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PO Collinson, DC Gaze, P Thokala and S Goodacre

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PO Collinson,* DC Gaze, P Thokala and S Goodacre

Clinical Blood Sciences Laboratory, St George's Hospital, London, UK

*Corresponding author

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Editorial contact: nihredit@southampton.ac.uk

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Abstract

Randomised Assessment of Treatment using Panel Assay of Cardiac markers – Contemporary Biomarker Evaluation (RATPAC CBE)

PO Collinson,* DC Gaze, P Thokala and S Goodacre

Clinical Blood Sciences Laboratory, St George's Hospital, London, UK

*Corresponding author paul.collinson@stgeorges.nhs.uk

Objectives: To test the diagnostic accuracy for detecting an acute myocardial infarction (AMI) using highly sensitive troponin assays and a range of new cardiac biomarkers of plaque destabilisation, myocardial ischaemia and necrosis; to test the prognostic accuracy for detecting adverse cardiac events using highly sensitive troponin assays and this range of new cardiac biomarkers; and to estimate the cost-effectiveness of using highly sensitive troponin assays or this range of new cardiac biomarkers instead of an admission and 10- to 12-hour troponin measurement.

Design: Substudy of the point-of-care arm of the RATPAC (Randomised Assessment of Treatment using Panel Assay of Cardiac markers) trial.

Setting: The emergency departments of six hospitals.

Participants: Prospective admissions with chest pain and a non-diagnostic electrocardiogram randomised to point-of-care assessment or conventional management.

Interventions: Blood samples taken on admission and 90 minutes from admission for measurement of cardiac markers [cardiac troponin I (cTnI), myoglobin and creatine kinase MB isoenzyme (CK-MB)] by point-of-care testing. An additional blood sample was taken at admission and 90 minutes from admission for analysis of high-sensitivity cTnI (two methods) and cardiac troponin T (cTnT), myoglobin, heart-type fatty acid-binding protein (H-FABP), copeptin and B-type natriuretic peptide (NTproBNP).

Main outcome measures: 1. Diagnostic accuracy compared with the universal definition of myocardial infarction utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory. 2. Ability of biomarker measurements to predict major adverse cardiac events (death, non-fatal AMI, emergency revascularisation or hospitalisation for myocardial ischaemia) at 3 months' follow-up. 3. Comparison of incremental cost per quality-adjusted life-year (QALY) of different biomarker measurement strategies for the diagnosis of myocardial infarction.

Results: Samples were available from 850 out of 1132 patients enrolled in the study. Measurement of admission myoglobin [area under the curve (AUC) 0.76] and CK-MB (AUC 0.84) was diagnostically inferior and did not add to the diagnostic efficiency of cTnI (AUC 0.90–0.94) or cTnT (AUC 0.92) measurement on admission. Simultaneous measurement of H-FABP and cTnT or cTnI did improve admission diagnostic sensitivity to 0.78–0.92, but only to the same level as that achieved with troponin measured on admission and at 90 minutes from admission (0.78–0.95). Copeptin (AUC 0.62) and NTproBNP (AUC 0.85) measured on admission were not useful as diagnostic markers. As a prognostic marker, troponin measured on

admission using a high-sensitivity assay (AUC 0.73–0.83) was equivalent to NTproBNP measurement (AUC 0.77) on admission, but superior to copeptin measurement (AUC 0.58). From modelling, 10-hour troponin measurement is likely to be cost-effective compared with rapid rule-out strategies only if a £30,000 per QALY threshold is used and patients can be discharged as soon as a negative result is available.

Conclusions: The measurement of high-sensitivity cardiac troponin is the best single marker in patients presenting with chest pain. Additional measurements of myoglobin or CK-MB are not clinically effective or cost-effective. The optimal timing for measurement of cardiac troponin remains to be defined. Copeptin measurement is not recommended. H-FABP requires further investigation before it can be recommended for simultaneous measurement with high-sensitivity troponin in patients with acute chest pain.

Trial registration: ISRCTN37823923.

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Contents

Glossary

Area under the curve The calculated integral underneath a plot of test sensitivity against (1 – test specificity).

Biomarker A metabolic intermediary molecule, signal molecule, enzyme or protein that significantly changes in response to a disease state and can be used to monitor onset or progress of disease, or predict outcome in response to treatment.

Cost-effectiveness acceptability curve A way of illustrating cost-effectiveness results by plotting the probability that the intervention is cost-effective (*y*-axis) against the maximum that society is willing to pay for an improvement in health (*x*-axis).

Cost-effectiveness plane A way of illustrating cost-effectiveness results by plotting the mean incremental cost and effectiveness on a four-quadrant graph. Interventions that are more costly and more effective fall in the north-east quadrant.

Dalton Unit of atomic mass.

False-negative A test result erroneously indicating that a patient with a condition does not have that condition.

False-positive A test result erroneously indicating that a patient without a condition does have that condition.

Incremental cost-effectiveness ratio The difference in costs between one intervention and an alternative, divided by the difference in outcomes.

Likelihood ratio Describes how many times more likely a person with a disease is to receive a particular test result than a person without the disease. A likelihood ratio of a positive test result is usually a number > 1; a likelihood ratio of a negative test result usually lies between 0 and 1.

Quality-adjusted life-year A measure of the benefit of health care combining the impact of both expected length of life and quality of life.

Receiver operating characteristic curve Graphical representation of the relationship between 'true-positive fraction' (sensitivity) and 'false-positive fraction' (1 – specificity). It displays the trade-offs between sensitivity and specificity as a result of varying the cut-off value for positivity in case of a continuous test result.

Reference standard Established test(s) against which the accuracy of a new test for detecting a particular condition can be evaluated.

Sensitivity (true-positive rate) The proportion of individuals with the target condition in a population who are correctly identified by a diagnostic test.

Specificity (true-negative rate) The proportion of individuals free of the target condition in a population who are correctly identified by a diagnostic test.

Test accuracy The proportion of test results that are correctly identified by the test.

True-negative A test result correctly identifying that a patient without a condition does not have that condition.

True-positive A test result correctly identifying that a patient with a condition has that condition.

List of abbreviations

ACS	acute coronary syndrome
AM	adrenomedullin
AMI	acute myocardial infarction
ANP	atrial natriuretic peptide
AUC	area under the curve
AVP	arginine vasopressin
BNP	B-type natriuretic peptide
CK	creatine kinase
CK-MB	creatine kinase MB isoenzyme
CRP	C-reactive protein
cTnI	cardiac troponin I
cTnT	cardiac troponin T
CTU	Clinical Trials Unit
CV	coefficient of variation
ECG	electrocardiogram
ED	emergency department
EDTA	ethylenediaminetetraacetic acid
FABP	fatty acid-binding protein
GDF15	growth differentiation factor 15
GRACE	Global Registry of Acute Coronary Events
H-FABP	heart-type fatty acid-binding protein
HTA	Health Technology Assessment
ICER	incremental cost-effectiveness ratio
IL	interleukin
IL-1	interleukin 1
IL-1β	interleukin 1 beta
IL-33	interleukin 33
IL-6	interleukin 6
IL-6R	interleukin-6 receptor
IMA	ischaemia-modified albumin
IQR	interquartile range
MACE	major adverse cardiac event
MMP	matrix metalloproteinase
MPO	myeloperoxidase
NICE	National Institute for Health and Care Excellence
NSTEMI	non-ST-segment elevation myocardial infarction
NTproBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
PAPP-A	pregnancy-associated plasma protein A
QALY	quality-adjusted life-year
RATPAC	Randomised Assessment of Treatment using Panel Assay of Cardiac markers
RATPAC-CBE	Randomised Assessment of Treatment using Panel Assay of Cardiac markers – Contemporary Biomarker Evaluation
RCV	relative change value
ROC	receiver operating characteristic
sIL-6R	soluble interleukin-6 receptor
STEMI	ST-segment elevation myocardial infarction
TIMP	tissue inhibitor of metalloproteinase

TnC	troponin C	TnT	troponin T
TnI	troponin I	WHO	World Health Organization

All abbreviations that have been used in this report are listed here unless the abbreviation is well known (e.g. NHS), or it has been used only once, or it is a non-standard abbreviation used only in figures/tables/appendices in which case the abbreviation is defined in the figure legend or at the end of the table.

Executive summary

Background

Patients admitted with chest pain and a suspected diagnosis of acute coronary syndrome (heart attack or unstable angina, usually referred to as ACS) constitute the largest single group of individuals attending a hospital emergency department. The majority of such individuals do not have a final diagnosis of coronary artery disease and are retained in hospital unnecessarily. Conversely, a proportion of patients attending with chest pain have had a heart attack (acute myocardial infarction, AMI) and are inappropriately sent home. For the clinician the challenge is to identify those patients at the highest risk of having had an AMI for further investigation and to discharge home those at low risk.

The diagnosis of an AMI is based on three factors: the history and clinical features of the patient, the findings on performing an electrocardiogram (ECG) and the results of laboratory investigations (measurement of cardiac biomarkers). History and clinical features can be incorporated into formal risk-scoring methods. Risk-scoring systems have limited diagnostic efficiency in patients attending the emergency department with chest pain. In the majority of patients, the ECG may be entirely normal or ECG changes are non-specific. The ECG is useful only if there are typical features that support the diagnosis of an AMI, such as ST-segment elevation or changes suggestive of ACS. These patients constitute a high-risk group and require admission and further investigation. In the majority of patients the ECG does not show a high-risk pattern and laboratory investigation is required to determine whether or not an AMI has occurred. The key test for diagnosis of an AMI is the measurement of one of the cardiac structural proteins of the heart, cardiac troponin. There are two cardiac troponins, cardiac troponin T (cTnT) and cardiac troponin I (cTnI). Elevation of cTnT or cTnI is absolutely specific for myocardial injury and is included as the gold standard biochemical test in the universal definition of myocardial infarction. Current guidelines recommend keeping patients in hospital for up to 12 hours for repeat measurement of cTnT or cTnI to confirm or exclude the diagnosis of an AMI.

To speed up diagnosis it has been suggested that other biomarkers, said to be more sensitive in the early phases of an AMI, might be combined with cTnT or cTnI measurement. Other proteins present within the myocardial cell cytoplasm – cytoplasmic markers – may be released into the circulation earlier than troponin following myocardial injury. An alternative strategy would be to measure hormones affected by myocardial injury (neurohormones). Neurohormones are produced either directly by the heart [B-type natriuretic peptide (BNP)] or in response to circulatory stress (copeptin). Finally, it has been suggested that, as the formation of an atheromatous plaque is the underlying cause of an AMI, novel markers which indicate that an individual is at high risk for rupture of an atheromatous plaque might also be useful. The strategy that has been proposed is the combined measurement of existing or new markers on admission to hospital. The hypothesis is that, if neither troponin nor one of the novel markers is elevated, the patient could be immediately discharged from hospital safely.

The concept of measurement of a number of different biomarkers, in addition to troponin, is based on the apparent inability to detect troponin elevation soon after myocardial infarction has occurred. Failure to detect early troponin rise was due to the relative insensitivity of the methods for troponin measurement in use. This is no longer the case. There has been progressive improvement in the laboratory methodology for cTnT and cTnI measurement and methods are now highly sensitive. The role of such sensitive troponin measurement methods is in the process of being evaluated with a view to their widespread introduction into clinical practice. Preliminary evidence suggests that these new sensitive methods will detect troponin elevation very early after an AMI has occurred, possibly at the time of hospital admission.

The progressive improvement in the sensitivity of troponin measurement methods has been accompanied by an increase in the number of clinical conditions, apart from an AMI, in which measurable troponin elevation occurs. This varies from more obvious clinical conditions, such as direct myocardial injury from trauma caused by road traffic accidents or stabbing, to more subtle injury, such as ingesting cocaine or pulmonary embolus. Concerns have, therefore, been expressed that the widespread use of sensitive troponin assays will result in an increase in the number of patients inappropriately retained in hospital for investigation for suspected cardiac disease.

The role of additional markers of myocardial injury and sensitive troponin measurements for the differential diagnosis of the patient presenting with chest pain remains an area of ongoing study. The challenge is to reduce the time within which a definitive diagnosis can be obtained, which will ensure prompt discharge from hospital of patients without an AMI while retaining only those patients at high risk of cardiac disease for further investigation and treatment. However, measurement of additional biomarkers in addition to troponin measurement alone has an increased cost and so would have to be cost-effective.

Objectives

The objective of the study was to examine the role of combinations of existing laboratory tests for the diagnosis of an AMI together with a range of new tests that have been proposed for this role. In addition, the role of the newer, sensitive troponin assays compared with more conventional but less sensitive assays would be studied.

The particular questions to be answered were:

1. Should troponin measurement be combined with measurement of two well-established cytoplasmic markers of myocardial injury, myoglobin and the MB isoenzyme of creatine kinase (CK-MB), to achieve an earlier diagnosis than that recommended in current guidelines?
2. Should troponin measurement be combined with measurement of new markers said to be very sensitive for myocardial infarction and an already existing marker, N-terminal pro-B-type natriuretic peptide (NTproBNP), to achieve an earlier diagnosis than that recommended in current guidelines?
3. Is there any diagnostic advantage to using the newer, more sensitive methods for troponin measurement rather than the already well-established troponin measurement methods?
4. How good were both the established and new cytoplasmic markers and the newer troponin measurement methods at predicting risk of a major adverse cardiac event (MACE) over the follow-up period to allow early, safe discharge of patients admitted with chest pain but considered not to have had an AMI?
5. What would be the cost-effectiveness of measuring a combination of biomarkers compared with measurement of cardiac troponin alone?

Methods

The population studied was recruited as part of a multicentre trial comparing point-of-care testing with conventional hospital management of patients with chest pain. This clinical trial, the Randomised Assessment of Treatment using Panel Assay of Cardiac markers (RATPAC), has been reported in full and was performed at six emergency departments in hospitals throughout the UK. Patients were recruited to the trial if they had chest pain but no clinical or ECG evidence of an AMI and would be admitted for exclusion of an AMI by the measurement of cTnT or cTnI according to current guidelines. All patients who consented and did not meet trial exclusion criteria were randomised to measurement of a panel of cardiac biomarkers by point-of-care testing on admission and 90 minutes from admission or the conventional pathway in the participating hospital for management of chest pain. Patients randomised to point-of-care testing had additional blood samples taken on admission and 90 minutes from admission that were separated

and frozen for subsequent analysis. A protocol was used to interpret the point-of-care testing results for cTnI, myoglobin and CK-MB. Patients showing an elevation of any of these markers consistent with AMI were admitted to hospital. Failure of the markers to rise by 90 minutes from admission was considered to exclude an AMI. The subsequent decision by the attending physician to admit or discharge the patient was on the basis of the results of point-of-care testing plus clinical features. RATPAC was a pragmatic clinical trial with the objective of comparing the management of patients for whom test results were available by point-of-care testing on admission and at 90 minutes from admission with the management of those managed conventionally. The RATPAC study found that patients randomised to point-of-care testing were discharged earlier than those managed conventionally, with an equivalent rate of MACE during the follow-up period. Blood samples from patients randomised to the point-of-care testing arm of the trial were available for further study. Patient recruitment was prospective, but subsequent biomarker analysis was retrospective. The patients had been fully characterised and followed up so provided an ideal cohort for assessment of the role of existing and novel cytoplasmic markers of myocardial injury, the role of sensitive troponin measurement methods and the role of neurohormones for the diagnosis and prognostic risk assessment of patients admitted with chest pain.

The population was representative of the patients seen in routine clinical practice. The population studied did not include patients at high risk as determined by ECG changes characteristic of an AMI. Biochemical laboratory tests are not required for this group as they have a presumptive diagnosis of an AMI (ST-segment elevation myocardial infarction, STEMI) that requires immediate hospital admission. Patients with STEMI are often inappropriately included in diagnostic studies of laboratory testing. In addition, the study excluded patients with ECG changes that would automatically suggest that myocardial injury was a high probability, patients with a very high risk of ACS who would also be admitted to hospital.

An extensive and detailed literature review was performed of the existing evidence for both current and novel biomarkers for the detection of myocardial injury. In choosing the tests to be evaluated it was important that the existing sample was appropriate for the analysis to be performed. The method selected also needed to have the potential for automation and introduction into routine clinical practice. Ideally, an automated method would already be available. Finally, the review of the literature needed to show that there was consistent evidence that the biomarker might be useful in routine clinical practice. The final choice of biomarkers for measurement was as follows. Two existing cytoplasmic biomarkers were selected as a reference standard. These were CK-MB by mass measurement, as these data were already available from the RATPAC study, and myoglobin, both from the original RATPAC study data and using a different method. Myoglobin is considered the prototype for a rapid release cytoplasmic marker and could be measured by a multiplex technique without further sample loss. As a novel cytoplasmic biomarker, heart-type fatty acid-binding protein (H-FABP) was measured simultaneously with myoglobin (Randox laboratories, Crumlin, Co Antrim, UK) using the same sample. Data from one conventional troponin assay used in the original RATPAC study – cTnI measured on the Stratus® CS analyser (Siemens Healthcare Diagnostics, Camberley, Surrey, UK) – were available. Three contemporary sensitive troponin measurement methods were studied: measurement of cTnI using the Siemens Ultra assay (Siemens Healthcare Diagnostics, Camberley, Surrey, UK) and the Beckman AccuTnI™ enhanced assay (Beckman-Coulter, High Wycombe, Buckinghamshire, UK) and measurement of cTnT using a new high-sensitivity assay (Roche Diagnostics, Burgess Hill, West Sussex, UK). The novel marker copeptin (B·R·A·H·M·S ThermoFisher, Cambridge, UK) was measured together with NTpro-BNP (Roche Diagnostics, Burgess Hill, West Sussex, UK). The literature review suggested that there was insufficient evidence to support the measurement of potential markers of plaque destabilisation for use as a diagnostic test in patients with acute chest pain or that the sample collected would be suitable.

The final diagnosis on all patients studied was performed by two independent reviewers, who examined the original diagnosis from the RATPAC study, all of the available clinical information and the results of cardiac troponin measurement from the original trial sites and that performed in the core clinical laboratory. Diagnosis utilised the universal definition of myocardial infarction based on the 99th percentile of the troponin method in use at the trial sites combined with troponin measurement on the study

samples performed using the Siemens Ultra assay, as this is known to achieve the performance criteria recommended for sensitive troponin assay. Patients with a troponin rise and a final diagnosis other than ACS or an AMI were reviewed in detail and it was decided whether or not an AMI was the most likely diagnosis. Disagreements were resolved by discussion and patients were classified as having an AMI or not.

The diagnostic performance of the biomarkers was examined using two different techniques. Individual biomarkers were assessed by construction of receiver operating characteristic (ROC) curves, a continuous plot of sensitivity against specificity utilising the final diagnosis as the classifier. Statistical analysis was by comparison of the area under the curve (AUC). Individual biomarkers and the biomarker combinations were then examined using prespecified diagnostic thresholds to classify patients into those with or those without an AMI. Patients with one biomarker value exceeding the threshold were classified as an AMI. When a combination of biomarkers was used, any one biomarker value exceeding the threshold was considered to classify the patient as having had an AMI. Comparison between strategies was then performed by construction of 2×2 tables and comparison by Fisher's exact probability test. Statistical analysis was performed using a commercially available Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) add-in, Analyse-it (version 2.2.1; www.analyse-it.com).

A decision-analysis model developed for another project [HTA 09/22/21: Goodacre S, Thokala P, Carroll C, Stevens JW, Leaviss J, Al Khalaf M, *et al*. Systematic review, meta-analysis and economic modelling of diagnostic strategies for suspected acute coronary syndrome. *Health Technol Assess* 2013;**17**(1)] was used to evaluate the cost-effectiveness of a 10-hour troponin strategy and the most promising early biomarker strategies identified in this study. The model applied diagnostic strategies to a hypothetical cohort of patients with a suspected AMI to determine the costs and outcomes associated with each strategy. We tested the model in three different scenarios, depending on the availability of doctors to act on 10-hour troponin results. Cost and utility estimates were derived from previous studies and routine data sources. The economic model was developed using Simul8 software (Simul8 Corporation, Boston, MA, USA), taking a health service perspective and a lifetime horizon with mean life expectancy based on UK interim life tables. Deterministic and probabilistic analyses were undertaken.

Results

Samples were obtained from 850 out of 1132 patients enrolled in the RATPAC study. Measurement of the conventional cytoplasmic biomarkers myoglobin and CK-MB did not significantly improve diagnostic sensitivity compared with measurement of cTnT or cTnI by any of the methods examined. Measurement of cTnT and cTnI was a significantly better outcome predictor than measurement of the conventional cytoplasmic biomarkers. As there is no diagnostic efficiency gained from measurement of myoglobin and CK-MB in addition to troponin, simultaneous measurement of all three markers would not be a cost-effective strategy. Measurement of H-FABP and troponin using a high-sensitivity assay did improve diagnostic sensitivity compared with measurement of troponin alone using a high-sensitivity assay. However, this was equivalent to measurement of troponin on admission and at 90 minutes from admission. Combined measurement on admission of both H-FABP and troponin does not achieve 100% sensitivity for rule-out of an AMI on admission testing. Measurement of copeptin was not useful as a diagnostic or prognostic test, so by cost minimisation was not cost-efficient.

Cost-effectiveness analysis compared the following strategies: no testing, high-sensitivity cTnT testing at presentation, high-sensitivity cTnT testing at presentation and at 90 minutes from presentation, high-sensitivity cTnT and H-FABP testing at presentation, and 10-hour troponin testing. At the £20,000 per quality-adjusted life-year (QALY) threshold, 10-hour troponin testing was cost-effective (£12,090 per QALY) if the patient can be discharged as soon as a negative troponin result is available (doctor-on-demand scenario) but not in the other scenarios (once-daily ward round and twice-daily ward rounds), when high-sensitivity cTnT and H-FABP measurement at presentation was cost-effective. At the £30,000 per QALY threshold, 10-hour troponin testing was cost-effective in the doctor-on-demand scenario and

twice-daily ward rounds scenario (£24,600 per QALY), whereas the troponin T and H-FABP measurement at presentation strategy was cost-effective (£14,806 per QALY) in the once-daily ward round scenario. Secondary analysis using cTnI (measured on the Stratus CS) instead of cTnT showed that cTnI testing at presentation and at 90 minutes was cost-effective in all three scenarios at the £20,000 per QALY threshold and in two of the scenarios at the £30,000 per QALY threshold, with 10-hour troponin being cost-effective only in the doctor-on-demand scenario at the £30,000 per QALY threshold (£24,327 per QALY).

Conclusions

Measurement of cardiac troponin using a sensitive method was the best test for the early diagnosis of an AMI. Although the study showed that diagnosis 90 minutes from admission was safe, 100% diagnostic sensitivity was not achieved at that time point and further studies are required to determine the optimal earliest time point when acceptable diagnostic sensitivity can be obtained. Measurement of myoglobin or CK-MB in addition to a sensitive troponin test is not recommended. H-FABP shows promise as an early marker and requires further study. Measurement of copeptin is not recommended as a routine test in patients presenting with acute chest pain. Ten-hour troponin testing is likely to be cost-effective compared with rapid rule-out strategies only if patients can be discharged as soon as a negative result is available and a £30,000 per QALY threshold is used.

Trial registration

This trial is registered as ISRCTN37823923.

Funding

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Chapter 1 Background

DESCRIPTION OF THE HEALTH PROBLEM

Introduction

Patients with chest pain constitute the largest single category of patients admitted to hospitals in the UK.[1] They are also diagnostically challenging. The majority of patients admitted have either stable ischaemic heart disease or no ischaemic heart disease.[2] Such admissions are often short and clinically inappropriate. Conversely, it has been estimated that 2–7% of patients with an acute myocardial infarction (AMI) are inappropriately discharged from the emergency department.[3,4] Attempts to improve diagnosis have included risk-scoring systems,[5] computerised decision support[6,7] and automated electrocardiographic interpretation.[8] Although clinical assessment remains integral to the assessment of patients with chest pain, biomarker measurement has become an essential component.

Current service provision

Development of biomarker measurement in patients with chest pain and suspected acute coronary syndrome

The development of immunoassays for cardiac-specific proteins resulted in a paradigm shift in the role of biomarker measurement in patients presenting with acute chest pain.[9–11] Before the development of immunoassays for the cardiac troponins, cardiac troponin T (cTnT) and cardiac troponin I (cTnI), biomarker measurement had been used for retrospective confirmation and was limited by the lack of cardiospecificity of the biomarkers. Diagnosis was orientated towards clinical features and the electrocardiogram (ECG), despite the known limitations of the ECG. The use of rapid serial measurement of creatine kinase (CK) improved the timeliness of diagnosis,[12,13] and measurement of the MB isoenzyme of CK (CK-MB) improved cardiospecificity, especially when mass rather than activity measurements were introduced.[14,15] The development of rapid assay techniques and the use of serial measurements of CK and CK-MB improved both speed and diagnostic sensitivity. The diagnosis of an AMI was limited only by the ability to detect a significant change between consecutive measurements, the relative change value (RCV). The RCV is determined by the assay imprecision (typically $<5\%$) and the intra-individual biological variation of the marker. Over short time frames (2–6 hours) this intra-individual variation is small.[12,14] Diagnosis within 4–12 hours of admission was possible and diagnostic strategies were developed for early rule-out of an AMI and early discharge of patients when significant ischaemic heart disease could be excluded.[16–20]

Measurement of cTnT[21] and cTnI[22,23] was initially introduced as a totally cardiospecific marker to replace CK and CK-MB mass measurement, with diagnosis based on equivalence to existing World Health Organization (WHO) criteria for an AMI. Early studies of the diagnostic efficiency of cardiac troponin showed that, when the diagnosis of an AMI was based on WHO criteria utilising CK[24] or CK-MB[25–28] measurements, troponin measurement showed excellent diagnostic sensitivity. Specificity was variable with values from 46% to 98% reported in different studies. The paradigm shift occurred when studies were performed that examined not diagnostic efficiency but independent measures such as the major adverse cardiac events (MACE) of death, myocardial infarction, readmission with unstable angina or need for urgent revascularisation. Outcome studies demonstrated that patients with a final diagnosis that excluded an AMI on WHO diagnostic criteria based on clinical and ECG findings and measurement of CK[29] or CK-MB[30–32] (hence a final diagnosis of unstable angina), but with detectable cTnT or cTnI, had a significantly higher incidence of a MACE. The finding of elevated cTnT and increased rate of MACE in patients with a WHO diagnosis of unstable angina was a consistent observation and confirmed by

meta-analysis.[33] The subsequent redefinition of myocardial infarction with troponin as the preferred biomarker represented the acceptance that measurement of cTnT or cTnI is the biochemical arbiter of myocardial injury and a prerequisite for the diagnosis of myocardial infarction.[9–11]

The clinical need for other biomarkers of cardiomyocyte necrosis

The first generation of cardiac troponin assays were relatively insensitive.[34] Comparison of the time course of release of markers of myocardial necrosis suggested that cytoplasmic markers such as myoglobin, CK and CK-MB were released earlier than cardiac structural proteins such as cTnT and cTnI.[9] The apparent earlier release of cytoplasmic markers had two practical clinical consequences. First, the consensus statement on the use of biomarkers in patients for the diagnosis of an AMI, which contributed to the redefinition of AMI, recommended blood sampling at 10–12 hours from admission to achieve optimal diagnostic sensitivity.[9,35] Second was a recommendation that measurement of a cytoplasmic marker in the early time period following chest pain admission should be used to cover the period of diagnostic insensitivity of cTnT and cTnI, the time period of 'troponin blindness'.[35] A practical consequence of the latter was the recommendation of measurement of myoglobin and/or CK-MB as early markers with measurement of cTnT and cTnI at 6–9 and 12–24 hours. The combined biomarker strategy led to the production of point-of-care diagnostic devices incorporating panels of markers to cover the different time windows.[36] Hence, myoglobin for early sensitivity, CK-MB for early sensitivity and specificity and cardiac troponin for specificity. Progressive improvement of troponin assays showed comparable sensitivity of troponin and myoglobin for early diagnosis, challenging the troponin blindness concept,[37] whereas the advent of sensitive troponin assays suggests that troponin may be detectable in the early stages of myocardial necrosis before myoglobin.[38]

The role of biomarkers in the diagnosis and management of the chest pain patient

The measurement of cardiac biomarkers combined with clinical symptoms and the ECG forms part of the assessment of the patient with chest pain, but the different diagnostic modalities are used in different ways. In the patient presenting with chest pain and ST-segment elevation on the ECG, management is by revascularisation by thrombolysis or preferably by percutaneous coronary intervention and stent placement.[39,40] In this patient group, the role of measurement of cardiac biomarkers is to provide confirmation of diagnosis for audit of diagnostic accuracy and to provide a degree of quantitation of infarct size.[41] In patients presenting without definitive ECG changes, measurement of cardiac biomarkers is crucial to subsequent management. Elevation of cardiac troponin confirms non-ST-segment elevation myocardial infarction (NSTEMI) and defines subsequent management, including therapy with antiplatelet agents and subsequent angiography.[42] As the majority of patients presenting with chest pain do not have a final diagnosis of NSTEMI, the objective is to achieve diagnosis as rapidly as possible.

The role of risk stratification in chest pain diagnosis

The clinical decisions that are required in the assessment of the chest pain patient are different according to whose perspective is taken – that of emergency department clinician or that of the cardiologist. The emergency department clinician is most concerned with the early exclusion of a myocardial infarction – rule-out of a myocardial infarction – whereas the cardiologist is most concerned with confirmation of myocardial infarction – rule-in of a myocardial infarction. In patients with a diagnostic ECG [i.e. ST-segment elevation myocardial infarction (STEMI) patients] there will be immediate assessment and transfer to the coronary care unit. Patients not showing characteristic ECG changes are assessed on the basis of the ECG and clinical features into high-, medium- and low-risk groups. High-risk patients are those with clinical or ECG evidence suggestive of myocardial ischaemia. Such patients will be admitted and further investigated. Medium- or low-risk patients are those without clinical or ECG evidence of ischaemia who require biomarker measurement to exclude an AMI. Confirmation of myocardial infarction requires measurement of troponin levels at a time point when 100% diagnostic sensitivity is obtained. On the basis of previous studies a measurement at 12–24 hours was considered appropriate, and current guidelines recommend 6–9 and 12 hours. Utilising a more sensitive assay should bring this time window forward.[43]

In addition to ruling in or ruling out myocardial infarction, it has been proposed that measurement of biomarkers of myocardial necrosis could be combined with measurement of other markers to allow either earlier diagnosis or risk stratification. Earlier diagnosis is based on the concept that myocardial injury will affect myocardial function. Impaired myocardial function could then be assessed either directly by measurement of biomarkers of myocardial function[44] or indirectly by measurement of biomarkers of circulatory stress.[45] The understanding of atherothrombotic disease as a disease of plaque rupture[46,47] and the appreciation of the role of plaque instability[48] was a paradigm shift in the understanding of the pathophysiology of acute ischaemic heart disease. The measurement of risk stratification biomarkers that would reflect plaque instability is therefore attractive.[49] The idea is that these markers would define high-risk groups requiring further investigation and low-risk groups who could be promptly and safely discharged.

Description of technologies for consideration for this assessment

Biomarkers for the differential diagnosis of the patient presenting with chest pain may be considered under the following categories.

Markers of cardiomyocyte necrosis

Cytoplasmic markers

Myoglobin

Myoglobin (molecular weight 16.7 kDa) is a single-chain globular protein containing a haem prosthetic group and is the primary oxygen-carrying pigment of muscle tissues. It is found in the cytoplasm and this, combined with its low molecular weight, means that it would theoretically be released earlier than other cytoplasmic biomarkers following myocyte necrosis. Initial studies showed that this was the case and myoglobin measurement has been proposed as an early marker for an AMI.[50–52] Comparison with the kinetics and cardiospecificity of other markers suggested that myoglobin measurement could be combined with other cardiac biomarkers in a panel for early diagnosis of an AMI,[53–55] especially in the setting of point-of-care testing.[36,56–58]

Creatine kinase MB isoenzyme

Creatine kinase MB isoenzyme is the more cardiac-specific isoenzyme of CK. It is found in the cytoplasm and comprises 5–50% of the CK found in the myocardium. CK was one of the earliest cardiac biomarkers used for the biochemical detection of an AMI.[59–61] Although initially measured by immunoinhibition, mass assays for CK-MB were developed and automated and form the basis of current methodology.[62–64] Measurement of CK-MB mass is the most established biomarker of an AMI and is still recognised in the universal definition of myocardial infarction; the only perceived advantage is an earlier rise of CK-MB than of cardiac troponin.[15]

Fatty acid-binding protein

Fatty acid-binding proteins (FABPs) are relatively small (15 kDa) proteins of 126–137 amino acids present in tissues with an active fatty acid metabolism, such as heart, liver and intestine. They reversibly bind long-chain fatty acids to facilitate their intracellular translocation. Nine distinct FABP types have been identified. Each type has a characteristic pattern of tissue distribution and a stable intracellular half-life of 2–3 days.[65] The myocardial isoform, heart-type fatty acid-binding protein (H-FABP; 132 amino acids), is present predominantly in the heart, but is also found in other tissues including skeletal muscle and the distal tubal cells in the kidney. A number of studies have examined the potential role of H-FABP in the diagnosis of myocardial infarction.[66–68] The interest is that H-FABP may be an early cytoplasmic marker of myocardial ischaemia and myocardial injury.

TABLE 1 Summary of H-FABP studies and results

Study	H-FABP			cTnT	
	Sensitivity (%)	Specificity (%)	H-FABP assay	Sensitivity cTnT (%)	cTnT assay
McCann 2008,[69] McCann 2009[70]	73	71	ELISA	55	Fourth-generation cTnT
Gururajan 2010[71]	87	93	ELISA	54	Abbott Diagnostics, Maidenhead, Berkshire
Garcia-Valdecasas 2011[72]	81	53	?	Not stated	Unknown
Body 2011[73]	75	89	ELISA	42	Unknown

ELISA, enzyme-linked immunosorbent assay.

Studies on H-FABP have concentrated on its potential as a very early marker when combined with troponin (*Table 1*). When compared with a conventional troponin assay, measurement of H-FABP was found to provide additional diagnostic sensitivity for early presentation.[69,71,72] However, the reported sensitivity of the cardiac troponin assays are low and the specificity of H-FABP is also low. Two meta-analyses have suggested that H-FABP does not meet the criteria for an early diagnostic test.[74,75] H-FABP has been shown to be a prognostic marker in patients with chest pain and suspected acute coronary syndrome (ACS).[70,76,77]

Studies using contemporary high-sensitivity troponin assays have suggested that there is no additional value of H-FABP measurement.[78–81] Three of these studies used a sensitive H-FABP assay.[79–81]

Cardiac structural proteins

Cardiac troponins

The cardiac troponins form part of the cardiac contractile apparatus, the troponin–tropomyosin complex. This is found within the sarcomere of all types of striated muscle but not in smooth muscle. The troponin–tropomyosin complex acts to regulate muscular contraction and comprises three troponins: troponin C (TnC;18 kDa), troponin I (TnI; 22 kDa) and troponin T (TnT; 37 kDa), plus tropomyosin. There are three isoforms of TnT and TnI, found in cardiac muscle, fast-twitch muscle and slow-twitch muscle, encoded by individual genes. There is only one isoform of TnC, which is common to all types of muscle. The cardiac isoforms of troponin, cTnT (chromosome 1q32) and cTnI (chromosome 19q13.3), have unique sequences and hence unique amino acid compositions and structures. After development of the preliminary immunoassays for cTnT[21] and cTnI,[23,82] measurement of both troponins was introduced into routine clinical practice and cardiac troponin became the recommended biomarker for diagnosis of an AMI.[9,11,83] Progressive assay improvement[34] has now resulted in the ability to measure cardiac troponin in normal populations, producing the current generation of high-sensitivity troponin assays.[38,84]

Myocardial function markers

Natriuretic peptides

The natriuretic peptides form a family of phylogenetically highly conserved bioactive peptides that have effects on sodium and water balance. These effects may be systemic, autocrine or paracrine or a combination of all three depending on the type of natriuretic peptide. Three natriuretic peptides are found in humans. Atrial natriuretic peptide (ANP) is found in storage granules in the atria and release occurs in response to changes in vascular pressure. B-type natriuretic peptide (BNP; 3.5 kDa), originally known as brain-type natriuretic peptide, is found in both atria and ventricles and is produced in response to tension in the atrial and ventricular walls. C-type natriuretic peptide (CNP) is produced by the endothelial cells as

a vasodilator. Currently, routine measurement of BNP is performed and, recently, a method for measuring ANP that may be suitable for routine clinical use has been developed.[85]

B-type natriuretic peptide is not stored, but undergoes continuous transcription and translation. Increases in wall tension stretch the cardiac myocytes and result in upregulation of BNP production. In addition, BNP responds to a range of other neuroendocrine and inflammatory stimuli.[86] BNP is secreted as a prohormone, pro-BNP. This then undergoes cleavage to produce the N-terminal fragment of the prohormone (NTproBNP; 8.5 kDa) and the active BNP.[87] The role of BNP measurements is in the differential diagnosis of breathlessness in patients suspected of acute[88–90] or chronic heart failure.[91,92] It has been suggested that BNP is elevated in patients with chest pain and suspected ischaemic heart disease as a consequence of myocardial ischaemia.[44,93] In addition, measurement has been shown to have prognostic value in patients presenting with ACS[94,95] and to be a predictive risk marker for recurrent cardiac events.[96] It has been suggested in guidelines that measurement may be useful in patients with chest pain and suspected ACS.[97]

Vascular stress markers

Copeptin

In view of the important role of arginine vasopressin (AVP) in acute and chronic disease, knowledge of endogenous plasma AVP concentrations may be helpful in the diagnosis of chest pain and suspected cardiovascular diseases and the monitoring of treatment. Copeptin (5 kDa) is a 39-amino-acid AVP-associated glycopeptide that contains a leucine-rich core segment. Together with AVP, copeptin is derived from a 164-amino-acid precursor termed preprovasopressin, which consists of a signal peptide, AVP, neurophysin II and copeptin; thus, copeptin is the C-terminal part of pro-AVP.[45] Copeptin levels are elevated after an AMI[98] and are associated with left ventricular dysfunction and remodelling and clinical heart failure post AMI.[99] Copeptin levels are affected by gender and renal function.[100] Copeptin may be elevated as a consequence of ischaemia and studies have been performed which suggest that measurement of copeptin is useful in patients with chest pain to rule in and rule out an AMI.[101,102]

Adrenomedullin

Adrenomedullin (AM) is a member of the calcitonin gene-related peptide family. It is synthesised as an immature 53-amino-acid precursor and is modified by amidation into a mature 52-amino-acid peptide with an intramolecular disulphide bond. In the heart, AM is present in ventricular tissue. Although mainly produced by vascular endothelial cells, vascular smooth muscle cells and macrophages, AM can also be produced by fibroblasts, adipocytes and cardiac myocytes. It does not appear to be stored so is probably regulated by transcription triggered by proinflammatory cytokines such as tumour necrosis factor-alpha, interleukin 1 beta (IL-1β), interferon gamma and nitrous oxide. The actions of AM are mediated by the seven transmembrane domain G protein-coupled calcitonin receptor-like receptors. The potential functions of AM include vasodilator, natriuretic, diuretic, antiapoptotic and prosurvival roles, angiogenesis and modulation of inflammation.[103] The predominant inotropic effect is on the atria.[104]

Adrenomedullin predicts the risk of future cardiovascular events, including heart failure, in an asymptomatic population [in which it was found to be superior to C-reactive protein (CRP) measurement][105] and following an AMI.[106–108] Prediction of risk and prognosis in heart failure post myocardial infarction seems to be the most effective role.[109] AM is raised in the circulation[110,111] and ventricular tissue[111] of patients with congestive heart failure and released from the lungs.[112] Values are proportional to the degree of heart failure, although elevation does not seem to be marked in New York Heart Association (NYHA) grade I heart failure.[110] AM has been studied as a prognostic marker in heart failure and has been compared with BNP. It has been demonstrated to be an independent risk predictor and synergistic with BNP in acute[113] and chronic heart failure.[114,115]

Plaque instability markers

Inflammatory markers

Pentraxins

The pentraxins are a superfamily of conserved proteins that are characterised by a cyclic multimeric structure. The classical short pentraxins, CRP and serum amyloid P component, are acute-phase proteins produced in the liver in response to inflammatory mediators. Long pentraxins have an unrelated, long amino-terminal domain coupled to the carboxy-terminal pentraxin domain.

