

# Single test rule-out of acute myocardial infarction using the limit of detection of a new high-sensitivity troponin I assay

**SHORT TITLE:**

Single test acute myocardial infarction rule-out with high-sensitivity troponin

**AUTHORS:**

Richard Body;<sup>a, b, c</sup> Niall Morris;<sup>a, b</sup> Denise Darby;<sup>d</sup> Emma Cowling;<sup>d</sup> Paul Collinson<sup>e</sup>

**Affiliations:**

a: Emergency Department, Central Manchester University Hospitals NHS Foundation Trust, Oxford Road, Manchester, M13 9WL, United Kingdom

b: Cardiovascular Sciences Research Group, The University of Manchester, Manchester Academic Health Science Centre, Oxford Road, Manchester, M13 9PL, United Kingdom

c: Healthcare Sciences Department, Manchester Metropolitan University, Oxford Road, Manchester, United Kingdom

d: Department of Clinical Biochemistry, Salford Royal NHS Foundation Trust, Stott Lane, Salford, M6 8HD, United Kingdom

e: Department of Chemical Pathology, St George's NHS Foundation Trust, Blackshaw Road, Totting, London, SW17 0QT, United Kingdom

**Corresponding author and address:**

Prof Richard Body

Professor and Honorary Consultant in Emergency Medicine

Emergency Department, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL, United Kingdom

**Email:** [richard.body@manchester.ac.uk](mailto:richard.body@manchester.ac.uk)

**Telephone:** 00 44 7880 712 929

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## Abstract

### Objectives

~~We aimed to~~ determine the diagnostic accuracy of a ~~new~~ high-sensitivity cardiac troponin I (hs-cTnI) assay in patients presenting to the Emergency Department (ED) with suspected acute coronary syndromes. Specifically, we evaluated the use of a single blood test at the time of arrival in the ED, using low hs-cTnI cut-offs.

### Methods

In a prospective diagnostic test accuracy study at 14 centers, we included patients presenting to the ED with suspected ACS within 12 hours of symptom onset. We drew blood for hs-cTnI (Siemens ADVIA Centaur, overall 99<sup>th</sup> percentile 47 ng/L, limit of quantification [LoQ] 2.50ng/L) on arrival.

Patients ~~also~~ underwent serial cardiac troponin testing over 3-6 hours. The primary outcome was an adjudicated diagnosis of acute myocardial infarction (AMI). We evaluated the incidence of major adverse cardiac events (MACE: death, AMI or revascularization) after 30 days. Test characteristics for hs-cTnI were calculated using ~~the previously reported cut-offs set at the LoQ and 5 ng/L cut-off and an optimised cut-off to achieve >99% sensitivity.~~

### Results

We included 999 patients, including 1312 (13.12%) with an adjudicated diagnosis of AMI. ~~Compared to the LoQ (100.0% sensitivity [95% CI 95.9-100.0%), 99.7% negative predictive value [NPV; 95% CI 97.6-100.0%), a 5 ng/L cut-off had slightly lower sensitivity (99.2%; 95% CI 95.8-100.0%) and similar NPV (99.8%; 95% CI 98.6-100.0%) but would rule out more patients (28.6% at the LoQ vs 50.4% at 5 ng/L). MACE occurred in 2 (0.7%) patients with hs-cTnI below the LoQ and 7 (1.4%) patients with hs-cTnI <5 ng/L. At the LoQ, the Siemens ADVIA Centaur hs-cTnI assay had a sensitivity of 99.2% (95% CI 95.9-100.0%) with 99.7% (95% CI 97.6-100.0%) negative predictive value (NPV), ruling out 28.6%~~

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181 patients with one test. MACE occurred in 3 (1.0%) patients with hs-cTnI <2.5 ng/L. At a previously  
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183 reported cut-off (5 ng/L), sensitivity was 98.5% (95% CI 94.6-99.8%) with 99.6% (95% CI 98.4-100.0%)  
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185 NPV, ruling out 50.4% patients with 1.6% incidence of MACE. Accounting for time from symptom  
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187 onset or ECG ischemia did not further improve sensitivity.  
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## 193 Conclusion

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195 The Siemens ADVIA Centaur hs-cTnI assay has high sensitivity and NPV to rule out AMI with a single  
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197 blood test in the ED. using the LoQ (3 ng/L) cut-off. At the LoQ cut-off a sensitivity >99% can be  
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199 achieved. At a 5 ng/L cut-off it may be possible to rule out AMI for over 50% patients with a single  
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201 test using a 5 ng/L cut-off.  
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## Background

Given the growing problem of Emergency Department (ED) crowding and its impact on patient outcomes [1,2], minimizing the time that patients spend in the ED awaiting the outcome of diagnostic tests is of critical importance for the sustainability of future healthcare. Chest pain is one of the most common reasons for ED attendance, yet the prevalence of acute myocardial infarction (AMI) in patients undergoing diagnostic evaluation is as low as 6% in some cohorts [3]. Safely reducing the need for serial troponin sampling has great potential to unburden crowded EDs, enabling earlier reassurance for patients and allowing clinicians to focus on other potential diagnoses at an earlier stage.

The detection of a rise and/or fall of cardiac troponin (cTn), with at least one concentration above the 99<sup>th</sup> percentile upper reference limit of the assay, is central to the diagnosis of AMI [4]. High sensitivity cardiac troponin (hs-cTn) assays can detect cTn in >50% of apparently healthy men and women with adequate precision (defined as a coefficient of variation <10% when measuring a sample with a cTn concentration equal to the 99<sup>th</sup> percentile upper reference limit) [5]. Because of the improved precision of hs-cTn assays, the time between serial samples can be reduced as there is a greater probability that a small observed change in cTn concentration is genuine, rather than being caused by the imprecision of the assay. Thus, AMI can be ruled out in over 60% patients using two tests taken as little as 1 hour apart [6].

