Highly sensitive cardiac troponin T assay for the diagnosis of Acute Myocardial Infarction in patients presenting to the Emergency Department with symptoms suggestive of Acute Coronary Syndrome—A systematic review of the literature and meta-analysis

Review protocol

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Background

**Target condition being diagnosed**

Coronary artery disease (CAD) is the leading cause of death in the developed countries and in the following few decades is expected to become the leading cause of death worldwide.\(^1\) CAD is the most common cause of death in the UK and accounts for 94 000 deaths each year, with approximately one in five men and one in seven women dying from the disease. It is also the most common cause of premature death, causing almost 31 000 premature deaths each year, which is approximately one fifth (19%) of premature deaths in men and one in ten (10%) premature deaths in women.\(^2\)

CAD is the result of a progressive accumulation of atheromatous plaque on the walls of the coronary arteries which, in its advanced stages, results in a narrowing of the vessels’ lumen—the free space in the arteries—and affects the coronary blood circulation causing myocardial ischemia. The most dramatic manifestation of CAD is the development of a thrombotic acute coronary syndrome (ACS), which occurs when atherosclerotic plaque gets ruptured or eroded and, through the processes of thrombogenesis and embolisation, leads to partial or total occlusion of an epicardial artery and occlusion of the smaller downstream vessels by debris from the plaque.\(^3\)

The usual presentation of ACS includes typical angina—substernal chest pain or constricting discomfort in the chest—which radiates to the arms, jaws and back and may be accompanied by other symptoms such as nausea, vomiting, dispnea (shortness of breath), diaphoresis (sweating), light-headedness and particularly a combination of those.\(^4,5\) Atypical presentation, which may not involve chest pain, occurs in up to 40% of the cases subsequently diagnosed with ACS and significantly increases the probability of misdiagnosis and suboptimal treatment.\(^6\)

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ACS covers both acute myocardial infarction (AMI) and unstable angina (UA). In AMI heart cells are dying as a result of interrupted blood supply, while in UA the insufficient supply of oxygen and nutrients causes chest pain and other symptoms associated with ACS, but without evidence of myocardial necrosis. Since patients with ACS may need urgent reperfusion therapy (e.g. fibrinolytic therapy and revascularisation) in order to avoid or minimise the damage to the heart muscle, timely diagnosis and risk stratification are of paramount importance. The diagnostic protocols currently used to triage patients admitted to the emergency department (ED) with symptoms suggestive of ACS combine information from the patient’s history and clinical examination, resting ECG and cardiac biomarkers (usually cardiac troponin). Upon admission to the ED patients undergo initial resting ECG and a blood sample is drawn to measure the level of cardiac troponin (cTn). Based on the results from the ECG, clinical examination and history patients are assigned to one of the following working diagnoses:

- ST-segment elevation myocardial infarction (STEMI);
- Non-ST-segment elevation ACS (NSTE ACS); and
- Non-ACS diagnosis.

Persistent ST-segment elevation is indicative of a total or nearly total occlusion of a coronary artery and most patients with ST-elevation will be referred for invasive coronary angiography (ICA) and will, eventually, undergo reperfusion therapy regardless of their troponin result. If ST-elevation is not sufficient to trigger PCI, patients would be treated as high-risk ACS patients, with ACS treatment and cardiology review/monitoring to watch for further elevation. Patients assigned to the NSTE ACS group may undergo further testing to confirm or exclude the diagnosis of ACS and, eventually, will be diagnosed as having:

- Non-ST-segment elevation myocardial infarction (NSTEMI) if the cTn results indicate myocardial necrosis in the clinical context of myocardial ischemia;
- UA if the troponin levels are normal but there is sufficient evidence of myocardial ischemia that explains the current symptoms; or,
- Non-ACS diagnosis.

The ability of the clinical symptoms, patient’s history, resting ECG and the previous generation of cTn assays to rule in or out the diagnosis of ACS in the early hours of the onset of the symptoms is limited. In order to avoid inappropriate discharge of patients with ACS, which may result in delayed treatment and major adverse coronary events (MACE), patients suspected to have ACS but with an initial negative cTn test and normal or non-diagnostic ECG are admitted to hospital for a short (usually up to 48 hours) period of clinical observation and undergo serial ECG and cTn tests. If the tests are negative, additional diagnostic tests, such as ETT, nuclear imaging and CCTA, may be considered to further risk stratify patients and rule out myocardial ischemia.

