

Diagnostic Accuracy of a High-sensitivity Cardiac Troponin Assay with a Single Serum Test in the Emergency Department

SHORT TITLE:

Accuracy of a novel high-sensitivity troponin assay in the Emergency Department

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Abstract

Objectives

To evaluate diagnostic accuracy of a high-sensitivity cardiac troponin I (hs-cTnI) assay for acute coronary syndromes (ACS) in the Emergency Department (ED). The assay has high precision at low concentrations and can detect cTnI in 96.8% of healthy individuals.

Methods

In successive prospective multi-center studies ('testing' and 'validation') we included ED patients with suspected ACS. We drew blood for hs-cTnI (Singulex Clarity® cTnI, 99th percentile 8.67ng/L, limit of detection [LoD] 0.08ng/L) on arrival. Patients also underwent hs-cTnT (Roche Elecsys) testing over ≥ 3 h. The primary outcome was an adjudicated diagnosis of ACS, defined as acute myocardial infarction (AMI; prevalent or incident), death, or revascularization within 30 days.

Results

The testing and validation studies included 665 and 2,470 patients respectively, of which 94 (14.1%) and 565 (22.9%) had ACS. At a 1.5ng/L cut-off, hs-cTnI had good sensitivity for AMI in both studies (98.7% and 98.1% respectively) and would have 'ruled out' 40.1% and 48.9% patients. However, sensitivity was lower for ACS (95.7% and 90.6% respectively). At a 0.8ng/L cut-off sensitivity for ACS was higher (97.5% and 97.9%, 'ruling out' 28.6% patients in each cohort). The hs-cTnT assay had very similar performance at the LoD (24.6% 'ruled out', 97.2% sensitivity for ACS).

Conclusion

The hs-cTnI assay could immediately 'rule out' AMI in 40% patients and ACS in over 25%, with similar accuracy to hs-cTnT at the LoD. Because of its high precision at low concentrations, this hs-cTnI assay has favourable characteristics for this clinical application.

Background

Chest pain accounts for approximately 6% of all ED attendances and for over one quarter of acute medical hospital admissions (1). Acute coronary syndrome (ACS) is the most common diagnosis suspected, which usually requires patients to undergo serial cardiac troponin (cTn) testing over several hours. As the majority of patients have non-cardiac diagnoses, there is great potential to reduce unnecessary resource utilization (2).

With high-sensitivity cTn assays (hs-cTn), the diagnosis of acute myocardial infarction (AMI) can be excluded in some patients using a single blood test at the time patients arrive in the Emergency Department (ED). Existing evidence suggests that AMI can be 'ruled out' in patients with hs-cTn concentrations below the limit of detection (LoD) of the assay, who have no evidence of ECG ischaemia, especially if time from symptom onset is >3 hours (3–8). This strategy relies on the use of hs-cTn concentrations below the functional sensitivity of the assay, meaning that the precision is suboptimal. This is a concern for laboratories, which face a substantial challenge to ensure appropriate quality control. To overcome this challenge, we will require hs-cTn assays with improved precision at low concentrations.

In this work, we aimed to evaluate the diagnostic accuracy of a one-test 'rule out' strategy using the Singulex hs-cTnI assay (Singulex Clarity, Alameda, United States), which has excellent precision at very low cardiac troponin concentrations, using thresholds below the 99th percentile in the ED.

Methods

Design and setting

Testing study

We conducted a prospective diagnostic test accuracy study at two centres in the United Kingdom. The current analysis is a pre-planned sub-study within a wider programme of research, called the Bedside Evaluation of Sensitive Troponin (BEST) study. The National Research Ethics Service granted ethical approval (reference 14/NW/1344) and all participants provided written informed consent. The study was prospectively registered on the UK National Institute for Health Research Portfolio (reference UKCRN 18000).

Validation study

We validated our findings using data from the Advantageous Predictors of Acute Coronary Syndromes (APACE) study, which is also a prospective diagnostic test accuracy study at 12 centers in 5 European countries (trial registration NCT00470587).