With the high analytical sensitivity of hs-cTn assays, over one third of patients can also have the diagnosis of AMI 'ruled out' following a single test at the time of arrival in the ED [7-9]. To do this, the cut-off has previously been set at the limit of detection (LoD) of the assay, which is considerably lower than the conventional upper reference limit (the 99<sup>th</sup> percentile of concentrations measured in

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299 a healthy reference population). In some cohorts, this algorithm does not achieve adequate  
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301 diagnostic sensitivity unless AMI is only 'ruled out' in the absence of ECG ischemia [8,10,11]. Such an  
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303 approach has been validated with several hs-cTn assays to date, but as each cTn assay is different,  
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305 the accuracy of this algorithm must be verified for each individual assay.  
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309 Siemens recently introduced a new, commercially available hs-cTnI assay (ADVIA Centaur High-  
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311 Sensitivity Troponin I assay). We sought to evaluate the diagnostic accuracy of that assay for acute  
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313 myocardial infarction (AMI) in patients presenting to the ED with suspected acute coronary  
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315 syndromes. Specifically, we aimed to determine the test characteristics of this assay using cut-offs set  
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317 below the conventional 99<sup>th</sup> percentile upper reference limit of the assay with a single test at the  
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319 time of arrival in the ED.  
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## 322 323 324 325 **Methods**

### 326 327 328 329 **Design and setting**

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331 The Bedside Evaluation of Sensitive Troponin (BEST) study was a prospective diagnostic test accuracy  
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333 study at 18 centers in the United Kingdom. The BEST study incorporated six pre-planned  
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335 workstreams or substudies. The analysis presented here is one of those workstreams and includes  
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337 data from 14 of the 18 participating centers. A full list of the participating centers is available in the  
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339 Supplementary Appendix. Ethical approval was granted by the National Research Ethics Service  
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341 (14/NW/1344) and all participants provided written informed consent. The study was prospectively  
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343 registered on the National Institute for Health Research portfolio (UK CRN 18000). We have  
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345 published additional (separate) analyses from this study, including one analysis evaluating the  
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347 diagnostic accuracy of four decision aids using the same hs-cTnI assay evaluated here [12]. [The](#)  
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349 [sensitivity, specificity, PPV and NPV of hs-cTnI using the LoQ cut-off was reported in brief in that](#)  
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358 manuscript, but further detail, comparison with higher cut-offs and an evaluation of the influence of  
359 time from symptom onset and ECG ischemia on diagnostic accuracy are included in this further  
360 analysis.  
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## 370 **Study participants**

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372 We included adults aged >18 years who presented to the ED with pain, discomfort or pressure in the  
373 chest, epigastrium, neck, jaw or upper limb without an apparent non-cardiac source, which the  
374 treating physician suspected may have been caused by an acute coronary syndrome (ACS). Patients  
375 with peak symptoms >12 hours before presentation, those with definite ST elevation myocardial  
376 infarction, those who required medical admission for another reason, and those who could not  
377 provide written informed consent were excluded.  
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## 388 **Data collection and analytical procedures**

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390 Using a bespoke case report form, we recorded data on patient demographics, symptomatology, past  
391 medical history, current medications, risk factors for coronary artery disease, physical examination,  
392 electrocardiogram (ECG) interpretation, patient disposition, the times of blood sampling and the  
393 results of initial investigations. We drew blood at the time of arrival in the ED. To avoid delay and to  
394 give patients time to consider participation in the study, the initial blood sample could be drawn at  
395 the same time as routine clinical samples, prior to obtaining informed consent. If written informed  
396 consent was not subsequently obtained, all samples were discarded. A second blood sample was  
397 drawn 3 hours (+/- 30 minutes) after the initial sample. Within 30 minutes of collection, all blood  
398 samples were centrifuged at 2,500G for 10 minutes. Serum was then extracted and frozen at -80°C  
399 until analysis or at -20°C for up to 4 weeks and -80°C thereafter (at the Royal Bolton Hospital).  
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## **Analytical validation**

### ***Assay performance characteristics***

Limit of blank (LoB) and LoD determinations were based on the Clinical & Laboratory Standards Institute (CLSI) protocol EP17-A with 20 replicates of the calibration blank and calculated as mean + 1.645\*SD(calibration blank) [SD, standard deviation]. The LoD was estimated using 20 replicates of a low concentration sample and calculated as LoB + 1.645\*SD (low concentration sample).

Assay imprecision was assessed based on CLSI protocol EP15-A.14 using human serum sample pools. Serum pools were prepared from residual samples by selection of sera of known high cardiac troponin concentrations. A base serum pool was created and individual sample pools prepared by dilution with serum with undetectable troponin by measurement using a high-sensitivity cTnT assay (hs-cTnT, Roche Diagnostics). The objective was to cover the range of cTnI values if possible from close to the limit of detection (1.6 ng/L), across the expected 99th percentile, the World Health Organisation (WHO) diagnostic cut-off for AMI diagnosis and to a high value representative of unequivocal AMI diagnosis. A total of 5 pools were analysed (36 individual samples in total for each pool, a total of 180 samples) with 4 samples from each pool measured daily over 9 consecutive days, corresponding to the analytical runs of the clinical samples). All samples were stored frozen at - 20°C prior to analysis, then thawed, mixed and centrifuged prior to analysis.

### ***Clinical validation***

The previously unfrozen serum samples were tested in batches for hs-cTnI (Siemens ADVIA Centaur High-Sensitivity Troponin I assay) from December 2017 to January 2018. The assay has an overall 99<sup>th</sup>



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476 percentile upper reference limit of 47 ng/L (57 ng/L in men and 37 ng/L in women); the co-efficient  
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478 of variation is <10% at 6 ng/L; the LoD, as reported by the manufacturer, is 1.6 ng/L; and the limit of  
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480 quantification (LoQ), which is defined as the lowest concentration with co-efficient of variation <20%,  
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482 is reported by the manufacturer to be 2.5 ng/L [13]. The manufacturer recommends that hs-cTnI  
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484 concentrations are only reported down to the LoQ (2.5 ng/L) [14]. Based on the International  
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486 Federation of Clinical Chemistry Committee for Cardiac Biomarkers recommendation to report hs-  
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488 cTn assay concentrations in ng/L and in integers, for our primary analyses hs-cTnI concentrations  
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490 were rounded to the nearest integer (ng/L) [15]. Such rounding has no impact on the analysis at the  
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492 LoQ cut-off (as all concentrations  $\geq 2.5$  ng/L are rounded to 3ng/L, which is the nearest integer).  
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## 498 **Outcomes**

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500 For this analysis, our primary outcome was a diagnosis of AMI. This was defined in accordance with  
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502 the third universal definition of myocardial infarction, requiring a rise and/or fall of cTn with at least  
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504 one concentration exceeding the 99<sup>th</sup> percentile upper reference limit of the assay. In addition,  
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506 patients must have had at least one of: symptoms compatible with myocardial ischemia; ECG  
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508 changes compatible with ischemia/infarction; imaging evidence of new loss of viable myocardium; or  
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510 angiographic evidence of an intracoronary thrombus [4].  
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515 Secondary outcomes included major adverse cardiac events (MACE) occurring within 30 days. A  
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517 MACE was defined as death, prevalent or incident AMI or coronary revascularization.  
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521 The diagnosis of AMI was adjudicated based on cTn concentrations using the assay in clinical use at  
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523 each participating center (as listed in the Supplementary Appendix). To comply with the  
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525 requirements of the study protocol, centers using a contemporary cTn assay were required to  
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527 undertake cTn testing on arrival and either 6 hours later or 10-12 hours after symptom onset [16,17].  
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536 Those centers using hs-cTn assays were required to undertake cTn testing on arrival and at least 3  
537 hours later [16]. All diagnoses were adjudicated by two investigators, acting independently of each  
538 other, and blinded to the investigational hs-cTnI assay results.  
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## 543 544 **Follow-up**

