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Two hour algorithm for triage towards rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac Troponin T

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ABSTRACT

Background: High-sensitivity cardiac troponin (hs-cTn) may allow an earlier diagnosis of acute myocardial infarction (AMI).

Methods: We prospectively enrolled 1148 (derivation cohort) and 517 (external validation cohort) unselected patients presenting with suspected AMI to the emergency department. Final diagnosis was adjudicated by two independent cardiologists. Hs-cTnT was measured at presentation and after two hours. A diagnostic algorithm incorporating hs-cTnT values at presentation and absolute changes within the first two hours was derived.

Results: AMI was the final diagnosis in 16% of patients in the derivation and 9.1% in the validation cohort. The 2h algorithm developed in the derivation cohort classified 60% of patients as “rule-out”, 16% as “rule-in” and 24% in the “observational- zone”. Resulting sensitivity and negative predictive value (NPV) were 99.5% and 99.9% for rule-out, and specificity and positive predictive value (PPV) 96% and 78% for rule-in. Applying the 2h triage algorithm in the external validation cohort, 78% of patients could be classified as “rule-out”, 8% as “rule-in” and 14% in the “observational-zone”. Resulting sensitivity and NPV were 96% and 99.5% for rule-out and specificity and PPV 99% and 85% for rule-in. Cumulative 30-day survival rates were 100%, 98.9% and 95.2% ($p<0.001$) and 100%,100% and 95% ($p<0.001$) in patients classified as “rule-out”, “observational-zone” and “rule-in” in the two cohorts respectively.

Conclusions: A simple algorithm incorporating hs-cTnT baseline values and absolute changes over two hours allowed a triage towards safe rule-out, or accurate rule-in, of AMI in the vast majority of patients with only 20% requiring more prolonged monitoring and serial blood sampling.

Key Words: Acute myocardial infarction – high-sensitive Troponin – Diagnostic algorithm

Introduction

Patients with symptoms suggestive of acute myocardial infarction account for about 10% of all emergency department consultations.[1] Electrocardiography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstones and complement clinical assessment.[2-5] The major limitation of former generation cTn assays is a delayed increase of circulating levels mandating serial sampling for 6-12 hours, which can result in both delayed diagnosis of acute myocardial infarction ("rule-in") as well as delayed exclusion of acute myocardial infarction ("rule-out").[2, 4-7]

Sensitive and high-sensitivity cardiac troponin (hs-cTn) assays have enabled measurement of lower cTn concentrations.[8] These assays have been shown to improve the diagnostic accuracy for acute myocardial infarction at presentation, and it has been suggested that more rapid rule-in and rule-out of acute myocardial infarction might be feasible with those tests.[9-11] On the other hand, improvement in assay sensitivity significantly increases the proportion of positive hs-cTn tests due to various acute and chronic conditions with cardiac involvement other than acute myocardial infarction.[12-15] As a consequence, the positive predictive value (PPV) of an elevated hs-cTn level for acute myocardial infarction has decreased,[9, 10, 16, 17] causing confusion amongst physicians treating patients with possible acute myocardial infarction.[18]

We recently developed a simple algorithm using hs-cTnT levels that allowed a triage towards a safe rule-out as well as an accurate rule-in of acute myocardial infarction within one hour in 77% of unselected acute chest pain patients.[19] Some experts however were concerned that the one-hour approach may not be safe when used in different ED populations and others have questioned the practicability of a 1-hour algorithm. Furthermore, external validation of the algorithm is pending.[20]

The aim of this study therefore was to develop an algorithm for rapid rule-in and rule-out of acute myocardial infarction from a European multicentre cohort using hs-cTnT levels and absolute changes within the first two hours, and to externally validate the derived algorithm in a non-European cohort.

Methods

Study design and population derivation cohort

Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) is an ongoing prospective international multicenter study designed and coordinated by the University Hospital Basel (ClinicalTrials.gov registry, number NCT00470587).[9, 21] From April 2006 to August 2011, a total of 2195 unselected patients presenting to the emergency department with symptoms suggestive of acute myocardial infarction such as acute chest pain and angina pectoris with an onset or peak within the last 12 hours were recruited. Patients with terminal kidney failure requiring dialysis were excluded. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Written informed consent was obtained from all patients.