Study participants

In the testing (BEST) study, we included adults (aged >18 years) who presented to the ED with pain, discomfort or pressure in the chest, epigastrium, neck, jaw or upper limb without an apparent non-cardiac source, which warranted investigation for possible ACS in the opinion of the treating physician. Patients with peak symptoms occurring >12h before enrolment, those with unequivocal ST elevation myocardial infarction (STEMI), those with another medical condition requiring hospital admission and patients lacking the mental capacity to provide written informed consent were excluded.

The validation (APACE) study included patients aged >18 years presenting to the ED with chest pain at rest occurring within the previous 12 hours, who had a suspected diagnosis of ACS. Patients were excluded if they had cardiogenic shock or end-stage kidney disease requiring dialysis. All patients provided written informed consent.

Data collection and laboratory analysis

Testing study

We recorded comprehensive clinical data using a bespoke case report form, which the treating physician was asked to complete at the time of initial assessment. These data included details of patients' symptoms, previous history, vital signs, physical examination findings and ECG interpretation. Forms were scanned and data were automatically extracted using Teleform (OpenText, London). We then undertook manual source data verification for 100% of the data, followed by a further process of data validation and cleaning.

Blood was drawn at the time of arrival in the ED and at least 3 hours later. Routine clinical samples were analysed using the hs-cTnT assay (Roche Diagnostics Elecsys using the Cobas e602 or Cobas 801 instruments, 99th percentile 14ng/L overall, 16ng/L in males, 9ng/L in females (9); limit of detection 5ng/L; limit of blank 3ng/L; co-efficient of variation <10% at 5ng/L. Results were reported down to 3ng/L. During the study period, at Manchester Royal Infirmary a CV of 8.2% was achieved with Randox hs-cTnT control at a concentration of 11.5ng/L; a CV of 2.3% at a concentration of 29.2ng/L using Roche Precicontrol Troponin; and a CV of 1.9% at a concentration of 2230ng/L. At St George's NHS Foundation Trust, a CV of 8.3% was achieved at a mean hs-cTnT concentration of 6.1ng/L using the Randox hs-cTnT control).

For research purposes, additional blood samples were also drawn into serum blood collection tubes at the time of arrival in the ED and 3 hours (+/- 30 minutes) later. Within 30 minutes of

collection, the samples were centrifuged at 2,500xg for 10 minutes. Serum was aliquoted and stored at -70°C or below within 4 hours of blood collection. These previously unfrozen serum samples were then tested in batches for hs-cTnI using the Singulex Clarity® assay. This assay uses an innovative single molecule counting technology to achieve excellent analytical sensitivity and precision (99th percentile 8.67ng/L overall, 9.23ng/L in men and 8.76ng/L in women; LoD 0.08ng/L; co-efficient of variation <10% at 0.53ng/L (10)). The assay can detect cTnI concentrations in 96.8% of apparently healthy individuals (11). Manufacturer's instructions state that the assay is stable with up to three freeze-thaw cycles (12). Current practice recommendations state that hs-cTn concentrations should be reported in ng/L, in whole numbers (13). Because of the nature of the Singulex Clarity assay (which has an LoD of 0.08ng/L and previously validated cut-offs of 0.8ng/L and 1.5ng/L), we have reported results to two decimal places in this analysis.

Validation study

Data collection was similar in the validation study. Details of the patients' medical history, physical examination and ECG interpretation were prospectively recorded at the time of arrival. All patients underwent blood sampling at the time of ED arrival. Serum was extracted and frozen at -80°C pending subsequent analysis for Singulex Clarity hs-cTnI.

Outcomes

The primary outcome was a diagnosis of ACS. ACS was defined as either type 1 AMI occurring during the initial hospital admission (prevalent AMI) or incident major adverse cardiac events (MACE) occurring within 30 days. MACE included death (all cause), incident type 1 AMI and coronary revascularization (including either percutaneous coronary intervention or coronary artery bypass grafting). Outcomes were adjudicated by two independent investigators based on all available

clinical data up to 30 days after presentation but blinded to hs-cTnI concentrations. The diagnosis of type 1 AMI was assigned in accordance with the third universal definition (14), using hs-cTnT concentrations as the reference standard. The diagnosis of type 1 AMI alone was considered a secondary outcome.