C-reactive protein CRP (25 kDa) was originally isolated as a protein that binds to the C (capsular)-polysaccharide of the cell wall of pneumococcus. CRP is a pentraxin composed of five 23-kDa subunits that plays a key role in the innate immune response.[116,117] It is produced mainly by hepatocytes after stimulation by cytokines, of which interleukin 6 (IL-6) appears to be the major inducer. CRP levels increase 6 hours after acute stimuli, reaching a peak within 48 hours (up to 100-fold). With abrupt cessation of stimuli, values decrease exponentially at a rate close to the half-life of CRP (18–20 hours).[116] Population based cut-offs have been proposed for risk stratification.[118] Although no diurnal variation and no age or gender dependence were demonstrated in initial studies,[118,119] these reports were based on comparisons of CRP concentrations across dissimilar studies with heterogeneous populations. The Dallas Heart Study compared levels of CRP between different race and gender groups, and found race and gender effects.[120]

There is a large body of evidence in populations with and without prior cardiovascular disease that CRP measurement predicts risk of cardiovascular events, death and risk of developing cardiac failure.[116] Assessment of the additional independent prognostic value of CRP is not easy. In ACS patients, CRP is said to add to risk prediction.[121] Routine measurement of CRP has been said to be valuable across the range of cardiac disease[122] and has been included in guidelines,[97] but is not routinely used.

Cytokines

Interleukin 6

Interleukin 6 (24 kDa), a 185-amino-acid polypeptide, is a pleiotropic cytokine with a variety of biological activities.[123,124] IL-6 is secreted from several cell types, including endothelial cells, macrophages, lymphocytes and adipocytes. The IL-6 receptor complex consists of two membrane-bound glycoproteins, an 80-kDa ligand-binding component (termed IL-6R) and a 130-kDa signal-transducing component (termed gp130). IL-6 also activates a soluble IL-6R (sIL-6R). The activated IL-6–sIL-6R complex is a potent agonist that binds the signal-transducing component of the membrane-bound receptor, gp130, with high affinity. CRP is a physiological regulator of sIL-6R shedding in human neutrophils and markedly increases formation of the sIL-6R–IL-6 complex.[125] Along with adrenergic agonists, cytokines play a major role in inducing cardiac hypertrophy. The main hypertrophic cytokines are all members of the IL-6 family and include IL-6 itself, leukaemia-inhibitory factor (LIF) and cardiotrophin 1 (CT-1). All IL-6 cytokines utilise gp130 in combination with ligand-specific receptors and mediate their effects through intracellular signal transduction pathways.[124] As IL-6 is the primary cytokine of the inflammatory process[126] and inflammatory plaque destabilisation is key to plaque rupture, it has been suggested that IL-6 measurement may be useful in patients with ACS. IL-6 has been shown to be of cardiac origin in ACS[127] and to relate to other risk factors.[128] Studies have shown that IL-6 measurement is prognostic in patients with ACS,[129–131] but data are not consistent.[132]

Interleukin 33 and ST2 receptor

ST2 (556 amino acids; 63 kDa), also known as IL1RL1, DER4, T1 and FIT-1, is a member of the Toll-like/interleukin 1 (IL-1) receptor superfamily. Four isoforms of ST2 exist: sST2, ST2L, ST2v and ST2Lv. Soluble ST2 (sST2) lacks the transmembrane and cytoplasmic domains contained within the structure of ST2L and includes a unique nine-amino-acid carboxy-terminal sequence. The overall structure of ST2L is similar to the structure of the type I IL-1 receptors. The ligand for ST2 is an 18-kDa protein, interleukin 33

(IL-33; also known as IL-1F11), and a member of the IL-1 family. The mode by which IL-33 exerts its effect has not been fully established but it probably acts in a similar way to other members of the IL-1 family, specifically IL-1β and IL-18,[133] and appears to be anti-inflammatory.[134] IL-33 was originally described as a modulator of inflammation, but the IL-33/ST2 system might also participate in the fibrotic response to tissue injury. Expression of ST2 is markedly upregulated on the application of mechanical strain to cardiac myocytes.[135] IL-33/ST2 signalling is a mechanically activated, cardioprotective fibroblast–cardiomyocyte paracrine system.[136,137]

ST2 is elevated following myocardial infarction[138] and elevated levels predict an adverse outcome independently of NTproBNP.[139] The role appears to be predominantly prognostic,[140] but a role in the emergency department population has been questioned,[141] as has the concept of additional risk prediction over current markers.[142] The major role appears to be as a marker of myocardial remodelling[143–145] and hence dysfunction. In patients with acute heart failure, elevation of ST2 predicts an adverse outcome and is an independent prognostic marker.[146–149] Interestingly, ST2 predicts an adverse outcome in patients presenting with acute dyspnoea regardless of whether or not they have heart failure[146] and in patients with pulmonary disease.[150]

Growth differentiation factor 15

Growth differentiation factor 15 (GDF15), also known as MIC-1, is a secreted member of the transforming growth factor-beta superfamily. GDF15 is synthesised as a precursor protein that undergoes disulphide-linked dimerisation. Proteolysis cleaves the correctly folded GDF15 precursor protein to release the N-terminal propeptide from the mature GDF15 peptide, which is then secreted as a disulphide-linked dimer with an M_r of approximately 28,000.[151–153] GDF15 is detectable only in the liver and placenta,[154] but can be induced in the heart by myocardial infarction and pressure overload.[155,156] It has been proposed that GDF15 is a cytokine released in an auto- or paracrine way that displays antihypertrophic and cardioprotective features; in particular, it protects the heart from ischaemia/reperfusion injury.[156]

The preanalytical and population characteristics of GDF15 have been characterised. GDF15 correlates with CRP and cystatin C in the healthy elderly but not with NTproBNP.[157] The role of GDF15 measurement has been examined in patients with both NSTEMI and STEMI. In NSTEMI, GDF15 has been proposed as a selection criterion for invasive coronary intervention.[158] In prospective trials of ACS [the Global Utilization of Strategies To Open occluded arteries (GUSTO)-IV non-ST-elevation acute coronary syndrome trial[159] and the PRavastatin OR atorVastatin Evaluation and Infection Therapy – Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI 22) trial[160]] GDF15 has been shown to be a prognostic marker. GDF15 has been shown to be a long-term prognostic marker in patients admitted with NSTEMI[161] and a prognostic marker in STEMI[162,163] and in the general AMI population.[164] Measurement of GDF15 has been shown to be prognostic in the chest pain population[165] and to add to conventional risk scores such as the Global Registry of Acute Coronary Events (GRACE) score.[166] Although some workers have concluded that GDF15 measurement is useful across the range of coronary artery disease,[167] when examined as part of a multimarker strategy it was not considered useful above conventional risk factor prediction.[168] GDF15 is elevated in patients with chronic heart failure and correlates with NYHA class. Values correlate with NTproBNP, but are independent predictors of prognosis.[169] GDF15 may have a role to play in diagnosis and risk stratification in patients with cardiac failure, but more studies are required. The role of GDF15 appears to be associated with the remodelling of the heart;[170] the mechanism appears to be through the inhibition of integrins.[171]

Myeloperoxidase

Myeloperoxidase (MPO) is a peroxidase enzyme (EC 1.11.1.7) that is most abundant in neutrophil granulocytes (a subtype of white blood cells). The 150-kDa MPO protein is a dimer consisting of two 15-kDa light chains and two variable-weight glycosylated heavy chains bound to a prosthetic haem group. Three isoforms have been identified, differing only in the size of the heavy chains. The situation with MPO has been confusing (*Table 2*). Early studies in patients presenting with chest pain suggested that, in patients with undetectable troponin, MPO measurement predicted short-term risk of myocardial

infarction and risk of MACE.[172] Studies in chest pain and ACS patients confirmed the prognostic value of MPO measurements,[173–175] although not in all patients.[176] There are some major limitations of these published studies. First, not all studies have used a contemporary high-sensitivity troponin assay. Second, there are some significant preanalytical questions. MPO is present in significant amounts in leucocytes and is released with clotting. Only EDTA (ethylenediaminetetraacetic acid) plasma is a suitable sample matrix and serum should not be used. Mechanistically, one study has shown no relationship between MPO and coronary disease when assessed angiographically.[183]

TABLE 2 Summary of studies of MPO as a diagnostic and prognostic risk marker

Study	Early diagnosis	Prognosis	Contemporary troponin assay	Comments
Brennan 2003[172]		Admission values	Second-generation cTnT (Roche diagnostics, Indianapolis, IN)	Plasma ns, ACS patients
Baldus 2003[173]		Baseline samples	Third-generation cTnT (Roche diagnostics, Mannheim)	Serum, CAPTURE trail
Cavusolglu 2007[174]		Baseline samples, long-term prognosis		Matrix not stated, ACS patients
Apple 2007[176]		Admission sample, not significant	cTnI Dimension® (Dade-Behring, Glasgow, Delaware)	Lithium-heparin plasma, chest pain patients
Morrow 2008[175]		Baseline samples, short-term outcome		Citrated plasma ns, TACTICS TIMI 18
McCann 2008,[69] McCann 2009[70,76]	Admission sample, not significant	Admission sample, not significant	Third-generation cTnT (Roche diagnostics, Burgess Hill, Sussex)	Serum
Apple 2009[177]	Admission sample, not significant		cTnI Dimension, third-generation cTnT (Roche diagnostics, Indianapolis, IN)	Lithium-heparin plasma, chest pain patients
Rudolph 2010[178]	Diagnosis in the early phase of onset of chest pain in patients negative for cTnI			EDTA plasma
Scirica 2011[179]		Did not add to cTnI and BNP	Seimens Ultra cTnI (Siemens Healthcare Diagnostics, Deerfield, IL)	EDTA plasma, MERLIN-TIMI 36
Sawicki 2011[180]	Diagnosis in the early phase of onset of chest pain in patients negative for cTnI		Architect (Abbot Diagnostics, IL)	EDTA plasma, generalised chest pain
Oemrawsingh 2011[181]		Predictive value after NSTEMI		Serum and lithium-heparin plasma, CAPTURE trial
Apple 2011[182]		MPO is an independent risk predictor		EDTA plasma

CAPTURE, Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment trial; MERLIN, Metabolic Efficiency With Ranolazine for Less Ischemia in NSTE-ACS; ns, not specified; TACTICS, Treat Angina with Aggrastat and Determine Cost of Therapy with an Invasive or Conservative Strategy; TIMI, Thrombolysis In Myocardial Infarction.

Matrix metalloproteinases and their inhibitors

Matrix metalloproteinases (MMPs) are zinc-dependent endopepetidases collectively capable of degrading extracellular matrix proteins and processing bioactive molecules. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the Fas ligand) and chemokine activation and inactivation. They are therefore involved in both remodelling and inflammation. There are 28 MMPs (*Table 3*).

The MMPs are inhibited by specific endogenous tissue inhibitor of metalloproteinases (TIMPs), which comprise a family of four protease inhibitors: TIMP-1, TIMP-2, TIMP-3 and TIMP-4.

Matrix metalloproteinase 9 (MMP9) correlates with the extent of angiographically described coronary disease[184] and disease progression,[185] with similar data reported for MMP1,[186] whereas MMP2 has been reported to be associated with calcified plaque.[187] TIMP-1 has been reported to be a predictor of death

TABLE 3 Matrix metalloproteinases

Abbreviation	Other names
MMP1	Interstitial collagenase
MMP2	Gelatinase-A, 72-kDa gelatinase
MMP3	Stromelysin 1
MMP7	Matrilysin, PUMP 1
MMP8	Neutrophil collagenase
MMP9	Gelatinase-B, 92-kDa gelatinase
MMP10	Stromelysin 2
MMP11	Stromelysin 3
MMP12	Macrophage metalloelastase
MMP13	Collagenase 3
MMP14	MT1-MMP
MMP15	MT2-MMP
MMP16	MT3-MMP
MMP17	MT4-MMP
MMP18	Collagenase 4, xcol4, *Xenopus* collagenase
MMP19	RASI-1, occasionally referred to as stromelysin-4
MMP20	Enamelysin
MMP21	X-MMP
MMP23A	CA-MMP
MMP23B	
MMP24	MT5-MMP
MMP25	MT6-MMP
MMP26	Matrilysin-2, endometase
MMP27	MMP-22, C-MMP
MMP28	Epilysin

and myocardial infarction in patients with angiographically demonstrated coronary disease.[188] MMPs do not appear to be consistently good tests for myocardial infarction, but do appear to be prognostic markers.[69,177,189,190] MMP9 concentrations correlate with the extent of infarction.[191]

It is likely that MMP levels reflect remodelling rather than acute disease.[192,193] After myocardial infarction, MMP2 levels show an inverse correlation and MMP9 levels a positive correlation with ventricular dysfunction,[194] so MMPs are related to heart failure rather than ACS. Post-infarct survival and development of heart failure is predicted by MMP3,[195] MMP9[196,197] and TIMP-1.[197]

Studies in heart failure patients show variable concordance. Reports of changes in MMPs and TIMPs in patients with heart failure are generally consistent; reports of the ability to predict outcome are not. In heart failure, MMP1,[198] MMP2,[199,200] MMP9[199,200] and TIMP-1,[198,199,201–203] but not MMP3,[199] are reported as elevated. One large study has reported that MMP9 is not elevated in patients with heart failure after adjustment for other variables.[203] In other studies MMP1 levels are reduced,[201] but TIMP-2 is not elevated in heart failure.[200] Noradrenaline correlates with MMP2 levels in heart failure and appears to increase its synthesis.[200] MMP1 but not TIMP-1 has been shown to predict outcome.[201] TIMP-1 has also been shown to be an outcome predictor.[203] MMP2 (but not MMP3, MMP9 or TIMP-1) and BNP correlated with NYHA grade (although not with each other) and were independent outcome predictors.[199] Others have found MMP3, but not MMP2, and BNP to be independent outcome predictors.[204] MMP9, despite the ability to predict adverse effects after an AMI, appears consistently to be a poor outcome predictor[199,203–205] in heart failure patients, especially compared with BNP[205] or TIMP-1.[203]

Currently, MMPs and TIMPs remain poorly understood. Until reference interval values of MMPs and TIMPs are defined, including biological determinants of variation, methodological standardisation occurs, the pathophysiology is more clearly defined[206] and large studies are undertaken directly comparing the individual candidates using agreed and validated methods, it is unlikely that these analytes will achieve clinical application.

Pregnancy-associated plasma protein A

Pregnancy-associated plasma protein A (PAPP-A), also known as pappalysin 1, is a high-molecular-weight (200 kDa) zinc-binding metalloproteinase (EC 3.4.24.79) that cleaves insulin-like growth factor-binding proteins.[207,208] It has been suggested that PAPP-A is a marker of plaque instability. The initial study that documented increased levels of PAPP-A in atheromatous plaques also documented elevated serum levels. It was suggested that measurement of PAPP-A could be used as an additional diagnostic and prognostic biomarker in patients presenting with chest pain.[209] Early studies showed that there are more patterns in patients with chest pain and it was suggested that it might not prove to be a useful early biomarker for diagnosis[210] but does appear to be prognostic.[211,212] Elevations of PAPP-A were reported to correlate with the extent of both coronary[213] and peripheral[214] vascular disease.

There are a number of problems with the measurement of PAPP-A. It has been demonstrated that the molecular form circulating in patients with ACS is different from that measured in pregnancy. The ACS-related form circulates as a monomer not complexed with a proform of eosinophilic major basic protein. The pregnancy-related form circulates as a complex with eosinophilic major basic protein.[215–217] Different assays may therefore perform differently.[218] It has been shown that free PAPP-A is a better predictor than total PAPP-A.[219]

Finally, it has been demonstrated that administration of intravenous heparin causes a rise in PAPP-A due to release from the arterial wall.[220,221]

Table 4 provides a summary of studies of PAPP-A.

TABLE 4 Summary of studies of PAPP-A

Study	Early diagnosis of chest pain	Prognostic marker	Population	Comments
Dominguez-Rodriguez 2005[222]	No elevation in AMI patients		Comparison of levels in AMI patients and age-matched control subjects	
Elesber 2007[223]	Predicts a diagnosis of ACS		Chest pain patients	
Sanchis 2008[224]		Not prognostic	Chest pain patients	
Iversen 2008[225]	Early elevation in STEMI		STEMI patients	
Kavsak 2009[226]		Prognostic of long-term outcome	Chest pain patients	
Iversen 2009[227]		Risk of myocardial infarction and death in NSTE-ACS and death in STEMI patients	NSTE-ACS and STEMI patients	
Iversen 2010[228]			Chest pain, normal ECG, normal biomarkers	
Khan 2011[229]	cTnT superior to PAPP-A and MPO		Chest pain patients	High-sensitivity cTnT
Body 2011[230]		Not prognostic		

NSTE-ACS, non-ST elevation acute coronary syndrome.

Ischaemia markers

Ischaemia-modified albumin

The N-terminal portion of human serum albumin is known to be a binding site for transition metal ions, binding cobalt, copper and nickel in their (II) forms.[231] It is also known that the N-terminal portion of human serum albumin is susceptible to biochemical degradation and is less stable than the albumin of other species.[232] Ischaemia-modified albumin (IMA) is a form of human serum albumin in which the N-terminal amino acids have been affected so that they are unable to bind transition metals. Measurement of IMA is with the albumin cobalt-binding (ACB®, Ischemia Technologies, Denver, CO) test. This involves addition of a known amount of cobalt to a serum sample, addition of dithiothreitol to bind the unbound cobalt and measurement of the colorimetric change. As normal albumin will bind cobalt, the amount of free cobalt, hence the absorbance, will be proportional to the amount of IMA present.

The postulated mechanism is that localised ischaemia results in acidosis and release of copper(II) from weak binding sites on circulating proteins and peptides. This is then scavenged by albumin. Copper-bound albumin is then damaged by hydroxyl free radicals, causing removal of the three N-terminal amino acids and release of the copper(II) ion to repeat the process in a chain reaction.[233] This has not been confirmed, however. In a study of patients with increased IMA the N-terminal portion of albumin was sequenced and no evidence of N-terminal degradation or truncation was found.[234] Recent physicochemical studies of cobalt binding to human serum albumin have suggested a different explanation. Three binding sites for cobalt were identified, two of which showed greater avidity than the N-terminal binding site.[235] Fatty acid binding to albumin occurs at one of the additional cobalt binding sites with a negative allosteric interaction. It is hypothesised that in myocardial ischaemia, the release of fatty acids results in binding of fatty acids to albumin. This would then reduce the ability of albumin to take up cobalt and would account for the presence of IMA.[235] If this also produced a conformational change in the albumin affecting the

N-terminal site, it would also reduce cobalt binding. The most consistent finding across all studies of IMA is of a high negative predictive value. This has been highlighted in a recently published meta-analysis specifically examining the role of IMA as a rule-out test.[236] The role of IMA has been reviewed and it is not considered suitable for routine laboratory measurement.[237]

Chapter 2 Research objectives and research questions

The objectives of this study were:

1. to test the diagnostic accuracy for an AMI of highly sensitive troponin assays and a range of new cardiac biomarkers of plaque destabilisation, myocardial ischaemia and necrosis
2. to test the prognostic accuracy for adverse cardiac events of highly sensitive troponin assays and this range of new cardiac biomarkers
3. to estimate the potential cost-effectiveness of using highly sensitive troponin assays or this range of new cardiac biomarkers instead of admission and 12-hour troponin measurements.

These objectives were addressed with the following research questions:

1. Is a panel of cardiac markers, as currently available, required for early diagnosis of myocardial infarction?
2. Do novel cytoplasmic markers of myocardial damage contribute to the early differential diagnosis of patients presenting with chest pain?
3. Are all high-sensitivity cardiac troponin markers of equivalent diagnostic efficiency?
4. Do markers of myocardial dysfunction contribute to the early differential diagnosis of patients presenting with chest pain?
5. Do markers of vascular dysfunction contribute to the early differential diagnosis of patients presenting with chest pain?
6. What is the prognostic role of cytoplasmic markers of myocardial damage compared with troponin measurement?
7. What is the prognostic role of high-sensitivity troponin assays?
8. What is the prognostic role of myocardial dysfunction compared with troponin measurement?
9. What is the cost-effectiveness of the identified strategies?

Study rationale

The Randomised Assessment of Treatment using Panel Assay of Cardiac markers (RATPAC) trial was a multicentre pragmatic randomised controlled trial and economic evaluation of a point-of-care cardiac marker panel in the management of patients with a suspected, but not proven, AMI in six emergency departments in the UK. The RATPAC – Contemporary Biomarker Evaluation (RATPAC-CBE) study aimed to examine whether the biomarker panel measured by point-of-care testing was the most appropriate diagnostic strategy or whether other cardiac biomarkers could replace or supplement the point-of-care biomarker panel.

The archived blood samples from the RATPAC study represented an ideal opportunity to extend the findings of the RATPAC trial in a cost-effective way. The enrolled patients were fully characterised and were followed up for MACEs. The population was also unique as it represented one found within the emergency department and selected on the basis of low cardiac risk rather than one enrolled in a clinical trial with a high prior probability of cardiovascular disease. This is a major limitation of many existing biomarker studies and has been highlighted in recent editorials and the consensus statement[83] on biomarker series of the working group of the European Society of Cardiology. As with other biomarker studies of this type, patient enrolment was prospective, but analysis was retrospective.

Selection of biomarkers for investigation was based on evidence obtained from reviewing the existing literature (see *Chapter 1*) and from knowledge of current and potential future clinical practice. In selecting the biomarkers for evaluation, the most important criteria were that:

1. a validated automated assay was available for the biomarker, which could be used in the routine clinical laboratory
2. there was already existing evidence suggesting that the biomarker might be of value
3. a comprehensive comparative evaluation of the biomarker had not already been performed
4. an appropriate sample was available and there was adequate sample volume.

The biomarkers finally selected were CK-MB, myoglobin, cTnT and cTnI measured with a range of high-sensitivity assays, H-FABP, copeptin and BNP measured as NTproBNP. Although CRP was initially considered, the lack of clinical use, despite widespread availability of the assay and sample volume limitations, mitigated against its final inclusion.

Chapter 3 Methods

Population

The population was patients presenting to the emergency department with chest pain due to a suspected, but not proven, AMI in which cardiac biomarker measurement by point-of-care testing could potentially rule out an AMI and allow discharge home. All patients with chest pain were considered for participation, but were then excluded if they met any of the following criteria:

1. Diagnostic ECG changes for an AMI or high-risk ACS (>1 mm ST deviation or >3 mm inverted T waves). These patients are at high risk of adverse outcome and require inpatient care even if initial cardiac biomarker testing is negative.
2. Known coronary heart disease presenting with prolonged (>1 hour) or recurrent episodes of typical cardiac-type pain. These patients have unstable angina and require inpatient care for symptom control even if cardiac biomarker testing is negative.
3. Proven or suspected serious non-coronary pathology such as pulmonary embolus that requires inpatient care even if an AMI is ruled out.
4. Comorbidity or social problems that require hospital admission even if an AMI can be ruled out.
5. Patients with an obvious non-cardiac cause of chest pain such as pneumothorax or muscular pain, in whom an AMI can be excluded as a possible cause without resorting to further diagnostic testing.
6. Presentation >12 hours after the most significant episode of pain. In such patients a single troponin measurement would clearly be more appropriate than panel testing.
7. Previous participation in the RATPAC trial.
8. Inability to understand the trial information because of cognitive impairment.
9. Non-English-speaking patients for whom translation facilities were not available.

For every fourth week of trial recruitment the research nurse at each hospital examined emergency department attendance lists to identify patients attending with chest pain and record basic demographic details and reasons for exclusion. The huge number of attendances with chest pain meant that undertaking this process throughout the whole trial would have produced an excessive workload, whereas monitoring every fourth week achieved the aim of reporting sample selection within acceptable use of resources.

Recruitment and randomisation

Research nurses and emergency department staff identified eligible patients, provided trial information and obtained written consent. Participants were then randomly allocated to receive either (1) diagnostic assessment using the point-of-care biochemical marker panel or (2) conventional diagnostic assessment without the panel.

The Nottingham Clinical Trials Unit (CTU) generated a simple randomisation sequence, stratified by centre, which was not revealed to any person involved in patient recruitment. Recruiting doctors and research nurses accessed a secure website provided by the Nottingham CTU and entered participant details. The CTU revealed each participant's allocated treatment group to the emergency department only after the participant's details were entered, written consent was confirmed and the participant irrevocably entered into the trial.

Planned interventions

Participants were randomised to receive either

1. diagnostic assessment using the point-of-care biochemical marker panel or
2. conventional diagnostic assessment without the panel.

The only difference between the two arms of the trial was that patients in the intervention arm received testing with the point-of-care panel. The use of all other tests and treatments, and decision-making in the emergency department, was at the discretion of the attending clinician.

The point-of-care cardiac marker panel comprised CK-MB (mass), myoglobin and cTnI, measured at presentation and 90 minutes later, using the Stratus® CS analyser (Siemens Healthcare Diagnostics, Camberley, Surrey, UK). Clinical staff were trained to use the test and given guidance in interpretation of the results. A recommended protocol that advised a first panel test immediately after initial emergency department assessment and a second panel test 90 minutes later was used. The protocol then advised hospital admission or discharge on the basis of point-of-care results. Decisions were ultimately at the discretion of clinical staff in patients randomised to use of the point-of-care protocol and its use was not enforced.

In addition to obtaining consent, collecting data and random allocation to use of the point-of-care test, the only change to routine practice was that clinical staff took an additional blood sample for storage (without repeating venepuncture) each time a point-of-care blood sample was required. The additional blood remaining after point-of-care testing was transported to the hospital laboratory to be centrifuged and refrigerated. Batches of samples were then transported quarterly to St George's Hospital for storage and subsequent secondary analysis.

The RATPAC study was a pragmatic trial intended to determine whether or not point-of-care testing should be standard practice for patients presenting to the emergency department with a suspected AMI and was designed to compare two alternatives (management with and without point-of-care testing) under routine conditions. This pragmatic design had the following implications:

1. There was no attempt to blind clinical staff, patients or carers to the allocated treatment group after randomisation.
2. The point-of-care test was provided with a recommended protocol for use, but management decisions were ultimately at the discretion of the clinical staff.
3. All other diagnostic tests and the use of laboratory blood tests in the control group were at the discretion of the clinical staff.
4. Blood samples were taken only for the purposes of clinical management. Additional blood samples to evaluate theoretical management strategies or to evaluate the accuracy of diagnostic assessments were not taken. The additional blood samples taken at the time of blood draw for point-of-care tests were utilised. This allows direct comparison with conventional management strategies.

Outcome measures

The primary outcome in the RATPAC study was the proportion of patients successfully discharged home after emergency department assessment. To be considered successfully discharged the patient had to (1) either have left the hospital or be awaiting transport home with a discharge decision having been made at 4 hours after initial presentation and (2) suffer no adverse event (as defined below) during the following 3 months.

Secondary outcomes were:

1. reattendance at and/or readmission to hospital over the following 3 months
2. adverse events (death, non-fatal AMI, emergency revascularisation or hospitalisation for myocardial ischaemia)
3. the proportion of admitted patients ultimately diagnosed as having an AMI by the universal definition of myocardial infarction.[11]

Recruiting staff recorded baseline data, the results of initial assessment (including any biochemical cardiac tests) and admission or discharge from the emergency department. Research nurses then used emergency department and hospital inpatient notes to record management decisions at initial attendance and admission, extract resource-use data and identify subsequent attendances/admissions and adverse events up to 3 months.

Research nurses checked patient status (dead or alive) at 1 and 3 months using hospital information systems. Participants who were not recorded as dead were mailed a questionnaire at 1 and 3 months from the University of Sheffield to identify adverse events and hospital attendances.

Classification of cases of AMI and adverse events was carried out by blind independent review of the relevant data. A single reviewer blinded to treatment group classified emergency department reattendances, subsequent hospital admissions and outpatient reviews as either potentially chest pain related (including non-cardiac conditions that could have initially presented as chest pain) or clearly non-chest pain related.

Ethical arrangements

Ethical approval was granted by Leeds East Research Ethics Committee (07/Q1206/22) and review was provided by the local research ethics committee at each participating centre. The study was performed in accordance with the Declaration of Helsinki.[238] The trial was conducted in accordance with Medical Research Council *Guidelines for Good Clinical Practice in Clinical Trials*.[239] The University of Sheffield was the sponsor for the trial. The RATPAC trial was registered with the international clinical trials authority (ISRCTN378239293).

All participants were asked to provide written informed consent. Although participants were recruited in an emergency setting and there was only a limited amount of time available for considering trial information, the nature of the selected group (in particular the exclusion of people clearly requiring hospital treatment) ensured that eligible patients would not be incapacitated by their medical condition. No provision was made for recruitment of incapacitated patients by personal or professional legal representatives.

Blood samples for the subsequent analysis were made anonymous as follows. Each patient received a clinical trial number that was used as the prime identifier in all subsequent data analysis. For each participating site, test packs were prepared. One test pack was to be used for each patient entered into the trial. Each test pack contained the following: four primary sample tubes [two lithium-heparin tubes for point-of-care testing plus two serum separator gel tubes for additional sampling (Becton Dickinson, Oxford, UK)], four long-term storage tubes, preprinted barcode labels and a site-specific pro forma. On first presentation, one lithium-heparin tube was taken and used for point-of-care testing and one serum separator gel tube was taken and sent to the laboratory. The serum separator gel tube was allowed to clot, was centrifuged and the supernatant serum separated into two of the long-term storage tubes, which were labelled with a preprinted barcode. The same preprinted barcode was then used to label the site-specific pro forma, which was also labelled with the patient trial number but with no additional

information. At 90 minutes the process was repeated. The long-term storage tubes were frozen to –20 °C and then transferred to St George's Hospital for long-term storage until analysis.

Diagnostic criteria

The universal definition of myocardial infarction[11] was used to categorise patients into those with or without an AMI utilising clinical, ECG, trial and local laboratory-derived cardiac troponin values and troponin measurements subsequently performed in the trial central laboratory on the admission and 90 minute samples using the Siemens Ultra assay as the predicate troponin method.

The initial working diagnosis and final diagnosis were those recorded in the notes by a senior clinician at the end of the initial emergency department assessment and at the end of hospital admission, respectively, based on available information at that time. This included the results of point-of-care testing as well as the results of local laboratory troponin measurements. Patients were classified as having an AMI on the basis of appropriate clinical features, electrocardiographic changes and the presence of a rise in troponin level above the diagnostic discriminant of the relevant assay in use locally and no alternative clinical cause of a troponin rise. Patients with a troponin rise consistent with an AMI and a final diagnosis of ACS or an AMI were classified as having an AMI. Patients with no troponin rise consistent with an AMI and a final diagnosis that was neither ACS nor an AMI were classified as not having an AMI. Patients with a final diagnosis of ACS or an AMI but no troponin rise were assessed by a single reviewer blind to treatment group who reviewed the initial and next-day ECG and categorised these patients as having an AMI only if an ECG showed ST-segment elevation and coronary reperfusion was performed. Patients with a troponin rise and a final diagnosis other than ACS or an AMI were assessed by two reviewers blinded to treatment group who reviewed case details and decided whether or not an AMI was the most likely diagnosis. Disagreements were resolved by discussion and patients classified as having an AMI or not.

Diagnostic review was then performed by two independent clinicians with access to all of the relevant information, utilising the 99th percentile from the local laboratory and also including troponin measurements performed in the central laboratory and compared with the final diagnosis. The trial admitted patients suspected of having an AMI on the basis of a rise in levels of cTnI, CK-MB or myoglobin measured on the Stratus CS analyser. These measurements were not used for the final diagnostic classification. All patients with a cTnI (measured on the Siemens Ultra assay) exceeding the 99th percentile or a troponin measurement from the local laboratory exceeding the 99th percentile were reviewed and the final diagnosis confirmed. Patients with a troponin rise and a final diagnosis other than ACS or an AMI were reviewed to decide whether or not an AMI was the most likely diagnosis. Disagreements were resolved by discussion and patients classified as having an AMI or not. Patients were categorised as AMI (type 1 MI, primary ischaemic cardiac injury), troponin elevation not due to an AMI but with a probable background of underlying coronary atheroma (type 2 MI, secondary ischaemic cardiac injury) and no myocardial injury.

Data processing

Trial data were collected on the case report form and follow-up form and were then entered by the research nurses into an online database provided on a secure central sever by the Sheffield CTU. The system had a full electronic audit trail. Quality control procedures were applied to validate the trial data. Error reports were generated when data clarification was required. All activities were performed in accordance with Sheffield Clinical Trials Research Unit (CTRU) standard operating procedures.

Core patient data were maintained by the trial co-ordinator using a unique trial number. Demographic, risk factor and diagnostic data were extracted as CSV (comma-separated value) files and transferred for combination with the analytical data. All of the analytical data were stored in a relational database

(Microsoft Access, Microsoft Corporation, Redmond, WA, USA) using the combination of trial number and unique sample number as identifiers. Database queries were extracted into Microsoft Excel for statistical analysis.

Analytical methods

Biochemical measurements were performed at trial sites (cardiac troponin measurements for local diagnostic classification), were standardised across sites for point-of-care testing and were performed at the core laboratory.

Trial sites

Trial sites measured cardiac troponin as follows:

- Siemens cTnI Ultra® assay (three sites: Barnsley, Leeds and Leicester) – the cTnI Ultra measurements were performed using an ADVIA Centaur® XP system (Siemens Healthcare Diagnostics, Camberley, Surrey, UK). The detection limit of the instrument is 6 ng/l and the upper limit is 50,000 ng/l. The claimed 10% coefficient of variation (CV) is 30 ng/l with a 99th percentile of 40 ng/l. Decision limits for diagnosis of an AMI used at the three sites were as follows: Barnsley 200 ng/l, Leeds 50 ng/l and Leicester 60 ng/l.
- Abbott cTnI (one site: Edinburgh) was measured on an Architect i2000SR system® (Abbott Diagnostics). The detection limit of the instrument is 10 ng/l and the upper limit is 50,000 ng/l. The claimed 10% CV is 32 ng/l and the 99th percentile 12 ng/l. A decision limit for diagnosis of an AMI of 50 ng/l was used.
- Beckman AccuTnI™ enhanced assay (one site: Bristol Frenchay) measurements were performed using an Access® 2 system (Beckman-Coulter, High Wycombe, Buckinghamshire, UK). The detection limit of the instrument is 10 ng/l and the upper limit is 100,000 ng/l. The claimed 10% CV is 60 ng/l with a 99th percentile of 40 ng/l. A diagnostic discriminant for an AMI of 60 ng/l was used.
- Roche cTnT (one site: Plymouth Derriford) measurements were performed using a Modular® E170 system (Roche Diagnostics, Burgess Hill, Sussex). The detection limit of the assay is 10 ng/l with an upper limit of 25,000 ng/l. The claimed 10% CV is 30 ng/l with a 99th percentile of 10 ng/l. The 99th percentile was used for diagnosis.