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546 To determine the presence or absence of MACE, we followed up participants after 30 days. Follow-  
547 up involved three stages: (1) verification of the patient's mortality status (and cause of death if  
548 applicable) based on electronic records; (b) checking all available electronic patient records; and (c)  
549 contact with the patient, which was usually undertaken by telephone but could have been face to  
550 face, by email or letter. For patients who were persistently uncontactable, we contacted the primary  
551 care practitioner for information. In the United Kingdom the primary care practitioner receives  
552 copies of all hospital discharge summaries, outpatient correspondence and results of investigations  
553 undertaken in the community.  
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## 567 **Statistical analysis**

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569 Baseline characteristics were summarised using descriptive statistics. Analytical validation was  
570 performed using the Analyse-It add-in for Microsoft Excel for imprecision calculation. Data including  
571 confidence intervals was plotted and a best fit power function used to obtain a curve. Imprecision at  
572 10% and 20% was estimated by interpolation. Using data from the blood sample drawn at the time of  
573 arrival in the ED, we calculated test characteristics with 95% confidence intervals using the 99<sup>th</sup>  
574 percentile upper reference limit and LoQ thresholds. Because the manufacturer does not  
575 recommend reporting results lower than the LoQ (2.5 ng/L), we did not evaluate diagnostic accuracy  
576 at the LoD threshold (1.6 ng/L). We repeated the process to account for the presence or absence of  
577 ECG evidence of ischemia. We used receiver operating characteristic (ROC) curve analysis to evaluate  
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591 global diagnostic accuracy and calculated the area under the curve using the method described by De  
592 Long et al [18]. We also sought to determine diagnostic accuracy at a cut-off of 5ng/L, which has  
593 recently been reported in the literature as the 'HighSTEACS' cut-off [19] and subsequently validated  
594 in a patient cohort from the United States [20]. Finally, we evaluated diagnostic accuracy at the 'rule-  
595 in' cut-off of 120ng/L, which was also reported in both previous studies. Statistical analyses were  
596 undertaken using SPSS version 23.0 (SPSS Inc, Chicago, Illinois) and MedCalc version 13.1.2.0  
597 (Mariakerke, Belgium).

## 608 **Sample size**

609 Sample size in an observational study of this nature is determined by the required precision for  
610 estimates of diagnostic accuracy, as no hypothesis is being tested. The BEST study aimed to recruit a  
611 total of 1,575 participants to identify an algorithm with 90% specificity and 100% sensitivity. This  
612 would yield lower bounds of the 95% confidence intervals of at least 95% for sensitivity and 99% for  
613 negative predictive value, allowing for 5% loss to follow-up. While that overall sample size was  
614 exceeded for the entire study, sample size in this pre-planned substudy was dictated by the  
615 availability of samples for hs-cTnI (Siemens ADVIA Centaur) analysis.

## 626 **Results**

627 The LOB of the assay was 0.68 ng/L with LOD of 1.33 ng/L, in accordance with the manufacturer's  
628 specifications from the data sheet. Within run imprecision gave a 20% CV of 1.2 ng/L with a 10% CV  
629 of 3.1 ng/L. Total imprecision was 1.4 ng/L at 20% and 4.3 ng/L at 10% (supplementary figures 1 and  
630 2). All values exceeded the minimum specification provided by the manufacturer.

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654 Between February 2015 and June 2017 we included a total of 1,487 patients at the participating  
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656 centers, of which 999 had sufficient data for inclusion in this analysis (Figure 1). A total of 1312  
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658 (13.12%) patients had an adjudicated diagnosis of AMI. Of the patients who did not have AMI, a  
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660 further 21 developed at least one MACE within 30 days. The baseline characteristics of the included  
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662 participants and the flow of participants through the study have previously been reported in a  
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664 separate analysis from this cohort [12], are shown in Table 1.

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668 Overall, the Siemens hs-cTnI assay had an AUC of 0.96 (95% CI 0.94 – 0.98; Figure 1). The test  
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670 characteristics using cut-offs set at the 99<sup>th</sup> percentile upper reference limit and at the LoQ are  
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672 shown in Table 21. At the LoQ cut-off, hs-cTnI missed ~~one no~~ AMIs. ~~That patient, who underwent~~  
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674 ~~venepuncture 7.5 hours after symptom onset, had a single elevated high-sensitivity cardiac troponin~~  
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676 ~~T concentration at the time of arrival (44 ng/L; 99<sup>th</sup> percentile 14ng/L), which fell to 6 ng/L at 3 hours~~  
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678 ~~and 5 ng/L at 6 hours. The patient had no further major adverse cardiac events or cardiac~~  
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680 ~~investigations within the follow-up period and was discharged from hospital with a non-cardiac~~  
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682 ~~diagnosis. The adjudicated diagnosis of AMI was assigned because there was no other apparent~~  
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684 ~~explanation for the initial troponin elevation. That patient had a non-ischemic ECG and hs-cTnI~~  
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686 ~~concentrations of 1.6 and 2.2 ng/L on arrival and at 3 hours, respectively. Because that single patient~~  
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688 ~~did not have evidence of ECG ischemia no patients were missed at that threshold~~, accounting for ECG  
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690 changes did not yield a further improvement in sensitivity.

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695 At the previously reported 5 ng/L cut-off, we found a sensitivity of 99.28.5% (95% CI 95.84.6 –  
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697 100.099.8%) for AMI with an NPV of 99.86% (95% CI 98.64 – 100.099.9%). Accounting for ECG  
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699 ischemia did not further improve sensitivity.

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712 Including the initial diagnosis of AMI, ~~three-two~~ (0.71.0%) patients with hs-cTnI <3 ng/L developed  
713 MACE within 30 days. ~~Both o~~Of those, ~~there was one prevalent AMI and two~~ were patients who  
714 underwent percutaneous coronary intervention with no deaths or incident AMIs. None of the two  
715 patients with incident MACE had evidence of ECG ischemia at the time of presentation to the ED. A  
716 total of ~~eight seven~~ (1.46%) patients with hs-cTnI <58 ng/L had MACE within 30 days. ~~Two~~ One of  
717 those ~~were~~ was a prevalent AMIs. One patient did not have AMI at the time of initial presentation  
718 but had an AMI within 30 days of the initial presentation. The other five patients all underwent  
719 percutaneous coronary intervention within 30 days.  
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731 In this study, a total of 538 (53.9%) presented within 3 hours of symptom onset. The analysis of  
732 diagnostic accuracy stratified by time from symptom onset is shown in ~~Supplementary~~ Table ~~12~~. We  
733 did not identify any signal to suggest lower sensitivity and NPV, at any threshold, among patients  
734 who presented within 3 hours of symptom onset. However, given the smaller number of patients in  
735 this subgroup analysis, the 95% confidence intervals around sensitivity were relatively wide.  
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744 At the previously reported 'rule-in' cut-off (120 ng/L), the hs-cTnI assay had a specificity of 98.9%  
745 (95% CI 97.9 – 99.5%) with a positive predictive value of 88.4% (95% CI 80.1 – 93.5%), 'ruling in' AMI  
746 for 86 (8.6%) patients.  
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## 752 Discussion