Follow up

We followed up all patients after 30 days, by: (a) verifying mortality status based on electronic records and establishing the registered cause of death for patients who had died; (b) checking all available electronic patient records; and (c) personal contact by telephone, email or in person. If patients remained persistently uncontactable we contacted their general practitioner (GP). Follow up was considered appropriate if the patients GP had been in contact with the patient during the follow up period and was able to provide sufficient information regarding ED attendances, hospital admissions, investigations and episodes of chest pain.

Statistical analysis

We analysed the diagnostic accuracy of the hs-cTnI assay using the blood sample drawn at the time of arrival (T0). We evaluated the following cut-offs: the limit of detection of the assay (0.08ng/L); the optimal cut-off to 'rule out' stable coronary artery disease in two recent studies: 0.8ng/L (15) and 1.5ng/L (16); and the 99th percentile (8.67ng/L). For reference, we compared the diagnostic accuracy of the hs-cTnI assay to the hs-cTnT assay that was used in practice during the study (Roche Diagnostics Elecsys), using the T0 samples. For the hs-cTnT assay, we used the limit of detection (5ng/L) as the 'rule-out' cut-off, as has previously been extensively validated (5,17).

Test characteristics including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) together with respective 95% confidence intervals (95% CI) were

calculated to assess the diagnostic accuracy. Paired comparison of diagnostic accuracy measures was performed with McNemar's test. Additionally, we calculated areas under the receiver operating characteristic (ROC) curves according to the method described by De Long (18). Statistical analyses were undertaken using SPSS version 23.0 (SPSS Inc, Chicago, Illinois) and MedCalc version 13.1.2.0 (Mariakerke, Belgium).

Sample size

Assuming that the prevalence of the primary outcome is approximately 10%, that the specificity of a troponin-based algorithm is approximately 90% and that we would identify an algorithm with 100% sensitivity, a sample of 605 patients would ensure that the lower bounds of the 95% confidence intervals were >90% for sensitivity and >99% for negative predictive value. Estimating that 5% may be lost to follow up or have missing data, we therefore set out to include a minimum of 650 participants in the testing study. As we are presenting a secondary analysis from the validation study, no *a priori* sample calculation was undertaken for this analysis in the validation study.

Results

Testing study

We included a total of 722 patients, of which 665 had sufficient data for inclusion in this analysis. Of the eligible participants, 77 (11.5%) had an adjudicated diagnosis of AMI on the initial admission and a further 17 (2.6%) developed a major adverse cardiac event (death, AMI or coronary revascularization) within 30 days. Thus, a total of 94 (14.1%) patients were considered to have ACS (Figure 1). The baseline characteristics of participants are shown in Table 1.

All 665 patients had cTnI concentrations above the limit of detection of the hs-cTnI assay at the time of presentation to the ED. The test characteristics of the assay at the selected cut-offs are shown in Table 2. Using hs-cTnI alone (without accounting for ECG ischemia), a threshold of 1.5ng/L produced a sensitivity of 98.7% for AMI with 99.6% NPV. This strategy would have allowed 267 (40.2%) patients to have AMI 'ruled out' with a single blood test at time of presentation. If AMI was only ruled out in patients without ECG ischemia, sensitivity remained 98.7% but the proportion of patients 'ruled out' dropped marginally to 38.2%.

In comparison, an hs-cTnT concentration below 5ng/L at the time of arrival in the ED had a sensitivity of 98.6% (95% CI 92.6 – 100.0%) for AMI, with an NPV of 99.6% (95% CI 97.5 – 100.0%) and specificity 46.7% (95% CI 42.6 – 50.8%). This strategy would have allowed AMI to be immediately 'ruled out' in 40.9% patients. Considering the diagnosis of ACS, this strategy had a sensitivity of 97.8% (95% CI 92.2 – 99.7%) and NPV 99.3% (97.2 – 99.8%).