Patients with ST-segment elevation myocardial infarction (n=83) and missing samples (n=964) were excluded from this analysis, which left 1148 patients available. Baseline characteristics of patients with missing samples are shown in supplemental Table 1. These patients did not differ significantly from the study population with the exception of a slightly higher rate of acute myocardial infarction (20% vs. 16%, p=0.03). The most common reasons for missing samples after 2 hours were early transfer to the cath lab or CCU and diagnostic procedures around the 2h window that precluded blood draw at 2h, but not the draw of earlier or future follow-up samples.

Study design and population validation cohort

From November 2008 to February 2011, a total of 978 unselected patients presenting to the emergency department of the Royal Brisbane and Women's Hospital with symptoms of possible acute myocardial infarction were recruited. Criteria for enrollment included age ≥18 years of age, with at least 5 min of symptoms where the attending physician planned

to perform serial cTn tests. The American Heart Association case definitions for possible cardiac symptoms were used (i.e., acute chest, epigastric, neck, jaw, or arm pain; or discomfort or pressure without an apparent noncardiac source).[22] Patients were excluded for any of the following: a clear cause other than acute coronary syndrome for the symptoms (e.g., examination findings of pneumonia), inability to provide informed consent, staff considered recruitment to be inappropriate (e.g., receiving palliative treatment), transfer from another hospital, pregnancy, previous enrollment, or inability to be contacted after discharge. Perceived high risk was not used as an exclusion criterion. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Written informed consent was obtained from all patients. Patients with ST-segment elevation myocardial infarction (n=23), patients who stated that their first episode of pain commenced >12 hours before presentation (n=279) and patients with missing samples (n=159) were excluded from this analysis, which left 517 patients available. Baseline characteristics of patients with onset of symptoms >12 hours and missing samples are shown in supplemental Table 2. Those patients did not differ significantly from the study population (all p-values >0.05).

Routine clinical assessment

Patients were managed according to local hospital protocols, including clinical history, physical examination, 12-lead ECG, continuous ECG-monitoring, pulse oximetry, standard blood tests and chest radiography. Clinical blood draws for local cTn measurement were performed at presentation, and then 6-12 h afterwards. Management of patients was at the discretion of the attending physician.

Investigational hs-cTnT analysis

Blood samples for determination of hs-cTnT (Roche Diagnostics) were collected in serum tubes at presentation to the emergency department and after 2 hours. Serial sampling was discontinued when the diagnosis of acute myocardial infarction was certain and treatment required transferring the patient to the catheter laboratory or coronary care unit. After centrifugation, samples were frozen at -80℃ until assayed in a blinded fashion using the Elecsys 2010 (Roche Diagnostics) in a dedicated core laboratory. For hs–cTnT, limit of blank (LoB) and limit of detection (LoD) have been determined to be 3 ng/l and 5 ng/l, an imprecision corresponding to 10% coefficient of variation (CV) was reported at 13 ng/L and the 99th-percentile of a healthy reference population at 14 ng/L.[8] In 876 recruited patients (Patient 1196-2072) in the APACE cohort and in all 517 patients of the Brisbane cohort, hs-cTnT measurements were performed with hs-cTnT assay lots that required revision of the calibration curve. After consultation with the manufacturer (Roche Diagnostics), these affected hs-cTnT values were re-calculated using the most appropriate methods for both settings (non-linear regression in APACE and recalibration of the measurement platform in Brisbane).[23] Glomerular filtration rate was calculated using the abbreviated Modification of Diet in Renal Disease formula.[24]

Adjudication of final diagnosis

Final diagnoses were adjudicated by independent cardiologists not directly involved in patient care. Adjudication was based on all available medical records (including patient history, physical examination, all laboratory testing including cTn levels, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, discharge summary) pertaining to the patient from the time of emergency department presentation to 60-day follow-up. While discharge diagnoses often were

correct and in agreement with the final adjudicated diagnosis, there were also cases where those diagnoses needed to be revised, most often because more information became available from medical testing during early follow-up, and more rarely, because the discharge diagnosis was not in agreement with the Universal Definition of acute myocardial infarction.