This diagnostic algorithm, though allowing for a reliable exclusion of ACS as the cause of the initial symptoms, is highly inefficient since only about 20% of all patients admitted for clinical observation and serial testing are ultimately diagnosed with ACS. This puts a significant pressure on the healthcare system, since acute chest pain and other symptoms suggestive of ACS are one of the most common reasons for ED visits and account for approximately 5% of all visits to the ED and up to 40% of the emergency hospital admissions (NICE 2010b). Also, such algorithm may delay diagnosis and hold back optimal treatment, especially in patients with NSTEMI who benefit from early invasive strategy (<24 hrs) involving ICA and revascularisation. Advances in cardiac imaging and biomarkers have led to the development of new diagnostic protocols which may allow faster triage of patients provisionally
diagnosed with NSTE ACS. Some of these protocols take advantage of the improved sensitivity and analytical precision of the new generation of highly sensitive cardiac troponin (hs-cTn) assays, which may help emergency physicians to rule in or out the diagnosis of AMI in the first few hours of the patient’s presentation to the ED.

**Index test: highly sensitive cardiac troponin T assay (hs cTnT)**

At present, cTn are the preferred biochemical markers for myocardial injury and one of the main diagnostic and risk stratification tools used in the triage of patients suspected of ACS. An elevated concentration of cTn is also central to the universal definition of AMI, which requires a rise and/or fall of cTn with ≥1 value above the 99th percentile of the upper reference limit (URL) plus objective evidence of myocardial ischemia.

Cardiac troponins are proteins which control calcium mediated interaction of actin and myosin, thus enabling the contraction and relaxation of the cardiac muscle. The troponin complex is comprised of three different isoforms: troponin C, I and T. While troponin C is similar in cardiac and slow skeletal muscles, troponins I or T (cTnI and cTnT) are present only in the cardiac myocytes, which makes them highly specific biochemical markers of myocardial damage. They are not specific, however, to AMI and may be detected in other acute or chronic conditions in which some level of myocardial damage is present. Most of the cTn is bound in the contractile apparatus of the myocytes but a small proportion of the total cTn content (<10%) is also present free and unbound in the cytosol. Damage to the cardiac myocyte first leads to the release of cytoplasmic cTn followed by release of bound cTn units which contribute to the continuous rise of cTn in blood circulation. Serum concentrations of cTnI and cTnT in AMI patients show a main peak within one day after onset of symptoms and remain elevated for 4–7 days for cTnI and 10–14 days for cTnT.

Immunoassays for the detection of cTn were first developed in the late 1980s and since then a significant number of different research and commercial cTn assays have been introduced, each new generation demonstrating better diagnostic and analytical performance. All assays are of the capture type where an immobilised antibody specifically binds cTnI or cTnT present in the specimen, which could be either serum or heparin plasma. The captured cTn is then reacted with a second and, in some assays, a third antibody that is coupled to an indicator molecule. The assays differ from each other in the type of antigen used for calibration, the type of antibody and epitopes to which they bind, and in the type of indicator molecule used. They also use different means of detection which could be spectrophotometric, fluorescent, chemiluminescent and electrochemical. CTnI assays are marketed by different companies and use various standard materials for calibration and antibodies with different epitope specificities. As they are not standardised, different results may be obtained for the same patient sample depending on the specific cTnI assay and platform used. Therefore, their results are not interchangeable and reference values and decision limits need to be determined separately for each cTnI assay and platform. In contrast, the patent for the cTnT assay is held jointly by its developer Hugo Katus and Roche Diagnostics, the latter one being the sole manufacturer of this assay. The new highly sensitive cTnT assay has been harmonised with the earlier 4th generation cTnT assay at higher concentrations but has limited comparability at low cTnT concentrations (<100 ng/L), mainly due to difference in the detection limits of the two assays.