If only patients with no ECG ischemia were 'ruled out', this hs-cTnT cut-off gave a sensitivity of 98.6% (95% CI 92.6 – 100.0%), NPV 99.6% (95% CI 97.4 – 99.9%) and specificity 44.3% (95% CI 40.2 – 48.4%) for AMI. For ACS, the sensitivity was identical (97.8%, 95% CI 92.2 – 99.7%) and NPV 99.2% (95% CI 97.0 – 99.8%). This strategy would have allowed 38.9% patients to have AMI immediately 'ruled out'.

Stratifying the analysis by time from symptom onset, we did not identify any trend towards lower sensitivity and NPV when these rule-out strategies were employed in patients who presented within 3 hours of symptom onset (Supplementary Table 1). Similarly, there was no suggestion that restricting the use of these rule out strategies to those who presented >3 hours after symptom onset would increase sensitivity and NPV (Supplementary Table 2). There was also no suggestion that patient sex affected diagnostic accuracy, although a smaller proportion of men would have been 'ruled out' at each cut-off evaluated (Supplementary Table 3).

Validation study

A total of 2,470 patients were included in the validation study, of which 565 (22.9%) met criteria for ACS. Baseline characteristics are shown in Table 1 and were notably similar to those in the testing study, albeit with a higher prevalence of hypertension and more late presenters (>6h from symptom onset).

Diagnostic accuracy was also broadly similar to the testing study. At a 0.8ng/L cut-off, 28.6% patients would have been 'ruled out', achieving a sensitivity of 97.9% for ACS and 100.0% for AMI (Table 2). Using the 1.5ng/L cut-off, 48.9% patients would have been immediately 'ruled out'. Sensitivity remained high for AMI at 98.1% but was lower for ACS (90.6%). If only patients with no ECG ischemia were considered 'ruled out', sensitivity for ACS increased to 92.2% (Table 3). There was no suggestion that time from symptom onset affected diagnostic accuracy at these cut-offs (Supplementary Tables 1 and 2).

For comparison, using the LoD (5ng/L) of the Roche hs-cTnT assay would have 'ruled out' 24.6% patients, achieving a sensitivity of 97.2% (95% CI 95.4 – 98.4%) for ACS with an NPV of 97.4% (95% CI 95.8 – 98.4%). For AMI, this strategy had 99.7% sensitivity (95% CI 98.5 – 100.0%) with 99.8% NPV (95% CI 98.8 – 100.0%).

Discussion

Our findings demonstrate that the Singulex Clarity hs-cTnI assay could be used to rule out ACS in the ED following a single blood test at the time of arrival. In our initial testing study, a threshold of 1.5ng/L gave very similar sensitivity and NPV to the limit of detection (5ng/L) of the Roche hs-cTnT assay and would have 'ruled out' a very similar proportion of patients. In the validation study, the hs-cTnI assay had high sensitivity for AMI at 1.5ng/L but lower sensitivity for ACS (which, in this study, was defined as AMI or MACE within 30 days). However, the diagnostic accuracy of the hs-cTnI assay

at a 0.8ng/L cut-off was very similar to the Roche hs-cTnT assay at the LoD (5ng/L). With the Roche hs-cTnT assay, the European Society of Cardiology has recommended use of the 5ng/L cut-off in practice to immediately 'rule out' AMI (6), although this recommendation is restricted to patients who present >3 hours after symptom onset. Our analysis did not detect any signal to suggest that this diagnostic strategy had a lower sensitivity among patients who present within 3 hours of symptom onset, but that analysis did have limited statistical power. Therefore, it would still seem prudent to exercise caution in early presenters.

These findings demonstrate that the Singulex Clarity cTnI assay (hs-cTnI) can achieve similar diagnostic performance to the Roche hs-cTnT assay for single test 'rule out'. However, while both assays have similar diagnostic accuracy, the Singulex assay has the advantage of offering superior precision at low troponin concentrations. This is likely to help with the challenge of ensuring adequate quality control for high-sensitivity troponin assays at low concentrations, below the 99th percentile. Furthermore, this validation of the diagnostic performance of the hs-cTnI assay in the acute environment will facilitate its future use in routine clinical practice.