Point-of-care testing assays (all sites)

The cardiac panel measured was myoglobin, CK-MB and cTnI. Measurements were performed using the Stratus CS analyser. The analytical characteristics of the assays for each analyte were as follows. Myoglobin: detection limit 1 μg/l, analytical range 1–900 μg/l, interassay CV 1.9–12.7% (56–308 μg/l), 95% reference interval, males 21–98 μg/l, females 19–56 μg/l, combined 20–82 μg/l; CK-MB: detection limit 0.3 μg/l, analytical range 0.3–150 μg/l, interassay CV 0.15–1.27% (3.7–39.3 μg/l), 95% reference interval 0.6–3.5 μg/l; cTnI: detection limit 0.03 μg/l, analytical range 0.03–50 μg/l, interassay CV 4.0–8.2% (0.067–0.344 μg/l), the 99th percentile of the assay is 0.07 μg/l.

Core laboratory assays

Cardiac troponin

Three high-sensitivity cardiac troponin measurements were performed.

Cardiac troponin T

The Roche Diagnostics high-sensitivity cTnT assay was used. The high-sensitivity cTnT measurements were performed using an Elecsys® 2010 system (Roche Diagnostics). The detection limit of the assay is 3 ng/l with an upper limit of 10,000 ng/l. The claimed 10% CV is 13 ng/l with a 99th percentile of 14 ng/l.

Cardiac troponin I

Siemens cTnI Ultra The cTnI Ultra measurements were performed using an ADVIA Centaur® XP system. The detection limit of the instrument is 6 ng/l and the upper limit is 50,000 ng/l. The claimed 10% CV is 30 ng/l with a 99th percentile of 40 ng/l.[240]

Beckman AccuTnI enhanced The AccuTnI enhanced measurements were performed using an Access 2 system. The detection limit of the assay is 1 ng/l and the upper limit is 100,000 ng/l. The claimed 10% CV is 30 ng/l with a 99th percentile of 40 ng/l.

Heart-type fatty acid-binding protein

Heart-type fatty acid-binding protein measurements were performed using the Evidence Cardiac Array measured on the Evidence Investigator (Randox Laboratories, Crumlin, County Antrim, UK). The detection limit of the assay is 1.5 mg/l and the upper limit is 100 mg/l, with a CV of 9.1% at 3.1 mg/l, 7.5% at 17.6 mg/l and 9.8% at 44.1 mg/l. The 95th percentile is 2.5 mg/l and the 99th percentile is 3.0 mg/l.

Myoglobin

Myoglobin measurements were performed using the Evidence Cardiac Array measured on the Evidence Investigator. The detection limit of the assay is 1.8 mg/l and the upper limit is 700 mg/l, with a CV of 8.8% at 83 mg/l, 9.4% at 119 mg/l and 9.5% at 125.9 mg/l. The 97.5th percentile is 66 mg/l.

Neurohormones

Copeptin

Copeptin was measured by time-resolved amplified cryptate emission (TRACE), which measures the signal that is emitted from an immunocomplex with time delay, using the KRYPTOR compact system (Brahms, Hennigsdorf, Germany). The detection limit of the assay is 4.8 pmol/l. The analytical range is 4.8–500 pmol/l with a CV of 12–17% at 12–20 pmol/l, 6–12% at 20–50 pmol/l and 6% above 50 pmol/l. The functional sensitivity (20% CV) is < 12 pmol/l and the limit of quantitation (10% CV) is 14.1 pmol/l. The 97.5th percentile is 17.4 pmol/l (19.1 pmol/l male, 12.9 pmol/l female).

B-type natriuretic peptide by N-terminal pro-B-type natriuretic peptide measurement

N-terminal pro-B-type natriuretic peptide was measured using a solid-phase two-site chemiluminescent sandwich immunoassay using an Immulite 2500 (Siemens Healthcare Diagnostics, Camberley, Surrey, UK). The detection limit is 20 ng/l and the measuring range 20–35,000 ng/l. The interassay %CV is 5.0–4.0 in the concentration range 40.9–32,096 ng/l.

Statistical methods

Demographics and patient characteristics were analysed by non-parametric statistics. Diagnostic test comparison was performed using AMI or MACE as the dichotomous variable. Individual markers on admission and at 90 minutes from admission, the peak of the admission or 90-minute value and delta (90-minute value – admission value) values were examined by the construction of receiver operating characteristic (ROC) curves and calculation of the area under the curve (AUC). Integrated strategies utilising prespecified cut-off values plus delta values were compared by construction of contingency tables analysed by Fisher's exact test. In addition, 95% confidence intervals were calculated. All statistical analysis was performed using Analyse-it for Microsoft Excel (version 2.21; www.analyse-it.com).

Economic analysis

Economic analysis was predicated by two different scenarios. In the first scenario it was assumed that a number of biomarkers would have equivalent diagnostic and prognostic efficiency or that one single biomarker would be superior to all other single biomarkers or combinations of biomarkers. Cost minimisation analysis was used for economic modelling for this scenario. In the second scenario it was assumed that a single biomarker or combinations of biomarkers at different time points or combinations of markers at different time points would be used to achieve optimal diagnostic and prognostic patient categorisation. For this scenario a decision-analysis cost-effectiveness approach was utilised.

The decision-analysis model developed for a related Health Technology Assessment (HTA) project 'Cost-effectiveness of diagnostic strategies for suspected acute coronary syndrome (ACS)' (HTA 09/22/21) was used.[241] Full details of the model are given in the HTA journal publication for this project, but the essential details are as follows.

A decision tree model was developed using Simul8 software (Simul8 Corporation, Boston, MA, USA) to explore the costs and health outcomes associated with different diagnostic strategies. The model took an economic perspective of the NHS in England and Wales and a lifetime horizon with mean life expectancy based on UK interim lifetables.[242] The basic model structure is shown in *Figure 1*.

The model applied different testing strategies for myocardial infarction to a hypothetical cohort of patients presenting to hospital with symptoms suggestive of myocardial infarction but with no diagnostic ECG changes (ST deviation > 1 mm or T-wave inversion > 3 mm), no known history of coronary heart disease and no major comorbidities requiring inpatient treatment (such as heart failure or arrhythmia). Each patient entering the model had the following characteristics defined by sampling from the RATPAC trial population: age, gender, myocardial infarction present or not, time delay between onset of worst pain and arrival at hospital, and time of day.

The following diagnostic strategies were applied to each patient:

1. no testing: discharge all patients without treatment
2. high-sensitivity troponin at presentation: discharge home if test is negative or admit to hospital for troponin testing at 10–12 hours if positive
3. high-sensitivity troponin and a combination of cytoplasmic or neurohormone biomarkers at presentation: discharge home if both tests are negative or admit to hospital for troponin testing at 10–12 hours if either test is positive
4. high-sensitivity troponin at presentation and at 90 minutes as in the RATPAC protocol: discharge home if both tests are negative or admit to hospital for troponin testing at 10–12 hours if either test is positive
5. standard troponin testing at 10–12 hours.

Strategy 1 is a theoretical 'zero option' strategy designed to test whether or not any of the testing strategies are cost-effective. Strategy 5 is current standard practice as recommended in guidance from the National Institute for Health and Care Excellence (NICE).[243]

It was assumed that blood tests performed at presentation were undertaken in the emergency department and that results would be available and a decision made within 2 hours of sampling. Subsequent time delays are likely to depend on the system in place for managing admissions with chest pain and, so, three different scenarios with regard to troponin measurement at 10–12 hours were tested:

1. the 'doctor-on-demand' scenario in which medical staff were available 24 hours a day to make a disposition decision within 1 hour of the results being available

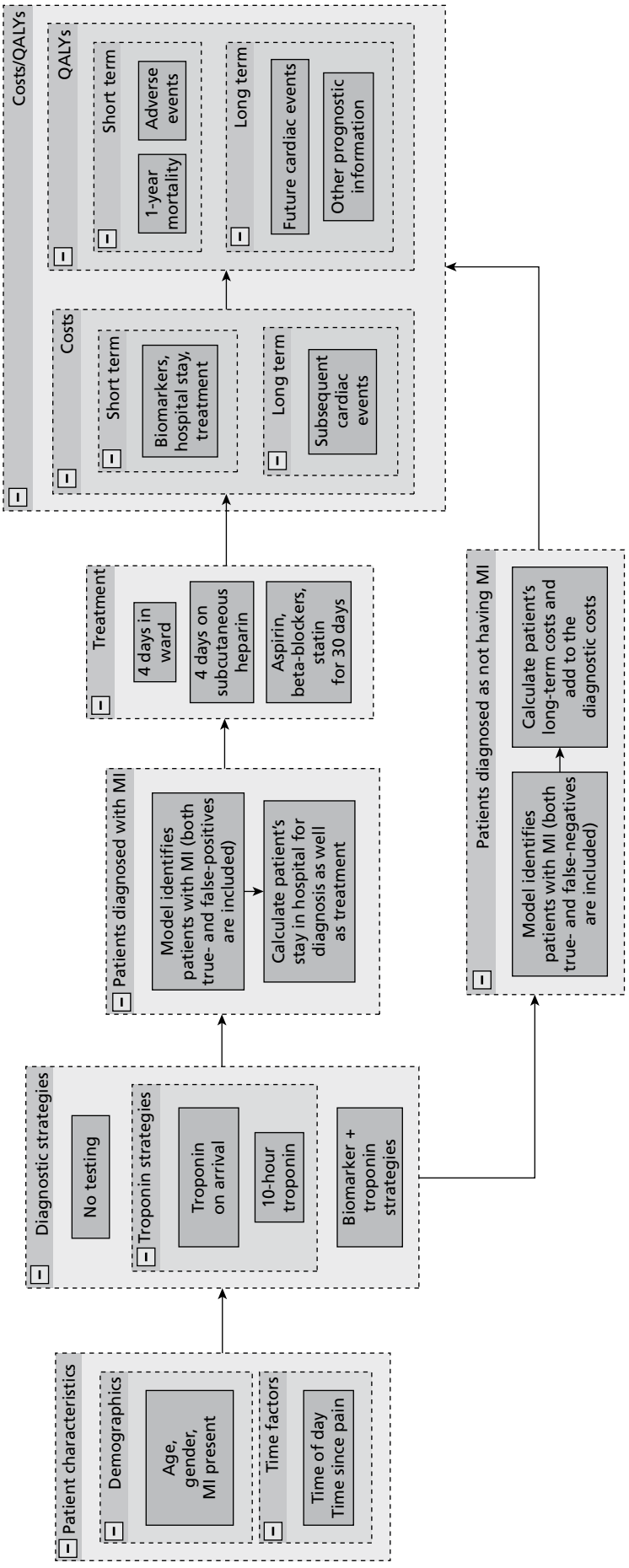

FIGURE 1 Basic model structure. MI, myocardial infarction.

2. the twice-daily ward round scenario in which medical staff were available only during twice-daily ward rounds (e.g. 0900 and 1800 hours) to make disposition decisions
3. the once-daily ward round scenario in which medical staff were available only during one daily ward round (e.g. 1400 hours) to make disposition decisions.

It was assumed that standard troponin measurement at 10–12 hours was the reference standard for myocardial infarction and was therefore effectively 100% sensitive and specific. All patients who were not discharged after presentation testing received testing at 10–12 hours to confirm or refute the diagnosis of myocardial infarction. Those with myocardial infarction were admitted to hospital and treated. Those without myocardial infarction were discharged home without treatment.

The sensitivity and specificity of presentation biochemical testing was estimated using data from this study. It was assumed that true-positives would be confirmed at 10–12 hours and admitted for treatment, false-positives would be admitted until a 10- to 12-hour troponin test ruled out myocardial infarction, and true-negatives and false-negatives would be discharged without treatment. Costs were accrued throughout the diagnostic process dependent on length of stay in hospital, number of biochemical tests received and receipt of treatment for myocardial infarction. It was assumed that all biochemical tests would cost £20 per test regardless of current availability or price. Current availability and price depend on current usage, which in turn depends on evidence of effectiveness and cost-effectiveness. It was assumed that any biochemical test that was shown convincingly to be effective and cost-effective would become widely available at a reasonable cost, regardless of current availability and cost.

Following diagnosis and treatment it was assumed that patients would die, suffer reinfarction or survive without reinfarction over the following year, depending on (1) whether or not they had myocardial infarction and (2) whether or not myocardial infarction was treated. The rates of death and reinfarction up to 1 year for patients with treated myocardial infarction, untreated myocardial infarction and no myocardial infarction were estimated using data from a cohort study of patients before and after implementation of a change of operational threshold.[244] The parameters used in the model are shown in *Table 5*.

It was assumed that survival and cardiac events after the first year would be independent of the diagnostic testing strategy at initial hospital admission, but that additional health-care costs and quality-adjusted life-years (QALYs) would be accrued by survivors and influenced by whether or not they suffered myocardial infarction and reinfarction. *Table 6* shows lifetime costs and QALYs for those with myocardial infarction. Patients without myocardial infarction were assumed to have normal quality-adjusted life expectancy and no additional health-care costs.

TABLE 5 Parameters used in the economic model and their derivation

Parameter	Estimate	Distribution	Source
Population characteristics			
Age (years), mean (SD)	53.0 (13.5)	SE 0.30	Goodacre *et al.*[245]
Male (%)	58.1	*n*/*N* = 1138/1958	
MI prevalence (%)	7.0	*n*/*N* = 137/1958	
Time delay (minutes), median (IQR)	132 (80–255)		
1-year probabilities of death and non-fatal MI (%)			
Death, treated MI	11	*n*/*N* = 9/80	Mills *et al.*[244]
Death, untreated MI	21	*n*/*N* = 19/90	
Reinfarction, treated MI	11	*n*/*N* = 9/80	
Reinfarction, untreated MI	29	*n*/*N* = 26/90	
Costs of tests, hospital stay and treatment (£)			
Treatment of MI (index or reinfarction)	3587	(3000,4000)	NHS reference costs[246]
Hospital stay (per hour) for testing	22	(20,30)	NHS reference costs for general medical ward[246]
Biochemical testing (per test)	20	(18,25)	Goodacre *et al.*[245]

IQR, interquartile range; MI, myocardial infarction; SD, standard deviation; SE, standard error.

TABLE 6 Lifetime cost and QALY estimates after myocardial infarction

Age (years)	Cost (£)	QALYs	QALYs with reinfarction
30–44	4012.5	12.20	9.76
45–54	3115	9.47	7.58
55–64	2215	6.73	5.39
65–74	1530	4.65	3.72
≥75	800	2.43	1.95

Chapter 4 Results

A total of 2263 participants were successfully recruited between 30 January 2007 and 2 June 2008; 1125 patients were successfully randomised to the point-of-care testing arm and 18 did not complete the 3-month follow-up. In the point-of-care testing arm there were 36 patients with events (3%): death 6 (1%); non-fatal myocardial infarction 5 (<1%); hospitalisation for ACS (without myocardial infarction) 18 (2%); life-threatening arrhythmia 6 (1%), emergency revascularisation 10 (1%). Event rates between the point-of-care testing arm and the central laboratory testing arm were not statistically different, although slightly more patients with an AMI were detected in the point-of-care testing arm (90/1125 vs 72/1118). Study enrolment is summarised in *Figure 2*.

Sample and result availability for the point-of-care arm and samples available from the subsequent extended biomarker phase are summarised in *Figures 3* and *4* respectively.

Demographics and patient characteristics in the point-of-care testing arm are summarised in *Table 7*.

The patient characteristics of the original data and the biomarker subset were not statistically significantly different. The diagnostic categorisation of patients on admission, following final diagnostic categorisation in the point-of-care arm of the RATPAC study and following review using the 99th percentile from the local laboratory and core laboratory troponin data are summarised in *Table 8*. In total, 847 out of 850 patients had laboratory troponin measurements performed. Median time from onset of chest pain to the last troponin measurement performed in the laboratory was 495 minutes [range 95–46,600 minutes, interquartile range (IQR) 310–738 minutes]. In total, 285 out of 850 of these samples (33.5%) were taken <6 hours from onset of chest pain, 556 (65.4%) were taken ≥6 hours from onset of chest pain and 364 (42.8%) were taken ≥10 hours from onset of chest pain. Hence, the majority of patients had a troponin measurement performed in accordance with the current recommendations of the European Society of Cardiology. Following a review of the laboratory troponin results and measurement of the admission and 90-minute samples using the Siemens Ultra assay three patients were reclassified as having an AMI.

The distribution of diagnoses in the final and review diagnosis groups was not statistically different.

Evaluation of the diagnostic accuracy for acute myocardial infarction of highly sensitive troponin assays and a range of new cardiac biomarkers of plaque destabilisation, myocardial ischaemia and necrosis

Is a panel of cardiac markers required for the early diagnosis of chest pain?

The cardiac troponin assay used in the RATPAC trial was the Stratus CS analyser. Although this is not considered to be a high-sensitivity troponin assay, it meets the criteria proposed as a guideline-acceptable assay.[84] The 10% CV is below the 99th percentile according to the manufacturer's data sheet. The need for additional cytoplasmic biomarkers as well as troponin is based on the argument that these will rise earlier than troponin as they are more readily released. The ability to detect troponin values around the 99th percentile may supersede the need for earlier markers as the time point for diagnostic insensitivity of troponin (the period of 'troponin blindness') may no longer occur. The markers traditionally suggested have been myoglobin and CK-MB. The first phase of the evaluation was therefore to assess the comparative diagnostic efficiency of cTnI measured on the Stratus CS alone utilising all of the available data for simultaneous real-time measurement of cTnI, myoglobin and CK-MB. The diagnostic efficiency of the panel approach was compared with that of single markers and single marker combinations utilising the final clinical diagnosis derived from the RATPAC study to allow comparison against the independent

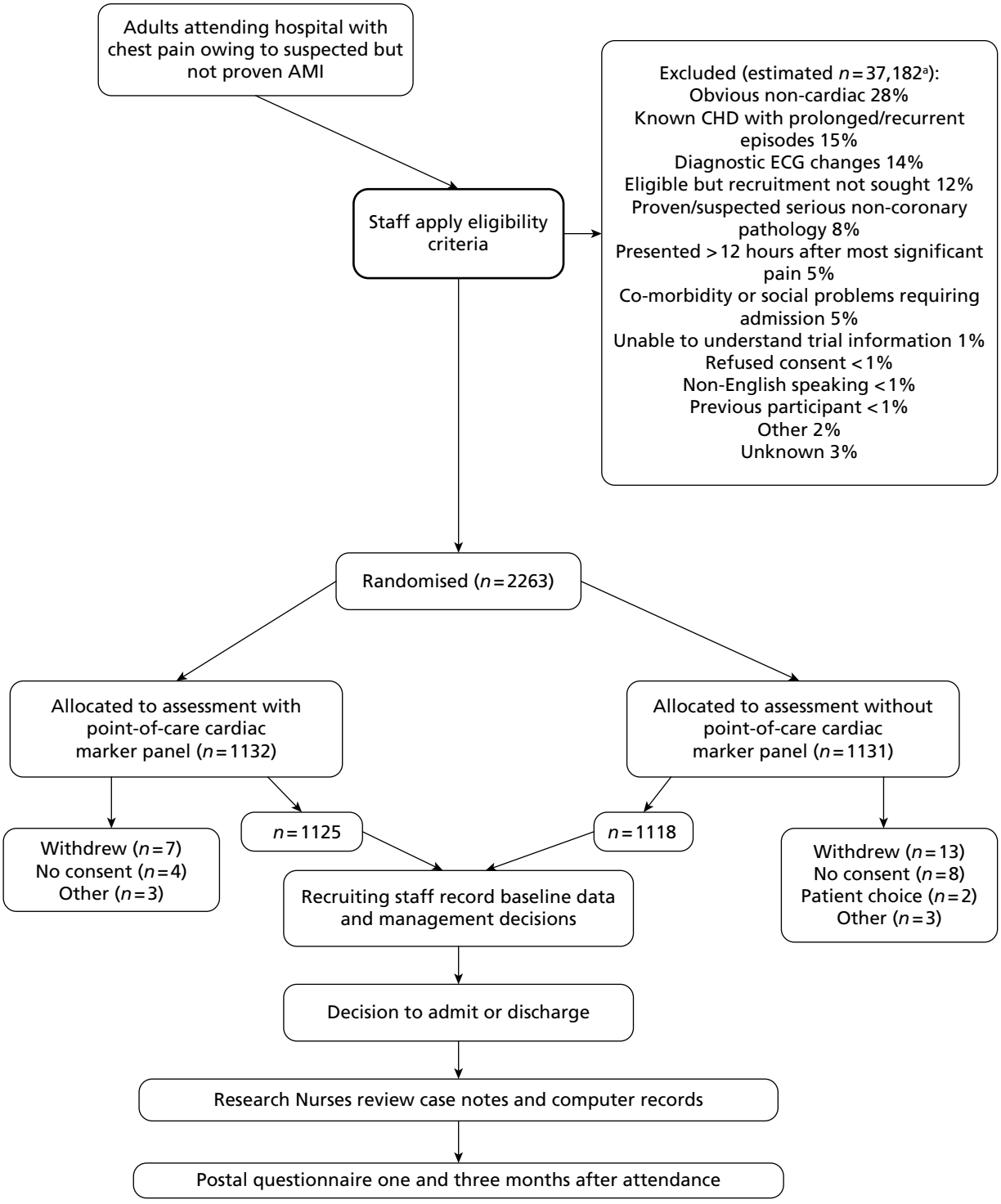

FIGURE 2 Trial enrolment. CHD, coronary heart disease. a, Patients were sampled on pre-determined screening days to assess the number of patients not recruited.

$$\text{Estimated number of patients not recruited} = \text{number not recruited on screening days} \times \frac{\text{total days recruiting}}{\text{total days screening}}.$$

Percentages are out of the total number of non-recruited patient notes screened ($n = 9109$).

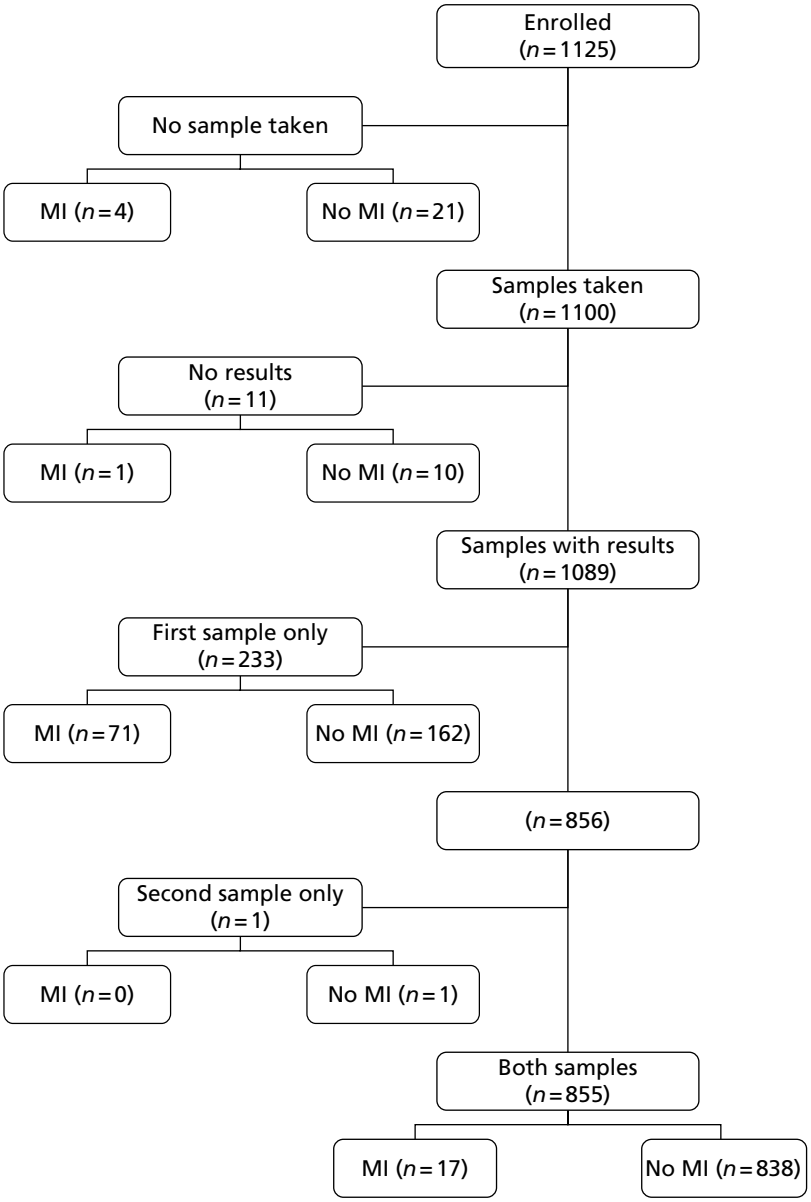

FIGURE 3 Sample and result availability for patients enrolled in the point-of-care arm of the study. MI, myocardial infarction.

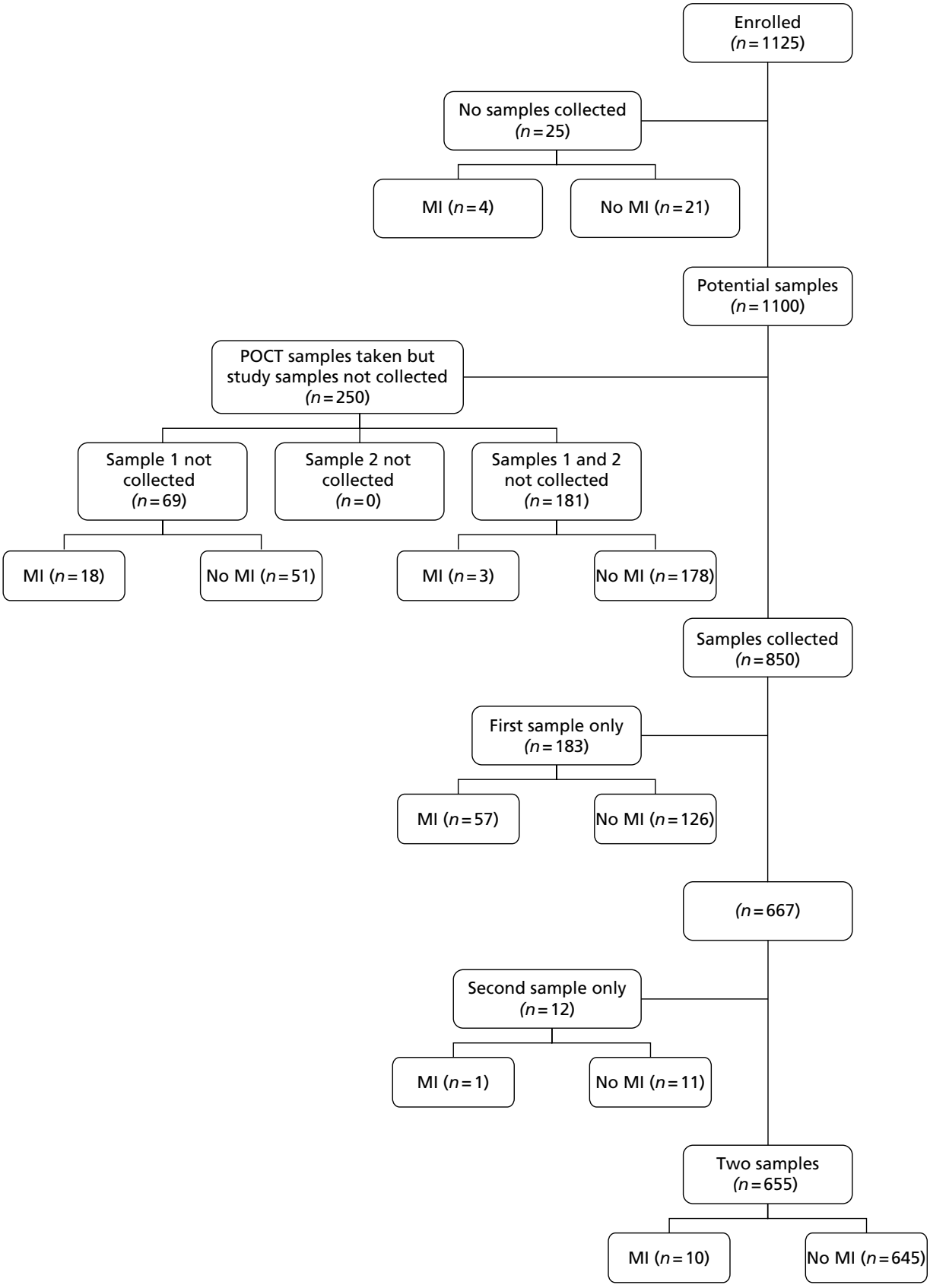

FIGURE 4 Samples available from the extended biomarker phase of the study. MI, myocardial infarction; POCT, point-of-care testing.

TABLE 7 Patient characteristics in the point-of-care testing arm

Point-of-care testing arm	All patients ($N = 1125$), *n* (%)	Biomarker subset ($N = 850$), *n* (%)
Median age (IQR) (years)	53.4 (44–64)	53.7 (44–64)
Min.–max. age (years)	21–92	23–92
Male	683 (61)	507 (60)
Female	442 (39)	343 (40)
Previous myocardial infarction	60 (5)	49 (6)
Angina + positive diagnostic test	46 (4)	32 (4)
Previous coronary artery bypass surgery	12 (1)	8 (1)
Angioplasty	37 (3)	32 (4)
Stenosis > 50% on angiography	14 (1)	8 (1)
Unproven clinical label of coronary heart disease	36 (3)	29 (3)
Diabetes	86 (8)	69 (8)
Hypertension	376 (33)	301 (35)
Hyperlipidaemia	271 (24)	201 (24)
Present smoker	310 (28)	242 (28)
Ex-smoker (last 10 years)	144 (13)	101 (12)
Cocaine abuse	6 (1)	6 (1)
First-degree relative with angina/myocardial infarction, onset age < 60 years	344 (31)	271 (32)
Use of aspirin in previous 7 days	207 (18)	162 (19)
More than one episode of rest angina in < 24 hours	75 (7)	52 (6)

IQR, interquartile range; max., maximum; min., minimum.

TABLE 8 Final diagnostic categorisation of patients

	All patients ($N = 1125$)		Biomarker subset ($N = 850$)
Diagnostic categorisation	Admission diagnosis, *n* (%)	Final diagnosis, *n* (%)	Review diagnosis, *n* (%)
Non-specific chest pain	233 (21)	361 (32)	279 (33)
Anxiety	51 (5)	36 (3)	31 (4)
Angina, no ACS	173 (15)	83 (7)	72 (9)
ACS (suspected AMI)	334 (30)	90 (8)	68 (8)
Gastro-oesophageal pain	117 (10)	124 (11)	100 (12)
Musculoskeletal pain	108 (10)	143 (13)	116 (14)
Other	86 (8)	228 (20)	154 (18)
Unknown	23 (2)	60 (5)	30 (3.5)

diagnostic standard used for patient management during the trial. The following diagnostic strategies were compared: individual marker values (cTnI > 99th percentile, CK-MB > 5 μg/l[20] and myoglobin > 95th percentile), delta CK-MB > 1.5 μg/l[20] and myoglobin (defined as percentage change from admission measurement > 25%). These were then compared with the combination of individual markers at presentation, the combination of individual markers at 90 minutes and the combination of admission and 90-minute marker values or delta values. For each combination, rule-in diagnosis of an AMI (specificity and positive predictive value) and rule-out diagnosis of an AMI (sensitivity and negative predictive value) were calculated.

Full data were available for 84 out of 90 patients of AMI and for 987 out of 1035 patients in whom an AMI was excluded. There were no interpretable results for cTnI in 45 patients (five AMI), for CK-MB in 47 patients (six AMI) and for myoglobin in 40 patients (six AMI). In the admission sample measurement of cTnI was the most diagnostically efficient, with an area under the ROC curve (95% confidence intervals in parentheses) of 0.96 (0.93 to 0.98) compared with 0.85 (0.80 to 0.90) for CK-MB and 0.75 (0.68 to 0.81) for myoglobin, and was statistically significantly greater than all other analytes, with a sensitivity of 0.845 (0.750 to 0.915) and a specificity of 0.976 (0.964 to 0.984). At 90 minutes, cTnI measurement had the highest AUC – 0.95 (0.87 to 1.00) compared with 0.86 (0.77 to 0.94) for CK-MB, 0.78 (0.65 to 0.91) for myoglobin, 0.83 (0.65 to 1.00) for delta CK-MB and 0.58 (0.35 to 0.81) for delta myoglobin – but was statistically significantly different only from delta myoglobin ($p = 0.0035$) and delta CK-MB ($p = 0.0064$). Comparison of final diagnoses showed that in one case there was no diagnostic elevation of cTnI, CK-MB or myoglobin on admission or at 90 minutes, although there was a clinical diagnosis of myocardial infarction and the patient was admitted. In one case CK-MB and myoglobin were elevated, but cTnI was not. The data are summarised in *Table 9*.

Optimal diagnostic performance for all markers was achieved by 90 minutes. Both on admission and at 90 minutes, measurement of cTnI was diagnostically significantly more sensitive (hence, would allow rule-out of an AMI) than CK-MB or myoglobin but was more specific (rule-in of an AMI) only than myoglobin. Examining the combination of peak values and peak values plus change (delta) showed that cTnI was superior to both CK-MB and myoglobin alone or in combination. Delta cTnI was less sensitive (12/16) than peak cTnI (83/85), but was more sensitive than CK-MB ($p = 0.018$) or myoglobin ($p = 0.018$).

Do cytoplasmic markers of myocardial damage contribute to the early differential diagnosis of patients presenting with chest pain?

The measurement of H-FABP has been proposed as a more sensitive marker because of the cytoplasmic location of H-FABP and its low molecular weight. However, the molecular weight (15 kDa) is not substantially less than that of myoglobin (16.7 kDa), although the tissue concentration is higher. The diagnostic performance of H-FABP was therefore compared with that of the other cytoplasmic markers of myocardial necrosis and cTnI measured using the Stratus CS analyser. H-FABP measurement was also compared with the other high-sensitivity cTnI and cTnT methods. ROC analysis used the diagnosis of myocardial infarction based on the review diagnosis utilising the cTnI Ultra assay as the reference standard to provide a degree of independence of the diagnostic classification from the biomarkers undergoing evaluation. ROC curves were constructed for markers measured on admission and for peak values (admission or 90 minutes from admission) and are shown in *Figures 5–8*. AUCs and comparison of AUCs are provided in *Tables 10* and *11*.

Cardiac troponin measurement was diagnostically superior to all of the other markers on admission as assessed by comparison of areas under the ROC curve. Both H-FABP and CK-MB were superior to myoglobin on admission. The diagnostic efficiencies of CK-MB and H-FABP were equivalent. The following diagnostic strategies were also compared: individual marker values [(cTnI > 99th percentile (0.07 μg/l), CK-MB > 5 μg/l[20] myoglobin > 95th percentile (66 mg/l), hFABP > 95th percentile (2.5 mg/l)]; delta CK-MB > 1.6 μg/l[20] and myoglobin (defined as % change from admission measurement > 25%); the combination of presentation or 90-minute value and the combination of presentation or 90-minute value plus delta value. These results are summarised in *Table 12*. As found previously, the diagnostic sensitivity of troponin

TABLE 9 Diagnostic efficiencies of individual markers on admission and at 90 minutes

Diagnosis	Positive test	cTnI	CK-MB	Myoglobin	cTnI or CK-MB	cTnI or myoglobin	Triple test	CK-MB or myoglobin
On admission								
MI (*n*)	Y	71	33	30	72	74	74	
	N	13	51	54	11	9	8	
No MI (*n*)	Y	24	31	50	47	67	82	
	N	966	959	942	933	912	889	
Sensitivity (95% CI)		0.845 (0.750 to 0.915)	0.393 (0.288 to 0.505)	0.357 (0.256 to 0.469)	0.867 (0.775 to 0.932)	0.892 (0.804 to 0.949)	0.902 (0.817 to 0.957)	
p-value			<0.0001	<0.0001	NS	NS	NS	
Specificity (95% CI)		0.976 (0.964 to 0.984)	0.969 (0.956 to 0.979)	0.950 (0.934 to 0.962)	0.952 (0.937 to 0.965)	0.932 (0.914 to 0.947)	0.916 (0.896 to 0.932)	
p-value			NS	0.003	0.0069	<0.0001	<0.0001	
PPV (95% CI)		0.747 (0.648 to 0.831)	0.516 (0.387 to 0.642)	0.375 (0.269 to 0.490)	0.605 (0.517 to 0.693)	0.525 (0.442 to 0.607)	0.474 (0.396 to 0.553)	
NPV (95% CI)		0.987 (0.977 to 0.993)	0.950 (0.934 to 0.962)	0.946 (0.930 to 0.959)	0.988 (0.979 to 0.994)	0.990 (0.982 to 0.996)	0.991 (0.982 to 0.996)	
At 90 minutes								
MI (*n*)	Y	16	3	4	12	11	11	
	N	1	9	7	0	0	0	
No MI (*n*)	Y	13	16	28	27	41	51	
	N	814	809	798	789	775	756	
Sensitivity (95% CI)		0.941 (0.713 to 0.999)	0.250 (0.055 to 0.572)	0.364 (0.109 to 0.692)	1.000 (0.735 to 1.000)	1.000 (0.715 to 1.000)	1.000 (0.715 to 1.000)	
p-value			0.0004	0.004	NS	NS	NS	
Specificity (95% CI)		0.984 (0.973 to 0.992)	0.981 (0.969 to 0.989)	0.966 (0.951 to 0.977)	0.967 (0.952 to 0.978)	0.950 (0.932 to 0.964)	0.937 (0.918 to 0.953)	

continued

TABLE 9 Diagnostic efficiencies of individual markers on admission and at 90 minutes (*continued*)

Diagnosis	Positive test	cTnI	CK-MB	Myoglobin	cTnI or CK-MB	cTnI or myoglobin	Triple test	CK-MB or myoglobin
p-value			NS	0.03	0.0337	0.0002	<0.0001	
PPV (95% CI)		0.552 (0.357 to 0.736)	0.158 (0.034 to 0.396)	0.125 (0.035 to 0.290)	0.308 (0.170 to 0.476)	0.212 (0.111 to 0.347)	0.177 (0.092 to 0.295)	
NPV (95% CI)		0.999 (0.993 to 1.000)	0.989 (0.979 to 0.995)	0.991 (0.982 to 0.996)	1.000 (0.995 to 1.000)	1.000 (0.995 to 1.000)	1.000 (0.995 to 1.000)	
Peak or delta								
MI (*n*)	Y	83	33	33	84	84	84	43
	N	2	51	51	1	1	1	42
No MI (*n*)	Y	37	31	127	60	155	170	145
	N	958	963	874	941	849	834	857
Sensitivity (95% CI)		0.976 (0.918 to 0.997)	0.393 (0.288 to 0.505)	0.393 (0.288 to 0.505)	0.988 (0.936 to 1.000)	0.988 (0.936 to 1.000)	0.988 (0.936 to 1.0)	0.506 (0.395 to 0.616)
p-value			<0.0001	<0.0001	NS	NS	NS	<0.0001
Specificity (95% CI)		0.963 (0.949 to 0.974)	0.969 (0.969 to 0.979)	0.873 (0.853 to 0.894)	0.940 (0.924 to 0.954)	0.846 (0.823 to 0.868)	0.831 (0.807 to 0.861)	0.855 (0.834 to 0.877)
p-value			NS	0.003	0.0239	<0.0001	<0.0001	<0.0001
PPV (95% CI)		0.692 (0.609 to 0.774)	0.516 (0.387 to 0.643)	0.206 (0.144 to 0.269)	0.583 (0.503 to 0.664)	0.351 (0.291 to 0.412)	0.331 (0.273 to 0.389)	0.229 (0.169 to 0.289)
NPV (95% CI)		0.998 (0.992 to 1.000)	0.950 (0.934 to 0.962)	0.945 (0.928 to 0.959)	0.999 (0.994 to 1.000)	0.999 (0.994 to 1.000)	0.999 (0.993 to 1.000)	0.953 (0.937 to 0.966)

MI, myocardial infarction; N, no; NPV, negative predictive value; NS, not significant; PPV, positive predictive value; Y, yes.

