Rapid Exclusion of Acute Myocardial Infarction in Patients With Undetectable Troponin Using a High-Sensitivity Assay

Richard Body, MB, CB, PhD,* Simon Carley, MD,† Garry McDowell, PhD,* Allan S. Jaffe, MD,‡ Michael France, MB BS,† Kennedy Cruickshank, MB, MD,* Christopher Wibberley, PhD,§ Michelle Nuttall,† Kevin Mackway-Jones, BM, BCH, MA†
Manchester, United Kingdom; and Rochester, Minnesota

JACC JOURNAL CME

This article has been selected as the month’s JACC Journal CME activity.

Accreditation and Designation Statement
The American College of Cardiology Foundation (ACCF) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

The ACCF designates this Journal-based CME activity for a maximum of 1 AMA PRA Category 1 Credit(s). Physicians should only claim credit commensurate with the extent of their participation in the activity.

Method of Participation and Receipt of CME Certificate
To obtain credit for JACC CME, you must:
1. Be an ACC member or JACC subscriber.
2. Carefully read the CME-designated article available online and in this issue of the journal.
3. Answer the post-test questions. At least 2 out of the 3 questions provided must be answered correctly to obtain CME credit.
4. Complete a brief evaluation.
5. Claim your CME credit and receive your certificate electronically by following the instructions given at the conclusion of the activity.

CME Objective for This Article: At the conclusion of this activity, the learner should be able to identify the role of hs-cTnT in ACS patients.

CME Editor Disclosure: JACC CME Editor Ajit Raisinghani, MD, FACC, reports that he has no financial relationships or interests to disclose.

Author Disclosures: Dr. Body has accepted travel and accommodation without honoraria from Roche Diagnostics and Randox Diagnostics for 2 conferences. Dr. Jaffe has accepted consulting fees/honoraria from most of the major diagnostic companies. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Medium of Participation: Print (article only); online (article and quiz)

CME Term of Approval:
Issue date: September 20, 2011
Expiration date: September 19, 2012

From the *University of Manchester, Manchester, United Kingdom; †Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom; ‡Mayo Clinic and Mayo College of Medicine, Rochester, Minnesota; and the §Faculty of Health, Psychology and Social Care, Manchester Metropolitan University, Manchester, United Kingdom. This study was funded by a grant from Central Manchester NHS Foundation Trust. For the cohort study, reagents for the high sensitivity troponin T assay were donated by Roche Diagnostics. Additional work in this cohort has been undertaken under collaborative agreements with Alere and Randox. All analyses were conducted independently of Roche Diagnostics, and no restrictions were placed on the freedom of the authors to publish the findings of this research. Dr. Body has accepted travel and accommodation without honoraria from Roche Diagnostics and Randox Diagnostics for 2 conferences. Dr. Jaffe has accepted consulting fees/honoraria from most of the major diagnostic companies. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received February 16, 2011; revised manuscript received May 26, 2011, accepted June 7, 2011.
## Rapid Exclusion of Acute Myocardial Infarction in Patients With Undetectable Troponin Using a High-Sensitivity Assay