As well as the potential value to rule out ACS in the ED, the favourable analytical characteristics of the hs-cTnI assay open other exciting possibilities for future patient care. For example, the ability to detect extremely low concentrations of cardiac troponin may allow clinicians to 'rule out' stable coronary artery disease with a single blood test in some patients, obviating the need for imaging (16). The assay may have value for the monitoring of apparently healthy individuals and predicting future cardiovascular risk (19,20). It may also help to identify patients most likely to respond to statin therapy (21). However, to maximize the potential for this assay to be used in acute settings, it is important to recognise that the Singulex Clarity® System requires additional development, including STAT capability and/or a tracking system, to achieve the required turnaround time for ED. Nevertheless, we have shown in this study that despite these additional future features it is possible to utilise the Singulex Clarity cTnI assay (hs-cTnI) for use in ED.

We note the following limitations. First, we used hs-cTnT as the reference standard troponin assay to adjudicate AMI. It is possible that the diagnostic performance of the Singulex Clarity hs-cTnI assay may have appeared better if the same hs-cTnI assay (or even another hs-cTnI assay) had been used for adjudication. Similarly, it is possible that the diagnostic performance of the hs-cTnT assay reported here may have been lower if a different assay had been used for adjudication. This will, at least, tend to provide a conservative estimate of the diagnostic accuracy of the hs-cTnI assay. However, as most missed events were MACE occurring within 30 days (and thus unrelated to hs-cTnT concentrations at the initial attendance), the impact on our findings is unlikely to be clinically important.

Second, our study is also limited by the short duration of follow-up (30 days). This short follow-up duration was used because the study primarily aimed to evaluate diagnostic accuracy, and 30-day MACE could be taken as a reasonable surrogate for unstable angina, in the absence of an accepted reference standard for that diagnosis. However, one key advantage of the Singulex hs-cTnI assay may be that detecting smaller cTn concentrations can enhance long-term risk stratification. This should be an important focus for future work.

In conclusion, With the use of a single blood test at the time of arrival in the ED, it is possible to 'rule out' the diagnosis of ACS in approximately on quarter of patients who have an hs-cTnI concentration $<0.8\text{ng/L}$ using the Singulex Clarity cTnI assay (hs-cTnI). At a cut-off of 1.5ng/L , the assay would 'rule out' over 40% patients and retained high sensitivity for AMI, although the assay had lower accuracy for MACE at 30 days. Given its high precision at low troponin concentrations, the assay has excellent potential for future clinical use in this context.

References

1. Goodacre S, Cross E, Arnold J, Angelini K, Capewell S, Nicholl J. The health care burden of acute chest pain. *Heart*. 2005;91:229–30.
2. McDevitt-Petrovic O, Kirby K, Shevlin M. The prevalence of non-cardiac chest pain (NCCP) using emergency department (ED) data: a Northern Ireland based study. *BMC Health Services Research* [Internet]. 2017 [cited 2018 Jan 22];17. Available from: <http://bmchealthservres.biomedcentral.com/articles/10.1186/s12913-017-2493-8>
3. Carlton E, Greenslade J, Cullen L, Body R, Than M, Pickering JW, et al. Evaluation of High-Sensitivity Cardiac Troponin I Levels in Patients With Suspected Acute Coronary Syndrome. *JAMA Cardiology*. 2016;1:405.
4. Chapman AR, Lee KK, McAllister DA, Cullen L, Greenslade JH, Parsonage W, et al. Association of High-Sensitivity Cardiac Troponin I Concentration With Cardiac Outcomes in Patients With Suspected Acute Coronary Syndrome. *JAMA*. 2017;
5. Pickering JW, Than MP, Cullen L, Aldous S, Ter Avest E, Body R, et al. Rapid Rule-out of Acute Myocardial Infarction With a Single High-Sensitivity Cardiac Troponin T Measurement Below the Limit of Detection: A Collaborative Meta-analysis. *Ann Intern Med*. 2017;166:715–24.
6. Roffi M, Patrono C, Collet J-P, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *European Heart Journal*. 2016;37:267–315.
7. Body R, Burrows G, Carley S, Cullen L, Than M, Jaffe AS, et al. High-Sensitivity Cardiac Troponin T Concentrations below the Limit of Detection to Exclude Acute Myocardial Infarction: A Prospective Evaluation. *Clinical Chemistry*. 2015;61:983–9.
8. Sandoval Y, Smith S, Shah A, Anand A, Chapman A, Love S, et al. Rapid rule out of acute myocardial injury using a single high-sensitivity cardiac troponin I measurement. *Clinical Chemistry*. 2017;63:369–76.
9. Saenger AK, Beyrau R, Braun S, Cooray R, Dolci A, Freidank H, et al. Multicenter analytical evaluation of a high-sensitivity troponin T assay. *Clinica Chimica Acta*. 2011;412:748–54.
10. IFCC Task Force on Clinical Applications of Cardiac Bio-markers (TF-CB). High sensitivity cardiac troponin I and T assay analytical characteristics designated by manufacturer [Internet]. International Federation of Clinical Chemistry; [cited 2018 Jul 10]. Available from: www.ifcc.org/media/463453/HighSensitivityCardiacTroponinI_T_AssayAnalyticalCharacteristics_v060617.pdf
11. Garcia-Osuna A, Gaze D, Grau-Agramunt M, Morris T, Telha C, Bartolome A, et al. Ultrasensitive quantification of cardiac troponin I by a Single Molecule Counting method: analytical validation and biological features. *Clinica Chimica Acta*. 2018;486:224–31.
12. Singulex, Inc. Sgx Clarity cTnI assay: instructions for use, 06-0019-01 [Internet]. 2017 [cited 2018 Nov 11]. Available from: <https://www.online->

