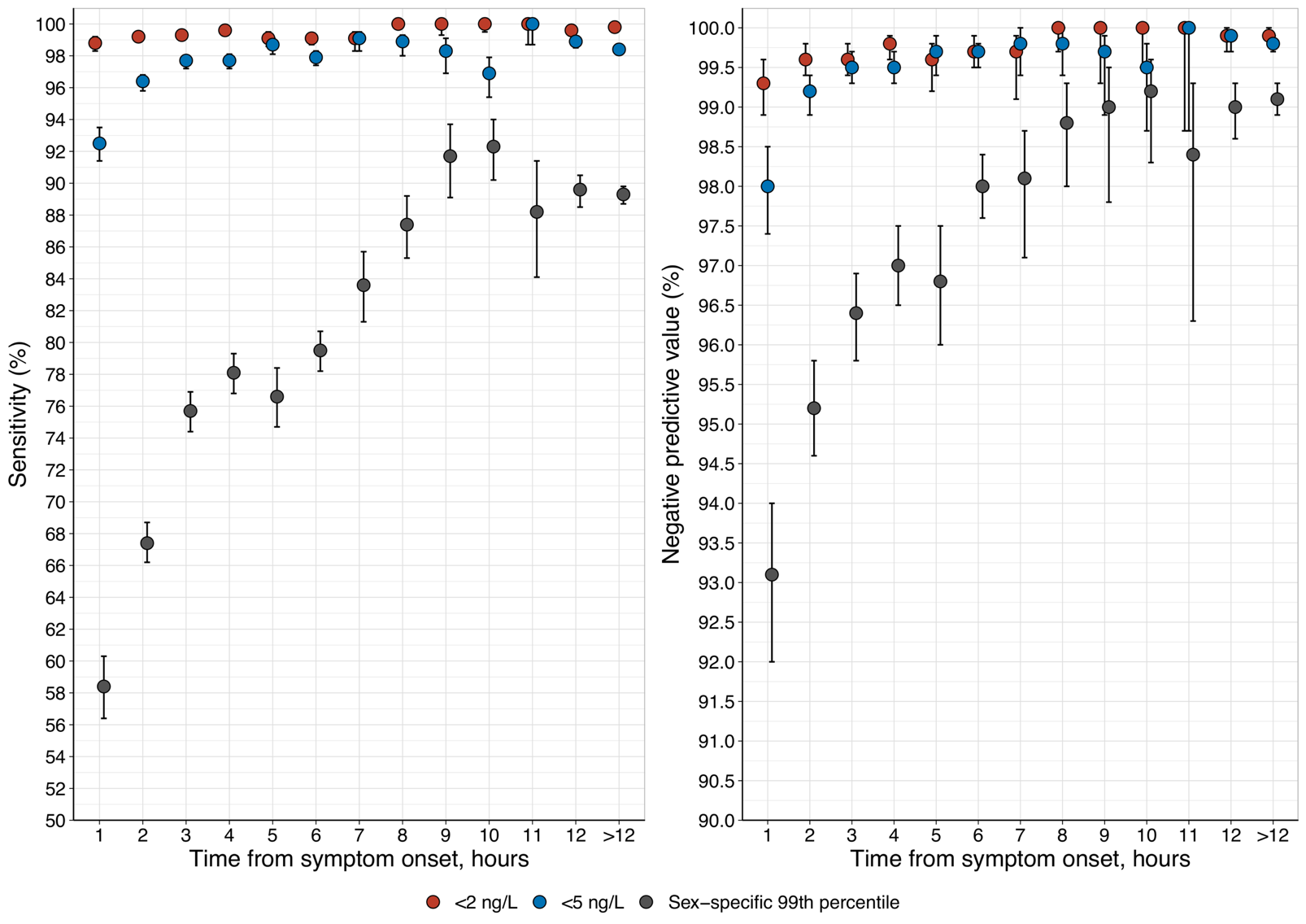
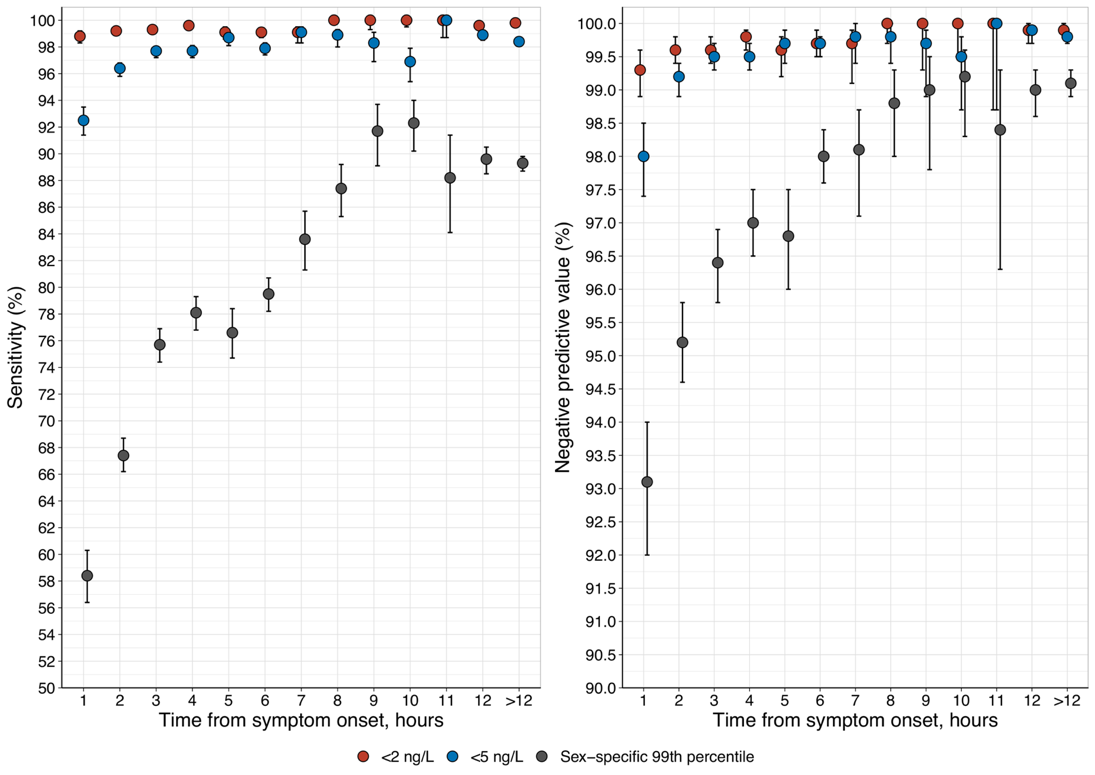
**Supplementary Figure 3. Density estimtate of troponin concentrations by time from symptom onset**