753 In this study we aimed to determine whether low concentrations of cardiac troponin, measured  
754 using an hs-cTnI assay (Siemens ADVIA Centaur High-Sensitivity Troponin I assay), could be used to  
755 safely 'rule out' the diagnosis of AMI using a single blood test at the time of arrival in the ED in  
756 routine clinical practice. We have confirmed the analytical claims for the assay in terms of limits of  
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772 detection and imprecision profile [21]. Our findings demonstrate that this assay can identify AMI  
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774 with high sensitivity and negative predictive value, setting the cut-off at the LoQ (2.5 ng/L, or  $\geq 3$  ng/L  
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776 considering that hs-cTn concentrations should be reported in integers) or potentially as high as 5  
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778 ng/L. At both cut-offs, the assay achieved a sensitivity and negative predictive value above 99%.  
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780 Setting the cut-off at the LoQ, approximately one in four patients (28.6%) could have been  
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782 discharged from the ED following a single blood test. The incidence of 30-day MACE in those patients  
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784 was low (~~1.00~~0.7%). At the previously reported 5 ng/L cut-off, 50.4% patients could have been  
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786 discharged from the ED after one test. The incidence of MACE in that group was higher (1.46%),  
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788 including one prevalent AMI, one incident AMI and five percutaneous coronary interventions.  
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790 However those procedures could arguably be undertaken on an outpatient basis without requiring  
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792 emergency hospital admission. ~~The only AMI missed by hs-cTnI at these cut-offs had an adjudicated~~  
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794 ~~diagnosis of AMI assigned based on a single elevation of high-sensitivity cardiac troponin T, although~~  
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796 ~~this was not felt to be significant by the treating clinicians and the patient had been discharged with~~  
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798 ~~a non-cardiac diagnosis.~~ These findings support clinical use of this hs-cTnI assay to 'rule out' AMI with  
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800 a single blood test.  
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806 Our findings are concordant with the results of previous work evaluating similar low cut-offs with  
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808 other high-sensitivity cardiac troponin assays. For example, in a collaborative meta-analysis involving  
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810 9,241 patients from 11 studies, hs-cTnT (Roche Elecsys) had a sensitivity of 98.7% (95% CI 96.6 –  
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812 99.5%) using a cut-off set at the LoD (5 ng/L) of the assay. At that cut-off, AMI would have been ruled  
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814 out in 30.6% of patients [7]. Similarly, in a study involving 12 centers in 9 countries, hs-cTnT <5 ng/L  
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816 achieved a sensitivity of 99.1%, ruling out 36% of patients with one test [8]. In a pooled analysis of 5  
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818 international studies including 3,155 patients, the Abbott ARCHITECT hs-cTnI assay had a sensitivity  
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820 of 99.0% (95% CI 96.8 – 99.7%) with NPV 99.5% (98.4 – 99.9%) using the LoD (1.2 ng/L) as a cut-off.  
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822 That strategy would have ruled out AMI in 18.8% patients [9]. However, in each of those studies, AMI  
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830 was only ruled out in patients with no ECG ischemia. Interestingly, in this work, hs-cTnI (Siemens  
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832 ADVIA Centaur High-Sensitivity Troponin I assay) achieved high sensitivity without the addition of  
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834 ECG interpretation, and taking account of ECG ischemia did not improve diagnostic accuracy.  
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839 At a cut-off set above the LoQ but below the 99<sup>th</sup> percentile upper reference limit (5 ng/L), hs-cTnI  
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841 could have 'ruled out' AMI in over 50% of patients. A similar approach has been taken with the  
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843 Abbott ARCHITECT hs-cTnI assay. Shah *et al* previously derived the optimal cut-off to rule out AMI  
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845 with a single blood test in the ED. Setting the cut-off at 5 ng/L yielded an NPV of 99.4% (95% CI 98.8 –  
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847 99.9%) on validation [22]. Sensitivity at that cut-off was, however, lower (94%), and evaluation in  
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849 external cohorts yielded similar findings (sensitivity 94.5%, 95% CI 91.1 – 96.7%) [9]. Using the same  
850  
851 5ng/L cut-off (the HighSTEACS cut-off) in this cohort had a similar effect. NPV remained high (at  
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853 ~~99.85%). S, although sensitivity dropped slightly, to 98.5% only marginally, to 99.2%,~~ and the  
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855 incidence of MACE at 30 days was ~~1.46%~~. If clinicians and patients are willing to accept that marginal  
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857 increase in risk, then this cut-off could yield even further diagnostic efficiency, allowing 50.4%  
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859 patients to have AMI ruled out with a single blood test. In this study, there was no suggestion that  
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861 restricting the use of this strategy to patients undergoing venepuncture  $\geq$ 3h after symptom onset  
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863 would improve sensitivity. However, applying that criterion would mean that only 20.4% of the entire  
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865 cohort would be 'ruled out' at the 5ng/L cut-off.  
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## 872 **Strengths and limitations**

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875 In this work, we have evaluated the diagnostic accuracy of the Siemens ADVIA Centaur hs-cTnI assay  
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877 in a carefully designed multi-center, prospective diagnostic test accuracy study. The multi-center  
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879 nature of this study increases the external validity of our findings. Because all patients underwent  
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881 reference standard delayed cardiac troponin testing following a protocol in accordance with current  
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889 national and international guidelines, there is no potential for verification bias. We also achieved a  
891 high follow-up completion rate, which enables us to be more confident about the incidence of MACE.  
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895 Our work does have some limitations that it is important to acknowledge. While our sample size was  
896 relatively large (n=999), we did not have serum samples available for analysis from approximately  
897 one third of the total cohort recruited to this multi-center study. This is, however, a common  
898 limitation in studies of this nature, and comparison of baseline characteristics between included and  
899 excluded participants does not suggest that this has introduced any systematic bias.  
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908 It is also important to recognise that samples in this study were analysed retrospectively in batches,  
909 with quality control procedures that are different to day to day practice in clinical laboratories. When  
910 used in routine practice, analytical performance may vary and the potential impact of batch to batch  
911 variation could not be studied. Finally, because the assay was not available until after the end of  
912 recruitment, the samples collected in this study were stored at -80°C for between six and 35 months  
913 prior to analysis. However the manufacturer's kit insert verifies that samples are stable at this  
914 temperature for up to 12 months and published data demonstrate the stability of the assay in  
915 samples that have been stored for up to 15 years at temperatures below -70°C [23].  
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## 928 **Conclusions**