<table>
<thead>
<tr>
<th>Objectives</th>
<th>This paper sought to evaluate whether high sensitivity troponin (hs-cTnT) can immediately exclude acute myocardial infarction (AMI) at a novel ‘rule out’ cut-off.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>Subgroup analysis of recent evidence suggests that undetectable hs-cTnT may exclude AMI at presentation.</td>
</tr>
<tr>
<td>Methods</td>
<td>In a cohort study, we prospectively enrolled patients with chest pain, evaluating them with standard troponin T and testing for hs-cTnT (Roche Diagnostics, Basel, Switzerland) at presentation. The primary outcome was a diagnosis of AMI. We also followed up patients for adverse events within 6 months. After subsequent clinical implementation of hs-cTnT, we again evaluated whether initially undetectable hs-cTnT ruled out a subsequent rise.</td>
</tr>
<tr>
<td>Results</td>
<td>Of 703 patients in the cohort study, 130 (18.5%) had AMI, none of whom initially had undetectable hs-cTnT (sensitivity: 100.0%, 95% confidence interval [CI]: 95.1% to 100.0%, negative predictive value: 100.0%, 95% CI: 98.1% to 100.0%). This strategy would rule out AMI in 27.7% of patients, 2 (1.0%) of whom died or had AMI within 6 months (1 periprocedural AMI, 1 noncardiac death). We evaluated this approach in an additional 915 patients in clinical practice. Only 1 patient (0.6%) with initially undetectable hs-cTnT had subsequent elevation (to 17 ng/l), giving a sensitivity of 99.8% (95% CI: 99.1% to 100.0%) and a negative predictive value of 99.4% (95% CI: 96.6% to 100.0%).</td>
</tr>
<tr>
<td>Conclusions</td>
<td>Undetectable hs-cTnT at presentation has very high negative predictive value, which may be considered to rule out AMI, identifying patients at low risk of adverse events. Pending further validation, this strategy may reduce the need for serial testing and empirical treatment, enabling earlier reassurance for patients and fewer unnecessary evaluations and hospital admissions. (J Am Coll Cardiol 2011;58:1332–9) © 2011 by the American College of Cardiology Foundation</td>
</tr>
</tbody>
</table>

Despite extensive research and evolving diagnostic technology, patients with symptoms possibly resulting from an acute coronary syndrome usually undergo investigation for at least 6 hours (and often longer) before acute myocardial infarction (AMI) can be excluded confidently (1–3). Thus in many centers, including ours, most patients (74% to 88%) are admitted to hospital, making up more than one-quarter of acute medical admissions. Only a minority (approximately 25%) ultimately are diagnosed with an acute coronary syndrome, and many fewer are diagnosed with AMI (4–6). A strategy that would enable AMI or acute coronary syndrome to be excluded at the time of presentation would be extremely helpful and likely would substantially reduce unnecessary hospital admissions. Clinical judgment alone is clearly insufficient to fulfill that goal, because up to 6% of patients with chest pain who are discharged from the emergency department (ED) have been reported to have had unrecognized AMI. Missed AMI in this setting has significant prognostic implications (7,8).

Recently, Reichlin et al. (9) and Keller et al. (10) reported that using more sensitive troponin assays can increase diagnostic accuracy by ruling in AMI for a greater proportion of patients at the time of initial presentation. However, using the conventional cutoff (the 99th percentile of a healthy reference population), the sensitivity of these novel assays was approximately 90%, meaning that serial testing remains necessary before AMI can be ruled out. Among the assays evaluated by Reichlin et al. (11) was the Elecsys high-sensitivity cardiac troponin T (hs-cTnT) assay (Roche Diagnostics). We noted that, using the lower detection limit of this assay as a cutoff, AMI seemed to be ruled out (sensitivity: 100%). If so, such a strategy would have enabled the exclusion of AMI in those with such low values (9.9% of the population) at the time of admission (12). If these findings could be validated, hs-cTnT could be used to reduce some of the unnecessary hospital admissions. Such an approach would lower resource utilization, would improve quality of care for patients, and would lead to earlier ED discharges. Accordingly, we set out to investigate whether AMI can be safely ruled out in patients with undetectable levels of hs-cTnT at the time of presentation to the ED.

### Methods

There were 2 parts to our investigation: an initial prospective cohort study to validate the findings of Reichlin et al. (12) in the context of a research environment and a prospective clinical audit to validate our findings in clinical practice. The prospective cohort study was approved by the local research ethics committee. The U.K. National Research Ethics Service confirmed that the clinical audit did not require ethical approval.

**Prospective cohort study.** The cohort study was a substudy of the Early Vascular Markers of Acute Coronary Syndromes project, which aims to develop a novel algorithm for the early exclusion of acute coronary syndromes in the
ED (13–17). We prospectively recruited patients who sought treatment at the ED with chest pain at Manchester Royal Infirmary, a university-affiliated teaching hospital with an annual ED census of approximately 145,000. We included patients who were older than 25 years and had chest pain within the previous 24 h that the initial treating physician suspected may be cardiac in nature. Patients were excluded if they had renal failure requiring dialysis, trauma with suspected myocardial contusion, or another medical condition mandating hospital admission or if they did not consent to and provide a blood sample for use by the research team.