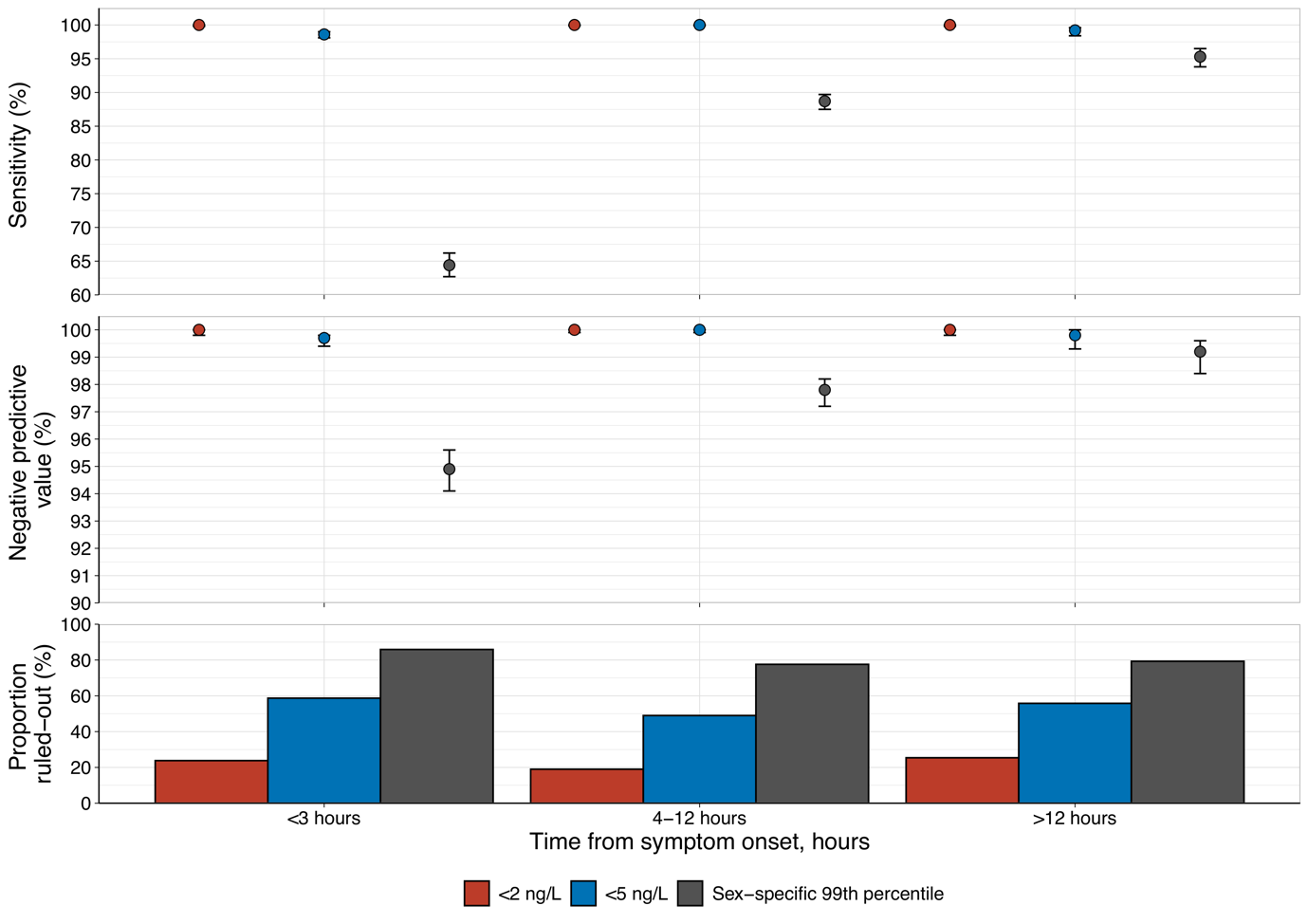
Density plot of the maximal cardiac troponin I concentration at presentation and following serial testing in patients with type 1 or type 4b myocardial infarction stratified by the time from symptom onset to the first troponin measurement.

**Chart, histogram

Description automatically generated**

**Supplementary Figure 4.** Scatter plot with 95% confidence intervals showing the sensitivity (A) and negative predictive value (B) for patients with cardiac troponin concentrations below 2 ng/L (red), 5 ng/L (blue) and the sex-specific 99th centile (grey) at presentation by time from symptom onset for an outcome of type 1 or 4b myocardial infarction or cardiovascular death within 30 days of the index presentation (n=41,103).



**Supplemental Figure 5:** Combined scatter and bar plot showing the sensitivity, negative predictive value and proportion of patients with cardiac troponin concentrations below 2 ng/L (red), 5 ng/L (blue) and the sex-specific 99th centile (grey) at presentation in the external validation cohort stratified by time from maximal symptom onset (n=7,088).