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930 We have identified that the Siemens ADVIA Centaur hs-cTnI assay has high sensitivity and NPV when  
931 used to rule out the diagnosis of AMI with a single test in the ED, using a cut-off set at the LoQ of the  
932 assay (<3 ng/L). The incidence of MACE in these patients is low, suggesting that this algorithm could  
933 be used to avoid unnecessary hospital admission for more than one quarter of patients. Our findings  
934 also suggest that this assay could be used to rule out AMI using a 5 ng/L cut-off, which could rule out  
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948 the diagnosis in over 50% of patients. Finally, a single hs-cTnI concentration of  $\geq 120$  ng/L in the ED  
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950 could be used to rule in AMI without serial sampling, achieving a positive predictive value of just  
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952 below 90%. This may help to rapidly triage patients to appropriate areas, unburdening crowded EDs.  
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## Legends to figures

Figure 1: Participant flow diagram

Figure 12: Receiver operating characteristic curve showing the overall diagnostic accuracy of the hs-cTnI assay (Siemens ADVIA Centaur), measured at the time of arrival in the ED, for a diagnosis of AMI

## Tables

**Table 1: Baseline characteristics of included patients**

	<b>Total (n=999)</b>	<b>AMI (n=132)</b>	<b>No ACS (n=867)</b>
Age in years, mean (SD)	58.1 (15.2)	64.9 (15.0)	57.0 (15.0)
Men (%)	630 (63.1)	92 (69.7)	539 (62.1)
Previous angina (%)	270 (27.0)	47 (35.6)	223 (25.7)
Previous myocardial infarction (%)	280 (28.0)	47 (35.6)	233 (26.9)
Previous coronary intervention (%)	228 (22.8)	191 (22.0)	37 (28.0)
Previous coronary artery bypass graft (%)	73 (7.3)	17 (12.9)	56 (6.5)
Hypertension (%)	483 (48.3)	77 (58.3)	406 (46.8)
Hyperlipidaemia (%)	387 (38.7)	64 (48.5)	323 (37.3)
Type 1 diabetes mellitus (%)	22 (2.2)	7 (5.3)	15 (1.7)
Type 2 diabetes mellitus (%)	198 (19.8)	35 (26.5)	163 (18.8)
Current smoking (%)	200 (20.0)	33 (25.0)	167 (19.3)
Time from symptom-onset to arrival in the ED, n (%):			
<3h	538 (53.9)	77 (58.3)	461 (53.2)
3–6h	199 (19.9)	23 (17.4)	176 (20.3)
>6h	197 (19.7)	25 (19.0)	172 (19.9)

**Table 12: Test characteristics of the Siemens ADVIA Centaur hs-cTnI assay, used alone at the time of arrival in the ED for a diagnosis of AMI**

Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+	LR-	Number (%) ruled out	30-day MACE in patients 'ruled out'
<b>HS-cTnI alone</b>								
LoQ ( $\geq 3$ ng/L)	<del>99.2</del> ( <del>95.9</del> - <del>100.0</del> ) <del>100.0</del> <del>0</del> (97.2 - 100.0)	<del>33.02</del> .9 (29.8 - 36.2)	18.4 (17.7 - 19.1)	<del>99.7</del> ( <del>97.6</del> - <del>100.0</del> ) <del>100.0</del> (N/A)	1.4 <del>98</del> (1.4 <del>21</del> - 1.5 <del>65</del> )	<del>0.02</del> ( <del>0.00</del> - <del>0.16</del> ) <del>0.00</del> (N/A)	286 (28.6)	<del>23</del> (0.7 <del>1.0</del> )
HighSTEACS ( $\geq 5$ ng/L)	<del>98.5</del> ( <del>94.6</del> - <del>99.8</del> ) <del>99.2</del> ( <del>95.8</del> - <del>100.0</del> )	57.8 (54.5 - 61.2)	26.2 (24.7 - 27.8)	<del>99.86</del> ( <del>98.64</del> - <del>99.9</del> ) <del>100.0</del> 0)	2.3 <del>53</del> (2.1 <del>75</del> - 2.5 <del>53</del> )	0.0 <del>13</del> (0.0 <del>01</del> - 0.0 <del>94</del> )	503 (50.4)	<del>78</del> (1.4 <del>6</del> )
Overall 99th percentile (47 ng/L)	74.6 <del>2</del> (66.2 <del>5.9</del> - 81.8 <del>5</del> )	96.5 (95.1 - 97.7)	76.4 <del>6</del> (69.2 <del>4</del> - 82.3 <del>5</del> )	96.2 <del>1</del> (95.0 <del>4.9</del> - 97.2 <del>4</del> )	21.5 <del>946</del> (14.9 <del>88</del> - 31.1 <del>20.93</del> )	0.2 <del>67</del> (0.20 - 0.3 <del>56</del> )	<del>5</del> 871 (87.2)	<del>50</del> 1 (5.7 <del>9</del> )
Sex-specific 99th percentiles	74.0 <del>3.5</del> (65.7 <del>1</del> - 81.3 <del>0.8</del> )	96.7 (95.2 - 97.8)	77.0 (69.8 - 82.9)	96.1 <del>0</del> (94.9 <del>7</del> - 97.1 <del>0</del> )	22.1 <del>61.97</del> (15.2 <del>814</del> - 32.1 <del>51.88</del> )	0.27 (0.20 <del>1</del> - 0.36)	873 (87.4)	53 (6.1)
<b>Only 'rule out' in the absence of ECG ischemia*</b>								
LoQ ( $\geq 3$ ng/L)	<del>99.2</del> ( <del>97.25</del> - <del>100.0</del> )	<del>32.12</del> (29.0 <del>1</del> - 35.3 <del>4</del> )	<del>17.98</del> .2 (17.2 <del>5</del> - 18.6 <del>9.0</del> )	<del>99.6</del> ( <del>97.5</del> - <del>100.0</del> ) (N/A)	1.4 <del>76</del> (1.4 <del>139</del> - 1.54)	0.0 <del>0</del> (N/A) <del>2</del> ( <del>0.00</del> - <del>0.17</del> )	<del>27</del> 880 (27.9 <del>8.0</del> )	<del>23</del> (0.7 <del>1.1</del> )
HighSTEACS ( $\geq 5$ ng/L)	<del>99.28</del> .5 ( <del>95.74</del> .6 - <del>100.0</del> ) <del>99.8</del>	56.5 <del>4</del> (53.1 <del>0</del> - 59.8)	25.2 <del>5</del> (23.8 <del>4.0</del> - 27.0)	<del>99.86</del> ( <del>98.64</del> - <del>100.0</del> ) <del>99.9</del> 9)	2.2 <del>86</del> (2.1 <del>109</del> - 2.4 <del>64</del> )	0.0 <del>13</del> (0.0 <del>01</del> - 0.1 <del>01</del> )	490 (49.2 <del>3</del> )	<del>78</del> (1.4 <del>6</del> )
Overall 99th percentile (47 ng/L)	78.9 <del>0</del> (70.8 <del>0</del> - 85.6 <del>4.8</del> )	92.5 (90.5 - 94.2)	60.8 <del>1.4</del> (54.7 <del>5.2</del> - 66.6 <del>7.1</del> )	96.7 <del>1</del> (95.5 <del>3</del> - 97.7 <del>5</del> )	10.5 <del>41</del> (8.1 <del>80</del> - 13.5 <del>037</del> )	0.2 <del>34</del> (0.1 <del>67</del> - 0.3 <del>23</del> )	<del>82</del> 830 (83.3 <del>1</del> )	<del>41</del> 3 (5.0 <del>2</del> )
Sex-specific 99th percentiles	78.1 <del>7.3</del> ( <del>70.06</del> 9.2 - 85.0 <del>4.1</del> )	92.4 (90.4 - 94.1)	60.2 <del>7</del> (54.1 <del>6</del> - 66.0 <del>5</del> )	96.6 <del>4</del> (95.4 <del>1</del> - 97.5 <del>3</del> )	10.3 <del>415</del> (7.9 <del>91</del> - 13.1 <del>503</del> )	0.2 <del>45</del> (0.1 <del>78</del> - 0.3 <del>34</del> )	<del>82</del> 831 (83.3 <del>2</del> )	<del>42</del> (5.1 <del>53</del> ) (5.3)