ifu.com/downloadPDF.aspx?GUID=83a15d00-fd3e-4481-85d3-64b8e8155925&lang=en-GB

13. Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, et al. Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. *Clinical Chemistry*. 2018;64:645–55.
14. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. *Eur Heart J*. 2012;33:2551–67.
15. Adamson P, Hunter A, Shah A, McAllister D, Pawade T, Williams M, et al. High-Sensitivity Cardiac Troponin I and the Diagnosis of Coronary Artery Disease in Patients with Suspected Angina Pectoris. *Heart, Lung and Circulation*. 2017;26:S79.
16. Lee G, Twerenbold R, Tanglay Y, Reichlin T, Honegger U, Wagener M, et al. Clinical benefit of high-sensitivity cardiac troponin I in the detection of exercise-induced myocardial ischemia. *American Heart Journal*. 2016;173:8–17.
17. Body R, Mueller C, Giannitsis E, Christ M, Ordonez-Llanos J, de Filippi CR, et al. The Use of Very Low Concentrations of High-sensitivity Troponin T to Rule Out Acute Myocardial Infarction Using a Single Blood Test. *Acad Emerg Med*. 2016;23:1004–13.
18. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44:837–45.
19. Neumann JT, Havulinna AS, Zeller T, Appelbaum S, Kunnas T, Nikkari S, et al. Comparison of Three Troponins as Predictors of Future Cardiovascular Events – Prospective Results from the FINRISK and BiomaCaRE Studies. Obukhov AG, editor. *PLoS ONE*. 2014;9:e90063.
20. Kaess BM, de Las Heras Gala T, Zierer A, Meisinger C, Wahl S, Peters A, et al. Ultra-sensitive troponin I is an independent predictor of incident coronary heart disease in the general population. *Eur J Epidemiol*. 2017;32:583–91.
21. Ford I, Shah ASV, Zhang R, McAllister DA, Strachan FE, Caslake M, et al. High-Sensitivity Cardiac Troponin, Statin Therapy, and Risk of Coronary Heart Disease. *Journal of the American College of Cardiology*. 2016;68:2719–28.