Earlier generations of cTn assays had low sensitivity in the early hours of the symptom onset and repeated testing was necessary to avoid inappropriate discharge of patients with NSTEMI. Also, these assays were unable to achieve the recommended analytical precisions of ≤10% Coefficient of Variation.
In order to overcome these limitations, hs-cTn assays have been developed, offering an improved precision and ability to detect even smaller concentrations of cTn. It has been demonstrated that hs-cTn assays are able to detect more cases of AMI earlier in the diagnostic process. Concerns have been raised, however, that this may also lead to overdiagnosing and unnecessary invasive treatment, since chronic elevations of cTn could be detected in a range of non-ACS conditions, such as stable CAD, chronic renal impairment, chronic heart failure and severe left ventricular hypertrophy. To overcome this problem, the importance of ‘delta troponin’ has been emphasised, which reflects the specific pattern of a rise and fall in cTn concentrations when the underlying cause is AMI. ‘Delta cTn’ is the difference in cTn concentration over a fixed period of time and could be characterised as a percentage increase or decrease, a change in absolute value or rate of change. Different definitions for ‘delta cTn’ have been proposed based on the analytical precision of the assay at the 99th percentile concentration, on patient outcomes or on the biological variation in a healthy reference population. Also, the definition of a ‘significant delta’ will depend on whether sensitivity or specificity of the test is more important in the specific decision-making context. Thus, a ‘minimum delta’ will be required if the focus is on ruling out AMI (in order to reduce the number of false negatives), while an ‘optimal delta’ will be needed to rule it in. Another important factor to be taken into account when using delta cTn is the timing of the symptom onset. Calculating delta cTn is useful in patients presenting early after the onset of symptoms as it indicates an evolving AMI but is of little value in late presenters to the ED in whom the cTn values are already markedly elevated, are near their peak or have reached a plateau and may not show significant changes. It is possible that delta cTn is also present in conditions other than ACS but at present no reliable data on this issue is available.

**Definition of AMI and reference standard**

The current universal definition for AMI postulates that this term should be used when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischemia. AMI due to a primary coronary event, such as plaque erosion or rupture, is defined as a rise and/or fall of cardiac biomarker values (preferably cardiac troponin) with at least one value above the 99th percentile of the upper reference limit (URL) and with at least one of the following:

- Symptoms of ischemia;
- New or presumed new significant ST-segment–T wave changes or new left bundle branch block (LBBB) in the electrocardiogram (ECG);
- Development of pathological Q waves in the ECG;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; and,
- Identification of an intracoronary thrombus by angiography or autopsy.

The universal definition of AMI does not specify, however, which generation of cTn assay should be used to determine the presence or absence of AMI.

The main issue in evaluating the diagnostic accuracy of hs-cTn assays is the fact that they are more sensitive than the standard cTn assays which are the current gold standard for the diagnosis of AMI. Since hs-cTn assays are usually evaluated with regards to their ability to identify evolving AMI in the first few hours of the patient’s presentation to the ED, diagnostic accuracy studies usually take advantage of the specific release kinetics that cTn shows in AMI and, more specifically, the progressive increase in cTn concentration over the first 24 hours from the symptom onset. Thus, to confirm or rule out the diagnosis of AMI, serial measurements over six or more hours from presentation are performed to ascertain that the magnitude and pattern of elevation is consistent with the one observed in AMI. Used in a serial manner, standard cTn assays are considered accurate enough to verify the diagnosis of AMI and, thus, to serve as part of the reference standard in diagnostic accuracy evaluations of hs-cTn. A recent
study, conducted by Aldous and colleagues, has demonstrated, however, that using a standard cTn assay, such as the fourth generation cTnT, as a reference standard may lead to an increased false positive rate and may, therefore, underestimate the specificity of the hs-cTn assay. They showed that the specificity of hs-cTnT improved considerably when serial measurements with the same assay were used as a reference standard; and that using hs-cTnT in a serial manner resulted in much higher rate of AMI (38.9%) than when the diagnosis was established using serial fourth generation cTnT (27.1%).\textsuperscript{41,42} This suggests that test accuracy evaluations may produce different accuracy estimates for hs-cTnT depending on the performance of the specific cTn assay used as part of the reference standard.