Blood was drawn at the time of ED presentation and immediately centrifuged. Serum was separated and frozen at \(-200^\circ\text{C}\) for up to 48 h and at \(-700^\circ\text{C}\) thereafter. Following an amendment to the initial protocol (also approved by the local research ethics committee), previously unthawed samples were tested for hs-cTnT (99th percentile: 14 ng/l, coefficient of variation <10% at 9 ng/l) and fourth-generation cardiac troponin T (cTnT) (99th percentile: 10 ng/l, coefficient of variation <10% at 30 ng/l) by laboratory personnel who were blinded to patient outcomes (11). Testing was completed in August 2009.

All patients underwent testing with the fourth-generation cTnT assay at least 12 h after the onset of the most significant symptoms. Patients then were followed up after 48 h, 30 days, and 6 months. At follow-up, the National Health Service Strategic Tracing Service database (a national database with mortality statistics updated on at least a weekly basis) initially was checked for mortality data. The cause of death was retrieved for all deceased patients. Electronic hospital records then were reviewed for every patient, including details of all subsequent ED attendances, hospital admissions, out-patient clinic appointments, and all investigations requested or undertaken. All living patients then were contacted by telephone. In the event that a patient could not be contacted after multiple attempts, their general practitioner was contacted. In the event that a patient was hospitalized at another hospital during the follow-up period, copies of relevant records were obtained.

Outcomes. The primary outcome was a diagnosis of AMI. This final diagnosis was adjudicated by 2 independent investigators (R.B. and S.C.) who had all clinical, laboratory, and imaging data available for review, but who were blinded to hs-cTnT levels. In accordance with the universal definition of AMI, patients were considered to fulfill the criteria for AMI if they had a rise or fall of cTnT, or both, above the 99th percentile (10 ng/l) in the appropriate clinical context (18). For patients with modest elevations of cTnT (<0.1 ng/ml) at baseline, an absolute difference of at least 20 ng/l on serial sampling was considered to represent a significant rise, fall, or both (19) based on the analytical performance of the cTnT assay. To ensure that we did not ignore significant cTnT rises that were not classified as AMI, we also undertook a sensitivity analysis to investigate the ability of admission hs-cTnT level to predict any subsequent cTnT elevation of more than 10 ng/l (the 99th percentile) with the standard assay, regardless of the cause. Secondary outcomes included death (all-cause) and incident AMI (i.e., not including the index event but including any evolving AMI that was identified more than 12 h after initial symptom onset) within 6 months.

Evaluation of hs-cTnT use in clinical practice. In October 2010, we introduced the Roche hs-cTnT assay into clinical practice at our institution. Clinical personnel were asked to draw blood for hs-cTnT testing at the time of presentation and (regardless of the initial level) at least 12 h after symptom onset. After 3 months, we identified all patients who had undergone at least 2 hs-cTnT tests during the same hospital admission since the introduction of the assay. We then calculated the sensitivity, specificity, and positive and negative predictive values of the initial hs-cTnT level for predicting any subsequent hs-cTnT elevation (>14 ng/l) on serial testing.

Statistical analysis. Because hs-cTnT levels showed poor conformity to the normal distribution (p < 0.05, 1-sample Kolmogorov-Smirnov test), nonparametric techniques were used to analyze the data. Data were summarized using the median and interquartile range. We used the Mann-Whitney U test to compare hs-cTnT levels between groups with and without each outcome.

We analyzed the overall diagnostic performance of hs-cTnT by calculating the area under the receiver-operating characteristic curve. The sensitivity and negative predictive value of both tests were calculated at the lower limit of detection of the assay (i.e., the predefined cutoff of 3 ng/l for hs-cTnT) and at the 99th percentile cutoff (14 ng/l for hs-cTnT; 10 ng/l for cTnT). Sensitivities and specificities were compared using McNemar’s test for paired proportions. Statistical analyses were undertaken using SPSS software version 16.0 (SPSS, Inc., Chicago, Illinois), except for calculation of sensitivity, specificity, positive predictive value, negative predictive value, and McNemar’s test, which were undertaken using MedCalc software version 11.3.6.0 (MedCalc Software, Mariakerke, Belgium).