Acute myocardial infarction was defined and cTn levels interpreted as recommended in current guidelines.[2, 3, 5, 25] In brief, acute myocardial infarction was diagnosed when there was evidence of myocardial necrosis with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Patients with acute myocardial infarction were further subdivided into acute myocardial infarction type 1 (primary coronary events) and acute myocardial infarction type 2 (ischemia due to increased demand or decreased supply, for example tachyarrhythmias or hypertensive crisis).[2, 3] Details on the adjudication in the derivation and validation cohort are given in the online supplemental appendix.

Follow-up and clinical endpoints

After hospital discharge, patients were contacted after 3 and 12 months (APACE) and at 6 weeks and 12 months (Brisbane) by telephone calls or in written form. Information regarding death was furthermore obtained from the patients' hospital notes, the family physician's records and the national registry on mortality. The primary prognostic endpoint was 30 days all-cause mortality.

Algorithm development and validation

The algorithm for use of hs-cTnT was developed in the derivation cohort (APACE). The algorithm incorporates both hs-cTnT levels and absolute hs-cTnT changes within the first two hours. Selection of these two parameters was based on the previously published very

high diagnostic accuracy of the combination of absolute levels and absolute changes.[21, 26] Optimal thresholds for rule-out were selected to allow for the highest sensitivity and negative predictive value (NPV) possible. Optimal thresholds for rule-in were obtained based on a classification and regression tree (CART) analysis.[27, 28] The CART algorithm provides a sequence of partitions of a given data set aimed at optimizing the prediction of a binary outcome variable. Each subsequent partition is obtained by splitting one of the preceding partition sets (nodes) into two parts. If quantitative predictor variables are used, a pair of new nodes is obtained by splitting an existing node at a given threshold value of one of these variables. The algorithm stops if no further improvement is possible or if any further split would violate a predefined criterion (e.g., on the minimal node size).[27, 28] Nodes in the CART tree were constrained to have a minimal number of cases of 20 in parent and child nodes. In addition to baseline hs-cTnT levels and absolute hs-cTnT changes within the first hour, age (as a continuous variable), gender, ECG features (signs of ischemia or not) and time since onset of symptoms (as a continuous variable) were included in the CART model as well.

The algorithm developed in the derivation cohort was then tested for diagnostic accuracy in the validation cohort (Brisbane). Subgroup analyses focused on the performance of the algorithm in patients presenting early (<6h) after the onset of symptoms, on patients with troponin levels below the 99th percentile and on patients with a TIMI risk score ≤ 1 versus and those with a TIMI risk >1.[29]

Statistical analysis

Continuous variables are described as mean ± SD or median with interquartile range (IQR), categorical variables by numbers and percentages. Differences in baseline characteristics between patients with and without acute myocardial infarction and between

patients in the derivation and validation cohort were assessed using the Mann-Whitney U test for continuous variables and the Pearson Chi-square test for categorical variables.

Survival during 30-days of follow up according to the classification provided by the hs-cTnT algorithm was plotted in Kaplan-Meier curves and the log-rank test was used to assess differences in survival between groups. Hazard ratios (HR) and 95% confidence intervals (CI) were obtained from Cox proportional hazard models to quantify the magnitudes of group differences.

All hypothesis testing was two-tailed and p-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS for Windows 19.0 (SPSS Inc, Chicago, IL).