**Rational**

The improved diagnostic and analytical performance of hs-cTn assays makes it possible to detect an evolving AMI early in the diagnostic process, thus providing an opportunity to speed up the triage of patients presenting to the ED with symptoms suggestive of ACS. Since approximately 80% of these patients will not have a final diagnosis of ACS, a highly sensitive test may help emergency physicians to rule out AMI in the first couple of hours after presentation and to look for alternative diagnosis or discharge the patient, if appropriate. On the other hand, hs-cTn assays will identify more patients with elevated cTn, which are due to conditions other than AMI. Although elevated cTn levels indicate increased long-term risk of death and cardiac events, this may not be of immediate concern to the emergency physicians, whose focus is on the short-term management of the patient. The calculation of delta cTn could help differentiate between chronic and acute cTn elevations but there is no consensus as to the definition of a significant delta change and the time interval for serial testing.\textsuperscript{33,36,43} The situation is further complicated by the number of different cTn assays in use, which are not standardised and their results are not comparable.\textsuperscript{44,45}

Given the significant level of uncertainty with regards to the use of hs-cTn in the triage of ED patients suspected of ACS, we decided to conduct a systematic review of the diagnostic accuracy of hs-cTn. Given the significant variability of cTnI assays, we decided to focus on hs-cTnT which is manufactured only by Roche\textsuperscript{®} Diagnostics and is the most common cTn assay used locally.

**Objectives**

**Primary objectives**

To determine the diagnostic accuracy of hs-cTnT (Roche\textsuperscript{®} Diagnostics) for the diagnosis of AMI in patients presenting to the ED with symptoms suggestive of ACS. The accuracy of a single troponin test on presentation, changes in troponin concentration over time (delta troponin) and a combination of them will be reviewed.

**Investigation of sources of heterogeneity**

Provided sufficient details are reported in the primary studies, we will study the following sources of heterogeneity:

- Different cut-off points. Time of sampling with regards to the time of symptom onset and time of admission.
- Sample type (serum or heparin plasma).\textsuperscript{46}
- Patients’ demographics such as age, gender, ethnicity.\textsuperscript{47,48}
- Clinical subgroups, such as patients with renal dysfunction, diabetes and CAD.\textsuperscript{49}
- Variations in the reference standard.
Methodological quality of the included studies.

Methods

Criteria for considering studies for this review

Type of studies
Eligible will be studies that assess the diagnostic accuracy of hs-cTnT assay, with either prospective or retrospective data collection. The hs-cTnT could be assessed alone or in combination with other tests.

Participants
Studies have to include patients presenting to the ED with symptoms suggestive of ACS.

Index test
The test under evaluation was a commercially available hs-cTnT assay (Roche® Diagnostics) performed in the first few hours from admission to the ED. The focus of the review is on the evaluation of the diagnostic accuracy of hs-cTnT as an early triage test that could inform clinical decision making in ED setting. The diagnostic accuracy of both a single troponin test on presentation, delta troponin and a combination of them will be reviewed. In the current NHS practice, the results from the troponin test are available within 2 hours of presentation and, therefore, studies in which the sample was drawn after this time may have limited applicability. Nevertheless, such studies will be included in the review and dealt with in the methodological quality assessment and analysis.

Target condition
AMI as defined by the joint ESC/ACCF/AHA/WHF task force for the universal definition of myocardial Infarction.25

Reference standard
We will include studies using any reference standard that is consistent with the universal definition of AMI.25 Differences in the reference standard will be used in the subgroup analysis.

Search methods for identification of studies

Electronic searches
- We will search Ovid MEDLINE and MEDLINE in-process (Appendix 1), Ovid EMBASE, Science Citation Index (SCI), Medion database, Database of Abstracts of Reviews of Effects (DARE), Cochrane Database of Systematic Reviews (CDSR), Research Portfolio Online Reporting Tools (RePORT, formally CRISP) and International Network of Agencies for Health Technology Assessment (INAHTA).
- The hs-cTnT assay was officially launched in February 2009.50 In order to capture any studies using pre-commercial versions of the test, we will search the period between 2006 and present.
- We will use the articles identified from the electronic sources to perform a cited reference search and related articles search to identify additional studies.
Searching other resources

- Specialists in the relevant fields will be consulted and, if recommended, additional sources and publications will be considered.
- The bibliographies of the identified studies will be hand searched for additional publications.