Abbreviations: PPV= positive predictive value, NPV= negative predictive value, LR+= positive

likelihood ratio, LR-= negative likelihood ratio

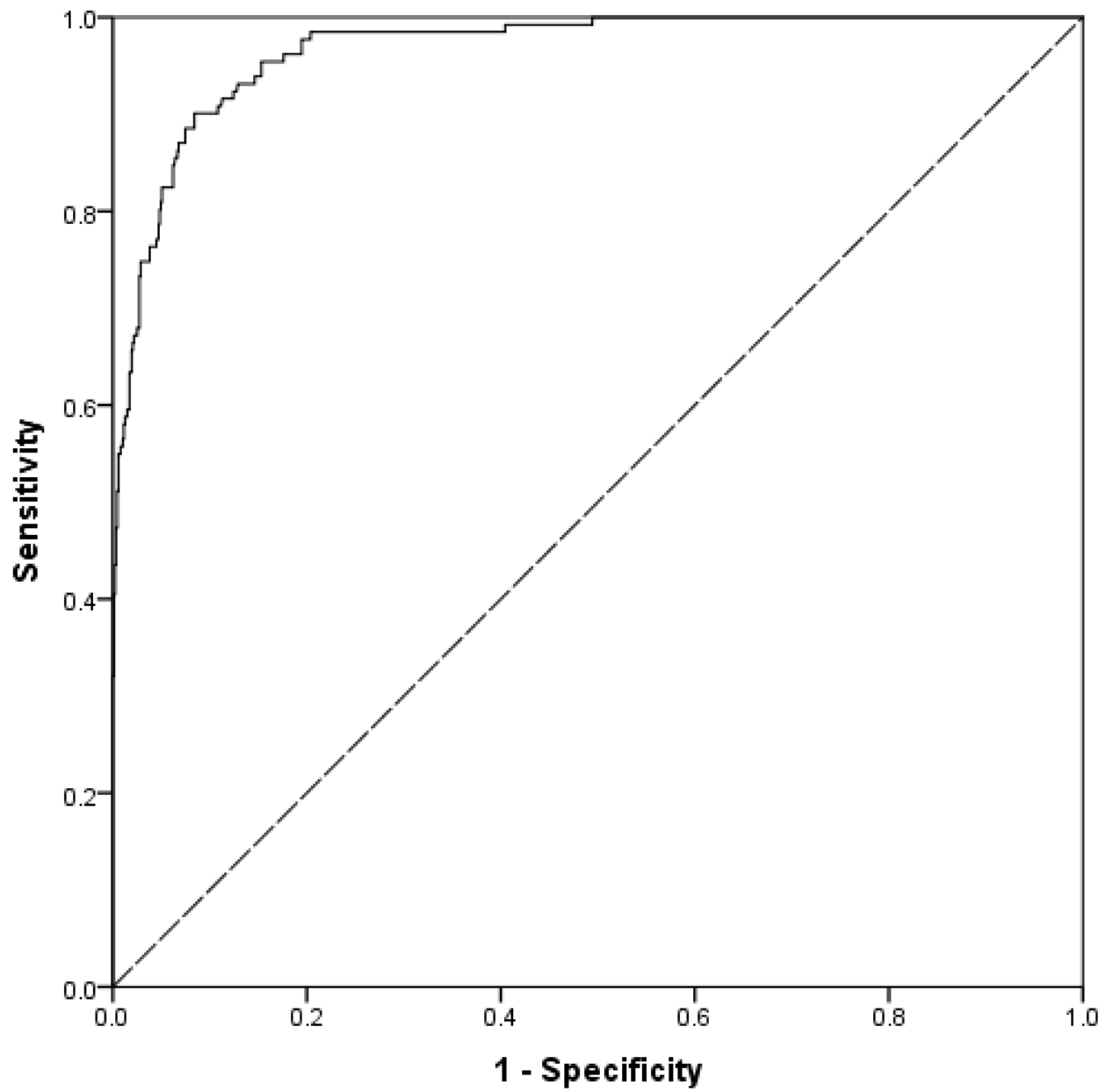


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\* Data for ECG ischemia were missing for five patients.