Results

Cohort study. In total, 804 patients consented to inclusion in the cohort study between January 2006 and February 2007, of whom 703 were eligible for inclusion in this analysis (Fig. 1). Baseline characteristics of included patients are shown in Table 1. The patients for whom no serum sample was available (n = 93) were more likely to have a history of hypertension, but were otherwise not significantly different to included patients. Follow-up was complete to 6 months for all 703 patients. The median time from symp-
tom onset to venipuncture was 3.5 h. One hundred thirty (18.5%) patients had an adjudicated diagnosis of AMI. After 6 months, 30 patients (4.3%) had died or had AMI. There were 15 deaths (2.1%) and 21 AMIs (3.0%); 6 patients with AMI died.

For establishing a diagnosis of AMI, the area under the receiver–operating characteristic curve of the initial hs-cTnT level was 0.94 (95% confidence interval [CI]: 0.91 to 0.96), which was significantly greater than that for cTnT (0.86, 95% CI: 0.82 to 0.91, p < 0.0001) (Fig. 2). No patients with an hs-cTnT value of <3 ng/l at the time of presentation had AMI, giving a sensitivity of 100.0% (95% CI: 97.2% to 100.0%) and a negative predictive value 100.0% (95% CI: 98.1% to 100.0%). The sensitivity for excluding AMI with these criteria remained 100.0% (95% CI: 95.1% to 100.0%) even among the 324 (46.1%) patients who had blood drawn within 3 h of symptom onset (Table 2). If this cutoff were used purely to enable early exclusion of AMI in some ED patients, 27.7% of patients could have AMI ruled out at the point of ED presentation. Specificity when using the 3-ng/l cutoff was low (34.0%, 95% CI: 30.2% to 38.1%). Using the conventional 99th percentile cutoff value (14 ng/l), sensitivity for excluding AMI fell, but remained significantly higher than for the initial sample cTnT (85.4% vs 75.2%, difference: 10.2%, p = 0.0002).

During the 6-month follow-up period, 2 (1.0%) patients with an hs-cTnT value of <3 ng/l experienced death or AMI. One of these patients, who initially gave a classical history of crescendo angina, continued to be asymptomatic and underwent inpatient percutaneous coronary intervention within 30 days, which was complicated by periprocedural AMI. The other patient died of noncardiac causes (esophageal carcinoma) within 6 months of presentation.

Using this strategy, patients with hs-cTnT of <3 ng/l at presentation would have had AMI ruled out a median of 9.6 h earlier (interquartile range: 6.0 to 10.8 h). Given that this is a substantial percentage of our population, this would have saved a cumulative total of 1,547 inpatient hours. It also would have avoided some empirical treatment. In this situation, 101 (51.8%) of the patients with hs-cTnT of <3 ng/l received treatment with clopidogrel in the ED and 84 (43.1%) received treatment with low molecular weight heparin.
Sensitivity analysis. For predicting any cTnT elevation of more than 10 ng/l regardless of the cause, the sensitivity of hs-cTnT at the time of presentation using the <3 ng/l cutoff value remained 100.0% (95% CI: 97.8% to 100.0%).

Prospective audit of hs-cTnT in clinical practice. We implemented the hs-cTnT assay in clinical practice on October 6, 2010, and collated the results on January 6, 2011. During that time, a total of 915 patients underwent at least 2 hs-cTnT tests during the same admission. The mean age of these patients was 65.9 years and 528 (57.7%) were male. The diagnostic performance of the initial hs-cTnT level for predicting a subsequent rise of hs-cTnT of more than 10 ng/l regardless of the cause, the sensitivity of the initial hs-cTnT level for predicting a subsequent rise of hs-cTnT of more than 10 ng/l subsequently had an elevation (to 17 ng/l). Thus, by ruling out AMI in patients with initial hs-cTnT of <3 ng/l, we would have avoided the need for serial hs-cTnT testing in 17.5% of patients, at a cost of missing 0.17% of subsequent hs-cTnT elevations. The only patient in whom subsequent hs-cTnT elevation would have been missed sought treatment within 1 h of symptom onset and was admitted to the coronary care unit. The patient was diagnosed with vasovagal syncope but died unexpectedly a week after discharge, with the registered cause of death being ischemic heart disease.