Results

Characteristics of patients

Baseline characteristics of the patients in the derivation cohort (n=1148) and the validation cohort (n=517) are shown in Tables 1 and 2. Acute myocardial infarction was the final diagnosis in 16.3% of patients in the derivation cohort (13.7% type 1 acute myocardial infarction, 2.6% type 2 acute myocardial infarction) and 9.1% in the validation cohort (5.6% type 1 acute myocardial infarction, 3.5% type 2 acute myocardial infarction). Of all patients, discharge within less than 24h occurred in 46% of patients in the derivation and 40% of the validation cohort. Time from onset of symptoms to first study blood draw was 3h (IQR 2-7) in the derivation cohort and 2h (IQR 1-5) in the validation cohort.

Hs-cTnT levels at presentation and after 2 hours

Levels of hs-cTnT at presentation and after 2 hours were significantly higher in patients with acute myocardial infarction compared to those without (Figure 1). Levels at presentation in men and women were similar in the overall cohorts and in the subset of patients with acute myocardial infarction (data not shown).

Of all patients, 36% (derivation cohort) and 22% (validation cohort) had hs-cTnT levels above the 99th percentile of healthy individuals at baseline or after 2 hours. Using this value as a qualitative cut-off level to diagnose acute myocardial infarction resulted in a sensitivity of 98%, a NPV of 99.6%, a specificity of 73% and a PPV of 42% in the derivation cohort and of 96%, 99.5%, 86% and 40% in the validation cohort.

Derivation of the hs-cTnT algorithm for the diagnosis of acute myocardial infarction

An algorithm incorporating hs-cTnT baseline values and absolute hs-cTnT changes within the first two hours was developed in the derivation cohort. For “rule-out” of acute

myocardial infarction, the optimal thresholds were selected to allow for the highest sensitivity and NPV possible. The rule-out criteria were defined as a maximal hs-cTnT level within the first 2 hours of <14 ng/l ***and*** an absolute change within the first two hours of <4 ng/l. For "rule-in" of acute myocardial infarction, the optimal thresholds as obtained by CART analysis were either a maximal hs-cTnT value within the first 2 hours of ≥53 ng/l ***or*** an absolute change in hs-cTnT within the first 2 hours of ≥10 ng/l. The additional variables in the CART analysis (age, gender, ischemic ECG changes and time since onset of symptoms) did not improve the accuracy and did not emerge as contributors to the final decision tree. Patients fulfilling neither of the above criteria for rule-in or for rule-out were classified in a third group called "observational zone". The diagnostic performance of the algorithm in the derivation cohort is shown in Figure 2 A. It classified 683 (60%) patients as "rule-out", 187 (16%) as "rule-in" and 278 (24%) patients in the "observational zone". Further details on the patients classified in the observational zone are given in the supplementary appendix. One patient with acute myocardial infarction was missed by the algorithm (see supplemental Table 3 for detailed patient characteristics), which resulted in a sensitivity and NPV of 99.5% and 99.9% for rule-out. Specificity and PPV for rule-in were 96% and 78%. The accuracy of the algorithm was very similar for men and women (Sensitivity/ NPV/ Specificity/ PPV 99.3 vs. 100%, 99.8 vs. 100%, 97 vs. 94% and 82 vs. 70% for male vs. female).

Validation of the hs-cTnT algorithm for the diagnosis of acute myocardial infarction

The algorithm was tested in the validation cohort. The performance in the validation cohort is depicted in Figure 2 B.

Applying the hs-cTnT algorithm to the validation cohort, 402 (78%) patients could be classified as "rule-out". Two patients with acute myocardial infarction were missed (see supplemental Table 3 for detailed patient characteristics), resulting in a sensitivity and

NPV of 96% and 99.5%, respectively. Additionally, 40 (8%) patients were classified as "rule-in", which resulted in a specificity and PPV of 99% and 85%. Taken together, the algorithm allowed a definite diagnosis after 2h in 86% of patients (either "rule-in" or "rule out"). The remaining 75 (14%) patients were classified in the "observational zone". Further details on the patients classified in the observational zone are given in the supplementary appendix. The final adjudicated diagnoses in patients falsely ruled-in for acute myocardial infarction (n=6) based on the algorithm were, acute heart failure (n=3), stable coronary artery disease (n=1) and pericarditis (n=2). The accuracy of the algorithm was similar for men and women. Sensitivity and NPV for rule out were 96% and 99.6% for male versus 96% and 99.4% for female; and specificity and PPV for rule in were 98% and 76% for male versus 100% and 100% for female. The algorithm performed equally well in patients presenting ≤ 6 hours or > 6 hours after the onset of symptoms, in those with a hs-cTnT level below the 99th percentile and in patients with a TIMI risk score ≤ 1 as well as those with a TIMI risk score >1 (see supplemental appendix for detailed results).