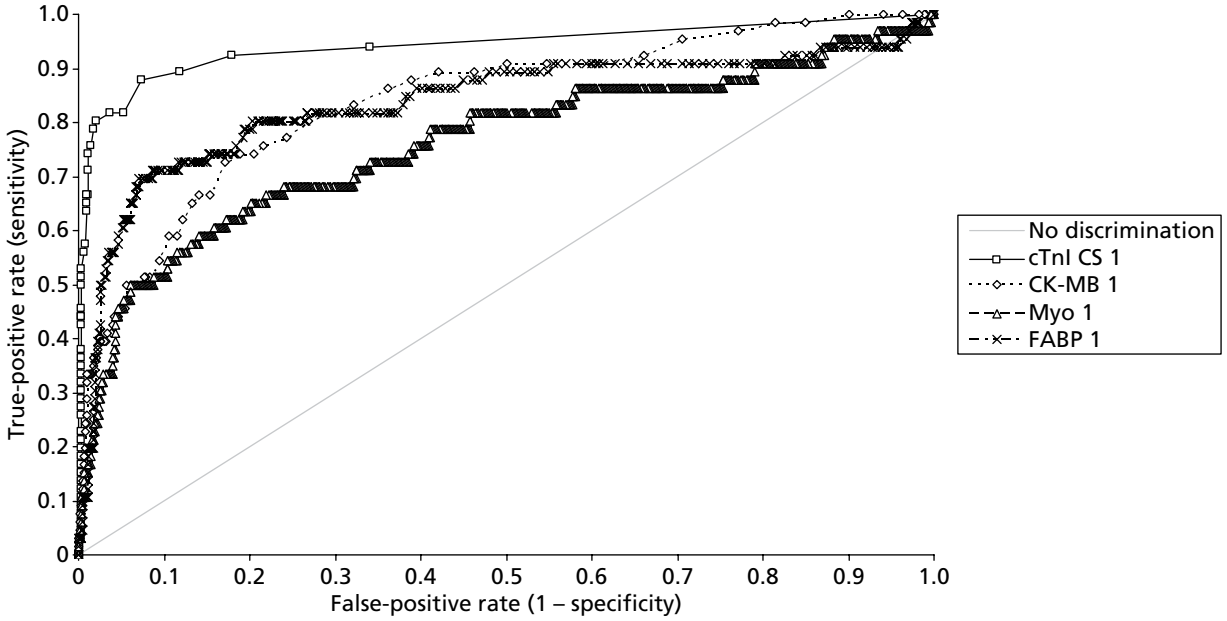

FIGURE 5 Receiver operating characteristic curves for cytoplasmic biomarkers compared with cTnI for the diagnosis of an AMI: markers measured on admission. cTnI CS 1, Stratus CS admission sample; CK-MB 1, CK-MB admission sample; Myo 1, myoglobin admission sample; FABP 1, H-FABP admission sample.

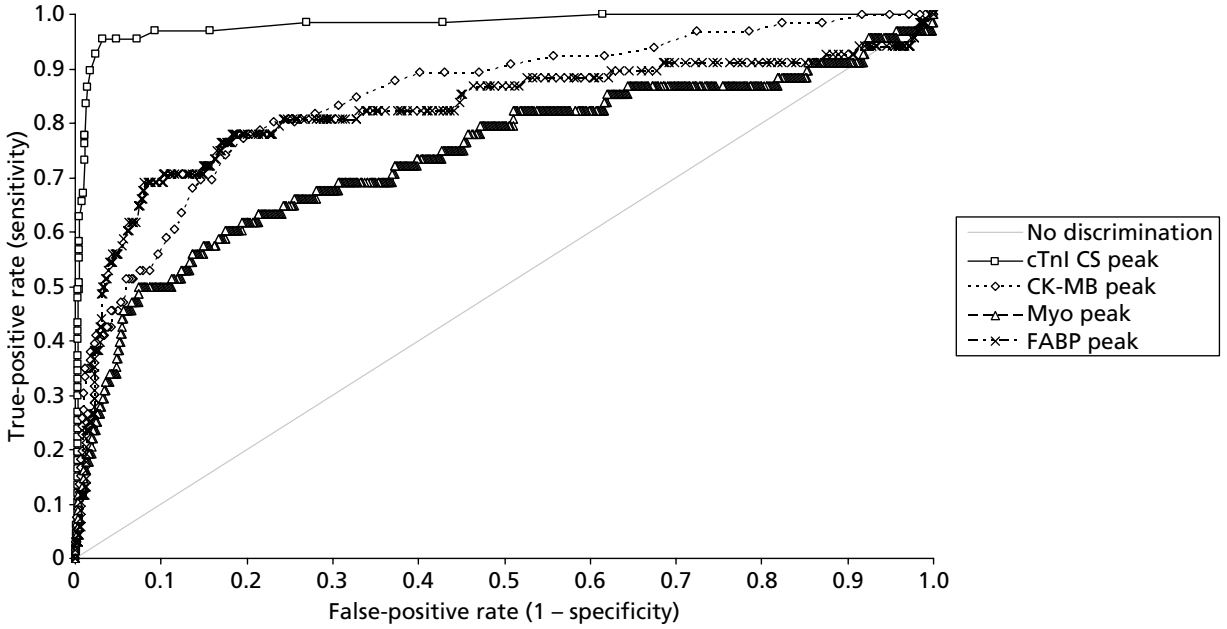

FIGURE 6 Receiver operating characteristic curves for cytoplasmic biomarkers compared with cTnI for the diagnosis of an AMI. Markers are peak values of measurement on admission or 90 minutes from admission. cTnI CS peak, Stratus CS peak value; CK-MB peak, CK-MB peak value; Myo peak, myoglobin peak value; FABP peak, H-FABP peak value.

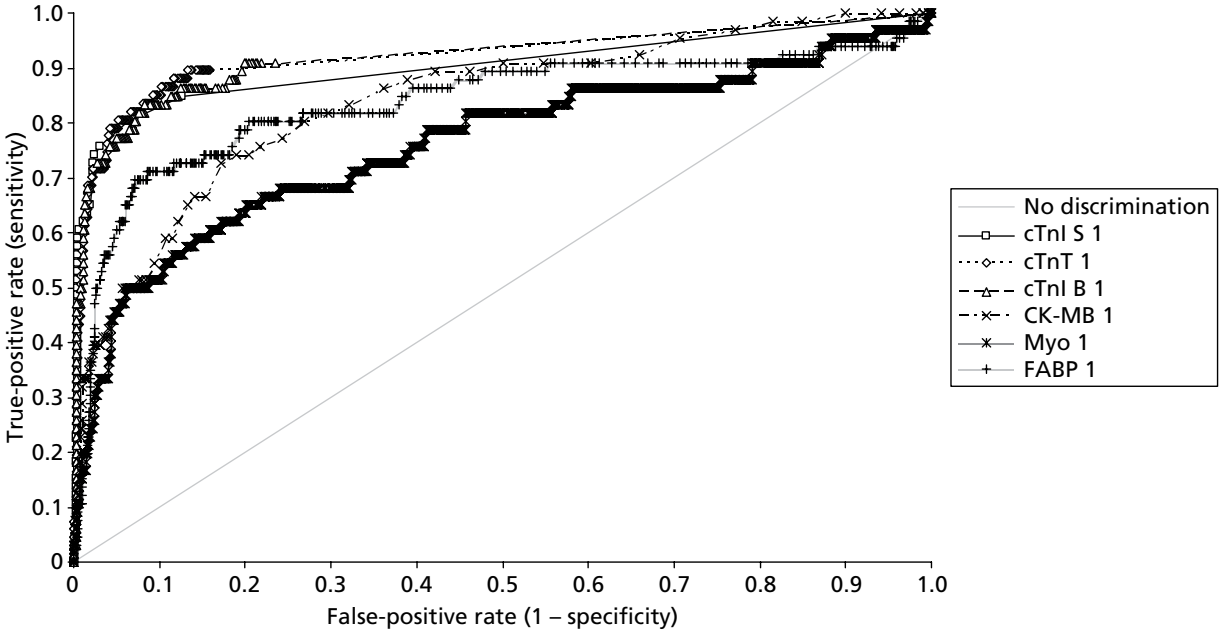

FIGURE 7 Receiver operating characteristic curves for cytoplasmic biomarkers compared with cTnI and cTnT for the diagnosis of an AMI: markers measured on admission. cTnI CS 1, Stratus CS admission sample; cTnT 1, Roche high-sensitivity cTnT admission value; cTnI B1, Beckman AccuTnI admission sample; CK-MB 1, CK-MB admission sample; Myo 1, myoglobin admission sample; FABP 1, H-FABP admission sample.

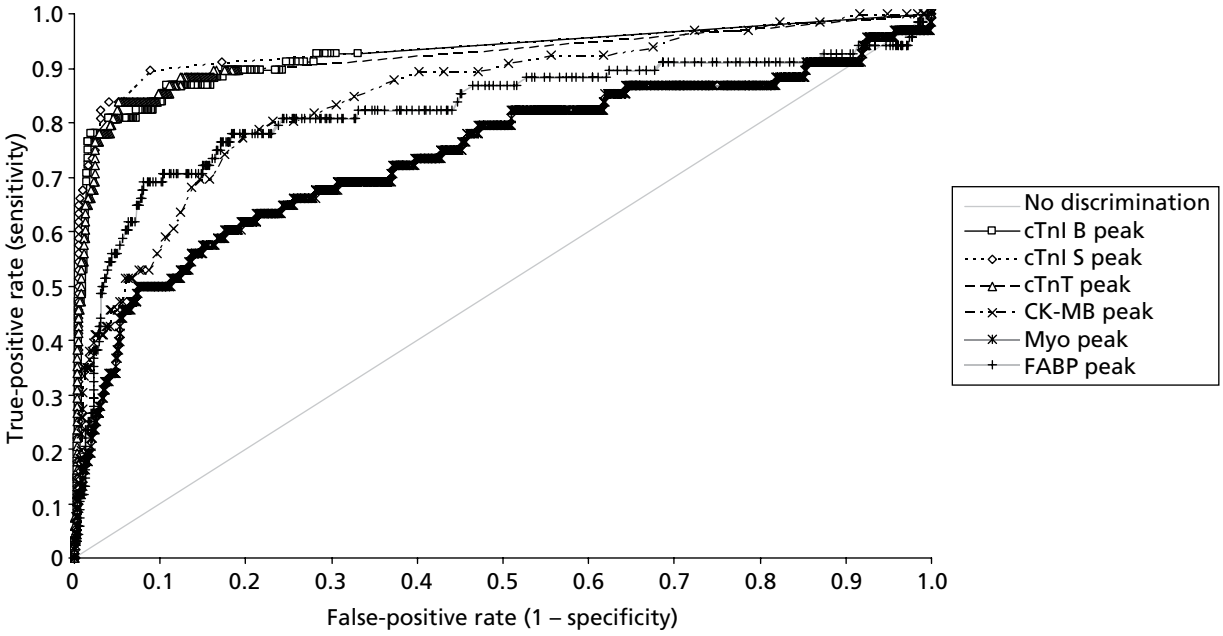

FIGURE 8 Receiver operating characteristic curves for cytoplasmic biomarkers compared with cTnI and cTnT for the diagnosis of an AMI. Markers are peak values of measurement on admission or 90 minutes from admission. cTnI B peak, Beckman AccuTnI peak value; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value; CK-MB peak, CK-MB peak value; Myo peak, myoglobin peak value; FABP peak, H-FABP peak value.

TABLE 10 Area under the ROC curve and comparisons of AUCs for cytoplasmic biomarkers compared with cTnI and cTnT for the diagnosis of an AMI: markers measured on admission

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS 1	0.94 (0.90 to 0.98)	cTnI CS 1 vs CK-MB 1	0.10 (0.04 to 0.16)	0.0023
CK-MB 1	0.84 (0.79 to 0.90)	cTnI CS 1 vs Myo 1	0.18 (0.10 to 0.26)	<0.0001
Myo 1	0.76 (0.69 to 0.84)	cTnI CS 1 vs FABP 1	0.10 (0.02 to 0.18)	0.0115
FABP 1	0.84 (0.77 to 0.90)	CK-MB 1 vs Myo 1	0.08 (0.02 to 0.14)	0.0107
cTnI B 1	0.92 (0.88 to 0.96)	CK-MB 1 vs FABP 1	0.00 (–0.05 to 0.05)	0.8579
cTnT 1	0.92 (0.88 to 0.96)	Myo 1 vs FABP 1	–0.08 (–0.14 to –0.01)	0.0146
cTnI S 1	0.90 (0.85 to 0.95)	cTnI B 1 vs CK-MB 1	0.08 (0.02 to 0.13)	0.0049
		cTnI B 1 vs Myo 1	0.16 (0.09 to 0.23)	<0.0001
		cTnI B 1 vs FABP 1	0.08 (0.02 to 0.14)	0.0054
		cTnI S 1 vs CK-MB 1	0.06 (0.01 to 0.11)	0.0267
		cTnI S 1 vs Myo 1	0.14 (0.07 to 0.21)	0.0002
		cTnI S 1 vs FABP 1	0.07 (0.01 to 0.12)	0.0317
		cTnT 1 vs CK-MB 1	0.08 (0.03 to 0.13)	0.0018
		cTnT 1 vs Myo 1	0.16 (0.09 to 0.23)	<0.0001
		cTnT 1 vs FABP 1	0.08 (0.03 to 0.14)	0.0036

CI, confidence interval; CK-MB 1, CK-MB admission sample; cTnI B 1, Beckman AccuTnI admission sample; cTnI CS 1, Stratus CS admission sample; cTnI S 1, Siemens Ultra admission sample; cTnT 1, Roche high-sensitivity cTnT admission sample; FABP 1, H-FABP admission sample; Myo 1, myoglobin admission sample.

was superior to that of all of the other biomarkers examined but its diagnostic specificity was not significantly different from that of CK-MB, although it was superior to that of the other two biomarkers. The use of a delta value for CK-MB, myoglobin and H-FABP did not significantly improve sensitivity but significantly worsened the specificity for myoglobin and H-FABP.

Are all high-sensitivity cardiac troponin methods of equivalent diagnostic efficiency?

The diagnostic performance of four methods of measuring cardiac troponin was compared utilising the review diagnosis as the gold standard. The four methods were cTnI measured using the Stratus CS analyser, the Beckman AccuTnI method and the Siemens Ultra assay and cTnT measured using the Roche high-sensitivity assay. Diagnosis was initially compared using the admission sample alone and then using the peak value of the admission or the 90-minute sample. The ROC curves obtained are shown in *Figures 9* and *10* with comparison of the AUCs in *Table 13*. Diagnosis based on classification using the 99th percentile value is summarised in *Table 14*.

There were no statistically significant differences between the AUCs or diagnostic categorisation for the admission samples for any of these troponin methods. For peak value, the Stratus CS seemed to be diagnostically superior to all of the other troponin methods. There were no differences between any of the other methods. This may well represent a selection bias in the analysis as patients with an elevated Stratus CS troponin level were admitted and a second sample was not taken.

The individual methods missed varying numbers of patients with a final diagnosis of myocardial infarction. The Stratus CS cTnI missed three patients. One was diagnosed on ECG criteria, but had an elevated cTnT. The other two patients had elevated cTnI by the Siemens assay and elevated cTnT. The Beckman cTnI assay

TABLE 11 Area under the ROC curve and comparisons of AUCs for cytoplasmic biomarkers compared with cTnI and cTnT for the diagnosis of an AMI: peak value of measurement on admission or 90 minutes from admission

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS peak	0.98 (0.96 to 1.00)	cTnI CS peak vs CK-MB peak	0.13 (0.08 to 0.19)	<0.0001
CK-MB peak	0.85 (0.80 to 0.90)	cTnI CS peak vs Myo peak	0.24 (0.16 to 0.32)	<0.0001
Myo peak	0.74 (0.67 to 0.82)	cTnI CS peak vs FABP peak	0.16 (0.09 to 0.23)	<0.0001
FABP peak	0.82 (0.75 to 0.89)	CK-MB peak vs Myo peak	0.11 (0.04 to 0.17)	0.0008
cTnI B peak	0.93 (0.88 to 0.97)	CK-MB peak vs FABP peak	0.03 (–0.03 to 0.08)	0.3328
cTnI S peak	0.94 (0.90 to 0.97)	Myo peak vs FABP peak	–0.08 (–0.15 to –0.02)	0.0109
cTnT peak	0.92 (0.88 to 0.97)	cTnI B peak vs CK-MB peak	0.08 (0.02 to 0.13)	0.0041
		cTnI B peak vs Myo peak	0.18 (0.11 to 0.26)	<0.0001
		cTnI B peak vs FABP peak	0.10 (0.04 to 0.16)	0.0005
		cTnI S peak vs CK-MB peak	0.09 (0.04 to 0.13)	0.0006
		cTnI S peak vs Myo peak	0.19 (0.12 to 0.26)	<0.0001
		cTnI S peak vs FABP peak	0.11 (0.06 to 0.17)	<0.0001
		cTnT peak vs CK-MB peak	0.07 (0.02 to 0.12)	0.0046
		cTnT peak vs Myo peak	0.18 (0.11 to 0.25)	<0.0001
		cTnT peak vs FABP peak	0.10 (0.04 to 0.16)	0.0019

CI, confidence interval; CK-MB peak, CK-MB peak value; cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak value; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value; FABP peak, H-FABP peak value; Myo peak, myoglobin peak value.

TABLE 12 Diagnostic efficiencies of individual markers on admission, individual markers at 90 minutes, peak values of individual markers and peak values of individual markers plus delta (change) for the diagnosis of an AMI

Diagnosis	Positive test	cTnI	CK-MB	Myoglobin	H-FABP
On admission					
MI (*n*)	Y	53	26	36	43
	N	13	40	30	23
No MI (*n*)	Y	16	23	81	48
	N	749	739	684	717
Sensitivity (95% CI)		0.803 (0.6867 to 0.891)	0.394 (0.276 to 0.522)	0.546 (0.418 to 0.669)	0.652 (0.524 to 0.765)
p-value[a]			<0.0001	0.0027	NS
Specificity (95% CI)		0.979 (0.966 to 0.988)	0.970 (0.955 to 0.981)	0.894 (0.870 to 0.915)	0.937 (0.918 to 0.953)
p-value[a]			NS	<0.0001	<0.0001
NPV (95% CI)		0.983 (0.971 to 0.991)	0.949 (0.931 to 0.963)	0.958 (0.941 to 0.971)	0.969 (0.954 to 0.980)

TABLE 12 Diagnostic efficiencies of individual markers on admission, individual markers at 90 minutes, peak values of individual markers and peak values of individual markers plus delta (change) for the diagnosis of an AMI (*continued*)

Diagnosis	Positive test	cTnI	CK-MB	Myoglobin	H-FABP
At 90 minutes					
MI (*n*)	Y	13	2	6	7
	N	1	8	5	4
No MI (*n*)	Y	9	14	57	20
	N	646	638	589	626
Sensitivity (95% CI)		0.929 (0.661 to 0.998)	0.200 (0.0252 to 0.556)	0.546 (0.234 to 0.833)	0.636 (0.308 to 0.891)
p-value[a]			0.0010	NS	NS
Specificity (95% CI)		0.986 (0.974 to 0.994)	0.979 (0.964 to 0.988)	0.912 (0.887 to 0.933)	0.969 (0.953 to 0.981)
p-value[a]			NS	<0.0001	NS
NPV (95% CI)		0.998 (0.991 to 1.000)	0.988 (0.976 to 0.995)	0.992 (0.980 to 0.997)	0.994 (0.984 to 0.998)
Peak value					
MI (*n*)	Y	64	27	38	44
	N	3	39	30	24
No MI (*n*)	Y	24	25	107	58
	N	742	740	673	722
Sensitivity (95% CI)		0.955 (0.875 to 0.991)	0.409 (0.289 to 0.537)	0.559 (0.433 to 0.679)	0.647 (0.522 to 0.759)
p-value[a]			<0.0001	<0.0001	<0.0001
Specificity (95% CI)		0.969 (0.954 to 0.980)	0.967 (0.952 to 0.979)	0.863 (0.839 to 0.887)	0.926 (0.905 to 0.943)
p-value[a]			NS	<0.0001	0.0003
Peak value or delta					
MI (*n*)	Y	64	27	38	46
	N	3	39	30	22
No MI (*n*)	Y	24	25	246	213
	N	742	740	534	567
Sensitivity (95% CI)		0.955 (0.875 to 0.991)	0.409 (0.289 to 0.537)	0.559 (0.433 to 0.679)	0.676 (0.552 to 0.785)
p-value[a]			<0.0001	<0.0001	<0.0001
Specificity (95% CI)		0.969 (0.954 to 0.980)	0.967 (0.952 to 0.979)	0.680 (0.647 to 0.713)	0.727 (0.696 to 0.758)
p-value[a]			NS	<0.0001	<0.0001

CI, confidence interval; MI, myocardial infarction; N, no; NPV, negative predictive value; NS, not significant, Y, yes.

a *p*-values are significance for diagnostic categorisation for each marker or marker combination compared with the diagnostic performance of cTnI measurement on the Stratus CS alone.

TABLE 13 Area under the ROC curve and comparison of AUCs for cardiac troponin measurements for the diagnosis of an AMI: markers measured on admission (1) or peak value of measurement on admission or at 90 minutes from admission (peak)

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS 1	0.94 (0.90 to 0.98)	cTnI CS 1 vs cTnI B 1	0.02 (–0.02 to 0.07)	0.3752
cTnI B 1	0.92 (0.88 to 0.96)	cTnI CS 1 vs cTnI S 1	0.04 (–0.01 to 0.08)	0.0947
cTnI S 1	0.90 (0.85 to 0.95)	cTnI CS 1 vs cTnT 1	0.02 (–0.03 to 0.07)	0.4610
cTnT 1	0.92 (0.88 to 0.96)	cTnI B 1 vs cTnI S 1	0.02 (–0.01 to 0.05)	0.2117
		cTnI B 1 vs cTnT 1	0.00 (–0.04 to 0.04)	0.9317
		cTnI S 1 vs cTnT 1	–0.02 (–0.05 to 0.01)	0.2494
cTnI CS peak	0.98 (0.96 to 1.00)	cTnI CS peak vs cTnI B peak	0.05 (0.02 to 0.09)	0.0056
cTnI B peak	0.93 (0.88 to 0.97)	cTnI CS peak vs cTnI S peak	0.05 (0.01 to 0.08)	0.0152
cTnI S peak	0.94 (0.90 to 0.97)	cTnI CS peak vs cTnT peak	0.06 (0.01 to 0.11)	0.0134
cTnT peak	0.92 (0.88 to 0.97)	cTnI B peak vs cTnI S peak	–0.01 (–0.02 to 0.01)	0.2340
		cTnI B peak vs cTnT peak	0.00 (–0.04 to 0.04)	0.8454
		cTnI S peak vs cTnT peak	0.01 (–0.02 to 0.05)	0.4760

CI, confidence interval; cTnI B 1, Beckman AccuTnI admission sample; cTnI B peak, Beckman AccuTnI peak value; cTnI CS 1, Stratus CS admission sample; cTnI CS peak, Stratus CS peak value; cTnI S 1, Siemens Ultra admission sample; cTnI S peak, Siemens Ultra peak value; cTnT 1, Roche high-sensitivity cTnT admission sample; cTnT peak, Roche high-sensitivity cTnT peak value.

missed 15 patients. In 13 patients there was no second sample but six showed elevation of cTnI measured by the Siemens assay and seven showed elevation of cTnT. The Siemens cTnI assay missed 11 patients, eight of whom had no second sample, but showed elevation of cTnI on the Beckman assay (two patients) and elevation of cTnT (four patients). Measurement of cTnT missed 11 patients, 10 of whom did not have a second sample. Three patients had an elevated cTnI on the Beckman and Siemens assays and one had an elevated cTnI on the Siemens assay alone.

Increased sensitivity of troponin methods is at the expense of specificity due to the detection of myocardial injury in a range of other non-ACS conditions. In the population where AMI was excluded, elevation of all four troponins occurred in three cases, two of which had myocarditis. Three patients showed elevation of all three of the troponin methods under investigation but not of cTnI measured by the Stratus CS. One of these patients had myocarditis. Elevation of cTnI measured by the Stratus CS occurred in 24/766 (3.1%) of cases with a final diagnosis that excluded myocardial infarction, two also showed elevation of cTnI by the Siemens Ultra, 1 by the Beckman method and eight showed elevation of cTnT. Elevation of troponin in patients without a final diagnosis of myocardial infarction occurred in 15/781 (1.9%) patients for cTnI measured by the Beckman method, 31 (31/782, 4.0%) patients for cTnI measured by the Siemens Ultra assay and 43/779 (5.5%) patients for cTnT. One in 15 with elevated cTnI by the Beckman method had elevation by the Stratus CS alone and 9/15 by the Siemens Ultra alone and 11/15 had an elevated cTnT. For the Siemens method, 16/31 also had elevation of cTnT. Detailed information on missed diagnosis of myocardial infarction for all four troponin methods and elevations in patients without a final diagnosis of myocardial infarction is summarised in *Appendices 1* and *2*.

TABLE 14 Diagnostic efficiencies of cardiac troponin measurements on admission and of peak values of individual markers for the diagnosis of an AMI

Diagnosis	cTnI CS	cTnI B	cTnI S	cTnT
On admission				
MI (*n*)	53	44	49	53
	13	22	17	14
No MI (*n*)	16	11	19	33
	749	758	751	733
Sensitivity (95% CI)	0.803 (0.687 to 0.891)	0.667 (0.540 to 0.778)	0.742 (0.620 to 0.842)	0.791 (0.674 to 0.881)
p-value[a]		NS	NS	NS
Specificity (95% CI)	0.979 (0.966 to 0.988)	0.986 (0.975 to 0.993)	0.975 (0.962 to 0.985)	0.957 (0.940 to 0.970)
p-value[a]		NS	NS	0.021
NPV (95% CI)	0.983 (0.971 to 0.991)	0.972 (0.958 to 0.982)	0.978 (0.965 to 0.987)	0.981 (0.969 to 0.990)
Peak value				
MI (*n*)	64	53	57	57
	3	15	11	11
No MI (*n*)	24	15	31	43
	742	766	751	736
Sensitivity (95% CI)	0.955 (0.875 to 0.991)	0.779 (0.662 to 0.871)	0.838 (0.729 to 0.916)	0.838 (0.729 to 0.916)
p-value[a]		0.005	0.048	0.048
Specificity (95% CI)	0.969 (0.954 to 0.980)	0.981 (0.969 to 0.989)	0.960 (0.944 to 0.973)	0.945 (0.926 to 0.960)
p-value[a]		NS	NS	0.029

CI, confidence interval; cTnI B, Beckman AccuTnI sample; cTnI CS, Stratus CS sample; cTnI S, Siemens Ultra sample; cTnT, Roche high-sensitivity cTnT sample; MI, myocardial infarction; NPV, negative predictive value; NS, not significant.

a *p*-values are significance for diagnostic categorisation for each marker compared with the diagnostic performance of cTnI measured using the Stratus CS.

Do markers of vascular dysfunction contribute to the early differential diagnosis of patients presenting with chest pain?

Measurement of cardiac troponin was compared with measurement of copeptin and NTproBNP on admission and for the peak of the admission or 90 minutes from admission value. The diagnostic efficiency was compared by ROC curve analysis using the review final diagnosis as above. The ROC curves are shown in *Figures 11* and *12* with the areas under the ROC curve and comparisons in *Table 15*. Measurement of NTproBNP was superior to copeptin measurement in the admission and peak samples. Measurement of cardiac troponin was superior to both NTproBNP and copeptin measurement on admission and in the peak sample.

Do combinations of biomarkers allow earlier rule-in or rule-out of an acute myocardial infarction in patients presenting with chest pain?

The rationale for the selection of other cytoplasmic biomarkers of cardiac necrosis or neurohormones is that they may provide earlier information or supplementary information to measurement of cardiac

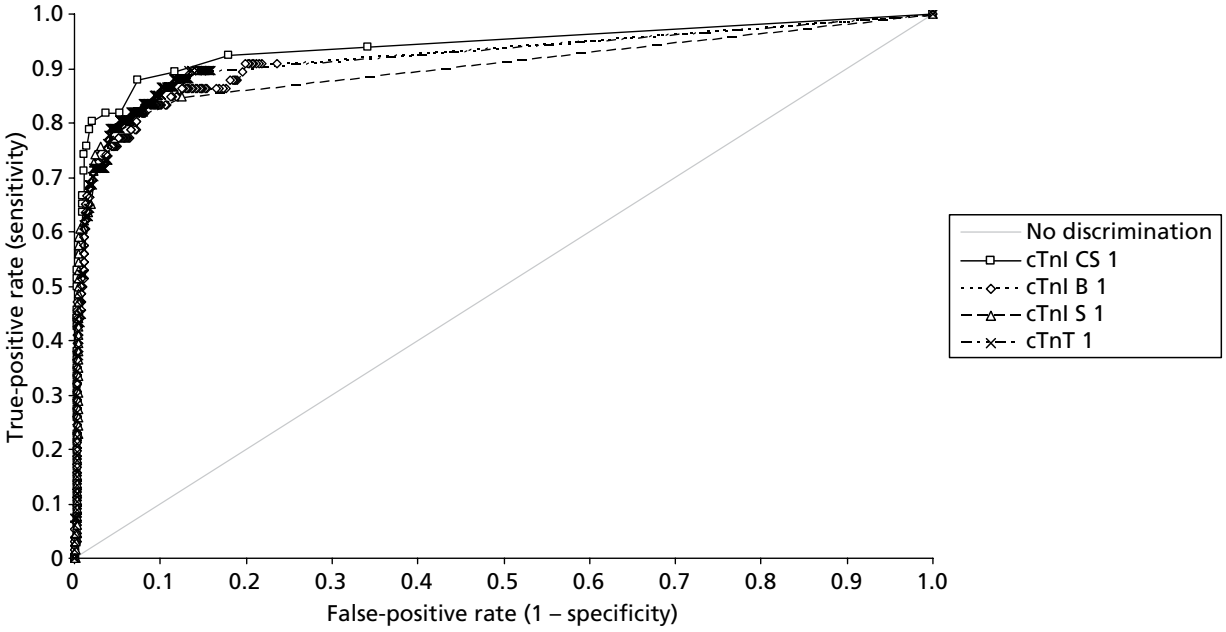

FIGURE 9 Receiver operating characteristic curves for troponin measurement for the diagnosis of an AMI: markers measured on admission. cTnI CS 1, Stratus CS admission sample; cTnI B 1, Beckman AccuTnI admission sample; cTnI S 1, Siemens Ultra admission sample; cTnT 1, Roche high-sensitivity cTnT admission sample.