Discussion

This research breaks new ground concerning the ability to use novel, high-sensitivity techniques to facilitate the early exclusion of AMI. Validating the findings presented by Reichlin et al. (12) in a separate investigation, our data from a total of more than 1600 patients demonstrate that, at the lower detection limit, this novel fifth-generation hs-cTnT assay has very high sensitivity at the time of presentation (100.0% [95% CI: 97.2% to 100.0%] and 99.8% [95% CI: 99.1% to 100.0%] in the cohort study and audit, respectively). We also demonstrated that these patients have a low subsequent 6-month event rate. If this strategy were relied on to exclude AMI immediately in practice, our data suggest that as many as 27.7% of patients could be reassured immediately.

Assuming that as many as 15 million patients seek treatment each year with chest pain at EDs (20), the strategy we report could avoid prolonged evaluation, including serial troponin testing, in approximately 4 million patients. This would obviate the need for serial troponin testing, would avoid unnecessary empirical treatment with its consequent risks, would enable clinicians to consider alternative diagnoses at an early stage, and would reduce unnecessary hospital admissions, unburdening EDs and easing overcrowding. Our study did not evaluate the cost-effectiveness of this strategy. There is clearly potential for substantial cost savings, and there is evidently a very low risk of missed AMI. However, a formal assessment of cost savings against the potential costs of missed AMI would be a worthwhile goal for future studies.

The novel high-sensitivity troponin assays are yet to be approved for clinical use in the United States, although the
hs-cTnT assay reported here has been approved and implemented in parts of Europe. There also have been reports of many other highly sensitive assays (21–24). If this approach is applicable to all such novel assays, it would have a profound impact on the ability to evaluate patients rapidly in the ED.

It is unlikely that any paradigm that is proposed will be perfect. Indeed, it should be recognized that the lower bounds of the 95% CIs for sensitivity extend to 97.2% in the cohort study and 99.1% in the audit. Research suggests that physicians will accept this level of risk (25), which may be less risk than in current practice (7,8). However, it is important to remember the potential medical and medicolegal implications of missed diagnoses of AMI. The risk-adjusted mortality rate for patients who are discharged with unrecognized AMI is almost twice as high as for hospitalized patients (8). Further, missed myocardial infarction is a leading cause for malpractice claims in the United States (26). The potential costs of 1 missed diagnosis therefore must be weighed against the costs of hospital admission for further testing, which may lead some to feel uncomfortable with implementing this strategy without further evidence. We currently are undertaking a further prospective validation study to address such concerns. In addition, it is important to acknowledge that troponins are markers of myocardial necrosis. Although our strategy may exclude AMI, some patients with unstable angina without AMI may not be identified. Thus, there will always be a role for careful evaluation of each individual patient.

Others may be concerned that a rapid rule-out protocol will miss AMI in those who seek treatment early and in patients with evolving AMI. The low incidence of adverse events in our study confirms that the risk of missing evolving AMI is small. In our evaluations, the only patient with undetectable initial hs-cTnT who had a subsequent rise sought treatment within 1 h of symptom onset. It therefore may be a prudent precaution to repeat testing after 1 to 2 h in patients seeking treatment so early, although this strategy warrants formal prospective evaluation.

These data, as with all research, inevitably will lead to further important questions. Because high-sensitivity assays will detect many more subtle elevations indicative of cardiac injury, the specificity of hs-cTnT for AMI as compared