Prognostic performance of the hs-cTnT algorithm to predict death during follow-up

In the derivation cohort, there were twelve deaths within 30 days and forty-seven within 1 year. Survival up to 30 days of follow-up was significantly associated with the categories "rule-out", "observational zone" and "rule-in" as classified by the hs-cTnT algorithm. Cumulative 30-days survival rates in Kaplan Meier curves were 100%, 98.9% and 95.2% ($p<0.001$ by log rank test) in the respective categories (Figure 3 A). This pattern continued up to a follow-up of 1 year with cumulative survival rates of 98.8%, 93.4% and 87.3% ($p<0.001$ by log rank test).

In the validation cohort, there were two deaths within 30 days and eight within 1 year. Survival up to 30 days of follow-up was significantly associated with the categories "rule-out", "observational zone" and "rule-in" as classified by the hs-cTnT algorithm. Cumulative 30-days survival rates in Kaplan Meier curves were 100%, 100% and 95% ($p<0.001$ by log rank test, Figure 3 B). Similarly, this pattern continued up to a follow-up of

1 year with cumulative survival rates of 100%, 96% and 87.5% (p=<0.001 by log rank test).

The combined endpoint of myocardial infarction and cardiac death within 30 days was also analyzed, which occurred in 191 patients in the derivation cohort and in 48 patients in the validation cohort. This endpoint was also significantly associated with the categories “rule-out”, “observational zone” and “rule-in” as classified by the algorithm and occurred in 0.4%, 15.5% and 77.5% of patients in the respective categories of the derivation cohort (p<0.001 by log rank test) and in 0.7%, 14.7% and 85.0% of patients the validation cohort (p<0.01 by log rank test).

Impact of Type 1 vs. Type 2 Acute Myocardial Infarction

There was a trend towards higher cardiac troponin levels at presentation in patients with acute myocardial infarction type 1 compared to acute myocardial infarction type 2 (63 ng/l (IQR 20-139) vs. 40 ng/l (IQR 19-88), p=0.17 in the derivation cohort; and 65 ng/l (IQR 36-107) vs. 27 ng/l (IQR 16-103), p=0.06 in the validation cohort). Absolute changes within the first 2 hours were significantly higher in acute myocardial infarction type 1 compared to type 2 patients in the derivation cohort (19 ng/l (IQR 8-62) vs. 11 ng/l (IQR 4-25), p=0.03), but not in the validation cohort (23 ng/l (IQR 7-96) vs. 20 ng/l (IQR 1-37), p=0.15). One year cumulative survival was comparable for patients with type 1 and type 2 acute myocardial infarction (89.4% vs. 86.1%, p=0.25 in the derivation cohort; and 86.2% vs. 88.9%, p=0.82 in the validation cohort).

Discussion

Using two large, independent and well-characterized prospective cohorts of unselected patients presenting with symptoms suggestive of acute myocardial infarction, this study aimed to develop an algorithm for rapid rule-in and rule-out of acute myocardial infarction from a European cohort using hs-cTnT baseline levels and absolute changes within the first two hours and to externally validate the derived algorithm in a non-European cohort. We report three major novel findings:

First, we developed and validated a simple algorithm incorporating hs-cTnT values at baseline and after two hours as well as the absolute changes over two hours.