Legends to figures

Figure 1: Participant flow diagram (derivation study)

Figure 2: Participant flow diagram (validation study)

Tables

Table 1: Baseline characteristics of included patients

	Testing study			Validation study		
	Total (n=665)	ACS (n=94)	No ACS (n=571)	Total (n=2,470)	ACS (n=565)	No ACS (n=1,905)
Age in years, mean (SD)	56 (15)	63 (14)	55 (15)	61 (16)	68 (13)	59 (16)
Men (%)	404 (60.8)	67 (71.3)	337 (59.0)	1,683 (68.1)	425 (75.2)	1,258 (66.0)
Previous angina (%)	181 (27.2)	33 (35.1)	148 (25.9)	NA	NA	NA
Previous myocardial infarction (%)	169 (25.4)	31 (33.0)	138 (24.2)	585 (23.7)	202 (35.8)	383 (20.1)
Previous coronary intervention (%)	161 (24.2)	30 (31.9)	131 (22.9)	604 (24.5)	200 (35.4)	404 (21.2)
Hypertension (%)	309 (46.5)	55 (58.5)	254 (44.5)	1,524 (61.7)	447 (79.1)	1,077 (56.5)
Hyperlipidaemia (%)	252 (37.9)	50 (53.2)	202 (35.4)	1,225 (49.6)	400 (70.8)	825 (43.3)
Type 1 diabetes mellitus (%)	8 (1.2)	2 (2.1)	6 (1.1)	415 (16.8)	157 (27.8)	258 (13.5)
Type 2 diabetes mellitus (%)	128 (19.2)	25 (26.6)	103 (18.0)			
Current smoking (%)	144 (21.7)	30 (31.9)	114 (20.0)	622 (25.2)	138 (24.4)	474 (25.4)

Time from symptom onset to arrival in the ED, n (%):*						
< 3h	379 (57.0)	47 (50.0)	328 (58.1)	584 (23.7)	121 (21.4)	463 (24.4)
3 – 6h	153 (23.0)	27 (28.7)	126 (22.1)	804 (32.7)	181 (32.0)	623 (32.9)
> 6h	131 (19.7)	19 (20.2)	112 (19.6)	1,071 (43.6)	263 (46.5)	810 (42.7)

* Time from symptom onset missing in 2 cases (testing study)

Table 2: Test characteristics of the Singulex Clarity hs-cTnI assay, used alone at the time of arrival in the ED in the testing and validation studies

	Cut-off	Study	Patients 'ruled out', n (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
For ACS	LoD (0.08ng/L)	Testing	0 (0.0)	100.0 (96.2 – 100.0)	0.0 (0.0 – 0.6)	14.1 (14.1 – 14.1)	100.0 (N/A)
		Validation	6 (0.2)	100.0 (99.4 – 100.0)	0.3 (0.1 – 0.7)	22.9 (21.2 – 24.6)	100.0 (N/A)
	0.8ng/L	Testing	114 (17.1)	97.9 (92.5 – 99.7)	19.6 (16.4 – 23.1)	16.7 (16.0 – 17.4)	98.3 (93.4 – 99.6)
		Validation	706 (28.6)	97.7 (96.1 – 98.8)	36.4 (34.2 – 38.6)	31.3 (30.5 – 32.1)	98.2 (96.9 – 98.9)
	1.5ng/L	Testing	267 (40.2)	95.7 (89.5 – 98.8)	46.1 (41.9 – 50.3)	22.6 (21.1 – 24.2)	98.5 (96.2 – 99.4)
		Validation	1,207 (48.9)	90.6 (87.9 – 92.9)	60.6 (58.3 – 62.8)	40.5 (39.1 – 42.0)	95.6 (94.4 – 96.6)
	99th percentile (8.67ng/L)	Testing	548 (82.4)	77.3 (67.7 – 85.2)	92.6 (90.1 – 94.6)	64.1 (56.7 – 70.9)	96.0 (94.3 – 97.2)
		Validation	1,963 (79.5)	63.5 (59.4 – 67.5)	92.2 (90.9 – 93.4)	70.8 (67.2 – 74.1)	89.5 (88.4 – 90.5)
For AMI	LoD (0.08ng/L)	Testing	0 (0.0)	100.0 (95.3 – 100.0)	0.0 (0.0 – 0.6)	11.6 (11.6 – 11.6)	N/A
		Validation	6 (0.2)	100.0 (99.0 – 100.0)	0.3 (0.1 – 0.6)	15.1 (15.1 – 15.2)	100.0 (N/A)
	0.8ng/L	Testing	114 (17.1)	100.0 (95.3 – 100.0)	19.4 (16.3 – 22.8)	14.0 (13.5 – 14.5)	100.0 (N/A)
		Validation	706 (28.6)	99.7 (98.5 – 100.0)	33.6 (31.6 – 35.7)	21.1 (20.6 – 21.6)	99.9 (99.0 – 100.0)
	1.5ng/L	Testing	267 (40.2)	98.7 (93.0 – 100.0)	45.2 (41.2 – 49.4)	19.1 (17.9 – 20.3)	99.6 (97.4 – 100.0)
		Validation	1,207 (38.9)	98.1 (96.2 – 99.2)	57.2 (55.1 – 59.4)	29.0 (27.9 – 30.1)	99.4 (98.8 – 99.7)
	99th percentile (8.67ng/L)	Testing	548 (82.4)	87.0 (77.4 – 94.0)	91.5 (88.9 – 93.6)	57.3 (50.4 – 63.9)	98.2 (96.8 – 99.0)
		Validation	1,963 (79.5)	82.8 (78.6 – 86.5)	90.6 (89.2 – 91.8)	61.0 (57.6 – 64.2)	96.7 (96.0 – 97.4)