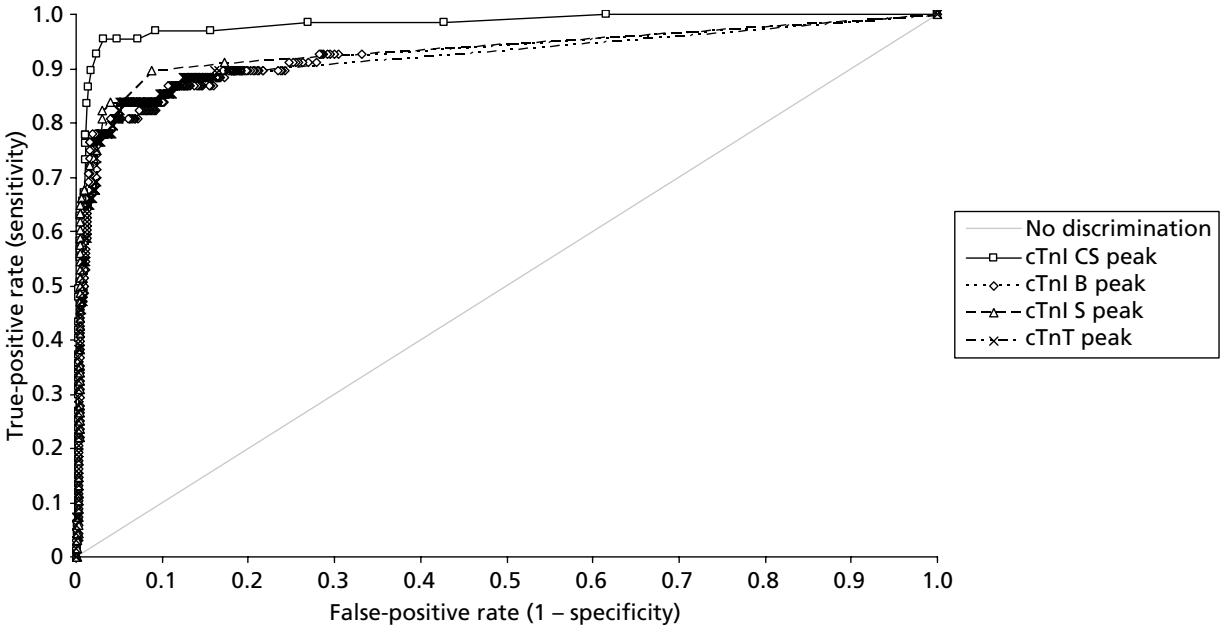

FIGURE 10 Receiver operating characteristic curves for troponin measurement for the diagnosis of an AMI: peak values on admission or at 90 minutes from admission. cTnI CS peak, Stratus CS peak value; cTnI B peak, Beckman AccuTnI peak value; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

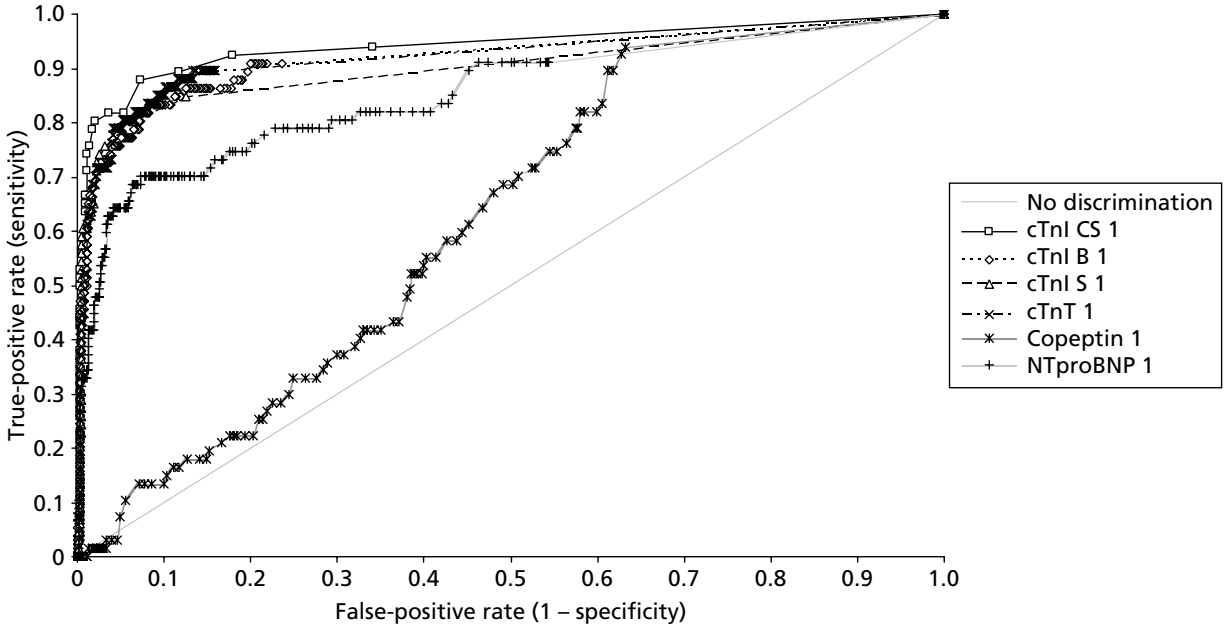

FIGURE 11 Receiver operating characteristic curves for vascular dysfunction markers compared with troponin measurements for the diagnosis of an AMI: markers measured on admission. cTnI CS 1, Stratus CS admission sample; cTnI B1, Beckman AccuTnI admission sample; cTnI S 1, Siemens Ultra admission sample; cTnT 1, Roche high-sensitivity cTnT admission value; copeptin 1, copeptin admission sample; NTproBNP 1, NTproBNP admission sample

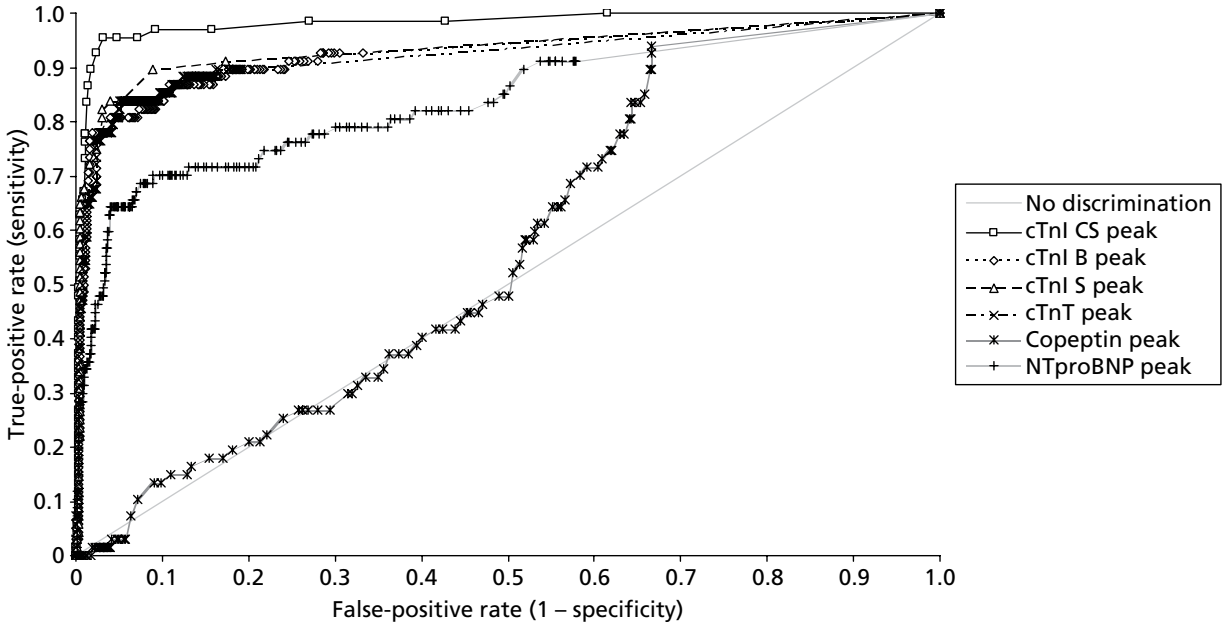

FIGURE 12 Receiver operating characteristic curves for vascular dysfunction markers compared with troponin measurements for the diagnosis of an AMI. Markers are peak values of measurement on admission or 90 minutes from admission. cTnI CS peak, Stratus CS peak sample; cTnI B peak, Beckman AccuTnI peak value; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cardiac cTnT peak value; copeptin peak, copeptin peak value; NTproBNP peak, NTproBNP peak value

TABLE 15 Area under the ROC curve and comparisons of AUCs for vascular dysfunction markers and cardiac troponin measurements for the diagnosis of an AMI on admission (1) and for peak values (peak)

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS 1	0.94 (0.90 to 0.98)	cTnI CS 1 vs copeptin 1	0.32 (0.26 to 0.38)	<0.0001
cTnI B 1	0.92 (0.88 to 0.96)	cTnI CS 1 vs NTproBNP 1	0.09 (0.03 to 0.14)	0.0029
cTnI S 1	0.90 (0.85 to 0.95)	cTnI B 1 vs copeptin 1	0.30 (0.23 to 0.36)	<0.0001
cTnT 1	0.92 (0.88 to 0.96)	cTnI B 1 vs NTproBNP 1	0.07 (0.02 to 0.12)	0.0101
Copeptin 1	0.62 (0.57 to 0.68)	cTnI S 1 vs copeptin 1	0.28 (0.22 to 0.34)	<0.0001
NTproBNP 1	0.85 (0.80 to 0.9)	cTnI S 1 vs NTproBNP 1	0.05 (0.01 to 0.09)	0.0273
		cTnT 1 vs copeptin 1	0.30 (0.24 to 0.36)	<0.0001
		cTnT 1 vs NTproBNP 1	0.07 (0.03 to 0.11)	0.0010
		Copeptin 1 vs NTproBNP 1	–0.23 (–0.30 to –0.16)	<0.0001
cTnI CS peak	0.98 (0.96 to 1.00)	cTnI CS peak vs copeptin peak	0.42 (0.35 to 0.48)	<0.0001
cTnI B peak	0.93 (0.88 to 0.97)	cTnI CS peak vs NTproBNP peak	0.14 (0.08 to 0.21)	<0.0001
cTnI S peak	0.94 (0.90 to 0.97)	cTnI B peak vs copeptin peak	0.36 (0.29 to 0.43)	<0.0001
cTnT peak	0.92 (0.88 to 0.97)	cTnI B peak vs NTproBNP peak	0.09 (0.03 to 0.15)	0.0044
Copeptin peak	0.56 (0.50 to 0.62)	cTnI S peak vs copeptin peak	0.37 (0.30 to 0.44)	<0.0001
NTproBNP peak	0.84 (0.78 to 0.90)	cTnI S peak vs NTproBNP peak	0.10 (0.04 to 0.16)	0.0014
		cTnT peak vs copeptin peak	0.36 (0.30 to 0.42)	<0.0001
		cTnT peak vs NTproBNP peak	0.08 (0.04 to 0.13)	0.0002
		Copeptin peak vs NTproBNP peak	–0.27 (–0.35 to –0.20)	<0.0001

CI, confidence interval; copeptin 1, copeptin admission sample; copeptin peak, copeptin peak value; cTnI B 1, Beckman AccuTnI admission sample; cTnI B peak, Beckman AccuTnI peak value; cTnI CS 1, Stratus CS admission sample; cTnI CS peak, Stratus CS peak value; cTnI S 1, Siemens Ultra admission sample; cTnI S peak, Siemens Ultra peak value; cTnT 1, Roche high-sensitivity cTnT admission sample; cTnT peak, Roche high-sensitivity cTnT peak value; NTproBNP 1, NTproBNP admission sample; NTproBNP peak, NTproBNP peak value.

troponin alone. A number of studies have suggested that the combination of either H-FABP or copeptin measurement with measurement of cardiac troponin on admission would allow very early diagnostic categorisation and potentially discharge solely on the basis of admission measurement. The combination of the different troponins with H-FABP or copeptin for rule-in or rule-out diagnosis was examined by construction of contingency tables utilising 99th percentile cut-offs for troponin and the 95th percentile cut-off for H-FABP. For copeptin, none of the values obtained exceeded the 95th percentile. In view of this an ROC optimised cut-off of 7.4 mg/l was used. The results of the analysis are summarised in *Table 16*. Measurement of H-FABP improved diagnostic sensitivity of all of the troponin measurements, but significantly reduced specificity. The increase in sensitivity obtained from combined measurement was equivalent to the sensitivity obtained from the combination of the measurement of troponin alone on admission and at 90 minutes from admission.

TABLE 16 Comparison of sensitivity and specificity of single biomarkers on admission and combination of measurements on admission with troponin measurement on admission and combined measurement of troponin on admission and 90 minutes from admission for the diagnosis of an AMI[a]

Diagnosis	Positive test	Admission sample	H-FABP	Combined	Copeptin	Combined	NTproBNP	Combined	Peak troponin sample
cTnI CS									
MI (*n*)	Y	50	41	58	34	57	44	56	60
	N	13	22	5	29	6	19	7	3
No MI (*n*)	Y	15	46	57	291	303	48	71	23
	N	725	694	683	449	437	692	669	717
Sensitivity (95% CI)		0.794 (0.673 to 0.885)	0.651 (0.520 to 0.767)	0.921 (0.824 to 0.974)	0.540 (0.409 to 0.666)	0.905 (0.804 to 0.964)	0.698 (0.570 to 0.808)	0.889 (0.784 to 0.954)	0.952 (0.867 to 0.990)
p-value			NS	NS	0.0043	NS		NS	NS
Specificity (95% CI)		0.980 (0.967 to 0.989)	0.938 (0.918 to 0.954)	0.923 (0.901 to 0.941)	0.607 (0.571 to 0.642)	0.591 (0.555 to 0.626)	0.935 (0.915 to 0.952)	0.904 (0.881 to 0.924)	0.969 (0.954 to 0.980)
p-value			<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	
NPV (95% CI)		0.982 (0.970 to 0.991)	0.969 (0.954 to 0.981)	0.993 (0.983 to 0.998)	0.939 (0.914 to 0.959)	0.986 (0.971 to 0.995)	0.973 (0.959 to 0.984)	0.990 (0.979 to 0.996)	0.996 (0.988 to 0.999)
cTnI S									
MI (*n*)	Y	46		54		52		52	52
	N	17		9		11		11	11
No MI (*n*)	Y	14		52		297		55	26
	N	726		688		443		685	714
Sensitivity (95% CI)		0.730 (0.603 to 0.834)		0.857 (0.746 to 0.933)		0.825 (0.709 to 0.909)		0.825 (0.709 to 0.909)	0.825 (0.709 to 0.909)

continued

TABLE 16 Comparison of sensitivity and specificity of single biomarkers on admission and combination of measurements on admission with troponin measurement on admission and combined measurement of troponin on admission and 90 minutes from admission for the diagnosis of an AMI[a] (*continued*)

Diagnosis	Positive test	Admission sample	H-FABP	Combined	Copeptin	Combined	NTproBNP	Combined	Peak troponin sample
p-value				NS		NS		NS	NS
Specificity (95% CI)		0.981 (0.968 to 0.990)		0.930 (0.909 to 0.947)		0.599 (0.563 to 0.634)		0.926 (0.904 to 0.944)	0.965 (0.949 to 0.977)
p-value				<0.0001		<0.0001		<0.0001	<0.0001
NPV (95% CI)		0.977 (0.964 to 0.987)		0.987 (0.976 to 0.994)		0.976 (0.957 to 0.988)		0.984 (0.972 to 0.992)	0.985 (0.973 to 0.992)
cTnT									
MI (*n*)	Y	49		54		53		53	52
	N	14		9		10		10	11
No MI (*n*)	Y	28		62		299		65	38
	N	712		678		441		675	702
Sensitivity (95% CI)		0.778 (0.655 to 0.873)		0.857 (0.746 to 0.933)		0.841 (0.727 to 0.921)		0.841 (0.727 to 0.921)	0.825 (0.709 to 0.909)
p-value				NS		NS		NS	NS
Specificity (95% CI)		0.962 (0.946 to 0.975)		0.916 (0.894 to 0.935)		0.596 (0.561 to 0.631)		0.912 (0.889 to 0.932)	0.949 (0.930 to 0.963)
p-value				0.0003		<0.0001		<0.0001	<0.0001
NPV (95% CI)		0.981 (0.968 to 0.989)		0.987 (0.975 to 0.994)		0.978 (0.969 to 0.989)		0.985 (0.973 to 0.993)	0.985 (0.974 to 0.993)

Diagnosis	Positive test	Admission sample	H-FABP	Combined	Copeptin	Combined	NTproBNP	Combined	Peak troponin sample
cTnI B									
MI (*n*)	Y	42		49		51		49	49
	N	21		14		12		14	14
No MI (*n*)	Y	11		53		296		53	15
	N	729		687		444		687	725
Sensitivity (95% CI)		0.667 (0.537 to 0.780)		0.778 (0.655 to 0.873)		0.841 (0.727 to 0.921)		0.778 (0.665 to 0.873)	0.778 (0.655 to 0.873)
p-value				NS		NS		NS	NS
Specificity (95% CI)		0.985 (0.974 to 0.993)		0.928 (0.907 to 0.946)		0.596 (0.561 to 0.631)		0.928 (0.907 to 0.946)	0.980 (0.980 to 0.989)
p-value				<0.0001		<0.0001		<0.0001	<0.0001
NPV (95% CI)				0.980 (0.967 to 0.989)		0.974 (0.955 to 0.986)			0.981 (0.968 to 0.990)

CI, confidence interval; cTnI B, cTnI Beckman sample; cTnI CS, cTnI Stratus CS sample; cTnI S, cTnI Siemens Ultra sample; cTnT, Roche high-sensitivity cTnT sample; MI, myocardial infarction; N, no; NPV, negative predictive value; NS, not significant; Y, yes.

a Each combination of troponin plus second marker measured on admission (H-FABP, copeptin or NTproBNP) is compared with troponin alone measured on admission for each of the individual troponin methods.

The prognostic accuracy for adverse cardiac events of highly sensitive troponin assays and the range of new cardiac biomarkers

What is the prognostic role of cytoplasmic markers of myocardial damage when compared with troponin measurement?

Receiver operating characteristic curves using the combined MACE of death, readmission with myocardial infarction, readmission with unstable angina or the need for urgent revascularisation as the dichotomous variable were constructed using the admission sample measurement and peak of the admission or 90-minute sample measurement comparing cytoplasmic markers with the four troponin measurement methods. The results are summarised in *Figures 13–16* with the AUCs and comparison of the AUCs in *Tables 17* and *18*.

Differences between the admission value and the peak value were observed. In the case of the admission measurement, cTnI measured on the Stratus CS was equivalent to all of the cytoplasmic markers (CK-MB, myoglobin and H-FABP) for outcome prediction. The three sensitive troponin methods demonstrated a greater AUC than the cytoplasmic markers, which was statistically significant for both cTnI methods but just failed to reach significance for cTnT for CK-MB and H-FABP. All of the cytoplasmic markers had equivalent ability to predict outcome on the admission sample measurement. When peak values were examined, troponin measurement was overall a significantly better outcome predictor than the cytoplasmic markers. Measurement of cTnI on the Stratus CS for the peak values was significantly better than measurement of all three cytoplasmic markers. The other cTnI methods also demonstrated improved outcome prediction with an increase in significance. Measurement of cTnT showed improvement in diagnostic performance only when compared with measurement of myoglobin when peak values were examined.

What is the prognostic role of high-sensitivity troponin assays?

Receiver operating characteristic curves using the combined MACEs of death, readmission with myocardial infarction, readmission with unstable angina or the need for urgent revascularisation as the dichotomous variable were constructed using the admission sample measurement and peak of the admission or 90-minute sample measurement comparing the four troponin measurement methods. The results are summarised in *Figures 14* and *16* with the AUCs and comparison of the AUCs in *Table 19*.

There was no statistically significant difference between the ability of the different troponin methods to predict MACEs on either admission or for peak sample measurements. Although cTnI measurement using the Stratus CS showed superior performance for the diagnosis of an AMI when assessed using peak values, the ability of the different troponin methods to predict MACEs was not statistically significantly different between admission values and peak values. The apparent superiority of the Stratus CS method for detecting an AMI may reflect selection bias in the population. A positive cTnI measured using the Stratus CS was used to select patients for admission. When an independent measure of performance was utilised, the ability to predict an adverse prognosis, this apparent discrepancy between the methods disappears.

What is the prognostic role of myocardial dysfunction markers compared with troponin measurement?

Receiver operating characteristic curves using the combined MACE of death, readmission with myocardial infarction, readmission with unstable angina or the need for urgent revascularisation as the dichotomous variable were constructed using the admission sample measurement and the peak of the admission or 90-minute sample measurement comparing the four troponin methods with neurohormone measurement. The results are summarised in *Figures 17* and *18* with the AUCs and comparison of the AUCs in *Table 20*.

When measured on admission, all four troponin methods and NTproBNP were significantly better outcome predictors than copeptin. On the admission sample, none of the four troponin methods predicted adverse events significantly better than measurement of NTproBNP. A different pattern was seen when examining

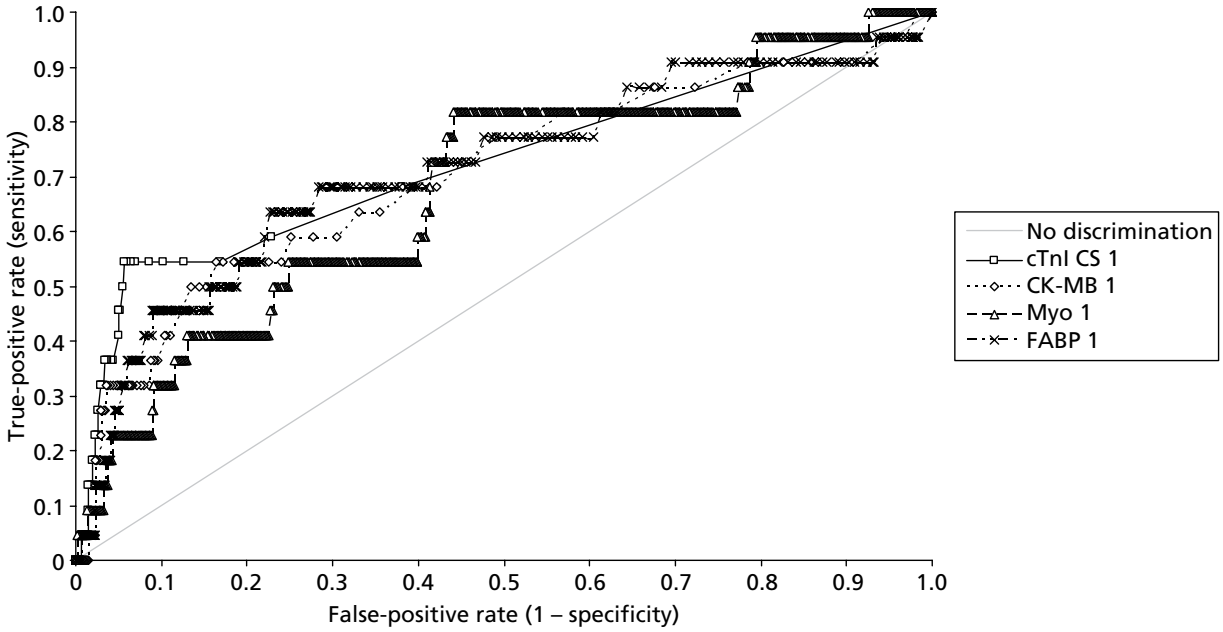

FIGURE 13 Receiver operating characteristic curves for cytoplasmic markers and troponin measurements for the prediction of MACE: markers measured on admission. cTnI CS 1, Stratus CS admission sample; CK-MB 1, CK-MB admission sample; Myo 1, myoglobin admission sample; FABP 1, H-FABP admission sample.

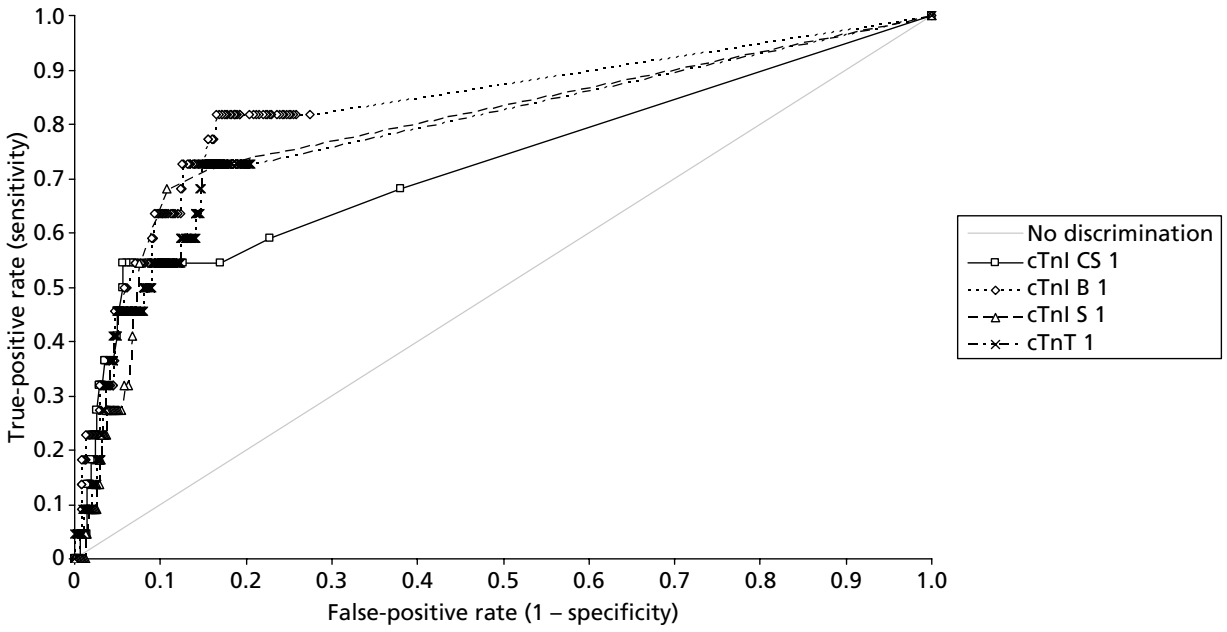

FIGURE 14 Receiver operating characteristic curves for troponin measurements for the prediction of MACE: markers measured on admission. cTnI CS 1, Stratus CS admission sample; cTnI B 1, Beckman AccuTnI admission sample; cTnI S 1, Siemens Ultra admission sample; cTnT 1, Roche high-sensitivity cTnT admission sample.

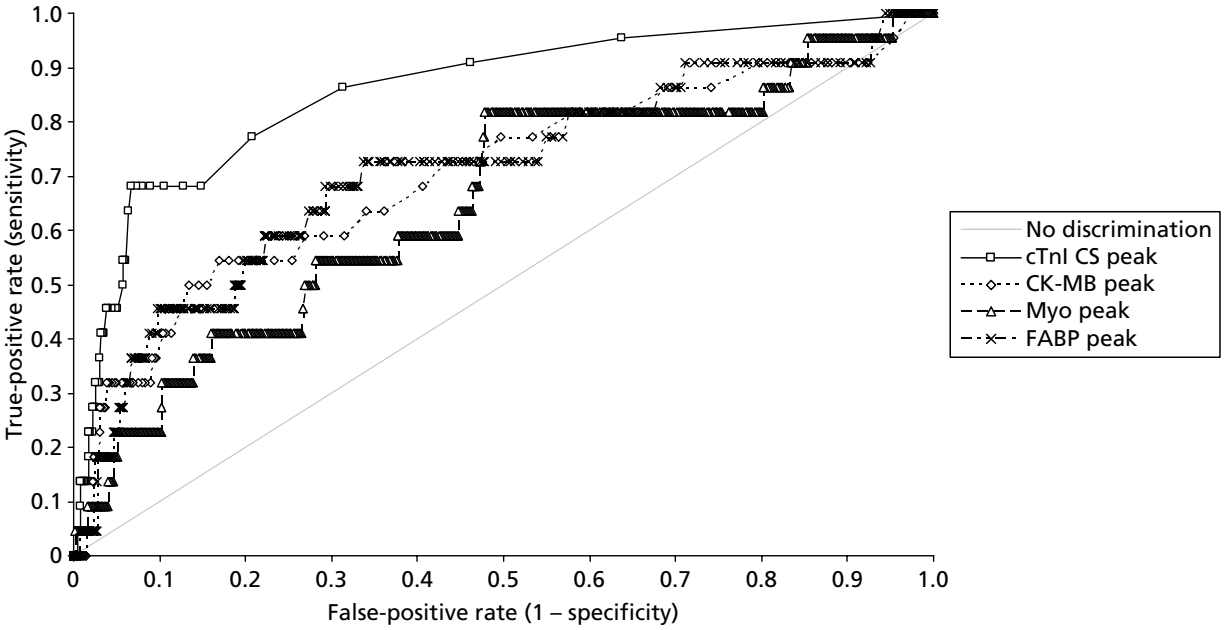

FIGURE 15 Receiver operating characteristic curves for cytoplasmic markers and troponin measurements for the prediction of MACE. Markers are peak values of measurement on admission or 90 minutes from admission. cTnI CS peak, Stratus CS peak value; CK-MB peak, CK-MB peak value; Myo peak, myoglobin peak value; FABP peak, H-FABP peak value.

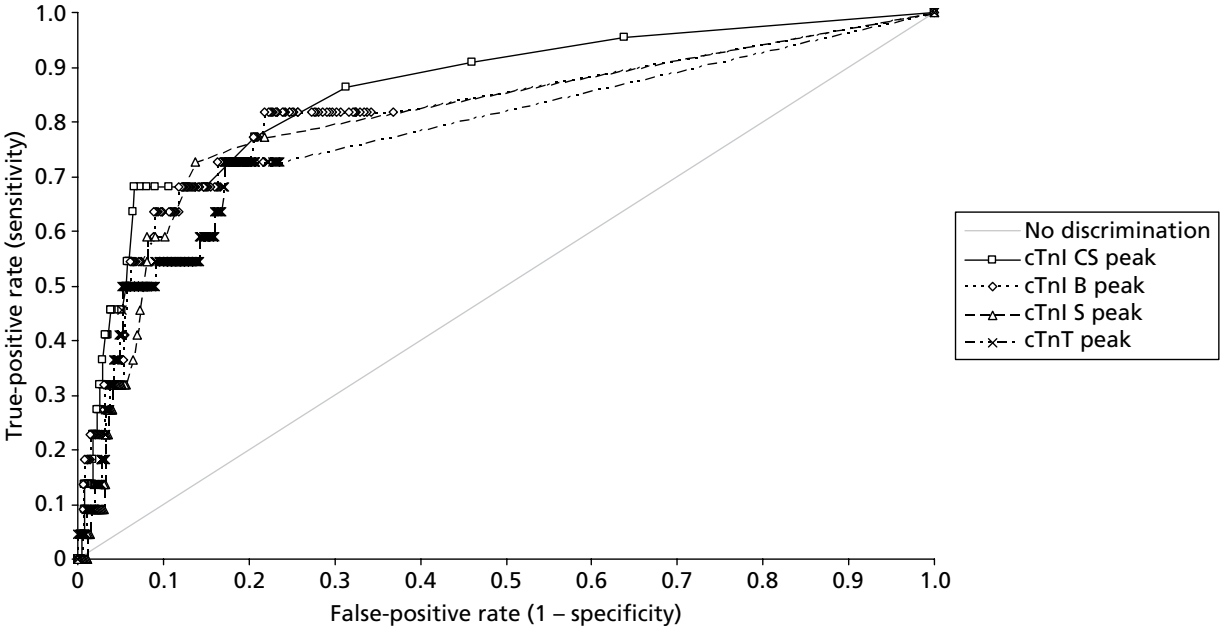

FIGURE 16 Receiver operating characteristic curves for troponin measurements for the prediction of MACE. Markers are peak values of measurement on admission or 90 minutes from admission. cTnI CS peak, Stratus CS peak value; cTnI B peak, Beckman AccuTnI peak value; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

TABLE 17 Area under the ROC curve and comparison of AUCs for cytoplasmic markers and cardiac troponin measurements for the prediction of MACE on admission

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS 1	0.73 (0.60 to 0.85)	cTnI CS 1 vs CK-MB 1	0.02 (–0.08 to 0.12)	0.6798
CK-MB 1	0.71(0.58 to 0.84)	cTnI CS 1 vs Myo 1	0.05 (–0.07 to 0.17)	0.4471
Myo 1	0.68 (0.56 to 0.80)	cTnI CS 1 vs FABP 1	0.01 (–0.10 to 0.12)	0.8484
FABP 1	0.72 (0.59 to 0.84)	CK-MB 1 vs Myo 1	0.03 (–0.07 to 0.12)	0.5908
cTnI B 1	0.83 (0.74 to 0.93)	CK-MB 1 vs FABP 1	–0.01 (–0.08 to 0.06)	0.7965
cTnI S 1	0.80 (0.70 to 0.90)	Myo 1 vs FABP 1	–0.04 (–0.12 to 0.05)	0.4295
cTnT 1	0.79 (0.68 to 0.89)	cTnI B 1 vs CK-MB 1	0.13 (0.06 to 0.19)	0.0001
		cTnI B 1 vs Myo 1	0.15 (0.07 to 0.24)	0.0004
		cTnI B 1 vs FABP 1	0.12 (0.05 to 0.19)	0.0012
		cTnI S 1 vs CK-MB 1	0.09 (0.02 to 0.16)	0.0093
		cTnI S 1 vs Myo 1	0.12 (0.03 to 0.21)	0.0126
		cTnI S 1 vs FABP 1	0.08 (0.01 to 0.16)	0.0337
		cTnT 1 vs CK-MB 1	0.08 (–0.01 to 0.18)	0.0810
		cTnT 1 vs FABP 1	0.07 (–0.01 to 0.16)	0.0982
		cTnT 1 vs Myo 1	0.11 (0.01 to 0.21)	0.0363

CI, confidence interval; CK-MB 1, CK-MB admission sample; cTnI B 1, Beckman AccuTnI admission sample; cTnI CS 1, Stratus CS admission sample; cTnI S 1, Siemens Ultra admission sample; cTnT 1, Roche high-sensitivity cTnT admission sample; FABP 1, H-FABP admission sample; Myo 1, myoglobin admission sample.

measurements of peak value. All four troponin methods were significantly better outcome predictors than NTproBNP when measured as the peak of the admission or 90-minute sample. Again, copeptin performed poorly and was significantly worse than NTproBNP.

Estimation of the potential economic impact (clinical effectiveness and cost-effectiveness) of using highly sensitive troponin assays or the range of new cardiac biomarkers instead of an admission and 12-hour troponin measurement

Economic impact of measuring a panel of contemporary cardiac markers compared with the measurement of cardiac troponin alone for early differential diagnosis in chest pain patients

Currently, the costs of performing troponin, CK-MB and myoglobin measurements are equivalent. All utilise the same equipment and measurement is performed by immunoassay. The measurement of troponin is required by the universal definition of myocardial infarction. Measurement of CK-MB and myoglobin in addition could be justified only if there was superior diagnostic performance of the panel of assays. As there was no diagnostic efficiency to be gained in measuring a panel compared with a single test, Cost minimisation analysis shows that measurement of cardiac troponin alone was the most cost-effective diagnostic strategy.

TABLE 18 Area under the ROC curve and comparison of AUCs for cytoplasmic markers and cardiac troponin measurements for the prediction of MACE for peak values (peak)

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS peak	0.86 (0.77 to 0.94)	cTnI CS peak vs CK-MB peak	0.15 (0.07 to 0.24)	0.0003
CK-MB peak	0.70 (0.57 to 0.83)	cTnI CS peak vs Myo peak	0.20 (0.09 to 0.32)	0.0003
Myo peak	0.65 (0.53 to 0.78)	cTnI CS peak vs FABP peak	0.15 (0.06 to 0.23)	0.0009
FABP peak	0.71 (0.58 to 0.84)	CK-MB peak vs Myo peak	0.05 (–0.05 to 0.15)	0.3486
cTnI B peak	0.82 (0.71 to 0.92)	CK-MB peak vs FABP peak	–0.01 (–0.08 to 0.06)	0.8263
cTnI S peak	0.81 (0.71 to 0.91)	Myo peak vs FABP peak	–0.06 (–0.15 to 0.03)	0.2153
cTnT peak	0.78 (0.67 to 0.89)	cTnI B peak vs CK-MB peak	0.12 (0.05 to 0.18)	0.0004
		cTnI B peak vs Myo peak	0.17 (0.08 to 0.25)	0.0003
		cTnI B peak vs FABP peak	0.11 (0.05 to 0.17)	0.0005
		cTnI S peak vs CK-MB peak	0.11 (0.04 to 0.17)	0.0021
		cTnI S peak vs Myo peak	0.16 (0.06 to 0.25)	0.0011
		cTnI S peak vs FABP peak	0.10 (0.04 to 0.16)	0.0018
		cTnT peak vs CK-MB peak	0.08 (–0.01 to 0.17)	0.0936
		cTnT peak vs Myo peak	0.13 (0.02 to 0.23)	0.0168
		cTnT peak vs FABP peak	0.07 (–0.02 to 0.16)	0.1108

CI, confidence interval; CK-MB peak, CK-MB peak value; cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak value; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value; FABP peak, H-FABP peak value; Myo peak, myoglobin peak value.

TABLE 19 Area under the ROC curve and comparison of AUCs for cardiac troponin measurements for the prediction of MACE on admission (1) and for peak values (peak)

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS 1	0.73 (0.60 to 0.85)	cTnI CS 1 vs cTnI B 1	–0.11 (–0.22 to 0.00)	0.0523
cTnI B 1	0.83 (0.74 to 0.93)	cTnI CS 1 vs cTnI S 1	–0.07 (–0.16 to 0.01)	0.1054
cTnI S 1	0.80 (0.70 to 0.90)	cTnI CS 1 vs cTnT 1	–0.06 (–0.16 to 0.04)	0.2112
cTnT 1	0.79 (0.68 to 0.89)	cTnI B 1 vs cTnI S 1	0.04 (–0.02 to 0.10)	0.2231
		cTnI B 1 vs cTnT 1	0.04 (–0.04 to 0.13)	0.3295
		cTnI S 1 vs cTnT 1	0.01 (–0.06 to 0.07)	0.8215
cTnI CS peak	0.86 (0.77 to 0.94)	cTnI CS peak vs cTnI B peak	0.04 (–0.02 to 0.10)	0.1791
cTnI B peak	0.82 (0.71 to 0.92)	cTnI CS peak vs cTnI S peak	0.05 (–0.01 to 0.11)	0.1109
cTnI S peak	0.81 (0.71 to 0.91)	cTnI CS peak vs cTnT peak	0.08 (–0.03 to 0.18)	0.1454
cTnT peak	0.78 (0.67 to 0.89)	cTnI B peak vs cTnI S peak	0.01 (–0.04 to 0.06)	0.6676
		cTnI B peak vs cTnT peak	0.04 (–0.06 to 0.13)	0.4538
		cTnI S peak vs cTnT peak	0.03 (–0.06 to 0.11)	0.5406

CI, confidence interval; cTnI B 1, Beckman AccuTnI admission sample; cTnI B peak, Beckman AccuTnI peak value; cTnI CS 1, Stratus CS admission sample; cTnI CS peak, Stratus CS peak value; cTnI S 1, Siemens Ultra admission sample; cTnI S peak, Siemens Ultra peak value; cTnT 1, Roche high-sensitivity cTnT admission sample; cTnT peak, Roche high-sensitivity cTnT peak value.

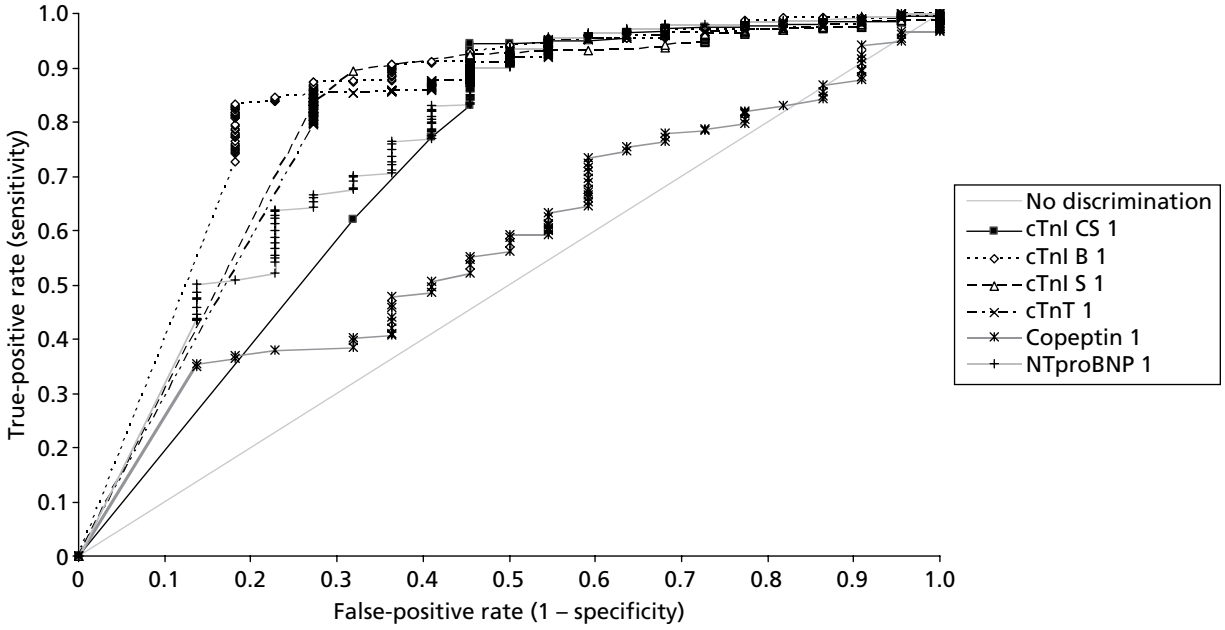

FIGURE 17 Receiver operating characteristic curves for troponin and neurohormone measurements for the prediction of MACE: markers measured on admission. cTnI CS 1, Stratus CS admission sample; cTnI B1, Beckman AccuTnI admission sample; cTnI S 1, Siemens Ultra admission sample; cTnT 1, Roche high-sensitivity cTnT admission value; copeptin 1, copeptin admission sample; NTproBNP 1, NTproBNP admission sample.