Using this algorithm in the validation cohort, a safe rule-out and accurate rule-in of acute myocardial infarction could be performed within 2 hours with a sensitivity and NPV of 96% and 99.5%, and a specificity of 99% and a PPV of 85%. Second, the use of this algorithm significantly shortens the time needed for rule-out and rule-in of acute myocardial infarction and may obviate the need for prolonged monitoring and serial blood sampling in 8 out of 10 unselected patients presenting with acute chest pain. Third, 30-days survival was 100% in patients ruled-out for acute myocardial infarction, which underscores the safety of early discharge for these patients with further out-patient management as deemed clinically appropriate.

Although the hs-cTn assays have been shown to increase the diagnostic accuracy at presentation,[9, 10] simple “how to use” instructions for clinical decision making are still lacking, but critically needed to take advantage of the high-sensitive assays and to shorten the time to rule-in and rule-out acute myocardial infarction.[2-5, 18] The current analyses corroborate and extend recent observations that a simple algorithm using hs-cTnT levels seems to allowed a safe rule-out as well as an accurate rule-in of acute myocardial infarction within one hour in many patients.[19] The external validation in a non-European

cohort resulting in similar accuracy and safety is reassuring and another important step towards a more widespread use in the emergency department.

With older cTn assays, the terms “troponin positive” and “troponin negative” were often used, and resulted in a high PPV and specificity, but low sensitivity. The hs-cTn assays are more sensitive and detect smaller amounts of cardiomyocyte damage more rapidly.[8] A “one size fits all” single cut-off criterion for simultaneous rule-in and rule-out would however result in either too many false positive results for patients with various acute and chronic conditions with cardiac involvement other than acute myocardial infarction, [12-15] or would not take advantage of the sensitivity of the hs-cTn assays and continue to miss small acute myocardial infarctions. Our study rather proposes the use of 2 different criteria for rule-in and rule-out resulting in 3 diagnostic groups, with only roughly 20% of the patients being classified in an “observational zone” requiring prolonged monitoring and serial blood sampling. This concept of a grey zone is well known from other fields of acute cardiac care such as the use of natriuretic peptides for the diagnosis of acute heart failure.[30]

The proportion of chest pain patients in an emergency department indeed suffering from acute myocardial infarction can vary remarkably according to the organization of the emergency medical services. Accordingly, acute myocardial infarction proportions reported from recent large chest pain cohort studies have ranged from 3% to 23%.[10, 31-35] It’s important for the generalizability of the algorithm that its performance was equally well in a higher risk cohort (derivation cohort, acute myocardial infarction rate 16%) and a lower risk cohort (validation cohort, acute myocardial infarction rate 9%).

For some but not all of the hs-cTn assays differences in the 99th percentile between women and men have been reported,[36, 37] which has recently brought up the option of possibly using a gender-specific 99th percentile. In our study, levels of hs-cTnT in patients

with symptoms suggestive of acute myocardial infarction were similar in men and women, and the accuracy of the algorithm was very similar for men and women. Furthermore, introduction of gender in CART analysis did not improve accuracy. Given that the current standard of care worldwide in the diagnosis of acute myocardial infarction is the use of uniform cut-off levels for hs-cTnT and that clinical applicability of the algorithm would be significantly lower if complexity was increased by introducing gender specific values, we elected to not use gender specific cut-off values for our algorithm.

This study complements prior work on a 2h algorithm that had used different diagnostic tests.[32,38] First, combining the TIMI scores and a point-of-care biomarker panel including standard cTn, creatine kinase MB, and myoglobin classified 10% of patients with chest pain as low-risk and suitable for early discharge.[32] Second, a combination of the TIMI score with sensitive cTn assays allowed 20% of chest pain patients to be identified as low risk.[38] The algorithm derived and externally validated in the present study clearly outperformed those previous algorithms: Rule-out of acute myocardial infarction was possible in 60% respectively 78% and rule-in of acute myocardial infarction in 16% respectively 8% of all chest pain patients within 2h in the two cohorts with very high diagnostic accuracy. Roughly 20% of the patients fulfilled neither criterion, were classified "observational zone" and would require more than 2h for assessment, and many of them probably will need additional diagnostic testing such as coronary angiography, exercise stress test or echocardiography. Compared to the 3-6h interval for a cTn follow-up sample recommended in current guidelines,[2-4] the shortening to a 2h follow-up period in the vast majority of patients represent a major advance in clinical care. It also complements our previously published 1h algorithm[19] as well as the recently presented but not yet published TRAPID-AMI study, both of which used a rapid triage algorithm concept similar to the one presented here. This study and the two other 1h-algorithm studies provide data driven