Abbreviations: PPV= positive predictive value, NPV= negative predictive value, LR+= positive likelihood ratio, LR-= negative likelihood ration, ACS= acute coronary syndromes, AMI= acute myocardial infarction

Table 3: Test characteristics of the Singulex Clarity hs-cTnI measured at the time of arrival in the ED, in combination with ECG findings: rule-out only if hs-cTnI below the stated cut-off and no ECG ischaemia

	Cut-off	Study	Patients 'ruled out', n (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
For ACS	0.8ng/L	Testing	110 (16.5)	97.9 (92.5 – 99.7)	18.9 (15.8 – 22.4)	16.6 (15.9 – 17.3)	98.2 (93.1 – 99.5)
		Validation	663 (26.8)	97.7 (96.1 – 98.8)	34.1 (32.0 – 36.3)	30.6 (29.8 – 31.3)	98.0 (96.7 – 98.9)
	1.5ng/L	Testing	254 (38.2)	95.7 (89.5 – 98.8)	43.8 (39.7 – 48.0)	21.9 (20.5 – 23.4)	98.4 (96.0 – 99.4)
		Validation	1,097 (44.4)	92.2 (89.7 – 94.3)	55.3 (53.0 – 57.5)	38.0 (36.7 – 39.3)	96.0 (94.7 – 97.0)
	99th percentile (8.67ng/L)	Testing	517 (77.8)	83.0 (73.8 – 90.0)	87.7 (84.8 – 90.3)	52.7 (46.8 – 58.6)	96.9 (95.2 – 98.0)
		Validation	1,655 (67.0)	76.5 (72.7 – 79.9)	79.9 (78.0 – 81.7)	53.0 (50.5 – 55.5)	92.0 (90.8 – 93.0)
For AMI	0.8ng/L	Testing	110 (16.5)	100.0 (95.3 – 100.0)	18.7 (15.6 – 22.1)	13.9 (13.4 – 14.3)	100.0 (N/A)
		Validation	663 (26.8)	99.7 (98.5 – 100.0)	31.6 (29.6 – 33.6)	20.6 (20.1 – 21.1)	99.9 (98.9 – 100.0)
	1.5ng/L	Testing	254 (38.2)	98.7 (93.0 – 100.0)	43.0 (39.0 – 47.1)	18.5 (17.4 – 19.7)	99.6 (97.3 – 99.9)
		Validation	1,097 (44.4)	89.0 (85.4 – 92.0)	77.0 (75.1 – 78.8)	40.7 (38.7 – 42.8)	97.5 (96.7 – 98.1)
	99th percentile (8.67ng/L)	Testing	517 (77.8)	89.6 (80.6 – 95.4)	86.6 (83.5 – 89.2)	46.6 (41.2 – 52.1)	98.5 (97.1 – 99.2)
		Validation	1,655 (67.0)	89.0 (85.4 – 92.0)	77.0 (75.1 – 78.8)	40.7 (38.7 – 42.8)	97.5 (96.7 – 98.1)