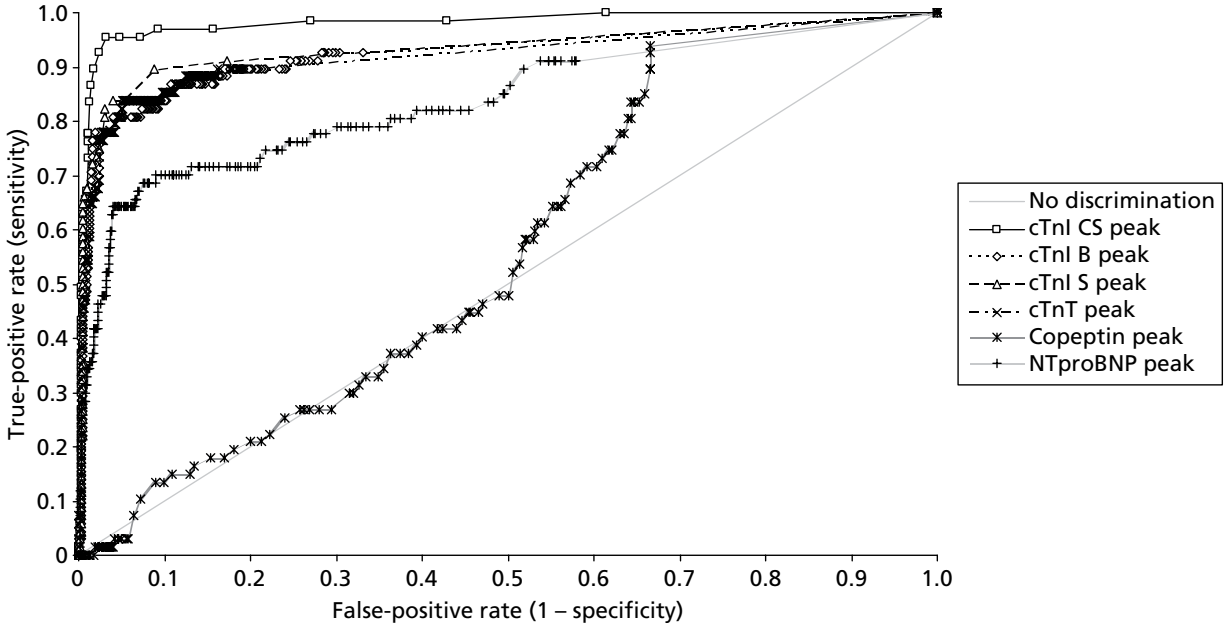

FIGURE 18 Receiver operating characteristic curves for troponin and neurohormone measurements for the prediction of MACE. Markers are peak values of measurement on admission or 90 minutes from admission. cTnI CS peak, Stratus CS peak sample; cTnI B peak, Beckman AccuTnI peak value; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cardiac cTnT peak value; copeptin peak, copeptin peak value; NTproBNP peak, NTproBNP peak value

TABLE 20 Area under the ROC curve and comparison of AUCs for cardiac troponin and neurohormone measurements for the prediction of MACE on admission (1) and for peak values (peak)

Test	Area (95% CI)	Contrast	Difference (95% CI)	*p*-value
cTnI CS 1	0.73 (0.60 to 0.85)	cTnI CS 1 vs copeptin 1	0.15 (0.02 to 0.28)	0.0291
cTnI B 1	0.83 (0.74 to 0.93)	cTnI CS 1 vs NTproBNP 1	–0.04 (–0.15 to 0.07)	0.4431
cTnI S 1	0.80 (0.70 to 0.90)	cTnI B 1 vs copeptin 1	0.25 (0.13 to 0.37)	<0.0001
cTnT 1	0.79 (0.68 to 0.89)	cTnI B 1 vs NTproBNP 1	0.06 (–0.04 to 0.17)	0.2496
Copeptin 1	0.58 (0.47 to 0.69)	cTnI S 1 vs copeptin 1	0.22 (0.10 to 0.34)	0.0004
NTproBNP 1	0.77 (0.65 to 0.89)	cTnI S 1 vs NTproBNP 1	0.03 (–0.07 to 0.13)	0.5951
		cTnT 1 vs copeptin 1	0.21 (0.09 to 0.33)	0.0006
		cTnT 1 vs NTproBNP 1	0.02 (–0.08 to 0.12)	0.6860
		Copeptin 1 vs NTproBNP 1	–0.19 (–0.30 to –0.09)	0.0004
cTnI CS peak	0.98 (0.96 to 1.00)	cTnI CS peak vs copeptin peak	0.42 (0.35 to 0.48)	<0.0001
cTnI B peak	0.93 (0.88 to 0.97)	cTnI CS peak vs NTproBNP peak	0.14 (0.08 to 0.21)	<0.0001
cTnI S peak	0.94 (0.90 to 0.97)	cTnI B peak vs copeptin peak	0.36 (0.29 to 0.43)	<0.0001
cTnT peak	0.92 (0.88 to 0.97)	cTnI B peak vs NTproBNP peak	0.09 (0.03 to 0.15)	0.0044
Copeptin peak	0.56 (0.50 to 0.62)	cTnI S peak vs copeptin peak	0.37 (0.30 to 0.44)	<0.0001
NTproBNP peak	0.84 (0.78 to 0.90)	cTnI S peak vs NTproBNP peak	0.10 (0.04 to 0.16)	0.0014
		cTnT peak vs copeptin peak	0.36 (0.30 to 0.42)	<0.0001
		cTnT peak vs NTproBNP peak	0.08 (0.04 to 0.13)	0.0002
		Copeptin peak vs NTproBNP peak	–0.27 (–0.35 to –0.20)	<0.0001

Copeptin 1, copeptin admission sample; copeptin peak, copeptin peak value; cTnI B 1, Beckman AccuTnI admission sample; cTnI B peak, Beckman AccuTnI peak value; cTnI CS 1, Stratus CS admission sample; cTnI CS peak, Stratus CS peak value; cTnI S 1, Siemens Ultra admission sample; cTnI S peak, Siemens Ultra peak value; cTnT 1, Roche high-sensitivity cTnT admission sample; cTnT peak, Roche high-sensitivity cTnT peak value; NTproBNP 1, NTproBNP admission sample; NTproBNP peak, NTproBNP peak value.

Economic impact of combining a novel biomarker of myocardial injury with cardiac troponin measurement

Objective

The objective was to estimate the potential cost-effectiveness of using highly sensitive troponin assays (at presentation alone or at presentation and 90 minutes later) and new cardiac biomarkers instead of a 10- to 12-hour troponin measurement.

After review of the results of the analytical part of the project, the following diagnostic strategies were applied to each patient:

1. no testing: discharge all patients without treatment
2. high-sensitivity troponin at presentation: discharge home if test is negative or admit to hospital for troponin testing at 10–12 hours if positive
3. high-sensitivity troponin and H-FABP at presentation: discharge home if both tests are negative or admit to hospital for troponin testing at 10–12 hours if either test is positive
4. high-sensitivity troponin at presentation and at 90 minutes: discharge home if both tests are negative or admit to hospital for troponin testing at 10–12 hours if either test is positive
5. standard troponin testing at 10–12 hours.

The cTnT and H-FABP data from this study were used to estimate the sensitivity and specificity of strategies 2–4. Only H-FABP in combination with troponin was tested because this combination was both more sensitive and more specific than other biomarker combinations (troponin with copeptin or troponin with NTproBNP) and so it would clearly dominate them if the costs of all biomarkers were assumed to be the same.

The main analysis used data from cTnT to estimate the sensitivity and specificity of cTnT at presentation, cTnT and H-FABP at presentation and cTnT at presentation and at 90 minutes. In a secondary analysis the same strategies were tested but using data from cTnI (Stratus CS), which analysis suggested may have superior diagnostic accuracy. The estimates used are presented in *Tables 21* and *22*.

Main analysis deterministic results

The results of the main deterministic analysis for cTnT strategies in a hypothetical cohort of patients ($n = 2240$) in the three scenarios tested, that is, doctor on demand, twice-daily ward round and once-daily ward round, are shown in *Table 23*. As expected, the effectiveness of the strategies (as measured by the total QALYs) increases in accordance with the strategy sensitivity, as an increase in sensitivity results in more patients of myocardial infarction being detected and treated. The total costs for each testing strategy increase as the specificity decreases and more patients require 10-hour troponin testing. However, the direct costs of measuring troponin at baseline and at 90 minutes exceed the costs of measuring troponin and H-FABP at baseline, so the former strategy is more expensive and thus dominated by the latter. The incremental cost-effectiveness ratio (ICER) reports the additional cost required using the strategy to accrue 1 additional QALY compared with the next most effective alternative. NICE decision-making suggests that a threshold of £20,000–30,000 per QALY is usually used, so a strategy is unlikely to be considered cost-effective if the ICER exceeds £20,000–30,000 per QALY.

At the £20,000 per QALY threshold, 10-hour troponin testing is cost-effective (£12,090 per QALY) in the doctor-on-demand scenario (if a decision can be made and the patient discharged as soon as a negative troponin result is available) but not in the other scenarios, where the ICER for 10-hour troponin, compared with high-sensitivity cTnT and H-FABP at presentation, exceeds £20,000 per QALY. In the other two scenarios (once-daily ward round and twice-daily ward rounds), the analysis shows that the strategies

TABLE 21 Sensitivity and specificity of strategies tested

Strategy	Sensitivity (95% CI)	Specificity (95% CI)
No testing	0	1
High-sensitivity cTnT at presentation	0.778 (0.655 to 0.873)	0.962 (0.946 to 0.975)
High-sensitivity cTnT and H-FABP at presentation	0.857 (0.746 to 0.933)	0.916 (0.894 to 0.935)
High-sensitivity cTnT at presentation and at 90 minutes	0.825 (0.709 to 0.909)	0.949 (0.930 to 0.963)
Troponin at 10–12 hours	1	1

TABLE 22 Sensitivity and specificity of strategies tested using cTnI Stratus CS instead of cTnT

Strategy	Sensitivity (95% CI)	Specificity (95% CI)
No testing	0	1
High-sensitivity cTnI at presentation	0.794 (0.673 to 0.885)	0.980 (0.967 to 0.989)
High-sensitivity cTnI and H-FABP at presentation	0.921 (0.824 to 0.974)	0.923 (0.901 to 0.941)
High-sensitivity cTnI at presentation and at 90 minutes	0.952(0.867 to 0.990)	0.969 (0.954 to 0.980)
Troponin at 10–12 hours	1	1

based on high-sensitivity cTnT and H-FABP at presentation are likely to be considered cost-effective compared with the next most effective alternative using a £20,000 per QALY threshold.

If a £30,000 per QALY threshold is used then 10-hour troponin testing is cost-effective in the doctor-on-demand scenario (£12,090 per QALY) and the twice-daily ward round scenario (£24,600 per QALY), whereas the high-sensitivity cTnT and H-FABP at presentation strategy is considered cost-effective in the once-daily ward round scenario (£14,806 per QALY).

Secondary analysis deterministic results

The results of the secondary analysis using cTnI are reported in *Table 24*. High-sensitivity cTnI at presentation and at 90 minutes was cost-effective in all three scenarios at the £20,000 per QALY threshold. At the £30,000 per QALY threshold, the 10-hour troponin strategy is cost-effective only in the doctor-on-demand scenario (£24,327 per QALY), with its ICER substantially exceeding the threshold in the other scenarios. High-sensitivity cTnI at presentation and at 90 minutes is cost-effective in the once-daily ward round scenario (£8310 per QALY) and the twice-daily ward round scenario (£7929 per QALY) at the £30,000 per QALY threshold.

Main probabilistic results

Probabilistic analysis incorporated uncertainty in the parameter estimates to provide estimates of the probability that each cTnT strategy would be cost-effective at different thresholds for willingness to pay for health gain. *Figure 19* shows the probability in the probabilistic sensitivity analyses that each strategy is optimal at thresholds of willingness to pay ranging from zero to £50,000 per QALY in the doctor-on-demand, twice-daily ward round and once-daily ward round scenarios. Tables containing the probabilities of cost-effectiveness of each cTnT strategy for the three scenarios at different willingness-to-pay thresholds are presented in *Appendix 3*.

In the doctor-on-demand scenario the high-sensitivity cTnT and H-FABP at presentation strategy has the highest probability of being optimal at thresholds between £6000 and £14,000 per QALY, whereas above £14,000 per QALY 10-hour troponin testing has the highest probability of being optimal. In the twice-daily ward round scenario, high-sensitivity cTnT and H-FABP at presentation has the highest probability of being optimal at thresholds between £8000 and £27,000 per QALY, whereas above £27,000 per QALY 10-hour troponin testing has the highest probability of being optimal. In the once-daily ward round scenario, high-sensitivity cTnT and H-FABP at presentation has the highest probability of being optimal at thresholds between £8000 and £37,000 per QALY, whereas 10-hour troponin testing is optimal only above thresholds of £37,000 per QALY. These results reflect the deterministic analysis and suggest that high-sensitivity cTnT and H-FABP at presentation has the highest probability of being cost-effective in most scenarios and at typically used thresholds for willingness to pay.

Secondary analysis probabilistic results

The secondary probabilistic analysis for cTnI strategies using the doctor-on-demand, twice-daily ward round and once-daily ward round scenarios is shown in *Figure 20*. Tables containing the probabilities of cost-effectiveness of each cTnI strategy for the three scenarios at different willingness-to-pay thresholds are presented in *Appendix 4*.

In the doctor-on-demand scenario the high-sensitivity cTnI and H-FABP at presentation strategy has the highest probability of being optimal at thresholds between £5000 and £12,000 per QALY; the high-sensitivity cTnI at presentation and at 90 minutes strategy is optimal between £12,000 and £28,000 per QALY; and 10-hour troponin testing is optimal above thresholds of £28,000 per QALY. In the twice-daily ward round and once-daily ward round scenarios, the high-sensitivity cTnI at presentation and at 90 minutes strategy has the highest probability of being optimal at all thresholds tested above £8000 per QALY and £7000 per QALY respectively. Again, these results reflect the deterministic analysis results in *Table 24* and suggest that the strategy using high-sensitivity cTnI on presentation and at 90 minutes has the highest probability of being cost-effective in most scenarios and at typically used thresholds for willingness to pay.

TABLE 23 Main analysis: deterministic results of the cost-effectiveness analysis using cTnT

	Doctor-on-demand scenario			Twice-daily ward round scenario			Once-daily ward round scenario		
	Total costs (£)	Total QALYs	ICER (£)	Total costs (£)	Total QALYs	ICER (£)	Total costs (£)	Total QALYs	ICER (£)
No testing	965,994	26,226.68	–	965,994	26,226.68	–	965,994	26,226.68	–
High-sensitivity cTnT at presentation	1,581,263	26,349.42	5012	1,614,048	26,349.42	5280	1,638,450	26,349.42	5479
High-sensitivity cTnT at presentation and at 90 minutes	1,715,256	26,354.37	Dominated	1,755,642	26,354.37	Dominated	1,785,754	26,354.37	Dominated
High-sensitivity cTnT and H-FABP at presentation	1,682,362	26,358.72	11,026	1,736,440	26,358.72	13,160	1,776,146	26,358.72	14,806
10-hour troponin	2,016,540	26,386.36	12,090	2,416,409	26,386.36	24,600	2,705,696	26,386.36	33,630

TABLE 24 Secondary analysis: deterministic results of the cost-effectiveness analysis using cTnI

	Doctor-on-demand scenario			Twice-daily ward round scenario			Once-daily ward round scenario		
	Total costs (£)	Total QALYs	ICER (£)	Total costs (£)	Total QALYs	ICER (£)	Total costs (£)	Total QALYs	ICER (£)
No testing	965,994	26,226.68	–	965,994	26,226.68	–	965,994	26,226.68	–
High-sensitivity cTnI at presentation	1,576,820	26,350.78	4922	1,609,240	26,350.78	5183	1,628,460	26,350.78	5338
High-sensitivity cTnI and H-FABP at presentation	1,711,921	26,370.19	6960	1,764,695	26,370.19	Extendedly dominated	1,803,022	26,370.19	Extendedly dominated
High-sensitivity cTnI at presentation and at 90 minutes	1,773,748	26,376.38	9988	1,812,245	26,376.38	7929	1,841,210	26,376.38	8310
10-hour troponin	2,016,540	26,386.36	24,327	2,416,409	26,386.36	60,537	2,705,696	26,386.36	86,621

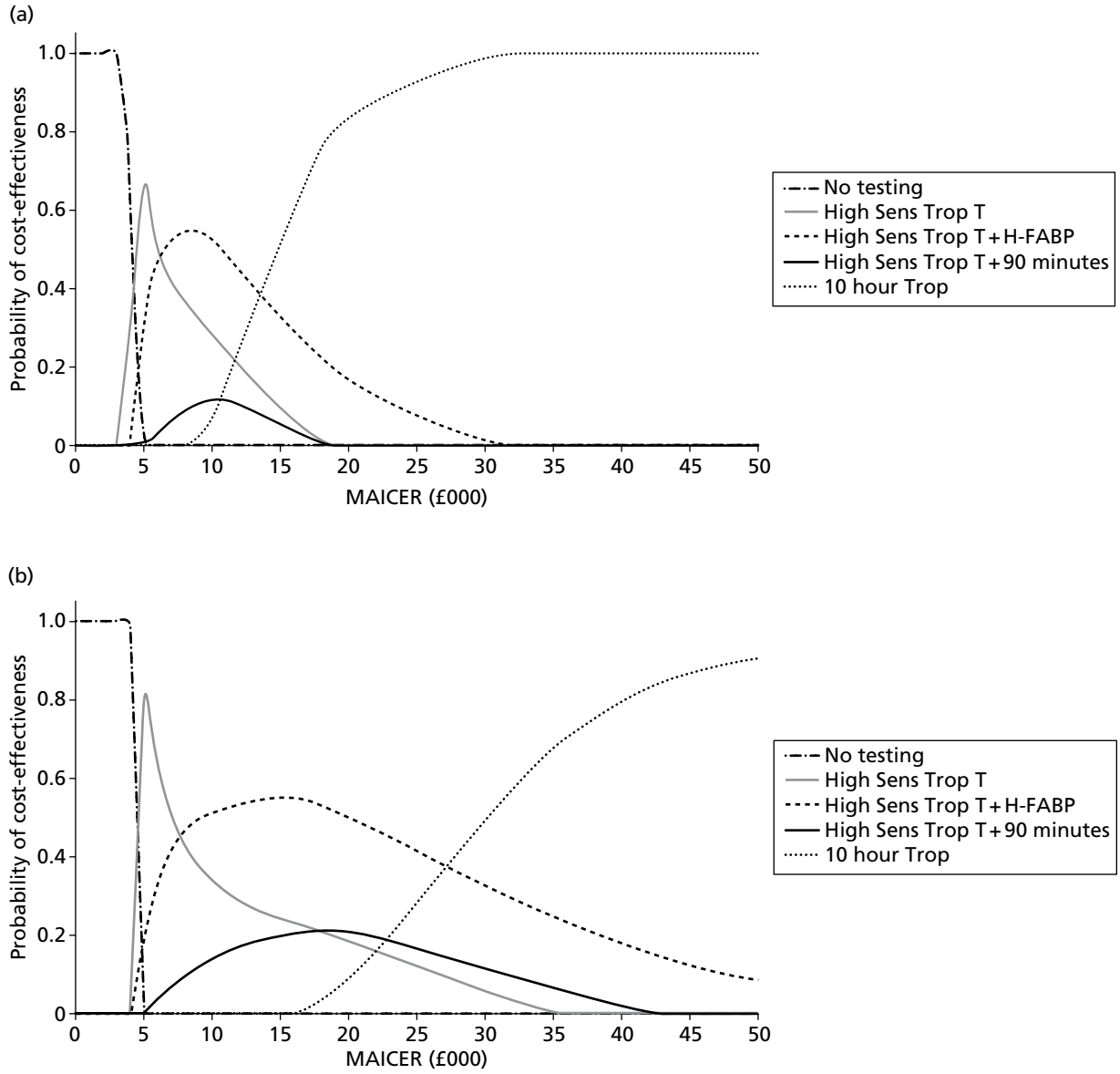

FIGURE 19 Cost-effectiveness acceptability curves of cTnT strategies. (a) Doctor-on-demand scenario; (b) twice-daily ward round scenario; (c) once-daily ward round scenario. MAICER, maximum acceptable incremental cost-effectiveness ratio; High Sens Trop T, high-sensitivity TnT at presentation; High Sens Trop T + H-FABP, high-sensitivity TnT and H-FABP at presentation; High Sens Trop T + 90 minutes, high-sensitivity TnT at presentation and 90 minutes; 10 hour Trop, 10-hour troponin.

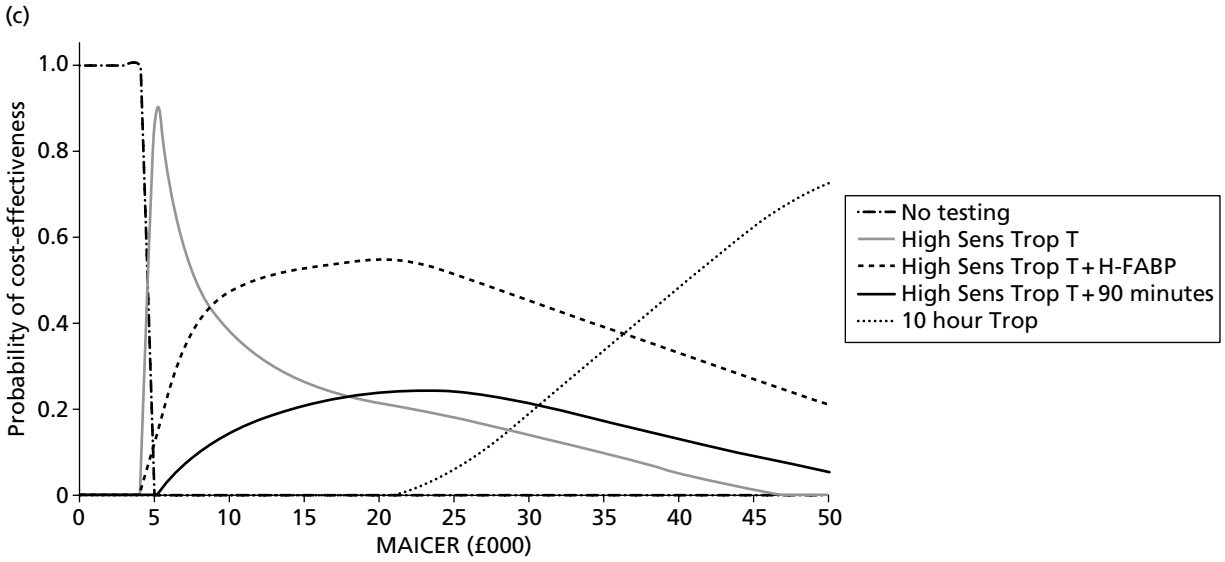

FIGURE 19 Cost-effectiveness acceptability curves of cTnT strategies. (a) Doctor-on-demand scenario; (b) twice-daily ward round scenario; (c) once-daily ward round scenario. MAICER, maximum acceptable incremental cost-effectiveness ratio; High Sens Trop T, high-sensitivity TnT at presentation; High Sens Trop T + H-FABP, high-sensitivity TnT and H-FABP at presentation; High Sens Trop T + 90 minutes, high-sensitivity TnT at presentation and 90 minutes; 10 hour Trop, 10-hour troponin (*continued*).

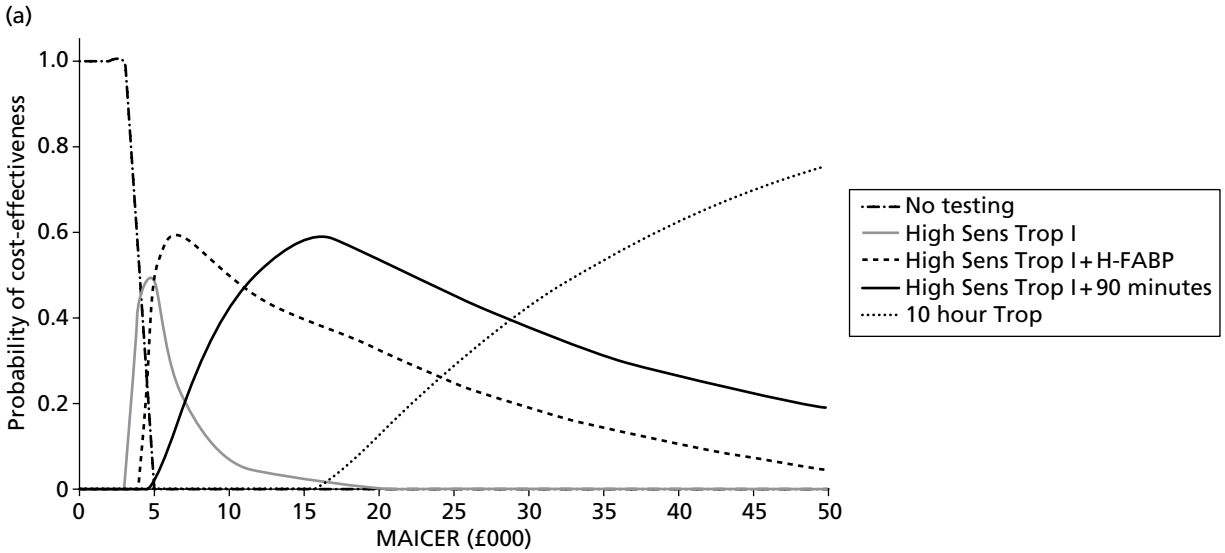

FIGURE 20 Cost-effectiveness acceptability curves of cTnI strategies. (a) Doctor-on-demand scenario; (b) twice-daily ward round scenario; (c) once-daily ward round scenario. MAICER, maximum acceptable incremental cost-effectiveness ratio; High Sens Trop I, high-sensitivity TnI at presentation; High Sens Trop I + H-FABP, high-sensitivity TnI and H-FABP at presentation; High Sens Trop I + 90 minutes, high-sensitivity TnI at presentation and 90 minutes; 10 hour Trop, troponin at 10–12 hours.

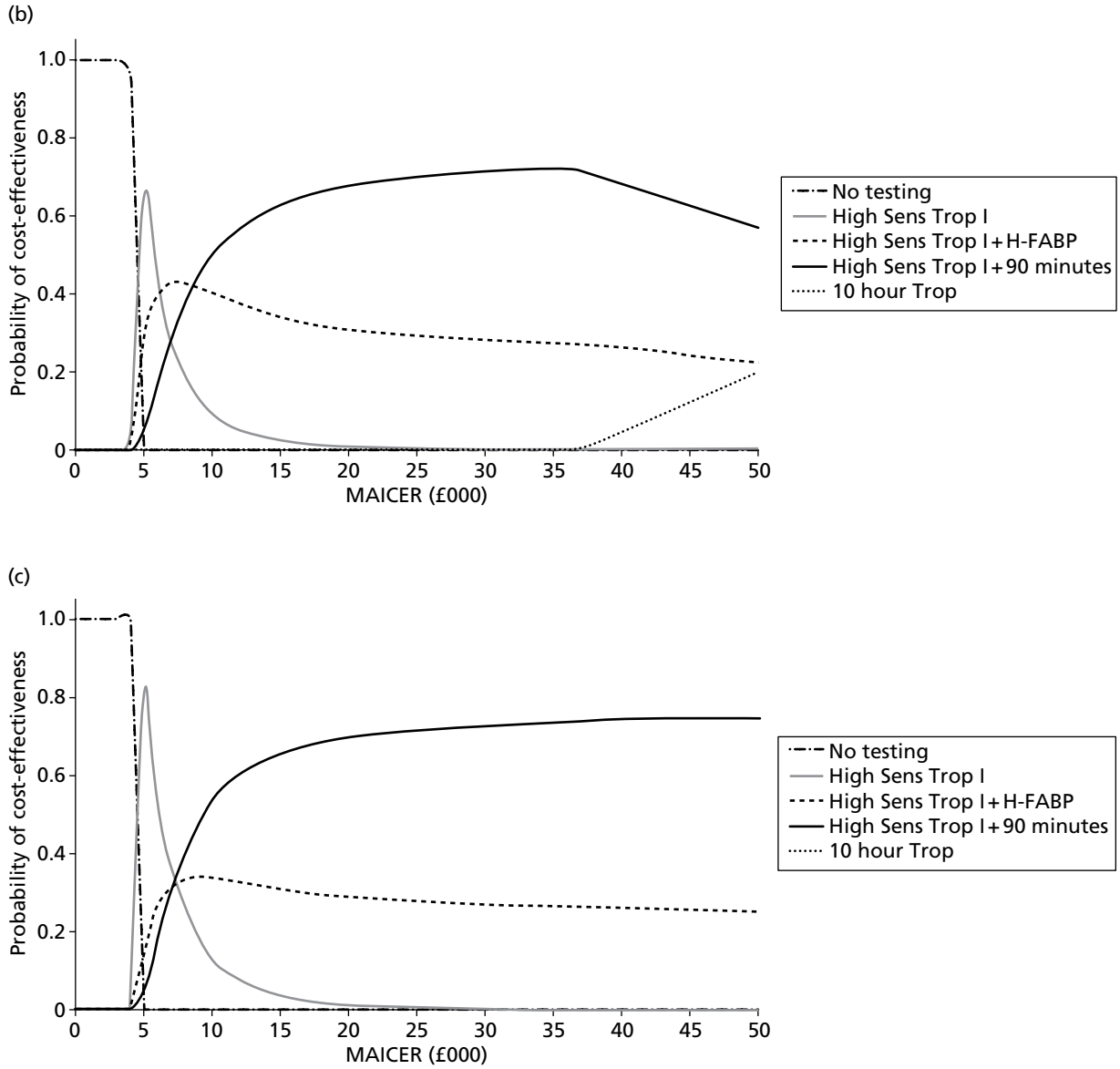

FIGURE 20 Cost-effectiveness acceptability curves of cTnI strategies. (a) Doctor-on-demand scenario; (b) twice-daily ward round scenario; (c) once-daily ward round scenario. MAICER, maximum acceptable incremental cost-effectiveness ratio; High Sens Trop I, high-sensitivity TnI at presentation; High Sens Trop I + H-FABP, high-sensitivity TnI and H-FABP at presentation; High Sens Trop I + 90 minutes, high-sensitivity TnI at presentation and 90 minutes; 10 hour Trop, troponin at 10–12 hours (*continued*).

Chapter 5 Discussion

Role of conventional biomarkers for diagnosis

The measurement of myoglobin or CK-MB as part of a cardiac marker panel did not contribute to the diagnostic efficiency in a population of low-risk chest pain patients. Previous studies which have demonstrated that the combination of cTnI, myoglobin and CK-MB is diagnostically efficient combined the two cytoplasmic markers with a low-sensitivity troponin assay.[36,56] A prospective study of chest pain patients with a third-generation cTnT assay utilising a 99th percentile cut-off demonstrated superior diagnostic sensitivity of cTnT measurement compared with CK-MB measurement for both rule-in and rule-out of an AMI.[247] The findings in RATPAC-CBE are in agreement with the findings of studies in which an appropriately sensitive cTnI method has been used and combined with a 99th percentile cut-off. In these studies cTnI measurement is superior to the combination of CK-MB and myoglobin measurement.[248,249] The use of rate of change (delta value) for myoglobin has been claimed to improve diagnostic sensitivity.[250] The addition of delta value served only to reduce the specificity of myoglobin measurement without a concomitant increase in sensitivity. Sensitive troponin measurement alone appears superior to measurement of CK-MB and myoglobin.[251] A recent systematic review has also shown that myoglobin and CK-MB do not have incremental value for early diagnosis of an AMI.[74] The most recently reported study of low-risk chest pain patients demonstrated that, even with a relatively low sensitivity cTnI measurement, additional measurement of CK-MB and myoglobin was not clinically useful.[252]

Role of novel cytoplasmic biomarkers

The measurement of H-FABP has been proposed to be a superior diagnostic marker to both CK-MB and myoglobin on the basis of its high tissue concentration and small molecular size. Admission measurement of H-FABP was diagnostically superior to measurement of myoglobin but not to measurement of CK-MB when assessed by ROC curve analysis. When prespecified diagnostic thresholds were used, H-FABP was superior to CK-MB but at the cost of a significantly lower specificity. When compared with all four troponin methods, measurement of H-FABP was diagnostically inferior. Measurement was statistically equivalent when using prespecified diagnostic thresholds; the failure to demonstrate diagnostic inferiority was due to the relatively small sample size of the population with a final diagnosis of myocardial infarction. As a single diagnostic test, measurement of H-FABP is not reliable for the diagnosis of patients presenting with acute chest pain and a suspected AMI. In RATPAC-CBE the diagnostic sensitivity of H-FABP and the AUC by ROC curve analysis are comparable to those reported previously[69,71–73,79] although there is one report that found much higher values.[71]

The role of H-FABP is therefore as a supplementary test. In the RATPAC-CBE population the combination of admission measurement of H-FABP and troponin by a high-sensitivity method improved diagnostic sensitivity when prespecified diagnostic thresholds were used. The improvement was not statistically significantly different from single marker measurement or from the diagnostic sensitivity achieved by combining admission and 90-minute troponin measurements. When compared by ROC curve analysis, sensitive troponin measurement was diagnostically and prognostically superior to that of H-FABP. This suggests that the combination of H-FABP with sensitive troponin measurement may not be diagnostically useful compared with repeating troponin measurement after a short time interval.

Assessment of the value of measurement of H-FABP as a supplementary test on the basis of the existing literature is difficult. Published studies showing improved diagnostic performance over the combination of H-FABP plus troponin have a number of methodological factors that make interpretation difficult. Some have included a mixed population of patients, including those with ST elevation myocardial infarction,[69,71]

and in some the population is not completely defined.[72,73] These studies have also used a relatively insensitive troponin assay rather than a current generation or high-sensitivity assay.[69,71–73]

The data from studies that have compared a high-sensitivity troponin assay with H-FABP measurement are also difficult to evaluate. All are in agreement that measurement of H-FABP does not add value above measurement of a high-sensitivity troponin alone. One study enrolled patients presenting with ST-segment elevation, for whom early diagnosis using biomarkers is not clinically useful.[81] Three studies used an appropriate chest pain population. In one, a semiquantitative method for measuring H-FABP was used.[78] The diagnostic threshold was 7 ng/l, a relatively high cut-off. Although evidence for the diagnostic performance of H-FABP measurement is presented in the paper, the power of combining troponin with H-FABP measurement is explored only for the diagnosis of non-ST-segment elevation ACS and not for NSTEMI. When high-sensitivity troponin and measurement of H-FABP by a sensitive method has been used, the diagnostic performance of the combination is not analysed.[79] A multicentre study of a high-sensitivity troponin assay compared with emerging biomarkers used an appropriate population and looked at the ability of new biomarkers to improve on diagnostic classification, but again a final diagnostic classification of ACS rather than myocardial infarction was used.[80] Only one study has reported both an appropriate population and the diagnostic ability of marker combinations. This study demonstrated that the addition of H-FABP to troponin measurement did not improve diagnostic performance.[253]

Role of high-sensitivity troponin assays

In RATPAC-CBE, slightly lower AUCs and diagnostic sensitivities for troponin measurement were obtained than in previously published studies. Studies published to date have used mixed populations that included patients with ST-segment elevation and have utilised a final diagnosis based on a less sensitive troponin method. Diagnostic sensitivities of 94–100% for troponin measured on admission have been reported in one study.[254] Diagnostic sensitivities of 82–84%, comparable with data from this study, have also been reported.[251,255] Neither of these two studies specifically addressed the problem of patients with NSTEMI. It is notable that, when patients considered to have undetectable troponin by conventional methods but detectable troponin by high-sensitivity methods are followed up, elevation of a sensitive troponin on admission is associated with an adverse prognosis.[256,257] This finding suggests that patients with NSTEMI are being missed when diagnosis is based on conventional troponin methods. The analogy would be the early literature on the evaluation of cTnT and cTnI in which diagnosis was based on measurement of CK-MB or CK, in which a high sensitivity but poor specificity were reported for troponin measurement. When a high-sensitivity cardiac troponin assay is used as the reference test for myocardial injury, the diagnostic sensitivity of previous-generation troponin assays is reduced.[258] When a different high-sensitivity troponin assay is used as the diagnostic gold standard biochemical test, the diagnostic sensitivity of a new high-sensitivity troponin assay may not be demonstrably statistically significantly different.[259] Moving the diagnostic threshold for cardiac troponin towards a lower diagnostic discriminant significantly improves clinical outcomes, supporting the view that lower is better and the diagnostic discriminant should be the 99th percentile.[244]

The optimal time point to undertake troponin measurement for diagnosis also remains undefined. Troponin measured on admission alone was not adequately sensitive; however, when combined with early serial measurement, rapid exclusion of myocardial infarction can be safely achieved within 2 hours in the low-risk chest pain population, as was demonstrated in RATPAC and other published studies.[252,260] Recent publications have suggested that, in a higher-risk population, diagnosis can be made with acceptable sensitivity by 3 hours from admission.[255,261] Questions remain as only one study was identified in the literature that specifically utilised a non-ST-segment elevation population and examined patients initially with a troponin below the 99th percentile on presentation.[262] This study demonstrated that follow-up to 6 hours was required before all patients with a final diagnosis of an AMI showed elevation of cTnT above the 99th percentile.