Data collection and analysis

Selection of studies
The first review author will review all titles and abstracts to identify relevant articles that will be retrieved for full text review. Full text articles will be reviewed independently by two researchers and disagreements will be settled through discussion. If necessary, the study authors will be contacted and asked to provide additional information to resolve the uncertainty. Since the review authors who will conduct the selection process are not familiar with this field, they will not be blinded to study authors, institution, and study results during the selection process.

Data extraction and management
Two review authors will independently extract data using a standardised data collection form developed by taking into account the Standards for Reporting of Diagnostic Accuracy (STARD) checklist. The form will be piloted in a small subset of studies and, if necessary, changes will be made. The absolute numbers of observations of true positives, false positives, true negatives, and false negatives must be specified or must be derivable from the available data. Study authors will be contacted to clarify any uncertainties and to obtain the complete data set. If the absolute number of observations cannot be obtained, despite contacting the authors, then the study will be excluded. Articles reanalysing or republishing data from a study population that has already been included in the review will be excluded.

Data abstracted by the two authors will be compared and any disagreements will be recorded and resolved through discussion. The raw test accuracy data will be used to construct 2 x 2 contingency tables and to calculate sensitivity and specificity for each subset of data using RevMan 5.1.6 or similar software.

The following additional data will be abstracted:
1. General information: author, title, journal (including volume and pages), year, institution, country and language.
2. Study design and population sampling (inclusion and exclusion criteria; clinical setting; referral and selection process; number of participants eligible, enrolled, completed index test and reference standard, number and reasons for drop out).
3. Participants’ characteristics (mean age; percentage of male patients enrolled; mean time from symptom onset to presentation; risk factors; risk score; clinical findings and other relevant information).
4. Performance of the index test.
6. QUADAS 2 items.

Assessment of methodological quality
The QUADAS 2 checklist will be adapted to the needs of the current review and two review authors will independently assess the methodological quality of all included studies with disagreements being resolved through discussion or arbitration. The results will be used to decide whether or not to perform a
meta-analysis of the included studies and, in a sensitivity analysis, to evaluate the impact of each methodological quality item on the final results.\textsuperscript{52}

**Statistical analysis and data synthesis**

We will construct 2x2 tables and calculate sensitivity and specificity, with 95% CIs, for each subset of data within the individual studies (cTn measurements at different time points and delta values). We will create coupled forest plots to evaluate the variation in sensitivity and specificity across all the studies included in a particular data set. We will plot the results in a receiver operator characteristic (ROC) space. Sensitivity will be used to define the y-axis, 1 - specificity will define the x-axis and each point on the plot will represent the proportion of true positives against the proportion of false positives for each subset of data.

If appropriate, we will conduct a meta-analysis of the pairs of sensitivity and specificity to create a summary ROC curve using the random-effects hierarchical SROC model of Rutter and Gatsonis.\textsuperscript{53} Covariates will be added as a source of heterogeneity.

**Sensitivity analysis**

In order to assess whether the methodological quality of the included studies influences the results, sensitivity analysis will be carried out using each individual quality item as a covariate in a bivariate regression model.

**Assessment of reporting bias**

We will contact the authors of those studies that were excluded because they did not report specific outcome measures of interest to inquire whether these data are available but had not been published. If data are available and meet the inclusion criteria, we will include them in our analysis. We will contact the authors of relevant papers to determine if they are aware of existing unpublished data that had not been included in the review. If appropriate, we will assess publication bias qualitatively.\textsuperscript{54}

**Appendix 1**

Proposed search strategy

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>

Search Strategy:

```
1 exp Chest Pain/ (50095)
2 (chest adj2 pain*).ti,ab. (22022)
3 chest discomfort.ti,ab. (834)
4 exp Acute Coronary Syndrome/ (5639)
5 acute coronary syndrome*.ti,ab. (15245)
6 ACS.ti,ab. (9120)
7 exp Coronary Artery Disease/ (32669)
8 coronary artery disease.ti,ab. (53984)
9 exp Angina, Unstable/ (9652)
```
10 unstable angina.ti,ab. (10337)
11 Myocardial Infarction/di [Diagnosis] (21760)
12 myocardial infarction.ti,ab. (120606)
13 heart attack.ti,ab. (2846)
14 or/1-13 (242235)
15 exp Troponin/du [Diagnostic Use] (104)
16 troponin*.ti,ab. (13969)
17 hs-ctn*.ti,ab. (93)
18 or/15-17 (13989)
19 exp Emergency Service, Hospital/ (43744)
20 (emergency adj (room* or department*)).ti,ab. (50646)
21 (ER or ED).ti,ab. (76453)
22 (presenting or presented).ti,ab. (818754)
23 or/19-22 (938321)
24 14 and 18 and 23 (1180)
25 limit 24 to yr="2006 -Current" (741)