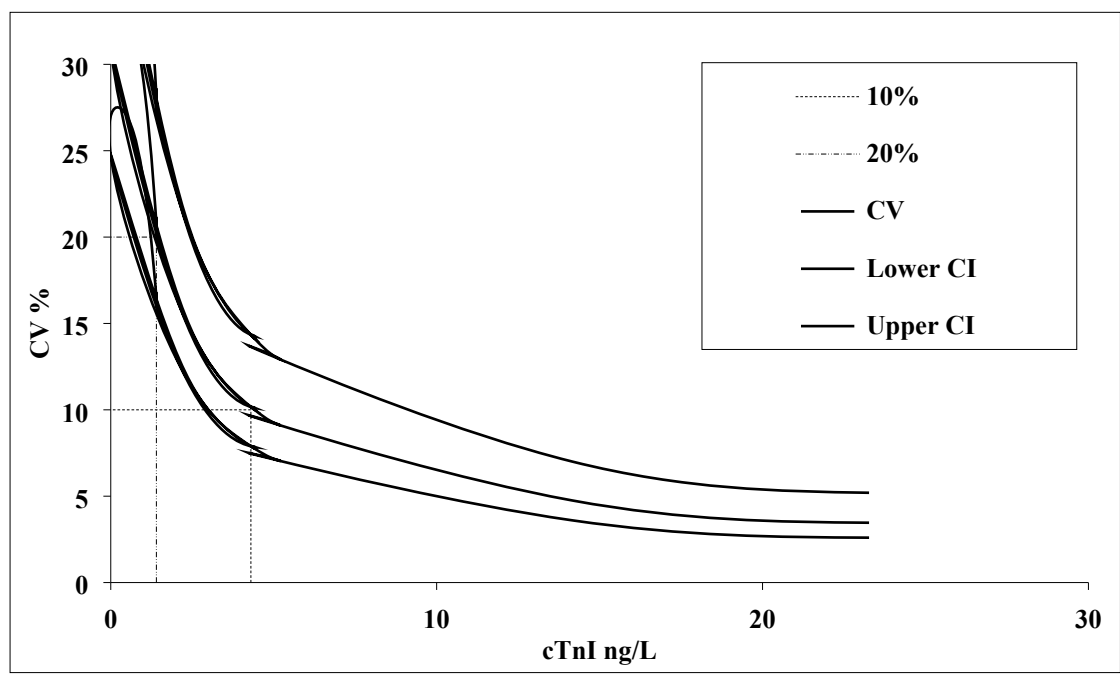
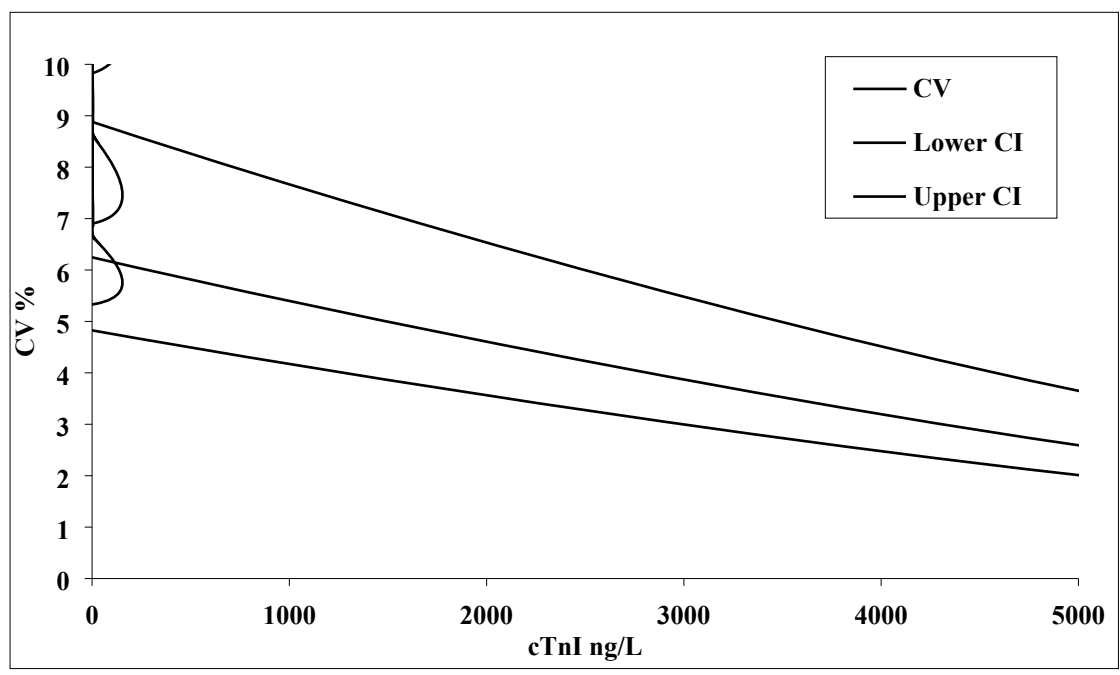
**Table 2: Sensitivity analysis. Test characteristics at selected cut-offs stratified by time from symptom onset to arrival in the ED**

<u>Cut-off</u>	<u>Sensitivity</u> <u>(95% CI)</u>	<u>Specificity</u> <u>(95% CI)</u>	<u>PPV</u> <u>(95% CI)</u>	<u>NPV</u> <u>(95% CI)</u>	<u>LR+</u>	<u>LR-</u>	<u>Number (%)</u> <u>ruled out</u>	<u>30-day</u> <u>MACE in</u> <u>patients</u> <u>'ruled out'</u>
<b>&lt;3h from symptom onset</b>								
<u>LoQ (≥3 ng/L)</u>	<u>100.0</u> <u>(95.3 -</u> <u>100.0)</u>	<u>34.7</u> <u>(30.4 -</u> <u>39.3)</u>	<u>20.4</u> <u>(19.3 -</u> <u>21.5)</u>	<u>100.0</u> <u>(N/A)</u>	<u>1.53</u> <u>(1.43 -</u> <u>1.64)</u>	<u>0.00</u> <u>(N/A)</u>	<u>160 (29.7)</u>	<u>1 (0.6)</u>
<u>HighSTEACS (≥5</u> <u>ng/L)</u>	<u>100.0</u> <u>(95.3 -</u> <u>100.0)</u>	<u>57.3</u> <u>(52.6 -</u> <u>61.8)</u>	<u>28.1</u> <u>(26.0 -</u> <u>30.3)</u>	<u>100.0</u> <u>(N/A)</u>	<u>2.34</u> <u>(2.11 -</u> <u>2.60)</u>	<u>0.00</u> <u>(N/A)</u>	<u>264 (49.1)</u>	<u>1 (0.4)</u>
<b>≥3h from symptom onset</b>								
<u>LoQ (≥3 ng/L)</u>	<u>100.0</u> <u>(92.5 -</u> <u>100.0)</u>	<u>30.7</u> <u>(25.9 -</u> <u>35.8)</u>	<u>16.3</u> <u>(15.3 -</u> <u>17.2)</u>	<u>100.0</u> <u>(N/A)</u>	<u>1.44</u> <u>(1.34 -</u> <u>1.55)</u>	<u>0.00</u> <u>(N/A)</u>	<u>107 (27.0)</u>	<u>1 (0.9)</u>
<u>HighSTEACS</u> <u>(≥5 ng/L)</u>	<u>97.9</u> <u>(88.7 -</u> <u>100.0)</u>	<u>58.2</u> <u>(52.8 -</u> <u>63.4)</u>	<u>24.0</u> <u>(21.7 -</u> <u>26.4)</u>	<u>99.5</u> <u>(96.7 -</u> <u>99.9)</u>	<u>2.34</u> <u>(2.05 -</u> <u>2.67)</u>	<u>0.04</u> <u>(0.01 -</u> <u>0.25)</u>	<u>204 (51.5)</u>	<u>5 (2.5)</u>