Role of neurohormones

Copeptin performed poorly both as a diagnostic test for myocardial infarction and as an outcome predictor. Copeptin was first developed as a stable peptide derived from the precursor of vasopressin that could be used to measure vasopressin levels without the problems of sample stability.[263] It was first systematically examined in a large series of patients following an AMI. The peak values of copeptin occurred at 24 hours and copeptin was elevated to a greater extent in patients with STEMI than in patients with NSTEMI. The study also found that copeptin levels predicted heart failure and death but not recurrent AMI.[98] Copeptin has been shown not to detect myocardial ischaemia.[102] There have been a number of reports on the role of copeptin for very early diagnosis of an AMI, in particular as a rule-out test. The results are contradictory, with some studies claiming high diagnostic sensitivity and others no diagnostic utility at all. The reasons for the discrepancies may relate to the populations studied and the troponin method used as the biochemical gold standard. The first group to report the value of copeptin examined consecutive chest pain admissions and utilised the fourth-generation cTnT assay as the biochemical gold standard at a diagnostic discriminant of 40 ng/l.[101] Copeptin was useful only in patients presenting < 10 hours of onset of chest pain. The AUC of copeptin for the diagnosis of an AMI was low (0.75) and was less than that of troponin alone (0.86). The combination of cTnT and copeptin improved the AUC for diagnosis to 0.97. Levels of copeptin were significantly lower in the NSTEMI population. Exclusion of patients with ST-segment elevation did not affect the diagnostic performance. A detailed examination of the data reported in this study shows that 362 of the 487 patients examined corresponded to a group comparable with that in the RATPAC study. In this group, 23 patients had a final diagnosis of myocardial infarction, of whom five (22%) had an undetectable cTnT (< 10 ng/l) and were detected by copeptin elevation. The study did not include any outcome data. The second large study that examined the role of copeptin again used a mixed population of patients with and without ST-segment elevation on presentation.[264] Diagnosis was based on a contemporary troponin method (not a high-sensitivity assay). Results were similar with troponin overall performing better than copeptin. The study reported that copeptin showed higher earlier diagnostic sensitivity than the other markers studied. When patients with ST-segment elevation were excluded, the admission AUC for troponin was 0.87, which improved to 0.93 on addition of copeptin. Data for a high-sensitivity assay were also reported. This yielded an admission AUC of 0.96, which improved to 0.97 on addition of copeptin. This improvement in AUC just achieved statistical significance. The reported experience with high-sensitivity troponin assays has been more mixed. A study of patients presenting to the emergency department with acute chest pain showed copeptin levels comparable to those found in the RATPAC study.[265] An additive value of copeptin and high-sensitivity cTnT was not demonstrated. A criticism of this study is that only a minority of patients had myocardial infarction. A second study examining patients presenting to a chest pain unit showed that the AUC for copeptin for diagnosis of an AMI was low (0.70) compared with that for high-sensitivity cTnT (0.90).[266] Exclusion of patients with ST-segment elevation showed no added benefit of using copeptin in combination with high-sensitivity cTnT when ROC curve analysis was used to assess diagnostic performance. Utilising a prespecified cut-off of the 99th percentile for cTnT (14 ng/l) and 14 pmol/l for copeptin improved diagnostic sensitivity but specificity was low at 0.56. It is possible that the poor sensitivity of copeptin was due to sample degradation; however, copeptin is known to be stable and no comparable problems were seen with other markers and the diagnostic sensitivity is comparable with that found in other studies. The role of copeptin to predict outcome is not well studied. In patients with established coronary artery disease, copeptin was not a strong predictor of cardiovascular outcome[168] and is probably a better predictor in patients with heart failure than in patients with chest pain.[267]

Measurement of BNP has been proposed as an early diagnostic test for patients presenting with chest pain.[44] Although studies have shown an additive value of simultaneous measurement of BNP and cardiac troponin,[268–270] increased sensitivity is achieved at the expense of diagnostic specificity.[268,269] Measurement of BNP is known to predict mortality across the entire spectrum, from normal populations[271] to patients with stable coronary artery disease[272] or presenting with ACS.[140,273] Similarly, in chest pain patients, BNP measurement is a risk predictor but is not useful for early diagnosis.[274–279]

Biomarkers for prognosis

As a prognostic marker, even in this low-risk population with a relatively small number of events, troponin measurement was superior to the cytoplasmic markers. This finding is in agreement with previous meta-analysis in the higher-risk ACS population.[33] All four troponin methods were equally efficient in predicting outcome. Copeptin was a poor outcome predictor. NTproBNP measurement is known to be a better predictor of mortality than troponin measurement, whereas troponin predicts risk of a repeat myocardial infarction.[280] In the RATPAC study, mortality was very low and cardiovascular events rather than cardiovascular death was the most common adverse event, which would account for the fact that the peak troponin value was the best predictor of MACEs.

Cost-effectiveness of biomarker strategies

The results showed that, as expected, effectiveness (QALYs) increased with increasing sensitivity and costs increased with decreasing specificity. At the £20,000 per QALY threshold, in all but one scenario a strategy of measuring high-sensitivity cTnT and H-FABP at presentation (with admission for 10-hour troponin testing if positive and discharge home if negative) was the optimal strategy; the 10-hour troponin strategy may have been optimal in the doctor-on-demand scenario. However, at the £30,000 per QALY threshold, 10-hour troponin testing is cost-effective in both the doctor-on-demand scenario and the twice-daily ward round scenario.

Sensitivity analyses considered alternative strategies using data from cTnI (Stratus CS), which our analysis suggested may have superior diagnostic accuracy. These analyses showed that delayed troponin testing is likely to be cost-effective only if patients with a negative test can be discharged as soon as the result is available and if a £30,000 per QALY threshold for willingness to pay is used. Thus, delayed troponin testing at 10 hours after symptom onset is unlikely to be cost-effective in most scenarios at commonly used thresholds of £20,000–30,000 per QALY. The implication of our analysis is that NICE guidance recommending 10- to 12-hour troponin testing, compared with high-sensitivity troponin at presentation combined with H-FABP or repeated at 90 minutes, does not appear to promote cost-effective use of NHS resources unless services are in place to allow rapid decision-making once delayed test results are available. However, there are a number of assumptions in the model that need to be taken into account when interpreting these findings.

Any modelling process involves simplifications and assumptions that may not accurately reflect clinical practice. It was assumed that delayed troponin testing is 100% sensitive and specific, reflecting its role as the reference standard for myocardial infarction. However, studies of presentation troponin testing report specificities of < 100%. It is known that not all troponin elevations represent myocardial infarction; however, modelling the effect of treating false-positive patients would be complex and require substantial assumptions with little supporting data. It was therefore assumed that sustained false-positive troponin elevations would affect presentation and delayed troponin testing equally, and that the only consequence of false-positive presentation troponin was the requirement for subsequent delayed testing. It was also assumed that a patient with a false-negative troponin at presentation would have the same prognosis (and thus ability to benefit from treatment) as a patient with a true-positive at presentation. This assumption may not hold if those patients with false-negative troponin at presentation have a smaller infarct and better prognosis. However, there was inadequate data available to test this assumption.

Also, our model assumes that patients waiting for troponin testing are cared for in hospital (even if not formally admitted) and therefore incur hospital costs. It could be argued that the benefits of delayed troponin testing could be accrued without most of the costs if patients were discharged home and asked to return for delayed testing. However, the feasibility and acceptability of this practice has not been tested and it is not routinely used.

This analysis is based on a model developed for a related project, 'Cost-effectiveness of diagnostic strategies for suspected acute coronary syndrome (ACS)' (HTA09/22/21), which used estimates of sensitivity and specificity for high-sensitivity troponin and H-FABP derived from a systematic literature review and meta-analysis.[241] The findings were similar, suggesting that a 10-hour troponin measurement is likely to be cost-effective only if a £30,000 per QALY threshold is used and the patient can be discharged as soon as the results are available. The combination of troponin with H-FABP or measurement of high-sensitivity troponin at baseline and at 90 minutes were both potentially cost-effective, but estimates of sensitivity and specificity in each case were derived from one study. The estimates of sensitivity for cTnT and cTnI derived from the meta-analysis were the opposite of the estimates in this study, with cTnT having higher sensitivity than cTnI. As a result, the secondary analysis with cTnI from the meta-analysis suggested that 10-hour troponin measurement was more cost-effective, whereas secondary analysis using cTnI in this study suggested that 10-hour troponin measurement was less cost-effective.

Overall, it appears that the sensitivity of the rapid rule-out strategy is the key determinant of whether or not 10-hour troponin measurement is, by comparison, cost-effective. If analysis includes a rapid rule-out strategy with a sensitivity of around 95% then a 10-hour troponin measurement is not cost-effective in most scenarios. If analysis includes a rapid rule-out strategy with a sensitivity of around 85% then a 10-hour troponin measurement is cost-effective in several scenarios. This should not be interpreted to mean that a rapid rule-out strategy with a sensitivity of 95% is 'acceptable' or 'safe', as both of these considerations involve value judgements that are separate from cost-effectiveness. What it does mean, however, is that if a rapid rule-out strategy is available with 95% sensitivity (and specificity of around 90%) then a 10-hour troponin strategy does not in comparison represent a worthwhile use of health-care resources.

The findings from this part of the study suggest that the following areas of future research may be worthwhile:

1. The sensitivity of troponin at baseline and at 90 minutes, and in combination with H-FABP, needs to be confirmed in further studies. Measurement at other time points for the optimisation of sensitivity without the requirement for hospital admission (such as at 120 minutes after presentation or 6 hours after symptom onset) could be explored.
2. Further modelling could be used to estimate the relative importance of sensitivity and specificity in determining cost-effectiveness. Alternative strategies, such as using the limit of detection to define a positive troponin test at baseline instead of the 99th percentile, could be used to optimise sensitivity at the expense of specificity. However, it is not clear how much specificity can be sacrificed to optimise sensitivity without adversely affecting cost-effectiveness. Modelling could be used to determine the relative effects of varying sensitivity and specificity of a hypothetical rapid rule-out strategy on cost-effectiveness.

General observations

Any biomarker must fulfil a number of criteria to achieve routine clinical use. These can be divided into three categories: analytical suitability, plausibility for clinical application and treatment impact.

Analytical suitability has three components. The population aspects of the test must be known, that is, the effect of age, gender and ethnicity and the influence of physiological and comorbid conditions. The preanalytical factors that may affect the results, such as collection conditions, anticoagulant requirements, preanalytical sample handling and stability in storage, need to be defined. A marker suitable for routine clinical use must be measurable in the routine clinical laboratory without special handling conditions. Finally, test performance needs to be adequate. In addition to the ability to measure the biomarker with precision and accuracy, the analysis must be simple and have a rapid turnaround time. Ideally, it should be

implemented on existing laboratory equipment rather than requiring additional apparatus. In practice, this means that a colorimetric or more likely an immunoassay for the marker is available.

Plausibility of a biomarker will involve the biological and clinical plausibility for the putative clinical role. Biological plausibility means that the pathobiology of the marker needs to be understood. This means an understanding of the genesis of the biomarker and of the relationship of the biomarker to the medical condition of interest. It is not sufficient to simply state that a negative result allows rule-out of the condition of interest. The positive results must also be explicable. Clinical plausibility for the putative clinical role requires sensitivity and specificity for the medical condition of interest in clinically appropriate populations, the patient groups in whom the test will be used in routine clinical practice. Many studies on biomarkers have evaluated diagnostic performance in clinical trial sample banks or highly selected patient groups. These populations do not constitute an appropriate environment to evaluate test performance as the disease prevalence is inappropriately high, often close to 100%. Such studies allow proof of concept but need to be followed by prospective evaluation in a clinically representative population. There is a lack of prospective observational studies of biomarkers performed using routine clinical populations with no exclusion criteria and well-documented final diagnoses and outcomes. Such studies are essential before any biomarker can be used in routine clinical practice.

Treatment effectiveness (evidence-based medicine) is one of the most important of the criteria. Any new biomarker must either offer a significant improvement in diagnostic efficiency or result in a change in treatment. In addition, it should demonstrate cost-effectiveness.

Strengths and weaknesses of the study

The RATPAC-CBE trial was a retrospective observational study of the point-of-care arm of a prospective randomised controlled trial. It therefore shares the same limitations of all such diagnostic studies – that although patient enrolment is prospective, biomarker analysis is retrospective. The strength of this design is that it represented real clinical practice. The population selected was a low-risk group that was managed according to a biomarker-based protocol. The population studied is therefore representative of the majority of patients who present for assessment of acute chest pain and a suspected AMI. Patient selection was randomised between conventional management and a rapid rule-out protocol. The equivalent rate of major adverse events between the point-of-care arm and the conventional management arm suggests that the population was well matched. The nature of the protocol means that an admission sample was obtained for the majority of patients but a follow-up 90-minute sample was obtained only when there was a much lower likelihood that the patient had an AMI. There is therefore a selection bias as patients with a final diagnosis of an AMI may be over-represented in the assessment of the 90-minute samples for diagnostic and prognostic ability. This was mitigated by analysing the peak of the presentation and 90-minute samples, both to increase numbers and to reduce the immediate temporal bias.

Ideally, the study would have had a 10- to 12-hour sample taken on all patients in the point-of-care arm for analysis in the core laboratory to provide a definitive diagnosis of myocardial injury. Three of the trial sites utilised a high-sensitivity troponin assay, whereas the other three used a contemporary assay but with a decision limit close to optimal. Nearly two-thirds of the patients studied had a troponin measurement at ≥6 hours from onset of chest pain and 42.8% had a troponin measurement at ≥10 hours from onset of chest pain. Samples were measured at presentation and at 90 minutes with a high-sensitivity assay. In addition, the Stratus CS analyser has analytical performance appropriate for a high-sensitivity assay. This means that the final diagnosis of myocardial infarction was based largely on a sensitive troponin measurement rather than on a conventional but lower-sensitivity troponin assay.

The use of a cohort study for the modelling also means that unanticipated effects cannot be evaluated.

Implications for practice

The findings of RATPAC-CBE support the widespread implementation of high-sensitivity troponin assays. They also support the use of troponin alone as the gold standard diagnostic test and suggest that additional measurement of myoglobin and CK-MB is not required. Copeptin cannot be recommended as a useful additional test for diagnostic or prognostic purposes. Although measurement of NTproBNP was confirmed as a useful prognostic test, it is no more useful than measurement of troponin and the measurement of both NTproBNP and cTnT or cTnI should not be undertaken.

The role of H-FABP remains unclear. Measurement of both a cardiac troponin and H-FABP does improve diagnostic performance but does not, on the basis of the evidence from RATPAC-CBE and the literature published to date, permit ruling out myocardial infarction based on a single measurement of both markers on admission.

Implications for future research

High-sensitivity troponin measurement is the best test for the assessment of patients with chest pain and for diagnosis of myocardial infarction. Recent guidelines have recommended the use of admission and 3-hour high-sensitivity troponin measurements for the diagnosis of an AMI,[281] but this recommendation is not truly evidence based. To date, no studies have been published that have combined clinical data and ECG data with a high-sensitivity troponin measurement performed at 10–12 hours from admission as the biochemical diagnostic gold standard to provide the definitive final diagnosis. Such a population would allow assessment of the earliest time point when it can be guaranteed that a rise in troponin can be detected with close to 100% diagnostic accuracy and definition of what would constitute an early diagnostic strategy.

A prospective observational study is required, combining clinical and ECG data with a high-sensitivity troponin measurement performed at 10–12 hours from admission as the biochemical diagnostic gold standard to define the kinetics of troponin release. Such a study would also support assessment of the diagnostic efficiency of H-FABP as well as any other putative new markers. This study should also address the prognostic utility of small troponin elevations between the 99th percentile and the limits determined from previous studies. Although it is assumed that low-level elevations of troponin predict an adverse short-term prognosis, the clinical effect of minor elevations on short-term prognosis is not known. In addition, the study should address to what extent short-term changes in troponin levels can be used to distinguish between acute and chronic elevations.

Elevations of troponin occur in the general population, associated with previous history of ischaemic disease, requirement for cardiac medication, coexistent conditions and evidence of echocardiographic abnormalities. These minor elevations of cardiac troponin in the general population are associated with an adverse long-term prognosis. In patients with chest pain they are associated with abnormalities in cardiac anatomy and evidence of atherosclerotic disease. The use of high-sensitivity troponin assays will inevitably detect small elevations in the population presenting to the emergency department that are representative of chronic rather than acute disease. Although data from RATPAC-CBE show that the number of such patients is relatively small, this number is significant. It is currently thought that change in troponin levels over a short time frame would allow the distinction between acute myocardial injury seen in AMI, associated with a poor prognosis, and more chronic elevations.

Studies on biological variation of troponin have been performed. There is some published evidence on change in troponin levels in patients presenting with suspected ACS, but again the studies have contained a mixed population of STEMI and NSTEMI patients. Data to determine what would be a discriminatory level of rate of change of troponin to distinguish between chronic elevation and acute NSTEMI in a more general chest pain population are currently lacking.

Conclusions

The measurement of cardiac troponin as cTnT or cTnI over a short time frame offers the best strategy for early confirmation or exclusion of an AMI. In this study, a low-risk group was successfully discharged on the basis of admission and 90-minute measurements. Questions remain as to what is the optimal timing for troponin measurement. In addition, troponin measurement needs to be incorporated within a clinical decision-making strategy that utilises clinical and ECG findings. Of all markers studied, only H-FABP appears to offer some improvement in diagnostic efficiency that might also be cost-effective. However, as yet, measurement of H-FABP is not carried out on routine clinical laboratory equipment suitable for a 24-hour diagnostic service.

Acknowledgements

Contribution of authors

Paul Collinson (Professor of Cardiovascular Biomarkers, St George's University of London) – chief investigator, conception and design of the study, analysis and interpretation of the data, writing and revising the report and final approval of the version to be published.

David Gaze (Clinical Research Scientist, St George's Hospital, London) – design of the study, analytical measurement of the biomarkers, data entry and data checking, drafting and revising the report and final approval of the version to be published.

Praveen Thokala (Research Fellow, Health Services Research, University of Sheffield) – health economic modelling and analysis, drafting and revising the report and final approval of the version to be published.

Steve Goodacre (Professor of Emergency Medicine, Health Services Research, University of Sheffield) – conception and design of the study, analysis and interpretation of the data, drafting and revising the report and final approval of the version to be published.

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Collinson P, Goodacre S, Gaze D, Gray A. Very early diagnosis of chest pain by point-of-care testing: comparison of the diagnostic efficiency of a panel of cardiac biomarkers compared with troponin measurement alone in the RATPAC trial. *Heart* 2012;**98**:312–18.

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Appendix 1 Detailed results of missed acute myocardial infarction cases by troponin measurement method

Missed acute myocardial infarction: Stratus CS analyser

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
2114	0.01	0.50	10	45.3	Angina, no ACS		0.07	cTnT
3193	0.03	390	2960	36.1	Non-specific		0.20	cTnI
6194	0.05	9.60	780	197.8	ACS		0.07	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Missed acute myocardial infarction: Beckman AccuTnI

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
1001	5.30	15.0	60	260.0	ACS		5.300	cTnI
2085		9.1	1690	189.4	Other	NSTEMI and new-onset non-insulin-dependent diabetes mellitus	0.048	cTnT
2114	0.01	0.5	10	45.3	Angina, no ACS		0.070	cTnT
3065	0.08	0.5	10	3.8	Angina, no ACS		0.200	cTnI
3427	0.08	30.0	30	19.2	ACS		0.080	cTnI
4048	8.76	10.9	70	1.5	Other	NSTEMI	8.760	cTnI
4160	0.10	30.0	60	29.0	Other	NSTEMI	6.400	cTnI
4270	0.18	0.5	10	1.5	Other	NSTEMI	0.580	cTnI
4286	0.16	5.0	20	6.5	Unknown		0.490	cTnI
5078	0.20	0.5	10	1.5	Other	Possible cardiac pain but a myocardial infarction ruled out	0.200	cTnI
6008	0.18	0.5	10	1.5	Gastro-oesophageal		0.180	cTnI
6036	0.10	1.9	60	15.9	Angina, no ACS		0.100	cTnI
6065	0.14	2.6	10	1.5	Non-specific		0.140	cTnI
6095	0.18	5.7	30	5.6	ACS		1.960	cTnI
6194	0.05	9.6	780	197.8	ACS		0.070	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Missed acute myocardial infarction: Siemens Ultra

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
1203	0.40	60.0	30	28.3	Non-specific		0.40	cTnI
2114	0.01	0.5	10	45.3	Angina, no ACS		0.07	cTnT
3065	0.08	0.5	10	3.8	Angina, no ACS		0.20	cTnI
3427	0.08	30.0	30	19.2	ACS		0.08	cTnI
4270	0.18	0.5	10	1.5	Other	NSTEMI	0.58	cTnI
4286	0.16	5.0	20	6.5	Unknown		0.49	cTnI
5078	0.20	0.5	10	1.5	Other	Possible cardiac pain but a myocardial infarction ruled out	0.20	cTnI
5113	0.37	60.0	30	81.0	Other	NSTEMI	1.35	cTnI
6008	0.18	0.5	10	1.5	Gastro-oesophageal		0.18	cTnI
6065	0.14	2.6	10	1.5	Non-specific		0.14	cTnI
6095	0.18	5.7	30	5.6	ACS		1.96	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Missed acute myocardial infarction: cardiac troponin T

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
2017	0.12	51.5	840	1.5	Other	Myocardial infarction	0.12	cTnT
2048	0.16	85.2	1290	1.5	ACS		0.16	cTnT
3065	0.08	0.5	10	3.8	Angina, no ACS		0.20	cTnI
4048	8.76	10.9	70	1.5	Other	NSTEMI	8.76	cTnI
4132	0.78	369.6	590	7.6	Other	NSTEMI	1.96	cTnI
4270	0.18	0.5	10	1.5	Other	NSTEMI	0.58	cTnI
4286	0.16	5.0	20	6.5	Unknown		0.49	cTnI
5078	0.20	0.5	10	1.5	Other	Possible cardiac pain but a myocardial infarction ruled out	0.20	cTnI
6008	0.18	0.5	10	1.5	Gastro-oesophageal		0.18	cTnI
6065	0.14	2.6	10	1.5	Non-specific		0.14	cTnI
6095	0.18	5.7	30	5.6	ACS		1.96	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Appendix 2 Detailed results of troponin elevation in non-acute coronary syndrome patients

Raised troponin: all methods

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
2304	4.36	1954.2	3690	236.3	Other	Focal myocarditis rather than coronary artery embolus causing myocardial scar on cardiac magnetic resonance imaging	4.36	cTnT
3025	0.09	55.0	80	33.0	Non-specific		0.20	cTnI
4091	8.45	2766.9	6690	314.7	Other	Pericarditis	8.45	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Raised troponin: Stratus CS analyser

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
1252	0.11	0.5	10	1.5	Non-specific		0.11	cTnI
2038	0.12	0.5	10	1.5	Non-specific		0.12	cTnT
2071	0.18	12.4	10	1.5	(Missing)		0.18	cTnT
2079	0.08	0.5	10	1.5	Non-specific		0.08	cTnT
2083	0.10	0.5	10	19.2	Musculoskeletal		0.10	cTnT
2087	0.13	20.3	80	96.2	Angina, no ACS		0.13	cTnT
2255	0.10	1.9	10	1.5	ACS		0.10	cTnT
2304	4.36	1954.2	3690	236.3	Other	Focal myocarditis rather than coronary artery embolus causing myocardial scar on cardiac magnetic resonance imaging	4.36	cTnT
2309	1.00	0.5	10	1.5	Other	Gallstones	0.07	cTnT
3017	0.20	0.5	10	1.5	Anxiety		0.20	cTnI
3025	0.09	55.0	80	33.0	Non-specific		0.20	cTnI
4091	8.45	2766.9	6690	314.7	Other	Pericarditis	8.45	cTnI
4134	0.08	0.5	10	1.5	Unknown		0.10	cTnI
4256	0.08	0.5	10	1.5	Gastro-oesophageal		0.08	cTnI
4285	0.10	20.0	10	13.2	Other	Urinary tract infection	0.10	cTnI
4308	0.08	0.5	10	1.5	Gastro-oesophageal		0.08	cTnI
4312	0.29	80.0	30	25.6	Other	Viral illness	0.07	cTnI
4316	0.08	0.5	10	1.5	Musculoskeletal		0.08	cTnI
4323	0.09	0.5	10	14.9	Musculoskeletal		0.09	cTnI
4333	0.09	0.5	10	1.5	Other	Syncope event	0.07	cTnI
5008	0.08	4.9	10	1.5	Non-specific		0.08	cTnI
5114	0.19	0.5	10	1.5	Non-specific		0.19	cTnI
6004	0.09	0.5	10	1.5	Non-specific		0.09	cTnI
6253	0.20	0.5	10	1.5	Musculoskeletal		0.07	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Raised troponin: all sensitive methods

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
2220	0.02	47.5	50	32.9	Non-specific		0.07	cTnT
3365	0.04	410.0	60	30.3	Non-specific		0.07	cTnI
4205	0.07	90.0	120	53.1	Other	Supraventricular tachycardia	0.07	cTnI
4464	0.07	50.0	50	30.4	Angina, no ACS		0.07	cTnI
6186	0.01	90.0	1640	46.0	Non-specific	Myocarditis	0.07	cTnI
6327	0.01	649.0	90	24.5	Gastro-oesophageal		0.07	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Raised troponin: Beckman AccuTnI

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
1018	0.01	132.7	90	6.6	Non-specific		0.07	cTnI
2220	0.02	47.5	50	32.9	Non-specific		0.07	cTnT
2304	4.36	1954.2	3690	236.3	Other	Focal myocarditis rather than coronary artery embolus causing myocardial scar on cardiac magnetic resonance imaging	4.36	cTnT
3025	0.09	55.0	80	33.0	Non-specific		0.20	cTnI
3223	0.07	47.0	80	11.0	Angina, no ACS		0.20	cTnI
3342	0.02	60.0	30	41.6	Gastro-oesophageal		0.07	cTnI
3365	0.04	410.0	60	30.3	Non-specific		0.07	cTnI
4070	0.03	106.1	110	5.4	Gastro-oesophageal		0.07	cTnI
4091	8.45	2766.9	6690	314.7	Other	Pericarditis	8.45	cTnI
4205	0.07	90.0	120	53.1	Other	Supraventricular tachycardia – rate-related ischaemia	0.07	cTnI
4312	0.29	80.0	30	25.6	Other	Viral illness	0.07	cTnI
4464	0.07	50.0	50	30.4	Angina, no ACS		0.07	cTnI
6087	0.01	128.6	10	3.7	Other	Postural drop secondary to amilodipine	0.07	cTnI
6186	0.01	90.0	1640	46.0	Non-specific		0.07	cTnI
6327	0.01	649.0	90	24.5	Gastro-oesophageal		0.07	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Raised troponin: Seimens Ultra

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
1018	0.01	132.7	90	6.6	Non-specific		0.07	cTnI
1219		15.0	60	76.1	Other	Supraventricular tachycardia	0.10	cTnI
2005	0.04	29.0	70	1.5	ACS		0.07	cTnT
2087	0.13	20.3	80	96.2	Angina, no ACS		0.13	cTnT
2170	0.01	13.9	60	1.5	Non-specific		0.07	cTnT
2220	0.02	47.5	50	32.9	Non-specific		0.07	cTnT
2284	0.01	7.5	60	1.5	Angina, no ACS		0.07	cTnT
2304	4.36	1954.2	3690	236.3	Other	Focal myocarditis rather than coronary artery embolus causing myocardial scar on cardiac magnetic resonance imaging	4.36	cTnT
2326	0.02	16.5	60	1.5	ACS		0.07	cTnT
3025	0.09	55.0	80	33.0	Non-specific		0.20	cTnI
3223	0.07	47.0	80	11.0	Angina, no ACS		0.20	cTnI
3365	0.04	410.0	60	30.3	Non-specific		0.07	cTnI
4021	0.01	19.2	90	8.9	Non-specific		0.07	cTnI
4029	0.06	29.1	50	1.5	ACS		0.07	cTnI
4070	0.03	106.1	110	5.4	Gastro-oesophageal		0.07	cTnI
4071	0.07	0.5	70	1.5	Gastro-oesophageal		0.07	cTnI
4091	8.45	2766.9	6690	314.7	Other	Pericarditis	8.45	cTnI
4103	0.04		3620	295.6	Musculoskeletal		0.07	cTnI
4126		0.5	50		Non-specific		0.06	cTnI
4205	0.07	90.0	120	53.1	Other	Supraventricular tachycardia – rate-related ischaemia	0.07	cTnI
4384	0.07	30.0	50	58.3	Other	Urinary tract infection	0.07	cTnI
4464	0.07	50.0	50	30.4	Angina, no ACS		0.07	cTnI
5081	0.02	13.3	50	1.5	Other	Possible cardiac pain but myocardial infarction ruled out	0.07	cTnI

APPENDIX 2

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
6013		5.1	120	34.7	Other	Lower respiratory tract infection	0.04	cTnI
6018	0.04	23.9	160	70.3	Non-specific		0.07	cTnI
6074	0.01	13.7	80	22.2	Angina, no ACS		0.07	cTnI
6123	0.03	4.3	70	9.3	Other	Microcytic anaemia	0.07	cTnI
6148	0.01	0.5	90	5.0	Musculoskeletal		0.07	cTnI
6186	0.01	90.0	1640	46.0	Non-specific		0.07	cTnI
6235	0.01	40.0	50	1.5	Musculoskeletal		0.07	cTnI
6327	0.01	649.0	90	24.5	Gastro-oesophageal		0.07	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Raised troponin: cTnT

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
1024	0.01	2.9	10	21.2	Musculoskeletal		0.07	cTnI
1041	0.02	22.4	30	17.7	Angina, no ACS		0.07	cTnI
1046	0.01	15.4	30	17.6	Other	Supraventricular tachycardia	0.07	cTnI
1219		15.0	60	76.1	Other	Supraventricular tachycardia	0.10	cTnI
2083	0.10	0.5	10	19.2	Musculoskeletal		0.10	cTnT
2087	0.13	20.3	80	96.2	Angina, no ACS		0.13	cTnT
2103	0.01	18.5	40	15.4	Non-specific		0.07	cTnT
2220	0.02	47.5	50	32.9	Non-specific		0.07	cTnT
2304	4.36	1954.2	3690	236.3	Other	Focal myocarditis rather than coronary artery embolus causing myocardial scar on cardiac magnetic resonance imaging	4.36	cTnT
3008	0.04	24.3	40	18.5	Musculoskeletal		0.20	cTnI
3025	0.09	55.0	80	33.0	Non-specific		0.20	cTnI
3076	0.01	31.0	40	22.7	Angina, no ACS		0.07	cTnI
3087	0.01	4.0	10	25.5	Musculoskeletal		0.07	cTnI
3138	0.04	0.5	10	15.9	Non-specific		0.20	cTnI
3276	0.02	2.7	30	19.4	Non-specific		0.07	cTnI
3311	0.06	10.0	30	20.1	Non-specific		0.07	cTnI
3342	0.02	60.0	30	41.6	Gastro-oesophageal		0.07	cTnI
3343	0.03	30.0	40	16.9	Non-specific		0.07	cTnI
3365	0.04	410.0	60	30.3	Non-specific		0.07	cTnI
3395	0.01	40.0	30	21.3	Anxiety		0.05	cTnI
3459	0.01	5.7	10	30.1	Non-specific		0.07	cTnI
4079	0.05	0.5	30	22.4	Other	Non-cardiac chest pain	0.07	cTnI

Study number	cTnI CS peak (μg/l)	cTnI B peak (ng/l)	cTnI S peak (ng/l)	cTnT peak (ng/l)	Final diagnosis	Comment	Peak troponin (μg/l) (local laboratory)	
4091	8.45	2766.9	6690	314.7	Other	Pericarditis	8.45	cTnI
4101		0.5	10	39.3	Angina, no ACS		0.06	cTnI
4103	0.04		3620	295.6	Musculoskeletal		0.07	cTnI
4205	0.07	90.0	120	53.1	Other	Supraventricular tachycardia – rate-related ischaemia	0.07	cTnI
4312	0.29	80.0	30	25.6	Other	Viral illness	0.07	cTnI
4313	0.06	30.0	30	29.4	Gastro-oesophageal		0.07	cTnI
4323	0.09	0.5	10	14.9	Musculoskeletal		0.09	cTnI
4332	0.04	30.0	40	22.8	Angina, no ACS		0.07	cTnI
4384	0.07	30.0	50	58.3	Other	Urinary tract infection	0.07	cTnI
4388	0.03	30.0	10	15.7	Non-specific		0.07	cTnI
4422	0.06	0.5	30	24.4	Non-specific		0.07	cTnI
4464	0.07	50.0	50	30.4	Angina, no ACS		0.07	cTnI
6013		5.1	120	34.7	Other	Lower respiratory tract infection	0.04	cTnI
6018	0.04	23.9	160	70.3	Non-specific		0.07	cTnI
6074	0.01	13.7	80	22.2	Angina, no ACS		0.07	cTnI
6081	0.01	20.0	20	57.9	Musculoskeletal		0.07	cTnI
6083	0.01	0.5	10	22.3	Musculoskeletal		0.07	cTnI
6138	0.03	0.5	10	25.7	Other	Bilateral pulmonary embolus	0.07	cTnI
6186	0.01	90.0	1640	46.0	Non-specific		0.07	cTnI
6210	0.04	39.0	40	22.0	ACS		0.07	cTnI
6327	0.01	649.0	90	24.5	Gastro-oesophageal		0.07	cTnI

cTnI B peak, Beckman AccuTnI peak value; cTnI CS peak, Stratus CS peak sample; cTnI S peak, Siemens Ultra peak value; cTnT peak, Roche high-sensitivity cTnT peak value.