<table>
<thead>
<tr>
<th>Time From Symptom Onset (h)</th>
<th>Assay and Cutoff</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>hs-cTnT, 3 ng/l*</td>
<td>100.0 (97.2–100.0)</td>
<td>34.0 (30.2–38.1)</td>
<td>25.6 (21.9–29.6)</td>
<td>100.0 (98.1–100.0)</td>
</tr>
<tr>
<td></td>
<td>hs-cTnT, 1.4 ng/l†</td>
<td>85.4 (78.1–91.0)</td>
<td>82.4 (79.0–85.4)</td>
<td>52.4 (45.4–59.2)</td>
<td>96.1 (94.0–97.7)</td>
</tr>
<tr>
<td></td>
<td>cTnT, 10 ng/l</td>
<td>75.2 (66.8–82.4)</td>
<td>94.6 (92.4–96.3)</td>
<td>75.8 (67.4–82.9)</td>
<td>94.4 (92.2–96.2)</td>
</tr>
<tr>
<td>&lt;3</td>
<td>hs-cTnT, 3 ng/l</td>
<td>100.0 (95.1–100.0)</td>
<td>63.8 (57.2–70.1)</td>
<td>47.7 (39.7–55.9)</td>
<td>100.0 (97.5–100.0)</td>
</tr>
<tr>
<td></td>
<td>hs-cTnT, 14 ng/l</td>
<td>79.7 (68.8–88.2)</td>
<td>83.0 (77.5–87.7)</td>
<td>60.8 (50.4–70.6)</td>
<td>92.5 (88.0–95.8)</td>
</tr>
<tr>
<td></td>
<td>cTnT, 10 ng/l</td>
<td>66.2 (54.3–76.8)</td>
<td>94.6 (90.8–97.2)</td>
<td>80.3 (68.2–89.4)</td>
<td>89.5 (84.8–93.1)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>hs-cTnT, 3 ng/l</td>
<td>100.0 (93.6–100.0)</td>
<td>32.7 (27.8–37.9)</td>
<td>19.2 (14.9–24.3)</td>
<td>100.0 (96.8–100.0)</td>
</tr>
<tr>
<td></td>
<td>hs-cTnT, 14 ng/l</td>
<td>92.9 (82.7–98.0)</td>
<td>82.0 (77.5–85.8)</td>
<td>45.2 (35.9–54.8)</td>
<td>98.6 (93.5–99.6)</td>
</tr>
<tr>
<td></td>
<td>cTnT, 10 ng/l</td>
<td>87.3 (75.5–94.7)</td>
<td>94.5 (91.6–96.7)</td>
<td>71.6 (59.3–82.0)</td>
<td>97.9 (95.8–99.2)</td>
</tr>
<tr>
<td>&lt;6</td>
<td>hs-cTnT, 3 ng/l</td>
<td>100.0 (97.3–100.0)</td>
<td>34.5 (29.7–39.4)</td>
<td>29.3 (24.7–34.3)</td>
<td>100.0 (97.3–100.0)</td>
</tr>
<tr>
<td></td>
<td>hs-cTnT, 14 ng/l</td>
<td>82.9 (74.3–89.5)</td>
<td>82.9 (78.8–86.5)</td>
<td>56.9 (48.6–64.8)</td>
<td>94.7 (91.7–96.8)</td>
</tr>
<tr>
<td></td>
<td>cTnT, 10 ng/l</td>
<td>71.2 (61.5–79.6)</td>
<td>95.3 (92.7–97.2)</td>
<td>80.4 (70.9–88.0)</td>
<td>92.5 (89.4–94.9)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>hs-cTnT, 3 ng/l</td>
<td>100.0 (94.2–100.0)</td>
<td>33.2 (26.5–40.4)</td>
<td>16.7 (11.1–23.6)</td>
<td>100.0 (94.2–100.0)</td>
</tr>
<tr>
<td></td>
<td>hs-cTnT, 14 ng/l</td>
<td>96.0 (79.7–99.9)</td>
<td>81.3 (74.9–86.6)</td>
<td>40.7 (28.1–54.3)</td>
<td>99.4 (96.4–100.0)</td>
</tr>
<tr>
<td></td>
<td>cTnT, 10 ng/l</td>
<td>92.0 (74.0–99.0)</td>
<td>93.0 (88.3–96.2)</td>
<td>63.4 (46.2–79.2)</td>
<td>98.9 (95.9–99.9)</td>
</tr>
</tbody>
</table>