results from real–world patients that to some extent may contradict theoretical considerations regarding the precision of the hs-cTnT assay and suggest that it allows the reliable detection of even small absolute changes.[5,26]

For the derivation cohort, specificity and positive predictive value were improved from 73% and 42% when only using absolute hs-cTnT levels to 96% and 78% respectively using the 2h hs-cTnT algorithm. Similarly, in the validation cohort, specificity and positive predictive value improved from 86% and 40% with absolute hs-cTnT levels to 99% and 85% respectively when using the 2h hs-cTnT algorithm. It is important to highlight that acute myocardial infarction remains a clinical diagnosis and that in clinical practice hs-cTnT levels are interpreted in conjunction with all other available information including 12-lead ECG, patient history and examination, and other diagnostic investigations. When felt appropriate, those parameters should over-rule the recommendation given by the algorithm. The accuracy of the algorithm in clinical practice, when used in conjunction with the above information and supported ideally by an automated electronic laboratory reporting system, will likely be even higher than reported in this hs-cTnT only analysis and will in especial further increase the PPV.

Potential limitations of the current study merit consideration. First, our study was conducted in emergency department patients with symptoms suggestive of acute myocardial infarction and the diagnostic accuracy of the algorithm will only be upheld in cohorts with a similar pretest probability. Second, the data presented was obtained from prospective observational studies. Studies applying the diagnostic algorithm prospectively for clinical decision-making are warranted. Third, different troponin assays were used clinically in the participating centres and might have influenced the clinical management of the patients. Fourth, we used one specific hs-cTn assay for derivation and validation of the algorithm (hs-cTnT). Different sensitive and high sensitive assays vary considerably with

regards to the amount of patients detected with elevated troponin levels. We hypothesize that similar algorithms can be developed for other hs-cTn assays,[39] but this requires similar derivation and validation in chest pain patient cohorts first.

In conclusion, using a simple algorithm incorporating hs-cTnT values at presentation and after two hours as well as absolute changes within the first two hours, a safe rule-out or accurate rule-in of acute myocardial infarction could be performed within two hours in the vast majority of patients presenting with chest pain. The use of this algorithm seems to be safe, significantly shortens the time needed for rule-out and rule-in of acute myocardial infarction and leaves only 20% of chest pain patients that require more prolonged monitoring and serial blood sampling.

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Conflict of interests

The authors designed the study, gathered and analyzed the data, vouch for the data and analysis, wrote the paper, and decided to publish. Drs. Reichlin and Mueller had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and approved the manuscript. The sponsors had no role in designing or conducting the study and no role in gathering or analyzing the data or writing the manuscript. The manuscript and its contents have not been published previously and are not being considered for publications elsewhere in whole or in part in any language, including publicly accessible web sites or e-print servers.

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All other authors declare that they have no conflict of interest with this study. The hs-cTnT assay was donated by Roche, who had no role in the design of the study, the analysis of the data, the preparation of the manuscript, or the decision to submit the manuscript for publication.

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Figure Legends

Figure 1 **Levels of hs-cTnT absolute changes within the first 2 hours**

Hs-cTnT levels at presentation to the emergency department and after 2 hours and absolute changes in patients with and without acute myocardial infarction (AMI) in the derivation (A) and validation cohort (B). Boxes represent IQR's, while whiskers display ranges (without outliers further than 1.5 IQR's from the respective end of the box).