Appendix 3 Probabilistic sensitivity analysis results for troponin T strategies

Probability of cost-effectiveness in doctor-on-demand scenario

	Probability of cost-effectiveness				
Lambda (l) (£)	No test	High-sensitivity cTnT	cTnT + H-FABP	cTnT at 0 + 90 minutes	10-hour troponin
0	1	0	0	0	0
1000	1	0	0	0	0
2000	1	0	0	0	0
3000	1	0	0	0	0
4000	0.7045	0.2955	0	0	0
5000	0	0.6626	0.3348	0.0026	0
6000	0	0.5077	0.4645	0.0278	0
7000	0	0.4194	0.5217	0.0589	0
8000	0	0.3640	0.5496	0.0864	0
9000	0	0.3246	0.5507	0.1080	0.0167
10,000	0	0.2846	0.5250	0.1156	0.0748
11,000	0	0.2414	0.4920	0.1140	0.1526
12,000	0	0.2010	0.4508	0.1040	0.2442
13,000	0	0.1651	0.4108	0.0896	0.3345
14,000	0	0.1261	0.3729	0.0730	0.4280
15,000	0	0.0958	0.3355	0.0549	0.5138
16,000	0	0.0649	0.2960	0.0386	0.6005
17,000	0	0.0363	0.2586	0.0213	0.6838
18,000	0	0.0115	0.2233	0.0045	0.7607
19,000	0	0	0.1949	0	0.8051
20,000	0	0	0.1690	0	0.8310
21,000	0	0	0.1437	0	0.8563
22,000	0	0	0.1225	0	0.8775
23,000	0	0	0.1042	0	0.8958
24,000	0	0	0.0888	0	0.9112
25,000	0	0	0.0741	0	0.9259
26,000	0	0	0.0604	0	0.9396
27,000	0	0	0.0468	0	0.9532
28,000	0	0	0.0358	0	0.9642

Lambda (l) (£)	Probability of cost-effectiveness				
	No test	High-sensitivity cTnT	cTnT + H-FABP	cTnT at 0 + 90 minutes	10-hour troponin
29,000	0	0	0.0239	0	0.9761
30,000	0	0	0.0145	0	0.9855
31,000	0	0	0.0052	0	0.9948
32,000	0	0	0.0006	0	0.9994
33,000	0	0	0	0	1
34,000	0	0	0	0	1
35,000	0	0	0	0	1
36,000	0	0	0	0	1
37,000	0	0	0	0	1
38,000	0	0	0	0	1
39,000	0	0	0	0	1
40,000	0	0	0	0	1
41,000	0	0	0	0	1
42,000	0	0	0	0	1
43,000	0	0	0	0	1
44,000	0	0	0	0	1
45,000	0	0	0	0	1
46,000	0	0	0	0	1
47,000	0	0	0	0	1
48,000	0	0	0	0	1
49,000	0	0	0	0	1
50,000	0	0	0	0	1

Probability of cost-effectiveness in twice-daily ward round scenario

	Probability of cost-effectiveness				
Lambda (l) (£)	No test	High-sensitivity cTnT	cTnT + H-FABP	cTnT at 0 + 90 minutes	10-hour troponin
0	1	0	0	0	0
1000	1	0	0	0	0
2000	1	0	0	0	0
3000	1	0	0	0	0
4000	0.9999	0.0001	0	0	0
5000	0	0.8002	0.1980	0.0018	0
6000	0	0.6298	0.3365	0.0337	0
7000	0	0.5078	0.4226	0.0696	0
8000	0	0.4327	0.4686	0.0987	0
9000	0	0.3772	0.5014	0.1214	0
10,000	0	0.3432	0.5156	0.1412	0
11,000	0	0.3143	0.5292	0.1565	0
12,000	0	0.2927	0.5392	0.1681	0
13,000	0	0.2713	0.5461	0.1826	0
14,000	0	0.2547	0.5523	0.1930	0
15,000	0	0.2434	0.5559	0.2007	0
16,000	0	0.2324	0.5567	0.2096	0.0013
17,000	0	0.2220	0.5487	0.2131	0.0162
18,000	0	0.2109	0.5377	0.2154	0.0360
19,000	0	0.2000	0.5227	0.2135	0.0638
20,000	0	0.1880	0.5064	0.2081	0.0975
21,000	0	0.1749	0.4912	0.2016	0.1323
22,000	0	0.1610	0.4727	0.1942	0.1721
23,000	0	0.1488	0.4531	0.1854	0.2127
24,000	0	0.1377	0.4352	0.1790	0.2481
25,000	0	0.1218	0.4170	0.1680	0.2932
26,000	0	0.1096	0.3990	0.1575	0.3339
27,000	0	0.0947	0.3819	0.1455	0.3779
28,000	0	0.0845	0.3656	0.1360	0.4139
29,000	0	0.0726	0.3478	0.1254	0.4542
30,000	0	0.0578	0.3316	0.1140	0.4966

Lambda (l) (£)	Probability of cost-effectiveness				
	No test	High-sensitivity cTnT	cTnT + H-FABP	cTnT at 0 + 90 minutes	10-hour troponin
31,000	0	0.0475	0.3136	0.1044	0.5345
32,000	0	0.0348	0.2969	0.0954	0.5729
33,000	0	0.0235	0.2791	0.0854	0.6120
34,000	0	0.0135	0.2645	0.0737	0.6483
35,000	0	0.0035	0.2478	0.0654	0.6833
36,000	0	0	0.2322	0.0558	0.7120
37,000	0	0	0.2180	0.0473	0.7347
38,000	0	0	0.2056	0.0392	0.7552
39,000	0	0	0.1922	0.0296	0.7782
40,000	0	0	0.1804	0.0215	0.7981
41,000	0	0	0.1703	0.0132	0.8165
42,000	0	0	0.1593	0.0049	0.8358
43,000	0	0	0.1481	0	0.8519
44,000	0	0	0.1371	0	0.8629
45,000	0	0	0.1275	0	0.8725
46,000	0	0	0.1188	0	0.8812
47,000	0	0	0.1106	0	0.8894
48,000	0	0	0.1023	0	0.8977
49,000	0	0	0.0959	0	0.9041
50,000	0	0	0.0886	0	0.9114

Probability of cost-effectiveness in once-daily ward round scenario

Lambda (l) (£)	Probability of cost-effectiveness				
	No test	High-sensitivity cTnT	cTnT + H-FABP	cTnT at 0 + 90 minutes	10-hour troponin
0	1	0	0	0	0
1000	1	0	0	0	0
2000	1	0	0	0	0
3000	1	0	0	0	0
4000	1	0	0	0	0
5000	0	0.8847	0.1145	0.0008	0
6000	0	0.7093	0.2558	0.0349	0
7000	0	0.5786	0.3468	0.0746	0
8000	0	0.4850	0.4092	0.1058	0
9000	0	0.4249	0.4485	0.1266	0
10,000	0	0.3779	0.4751	0.1470	0
11,000	0	0.3432	0.4934	0.1634	0
12,000	0	0.3201	0.5037	0.1762	0
13,000	0	0.2964	0.5142	0.1894	0
14,000	0	0.2785	0.5221	0.1994	0
15,000	0	0.2632	0.5292	0.2076	0
16,000	0	0.2501	0.5356	0.2143	0
17,000	0	0.2394	0.5401	0.2205	0
18,000	0	0.2320	0.5428	0.2252	0
19,000	0	0.2236	0.5456	0.2308	0
20,000	0	0.2153	0.5494	0.2353	0
21,000	0	0.2091	0.5496	0.2397	0.0016
22,000	0	0.2039	0.5431	0.2413	0.0117
23,000	0	0.1969	0.5346	0.2425	0.0260
24,000	0	0.1905	0.5267	0.2415	0.0413
25,000	0	0.1841	0.5151	0.2391	0.0617
26,000	0	0.1748	0.5066	0.2356	0.0830
27,000	0	0.1661	0.4943	0.2303	0.1093
28,000	0	0.1574	0.4824	0.2234	0.1368
29,000	0	0.1484	0.4697	0.2175	0.1644
30,000	0	0.1408	0.4548	0.2098	0.1946

Lambda (l) (£)	Probability of cost-effectiveness				
	No test	High-sensitivity cTnT	cTnT + H-FABP	cTnT at 0 + 90 minutes	10-hour troponin
31,000	0	0.1325	0.4408	0.2021	0.2246
32,000	0	0.1243	0.4288	0.1960	0.2509
33,000	0	0.1133	0.4157	0.1895	0.2815
34,000	0	0.1051	0.4019	0.1810	0.3120
35,000	0	0.0940	0.3908	0.1709	0.3443
36,000	0	0.0862	0.3783	0.1622	0.3733
37,000	0	0.0789	0.3663	0.1528	0.4020
38,000	0	0.0704	0.3537	0.1455	0.4304
39,000	0	0.0595	0.3415	0.1371	0.4619
40,000	0	0.0512	0.3295	0.1297	0.4896
41,000	0	0.0441	0.3177	0.1210	0.5172
42,000	0	0.0353	0.3035	0.1123	0.5489
43,000	0	0.0264	0.2923	0.1054	0.5759
44,000	0	0.0188	0.2788	0.0980	0.6044
45,000	0	0.0119	0.2669	0.0913	0.6299
46,000	0	0.0049	0.2563	0.0829	0.6559
47,000	0	0	0.2450	0.0738	0.6812
48,000	0	0	0.2327	0.0683	0.6990
49,000	0	0	0.2221	0.0621	0.7158
50,000	0	0	0.2132	0.0546	0.7322

Appendix 4 Probabilistic sensitivity analysis results for troponin I strategies

Probability of cost-effectiveness in doctor-on-demand scenario

	Probability of cost-effectiveness				
Lambda (l) (£)	No test	High-sensitivity cTnI	cTnI + H-FABP	cTnI at 0 + 90 minutes	10-hour troponin
0	1	0	0	0	0
1000	1	0	0	0	0
2000	1	0	0	0	0
3000	1	0	0	0	0
4000	0.5373	0.4615	0.0012	0	0
5000	0	0.4933	0.4910	0.0157	0
6000	0	0.3151	0.5870	0.0979	0
7000	0	0.2098	0.5912	0.1990	0
8000	0	0.1451	0.5637	0.2912	0
9000	0	0.0991	0.5313	0.3696	0
10,000	0	0.0733	0.4998	0.4269	0
11,000	0	0.0543	0.4723	0.4734	0
12,000	0	0.0424	0.4494	0.5082	0
13,000	0	0.0334	0.4311	0.5355	0
14,000	0	0.0268	0.4136	0.5596	0
15,000	0	0.0204	0.4004	0.5792	0
16,000	0	0.0163	0.3877	0.5940	0.0020
17,000	0	0.0139	0.3747	0.5857	0.0257
18,000	0	0.0109	0.3607	0.5735	0.0549
19,000	0	0.0070	0.3447	0.5601	0.0882
20,000	0	0.0025	0.3279	0.5428	0.1268
21,000	0	0	0.3110	0.5273	0.1617
22,000	0	0	0.2951	0.5079	0.1970
23,000	0	0	0.2805	0.4926	0.2269
24,000	0	0	0.2670	0.4721	0.2609
25,000	0	0	0.2531	0.4560	0.2909
26,000	0	0	0.2377	0.4401	0.3222
27,000	0	0	0.2246	0.4233	0.3521
28,000	0	0	0.2146	0.4067	0.3787

Lambda (l) (£)	Probability of cost-effectiveness				
	No test	High-sensitivity cTnI	cTnI + H-FABP	cTnI at 0 + 90 minutes	10-hour troponin
29,000	0	0	0.2038	0.3924	0.4038
30,000	0	0	0.1926	0.3794	0.4280
31,000	0	0	0.1816	0.3657	0.4527
32,000	0	0	0.1720	0.3519	0.4761
33,000	0	0	0.1617	0.3397	0.4986
34,000	0	0	0.1531	0.3287	0.5182
35,000	0	0	0.1449	0.3165	0.5386
36,000	0	0	0.1370	0.3038	0.5592
37,000	0	0	0.1284	0.2932	0.5784
38,000	0	0	0.1214	0.2843	0.5943
39,000	0	0	0.1142	0.2765	0.6093
40,000	0	0	0.1073	0.2663	0.6264
41,000	0	0	0.1010	0.2582	0.6408
42,000	0	0	0.0935	0.2494	0.6571
43,000	0	0	0.0874	0.2410	0.6716
44,000	0	0	0.0821	0.2330	0.6849
45,000	0	0	0.0754	0.2257	0.6989
46,000	0	0	0.0692	0.2184	0.7124
47,000	0	0	0.0630	0.2121	0.7249
48,000	0	0	0.0568	0.2043	0.7389
49,000	0	0	0.0521	0.1992	0.7487
50,000	0	0	0.0480	0.1937	0.7583

Probability of cost-effectiveness in twice-daily ward round scenario

	Probability of cost-effectiveness				
Lambda (l) (£)	No test	High-sensitivity cTnI	cTnI + H-FABP	cTnI at 0 + 90 minutes	10-hour troponin
0	1	0	0	0	0
1000	1	0	0	0	0
2000	1	0	0	0	0
3000	1	0	0	0	0
4000	0.9806	0.0194	0	0	0
5000	0	0.6587	0.2970	0.0443	0
6000	0	0.4282	0.4018	0.1700	0
7000	0	0.2847	0.4323	0.2830	0
8000	0	0.1932	0.4288	0.3780	0
9000	0	0.1313	0.4189	0.4498	0
10,000	0	0.0946	0.4051	0.5003	0
11,000	0	0.0703	0.3910	0.5387	0
12,000	0	0.0530	0.3772	0.5698	0
13,000	0	0.0410	0.3642	0.5948	0
14,000	0	0.0326	0.3538	0.6136	0
15,000	0	0.0249	0.3436	0.6315	0
16,000	0	0.0211	0.3346	0.6443	0
17,000	0	0.0180	0.3279	0.6541	0
18,000	0	0.0157	0.3213	0.6630	0
19,000	0	0.0129	0.3163	0.6708	0
20,000	0	0.0111	0.3108	0.6781	0
21,000	0	0.0093	0.3067	0.6840	0
22,000	0	0.0083	0.3035	0.6882	0
23,000	0	0.0071	0.3000	0.6929	0
24,000	0	0.0059	0.2978	0.6963	0
25,000	0	0.0047	0.2941	0.7012	0
26,000	0	0.0041	0.2930	0.7029	0
27,000	0	0.0036	0.2907	0.7057	0
28,000	0	0.0033	0.2882	0.7085	0
29,000	0	0.0021	0.2865	0.7114	0
30,000	0	0.0019	0.2837	0.7144	0

Lambda (l) (£)	Probability of cost-effectiveness				
	No test	High-sensitivity cTnI	cTnI + H-FABP	cTnI at 0 + 90 minutes	10-hour troponin
31,000	0	0.0018	0.2819	0.7163	0
32,000	0	0.0016	0.2801	0.7183	0
33,000	0	0.0016	0.2786	0.7198	0
34,000	0	0.0014	0.2773	0.7213	0
35,000	0	0.0013	0.2759	0.7228	0
36,000	0	0.0012	0.2747	0.7241	0
37,000	0	0.0011	0.2733	0.7174	0.0082
38,000	0	0.0007	0.2719	0.7061	0.0213
39,000	0	0.0001	0.2699	0.6940	0.0360
40,000	0	0	0.2665	0.6820	0.0515
41,000	0	0	0.2631	0.6704	0.0665
42,000	0	0	0.2588	0.6585	0.0827
43,000	0	0	0.2557	0.6471	0.0972
44,000	0	0	0.2513	0.6351	0.1136
45,000	0	0	0.2467	0.6240	0.1293
46,000	0	0	0.2422	0.6120	0.1458
47,000	0	0	0.2382	0.6020	0.1598
48,000	0	0	0.2340	0.5919	0.1741
49,000	0	0	0.2296	0.5817	0.1887
50,000	0	0	0.2258	0.5713	0.2029

Probability of cost-effectiveness in once-daily ward round scenario

	Probability of cost-effectiveness				
Lambda (l) (£)	No test	High-sensitivity cTnI	cTnI + H-FABP	cTnI at 0 + 90 minutes	10-hour troponin
0	1	0	0	0	0
1000	1	0	0	0	0
2000	1	0	0	0	0
3000	1	0	0	0	0
4000	1	0	0	0	0
5000	0	0.8123	0.1463	0.0414	0
6000	0	0.5346	0.2750	0.1904	0
7000	0	0.3680	0.3132	0.3188	0
8000	0	0.2588	0.3339	0.4073	0
9000	0	0.1831	0.3407	0.4762	0
10,000	0	0.1267	0.3391	0.5342	0
11,000	0	0.0939	0.3335	0.5726	0
12,000	0	0.0723	0.3264	0.6013	0
13,000	0	0.0562	0.3194	0.6244	0
14,000	0	0.0437	0.3139	0.6424	0
15,000	0	0.0351	0.3078	0.6571	0
16,000	0	0.0282	0.3018	0.6700	0
17,000	0	0.0229	0.2989	0.6782	0
18,000	0	0.0193	0.2953	0.6854	0
19,000	0	0.0170	0.2926	0.6904	0
20,000	0	0.0150	0.2894	0.6956	0
21,000	0	0.0134	0.2867	0.6999	0
22,000	0	0.0110	0.2836	0.7054	0
23,000	0	0.0093	0.2816	0.7091	0
24,000	0	0.0087	0.2784	0.7129	0
25,000	0	0.0076	0.2769	0.7155	0
26,000	0	0.0063	0.2754	0.7183	0
27,000	0	0.0051	0.2741	0.7208	0
28,000	0	0.0044	0.2724	0.7232	0
29,000	0	0.0038	0.2705	0.7257	0
30,000	0	0.0036	0.2692	0.7272	0

Lambda (l) (£)	Probability of cost-effectiveness				
	No test	High-sensitivity cTnI	cTnI + H-FABP	cTnI at 0 + 90 minutes	10-hour troponin
31,000	0	0.0025	0.2681	0.7294	0
32,000	0	0.0021	0.2667	0.7312	0
33,000	0	0.0020	0.2655	0.7325	0
34,000	0	0.0019	0.2645	0.7336	0
35,000	0	0.0016	0.2631	0.7353	0
36,000	0	0.0016	0.2623	0.7361	0
37,000	0	0.0014	0.2611	0.7375	0
38,000	0	0.0013	0.2604	0.7383	0
39,000	0	0.0013	0.2595	0.7392	0
40,000	0	0.0012	0.2586	0.7402	0
41,000	0	0.0012	0.2577	0.7411	0
42,000	0	0.0012	0.2566	0.7422	0
43,000	0	0.0011	0.2560	0.7429	0
44,000	0	0.0010	0.2554	0.7436	0
45,000	0	0.0008	0.2550	0.7442	0
46,000	0	0.0005	0.2542	0.7453	0
47,000	0	0.0004	0.2542	0.7454	0
48,000	0	0.0004	0.2542	0.7454	0
49,000	0	0.0004	0.2537	0.7459	0
50,000	0	0.0004	0.2534	0.7451	0.0011

Appendix 5 Study protocol

PROJECT TITLE: RATPAC CBE (RANDOMISED ASSESSMENT OF TREATMENT USING PANEL ASSAY OF CARDIAC MARKERS – CONTEMPORARY BIOMARKER EVALUATION)

Research objectives

1. To test the diagnostic accuracy for an AMI of highly sensitive troponin assays and a range of new cardiac biomarkers of plaque destabilisation, myocardial ischaemia and necrosis.
2. To test the prognostic accuracy for adverse cardiac events of highly sensitive troponin assays and this range of new cardiac biomarkers.
3. To estimate the potential economic impact (clinical effectiveness and cost-effectiveness) of using highly sensitive troponin assays or this range of new cardiac biomarkers instead of an admission and 12-hour troponin measurement.

Existing research

Chest pain due to suspected but not proven acute myocardial infarction (AMI) is responsible for a substantial number of emergency department attendances and emergency hospital admissions in the NHS[1]. Current recommendations suggest that these patients should receive diagnostic testing with measurement of cardiac troponin (now considered to be the definitive test of myocardial necrosis) in a sample taken 12 hours after symptom onset[2]. This delay is necessary because the diagnostic activity of troponin measurement using current assays does not reach peak diagnostic sensitivity until this time. This approach is inconvenient and potentially costly because it requires many patients to be unnecessarily admitted to hospital until the 12-hour sample can be obtained and measured. The majority of patients with a suspected AMI do not actually have an AMI, so their admission will ultimately prove avoidable. Cost-effectiveness analysis suggests that admitting patients for cardiac marker testing is not an efficient use of health service resources[3]. Evidence also suggests that these testing guidelines are often not followed in a busy emergency setting where acute beds are limited. Collinson *et al*[4] showed that 7% of patients discharged after emergency department assessment for acute chest pain had elevated troponin levels at follow-up two days later. Goodacre *et al*[5] showed that in the routine care arm of a randomised trial of a chest pain unit, 14% of patients with an elevated troponin level at two-day follow-up had been sent home from the emergency department.

To overcome the limitations of waiting for 12 hours a number of approaches have been suggested. These include rapid, early sampling[6], the use of cardiac marker panels, including markers which may be detected earlier than troponin[7] and the use of novel markers of ischaemia[8] or of plaque destabilization and rupture[9;10]. Early studies comparing cardiac troponin to other biomarkers suggest that measurement of cardiac troponin is equivalent to the other currently used markers such as myoglobin and the MB isoenzyme of creatine kinase[11]. Meta-analyses have estimated the diagnostic accuracy of individual cardiac markers[12], but there have been no systematic reviews of cardiac panels.

Recent developments have improved the measurement technology for cardiac troponins and have suggested that much earlier sampling with serial measurement and calculation of rate of rise[13] (the significant difference between two consecutive measurements) can be utilised[14]. To date there have been very few studies of the value of biomarker panels in the general chest pain population and none that have examined the new, more sensitive, troponin assays and compared them with biomarker panels. The recent National Institute of Clinical Excellence draft guidance on chest pain specifically identifies the need

for further research into high sensitivity troponin assays and recommends comparison with high sensitivity troponin assays as part of any biomarker evaluation in chest pain patients.

Research methods

Design

We plan to use blood samples collected from patients recruited to an HTA-funded trial of point of care cardiac markers to test the diagnostic and prognostic accuracy of new cardiac markers and highly sensitive troponin assays.

The RATPAC Trial (HTA 06/302/19) (Randomised Assessment of Treatment using Panel Assay of Cardiac markers) was a prospective randomised controlled trial of point-of-care cardiac markers in the emergency department. The research objectives of the study were to evaluate the clinical effectiveness and cost-effectiveness of the currently most promising point-of-care cardiac marker panel currently used in the emergency department.

In patients presenting to the emergency department with suspected but not proven acute myocardial infarction (AMI) the study measured the effect of using a point-of-care cardiac marker panel upon:

1. The proportion of patients successfully discharged home after emergency department assessment
2. Health utility and satisfaction with care
3. The use of coronary care beds and cardiac treatments.
4. Subsequent re-attendance at and/or re-admission to hospital
5. Major adverse events (death, non-fatal AMI, life-threatening arrhythmia, emergency revascularisation or hospitalisation for myocardial ischaemia)
6. Health and social care costs.

The study finished recruiting after external review carried out on behalf of the HTA suggested that the study had achieved its primary outcome.

Setting

The study was performed in six emergency departments in the United Kingdom. Emergency department staff identified eligible patients, provided trial information and obtained written consent. Participants were then randomly allocated to receive either: a) Diagnostic assessment using the point-of-care biochemical marker panel, or b) Conventional diagnostic assessment without the panel.

Target Population

People who presented to the emergency department with chest pain due to suspected but not proven AMI in whom a negative cardiac marker test measured by point-of-care marker testing could potentially rule out an AMI and allow discharge home. The following classes of patients were excluded.

1. Patients with diagnostic ECG changes for an AMI or high-risk acute coronary syndrome (>1 mm ST deviation or >3 mm inverted T waves). These patients have a presumptive diagnosis of myocardial infarction (both ST elevation myocardial infarction and non ST elevation myocardial infarction) and are at high risk of adverse outcome and require inpatient care even if marker tests are negative.
2. Patients with known coronary heart disease presenting with prolonged (>1 hour) or recurrent episodes of typical cardiac-type pain. These patients have unstable angina and require inpatient care for symptom control even if marker tests are negative.
3. Patients with proven or suspected serious non-coronary pathology (e.g. pulmonary embolus) that required inpatient care even if an AMI is ruled out.
4. Patients with co-morbidity or social problems that require hospital admission even if an AMI can be ruled out.

5. Patients with an obvious non-cardiac cause (e.g. pneumothorax or muscular pain), in whom an AMI could be excluded as a possible cause without resorting to further diagnostic testing.
6. Patients presenting more than 12 hours after their most significant episode of pain, for whom a single troponin measurement have been more appropriate than point-of-care panel testing.
7. Previous participation in the RATPAC trial.
8. Patients who were unable to understand the trial information due to cognitive impairment.
9. Non-English speaking patients for whom translation facilities were not available.

Participants were randomised to receive either: diagnostic assessment using the point-of-care biochemical marker panel, or conventional diagnostic assessment without the panel. The only difference between the two arms of the trial was that patients in the intervention arm received testing with the point-of-care panel. The use of all other tests and treatments, and decision-making in the emergency department, was at the discretion of the attending clinician.

The point-of-care cardiac marker panel utilised was CK-MB(mass), myoglobin and troponin I, measured at presentation and 90 minutes later, using the Stratus-CS point-of-care analyser. Of the systems currently available the latest version of the Siemens Stratus CS has the most data as an instrument suitable both for the emergency laboratory and for use as a POCT instrument. The troponin method available on this instrument also meets the criteria for diagnosis of an AMI according to the most recent criteria proposed by the European Society of Cardiology.

Patients randomised to the point of care arm had a blood sample taken at study enrolment and 90 minutes later for analysis by point of care testing. At the same time that blood was taken for point of care testing, they consented to allow (without the need for additional venopuncture) the clinical staff to take an extra tube of blood. The additional blood sample was transported to the hospital laboratory where it was allowed to clot, centrifuged, the serum separated into two aliquots and frozen to –20 °C in a timely manner. Other than obtaining consent, collecting data, and random allocation to use of the point-of-care test, the only change to routine practice was taking the additional blood sample for subsequent biomarker assessment.

Batches of samples were then transported frozen on dry ice to St Georges Hospital and are stored at –70 °C prior to analysis. Previous extensive stability studies have shown that cardiac biomarkers which are clinically useful and usable are fully stable with this storage regimen.

Follow-up

All participants were followed up until 90 days after initial attendance. A postal questionnaire consisting of the EQ-5D health utility questionnaire and a resource use questionnaire was sent to all participants at 30 and 90 days with one remailing to non-responders. Hospital records were reviewed at 90 days to identify all adverse events, hospital attendances and admissions.

Health Technologies Being Assessed

The archived blood samples from the RATPAC study represents an ideal opportunity to extend the findings of the RATPAC trial in a cost effective way. The existing patients enrolled are fully characterized and have been followed up for major adverse cardiac events. The population is also unique as it represents one found within the emergency department which has been selected on the basis of low cardiac risk rather than enrolled in a clinical trial with a high prior probability of cardiovascular disease. This is a major limitation of many existing biomarker studies and has been highlighted in recent editorials and the consensus statement on biomarker series of the working group of the European Society of Cardiology.

The samples will be analysed using state of the art high sensitivity troponin assays, two sensitive cardiac troponin I and one sensitive cardiac troponin T assays. These assays have been previously independently

analytically validated and the findings published in peer reviewed journals[15-17]. In addition, samples will be analysed for heart fatty acid binding protein, myoglobin, copeptin, interleukin-6, sensitive C-reactive protein and B-type natriuretic peptide (measured as the N terminal pro-hormone, NT pro-BNP). All of these assays are either commercially available or due to be launched on analytical platforms in wide spread clinical use in laboratories world wide. Finally, prior to project start the literature will be surveyed to see if there are any additional markers which need to be considered.

Measurement of myoglobin will allow independent confirmation of the results obtained by point of care testing and allow a definitive statement to be made as to the additional value, or not, of myoglobin measurement for the very early diagnosis of myocardial injury when compared with a sensitive troponin assay[18]. Heart fatty acid binding protein[19–21] and copeptin[22] have been proposed as alternative early markers to detect or exclude myocardial injury. Interleukin-6, sensitive C reactive protein and NT pro-BNP[23;24] have all been proposed and published in peer-reviewed literature as additional tests for risk stratification in patients presenting with chest pain. To date, no studies have been done in the general chest pain population to confirm or refute these claims.

Measurement of cost and outcomes

Diagnostic outcome

The study will examine the diagnostic performance based on the final consensus diagnosis from the RATPAC study. The diagnostic criteria used for acute myocardial infarction will be those recognised by the European Society of Cardiology in the new universal definition of myocardial infarction based on the troponin values obtained from the Stratus CS. This assay meets the performance characteristics recommended in the new universal definition of myocardial infarction. According to this definition, a troponin level above the 99th percentile of the values for a reference control group is considered positive, and in the context of a patient with ischaemic symptoms (i.e. chest pain) would satisfy the diagnosis for an AMI. This definition identifies patients who are most likely to benefit from treatments that usually require hospital admission. The individual diagnostic performance of each biomarker alone and in combination will be assessed by construction of receiver operator characteristic curves (ROC curves) and compared by calculation of the C statistic, the area under the curve. In addition, multivariate regression analysis will be performed to determine which marker or combination of markers will independently add significant diagnostic efficiency and predictive ability to obtain the final diagnosis.

Prognostic outcome

An independent assessment the ability to predict outcome in a multivariate risk model will be examined. This will include comparison with other risk predictive models (scoring systems derived from registry studies such as TIMI and GRACE risk scores). We will analyse the association between marker levels and adverse events within 30 days. The individual prognostic ability of each biomarker alone and in combination will be assessed by construction of ROC curves and compared by calculation of the C statistic. In addition, multivariate regression analysis will be performed to determine which marker or combination of markers is able to optimally predict outcome. The objective will be to determine whether the additional biomarker information helps diagnose patients or predict outcome by itself and whether they add to scoring systems (such as the TIMI & GRACE scores) and other clinical variables.

Economic analysis

The economic analysis will be based initially on cost minimisation analysis. The base case will be full laboratory cost to achieve diagnosis and comparison of costs for individual marker and marker panel strategies. Laboratory costs will be calculated using the ABC laboratory cost package and include cost per reportable result (including quality assurance and calibration based on routine laboratory performance) and total tests cost (NHS price) including staff and overhead costs. The laboratory at St George's hospital has already performed this work for it existing cardiac biomarker tests, so will be able to utilize the same methodology to allow direct comparison of the biomarker is included in the study. In addition, cost

modelling utilising hospital episode costs will be performed to estimate cost benefit of increased test costs compared with reduction in length of stay.

Sample Size

1132 patients were enrolled into the intervention arm of the study of whom 1076 had blood samples taken for measurement of biomarkers on at least one occasion. The incidence of acute myocardial infarction was 130/1076 (12.1%). This means that at conventional statistical significance, the study will be powered to detect the inability of the candidate tests to improve diagnostic sensitivity if they fail to detect more than five cases of myocardial infarction when compared to the predicate test. In total 2263 patients were recruited with follow up obtained (to date) in 1930 (85.3%) with 14 (0.7%) with non fatal MI and a death rate of 2 (0.1%) including an AMI.

Economic cost of chest pain

A typical District General Hospital will see around 6500 patients presenting with chest pain of suspected cardiac origin each year. In those admitted, 70% will have myocardial infarction excluded. Length of stay is typically 1 to 2 days. Only a minority of cases have active coronary artery disease requiring intervention. One study estimated that 50 to 75% of admissions did not require hospital stay. There is therefore a considerable economic cost in terms of bed occupancy and inappropriate investigation. A strategy which allows very rapid discharge of patients at low risk would result in significant improvement in the use of scarce NHS resources. A number of different strategies to this have been proposed including the use of multiple cardiac marker panels and novel cardiac markers.

Biomarker costs

A typical hospital laboratory within the UK will perform 20,000 troponin assays annually. The cost of troponin measurement has fallen substantially from a typical cost of £20 per test to £2–£3, so annual spend is £60,000, corresponding to a national cost of approximately £15 million. The use of a rapid troponin-based protocol which shortens hospital stay for chest pain to 2 to 3 hours from 12 to 24 hours would have significant economic benefit. Based on the average bed cost of £100 per day a typical hospital with 5000 chest pain attendances per annum could potentially exclude 2500 patients with a reduction in bed occupancy from 1250–2500 bed days to 312 bed days, a saving of £2.2 million. If it can be shown that a rapid biomarker based strategy utilising modern assay technology or new markers can be used safely to substantially reduce hospital stay nationwide considerable savings to the health-care budget could be made.

The use of a multiple marker panel or a new range of cardiac by markers would result in a substantial increase in laboratory costs. If this were truly offset by significant reduction in hospital stay, utilisation of novel biomarkers would be cost effective. If the use of more sensitive troponin assays makes measurement of novel biomarker or biomarker panels unnecessary, a significant waste of resources would occur. Assuming a typical market entry price for a novel biomarker of £20 or for a panel of tests, £20 per biomarker panel, and assuming the same pattern of utilisation as that used for troponin requesting, annual cost would be £400,000 for a typical hospital. On a UK wide basis it can be estimated that moving to a novel biomarker or a biomarker panel which is more expensive and not significantly diagnostically superior to optimal use of the existing testing, or utilization of the next generation of troponin assays, could cost an additional £20–£100 million per annum without conferring health-care benefits.

Market impact

The UK market for diagnostics has been estimated at between £100 and £250 million per annum. An evidence-based strategy for biomarker measurement would offer a significant competitive advantage for any UK based company.

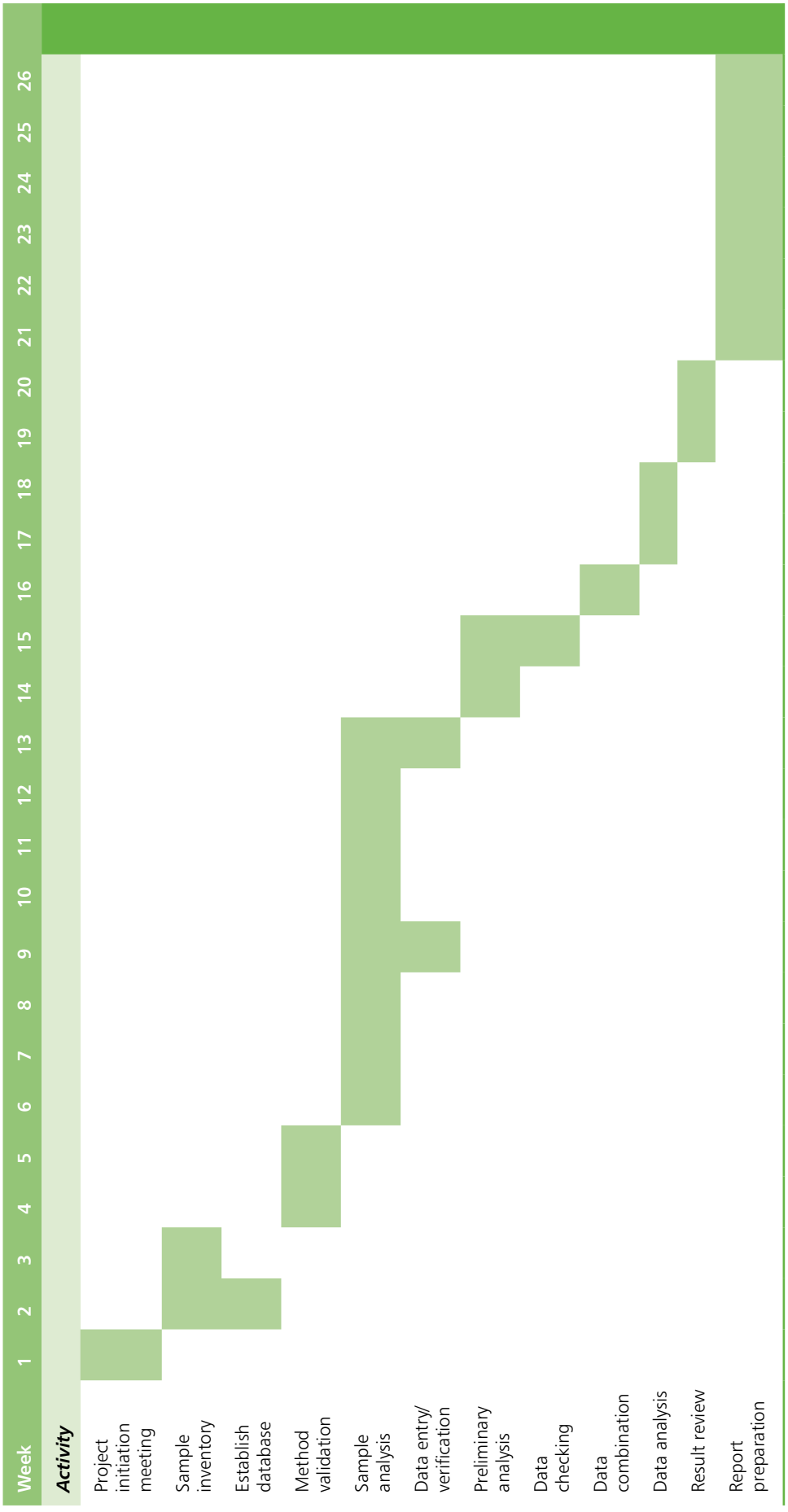
Relationship with evidence based practice

Finally, the UK is developing a national catalogue of laboratory tests. This will ultimately include assessment of cost effectiveness and test efficiency. This study will serve to inform this national initiative.

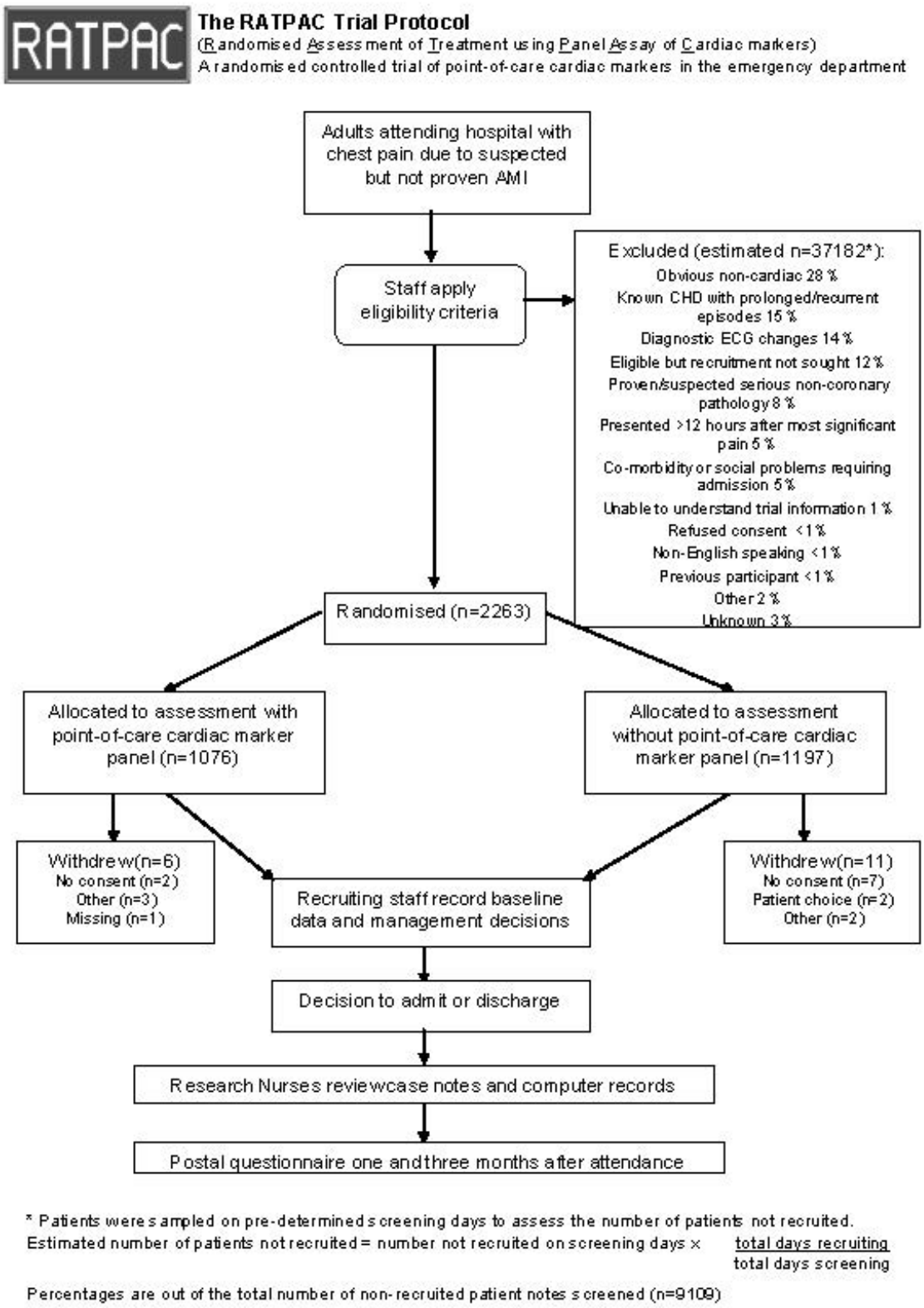
Outcomes

The objective of the study is to provide a benchmark for the new sensitive troponin assays. In addition, it will establish using a very well validated clinically relevant cohort the true role, or otherwise of the new proposed markers such as heart fatty acid binding protein. It will establish, for the first time, if inflammatory markers and markers of heart failure have a role to play in the general chest pain population. The routine and research laboratories at St. George's Hospital, contains a range of state of the art equipment representative of that seen in a typical hospital laboratory. The samples will therefore be analysed under typical laboratory conditions so that findings can be used throughout the UK and abroad.

Project Timetable



RATPAC recruitment chart



* Patients were sampled on pre-determined screening days to assess the number of patients not recruited.
Estimated number of patients not recruited = number not recruited on screening days × $\frac{\text{total days recruiting}}{\text{total days screening}}$

Percentages are out of the total number of non-recruited patient notes screened (n=9109)

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