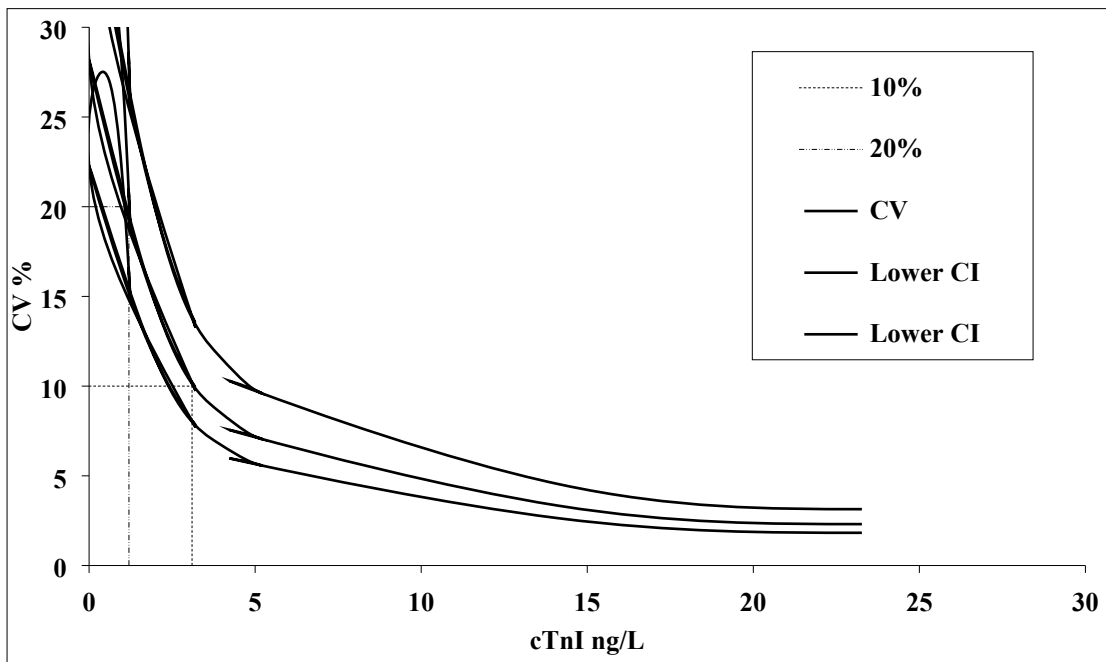
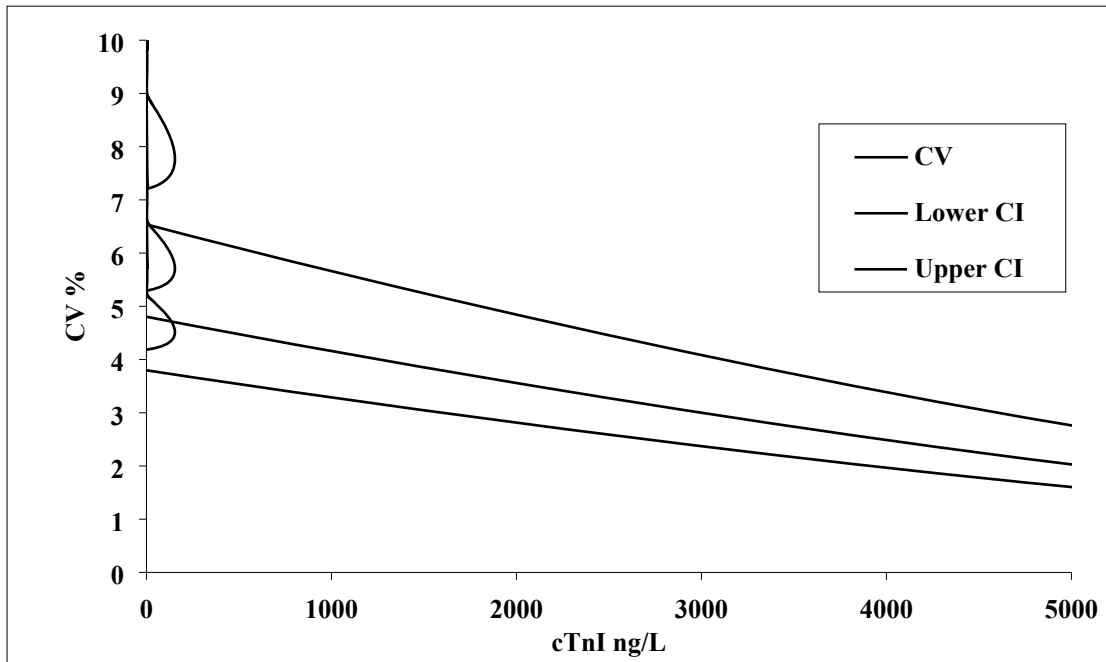
**Supplementary Figure 1**

Total assay imprecision covering the lower part of the analytical range (upper figure) and clinical decision points (lower figure). Both figures show the interpolated curve plus upper and lower confidence intervals. In the lower figure, the 10% CV and 20% CV are marked.



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62 **Supplementary Figure 2**  
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64 Within run imprecision covering the lower part of the analytical range (upper figure) and clinical  
65 decision points (lower figure). Both figures show the interpolated curve plus upper and lower  
66 confidence intervals. In the lower figure, the 10% CV and 20% CV are marked.  
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**Supplementary Table 1: Baseline characteristics of patients who were included in this analysis and those who were included in the BEST study at participating centers but had insufficient data for inclusion in the analysis**

	Included (n=999)	Not included (n=488)
Had AMI	132 (13.2)	68 (13.9)*
Age in years, mean (SD)	58.1 (15.2)	59.9 (15.5)
Men (%)	630 (63.1)	326 (66.8)
Previous angina (%)	270 (27.0)	160 (32.8)
Previous myocardial infarction (%)	280 (28.0)	128 (26.2)
Previous coronary intervention (%)	228 (22.8)	98 (20.1)
Previous coronary artery bypass graft (%)	73 (7.3)	38 (7.8)
Hypertension (%)	483 (48.3)	254 (52.0)
Hyperlipidaemia (%)	387 (38.7)	162 (33.2)
Type 1 diabetes mellitus (%)	22 (2.2)	8 (1.6)
Type 2 diabetes mellitus (%)	198 (19.8)	85 (17.4)
Current smoking (%)	200 (20.0)	94 (19.3)
Time from symptom onset to arrival in the ED, n (%):		
< 3h	538 (53.9)	267 (54.7)
3 – 6h	199 (19.9)	100 (20.5)
> 6h	197 (19.7)	84 (17.3)

\* Of 365 patients with full data to enable adjudication of AMI (i.e. patients who completed appropriate serial sampling)

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