*Rule out cutoff set at the lower limit of detection of the assay. †Cutoff set at the 99th percentile of a reference population. CI = confidence interval; cTnT = cardiac troponin T; hs-cTnT = high-sensitivity cardiac troponin T; NPV = negative predictive value; PPV = positive predictive value.
with other causes of myocardial damage may be lower (11). Some therefore may voice concern that using the lower detection limit as a cutoff will compromise specificity further. However, we should be clear that this is merely a rule-out cutoff value and should not be used to define abnormality. Thus, patients should not be labeled as abnormal at values of less than the 99th percentile of a reference population. For patients with detectable levels of hs-cTnT, AMI cannot be ruled out at the time of presentation; they should undergo further investigation, including further troponin testing. Many of these patients still can have AMI ruled out after subsequent hs-cTnT testing, but some will have AMI. This proposed use of hs-cTnT relies on a good understanding of the concepts ‘SpIn’ (‘Specificity’ rules ‘In’) and ‘SnOut’ (‘Sensitivity’ rules ‘Out’) (27). It bears similarities to our current use of D-dimer for exclusion of venous thromboembolism (28) and the rule-in and rule-out cutoffs suggested for natriuretic peptides in the evaluation of suspected acute decompensated heart failure (29).

It may be possible at some time to develop a strategy to evaluate a larger percentage of patients more rapidly than at 6 to 12 h after symptom onset, but our data do not provide insight into that issue. We have not investigated the added value of serial hs-cTnT estimation in this study. This is the goal of our future studies, to address 2 further important questions: (1) At what level of hs-cTnT can we consider AMI to be ruled in, provided there is a demonstrable rise or fall, or both, in concentrations? (2) For how long must patients undergo serial hs-cTnT estimations to differentiate robustly all patients with AMI from those without AMI?

Study limitations. For our initial analysis, we tested for hs-cTnT in samples that had been stored at −70°C (without freeze-thaw cycles) for approximately 2.5 years. There are currently no data about the stability of the assay under these conditions, which raises the possibility that levels in the cohort study were influenced by sample instability. However, troponin T as measured with this assay is known to be stable for 12 months after storage at −20°C (30), suggesting (but not confirming) that instability is unlikely to be an issue in this study. Further, the findings of our prospective evaluation (where testing was contemporaneous) suggest that sample instability is unlikely to have influenced our results significantly.

Some may be concerned that the analytical imprecision of the assay at this cutoff may lead to inaccurate results. Our findings clearly demonstrate that this does not lead to significant false negative results. However, it is possible that there were some false positive results. This may be why we could exclude only <30% of patients at the time of presentation. By achieving greater analytical sensitivity and precision through future assay development, perhaps this number could be increased still further.

Conclusions
Our findings validate previous work and demonstrate that, at the lower detection limit, hs-cTnT has very high sensitivity for AMI at the time of presentation, with the lower bounds of the 95% confidence intervals being 97.2% and 99.1% in our 2 cohorts. This strategy could enable clinicians immediately to exclude AMI in up to 27.7% of patients. These patients are at low risk of significant adverse events within 6 months. This strategy could be used to obviate the need for serial troponin testing and empirical treatment and to reduce unnecessary hospital admissions. Further validation is warranted to yield tighter confidence intervals and to establish firmly the earliest time point at which AMI may be excluded safely using this novel high-sensitivity assay.

Acknowledgments
The authors thank the staff in the emergency and biochemistry departments at Manchester Royal Infirmary for their assistance, the Manchester Biomedical Research Center for their support, and the Center for Effective Emergency Care at Manchester Metropolitan University for their support.

Reprints requests and correspondence: Dr. Richard Body, Cardiovascular Sciences, 3rd Floor, Core Technology Facility, University of Manchester, 46, Grafton Street, Manchester M13 9WL, United Kingdom. E-mail: richard.body@manchester.ac.uk.

REFERENCES

### Table 3

<table>
<thead>
<tr>
<th>hs-cTnT Cutoff</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ng/l</td>
<td>99.8 (99.1–100.0)</td>
<td>49.5 (43.9–55.1)</td>
<td>78.5 (75.4–81.4)</td>
<td>99.4 (96.6–100.0)</td>
</tr>
<tr>
<td>14 ng/l</td>
<td>98.4 (97.0–99.3)</td>
<td>87.9 (84.0–91.1)</td>
<td>92.8 (90.4–94.7)</td>
<td>97.2 (94.7–98.7)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 2.