Appendix 2
Data extraction form

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<th>Comments</th>
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<td>Author</td>
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<tr>
<td>Language</td>
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<tr>
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<td>Study design</td>
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<td>Exclusion criteria</td>
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<tr>
<td>Description of clinical setting and referral and selection process</td>
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<td>Number of participants:</td>
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<tr>
<td>completed index test</td>
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<tr>
<td>completed reference standard</td>
<td></td>
<td></td>
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<tr>
<td>excluded/dropped out</td>
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### Participants’ characteristics:

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<th>Description</th>
<th>Performance</th>
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<tr>
<td>Mean age</td>
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<tr>
<td>Sex (n/% of male patients enrolled)</td>
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<tr>
<td>Mean time from symptom onset to presentation</td>
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<tr>
<td>Risk score</td>
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<tr>
<td>Other relevant information</td>
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<tr>
<td>Index test</td>
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<tr>
<td>Reference standard</td>
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### Appendix 3

**Methodological quality assessment (QUADAS 2) checklist**

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<tr>
<th>Domain/Question</th>
<th>Qualifier</th>
<th>Comment</th>
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<tr>
<td>1. Patient selection</td>
<td>Yes/No/Unclear</td>
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<tr>
<td>1.1. Risk of bias: Could the selection of patients have introduced bias?</td>
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<tr>
<td>Was a consecutive or random sample of patients enrolled?</td>
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<td>Was a case-controlled design avoided?</td>
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<tr>
<td>Did the study avoid inappropriate exclusions?</td>
<td></td>
<td>Inappropriate exclusions are defined as any exclusions from the target population as defined below.</td>
</tr>
<tr>
<td>1.2. Applicability:</td>
<td></td>
<td>Relevant participants are patients presenting to the ED with symptoms suggestive of ACS.</td>
</tr>
<tr>
<td>Are there concerns that the included patients and setting do not match the review question?</td>
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<tr>
<td>2. Index test</td>
<td></td>
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<tr>
<td>2.1. Risk of bias: Could the conduct or interpretation of the index test have introduced bias?</td>
<td></td>
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<tr>
<td>If a threshold was used, was it pre-specified?</td>
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<tr>
<td>2.2. Applicability:</td>
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<tr>
<td>Are there concerns that the index test, its conduct, or its interpretation differ from the review question?</td>
<td></td>
<td>If the first sample is drawn &gt;2 hrs after presentation, the study will not reflect the</td>
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<tr>
<td>3. Reference standard</td>
<td>current UK practice.</td>
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<tr>
<td>3.1. Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?</td>
<td>For the purpose of this review, ‘acceptable reference standard’ will be defined as a combination of a validated troponin test and objective evidence of ischemia as recommended in the universal definition of AMI.</td>
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<tr>
<td>Is the reference standard likely to correctly classify the target condition?</td>
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<tr>
<td>Were the reference standard results interpreted without knowledge of the results of the index test?</td>
<td>Incorporation bias may be present if the results from the hs-cTnT test were included in the final diagnosis.</td>
<td></td>
</tr>
<tr>
<td>3.2. Applicability:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are there concerns that the target condition as defined by the reference standard does not match the question?</td>
<td>The definition of AMI should be consistent with the universal definition of AMI.</td>
<td></td>
</tr>
<tr>
<td>4. Flow and timing</td>
<td></td>
<td></td>
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<tr>
<td>4.1. Risk of bias:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was there an appropriate interval between the index test and reference standard?</td>
<td>Index test and reference standard should be performed within seven days from each other and patients should be followed up for at least 30 days to rule out the diagnosis of ACS.</td>
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<tr>
<td>Did all patients receive the same reference standard?</td>
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<tr>
<td>Were all patients included in the analysis?</td>
<td>If serial testing was performed, some patients might not have been included due to incomplete data sets</td>
<td></td>
</tr>
</tbody>
</table>

**Bibliography**