Figure 2 **Performance of the hs-cTnT algorithm for diagnosis of acute myocardial infarction in the derivation and validation cohort**

Performance of the algorithm classifying patients into "rule-out", "observational zone" and "rule-in". Results are displayed for the derivation cohort (Panel A) and for the validation cohort (Panel B). hs-cTnT values are presented in ng/l. 0h/2h = hs-cTnT at presentation and after 2 hours. Delta 2h = absolute change of hs-cTnT within the first two hours; NPV = negative predictive value; PPV = positive predictive value.

Figure 3 **Kaplan Meier curves for the cumulative survival according to classification provided by the hs-cTnT algorithm**

Kaplan Meier curves displaying survival during 30-days of follow-up in the derivation (Panel A) and validation (Panel B) cohort according to the classification into "rule-out", "observational zone" and "rule-in" provided by the hs-cTnT one hour algorithm. Differences in survival were assessed using the log-rank test.

Table 1 **Baseline Characteristics of the Patients in the Derivation Cohort**

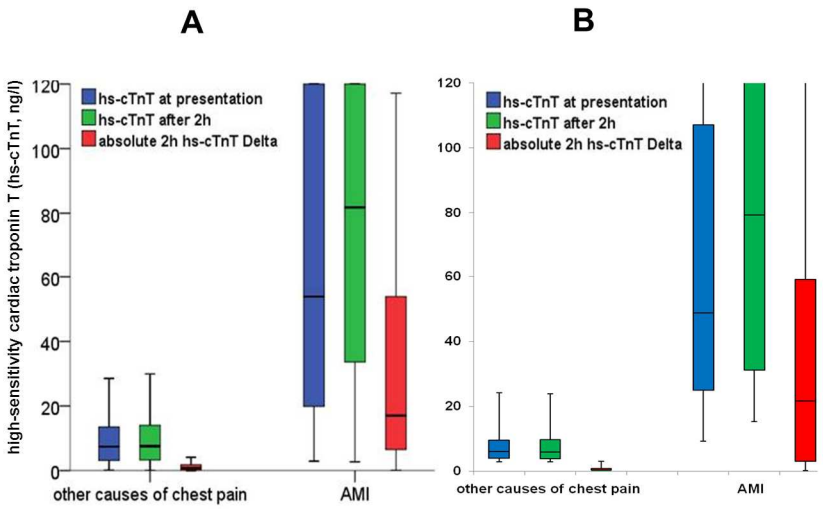
	All patients (n=1148)	Acute MI (n=187)	Others (n=961)	P Value
Age – yr	62 (51 – 74)	71 (59 – 79)	61 (50 – 73)	< 0.001
Male gender – no. (%)	795 (69)	139 (74)	656 (68)	0.58
Risk factors – no. (%)				
Hypertension	741 (65)	143 (77)	598 (62)	<0.001
Hypercholesterolemia	538(47)	109 (58)	429 (45)	0.001
Diabetes	215 (19)	48 (26)	167 (17)	0.008
Current smoking	292 (25)	46 (25)	246 (26)	0.80
History of smoking	427 (37)	79 (42)	348 (36)	0.11
History – no. (%)				
Coronary artery disease	412 (36)	91 (49)	321 (33)	<0.001
Previous myocardial infarction	280 (24)	60 (32)	220 (23)	0.007
Previous revascularization	325 (28)	61 (33)	264 (28)	0.15
Peripheral artery disease	78 (7)	23 (12)	55 (6)	0.001
Previous stroke	57 (5)	18 (10)	39 (4)	0.001
Creatinine clearance - (ml/min/m2)	91 (73 – 108)	83 (62 – 104)	92 (75 – 109)	< 0.001
ECG findings – no. (%)†				
Left bundle branch block	38 (2)	16 (9)	22 (2)	<0.001
ST-segment elevation	20 (2)	1 (1)	19 (2)	0.17
ST-segment depression	99 (9)	46 (25)	53 (6)	< 0.001
T-wave inversion	88 (8)	19 (10)	69 (7)	0.16
No significant ECG abnormalities	903 (79)	105 (56)	798 (83)	< 0.001

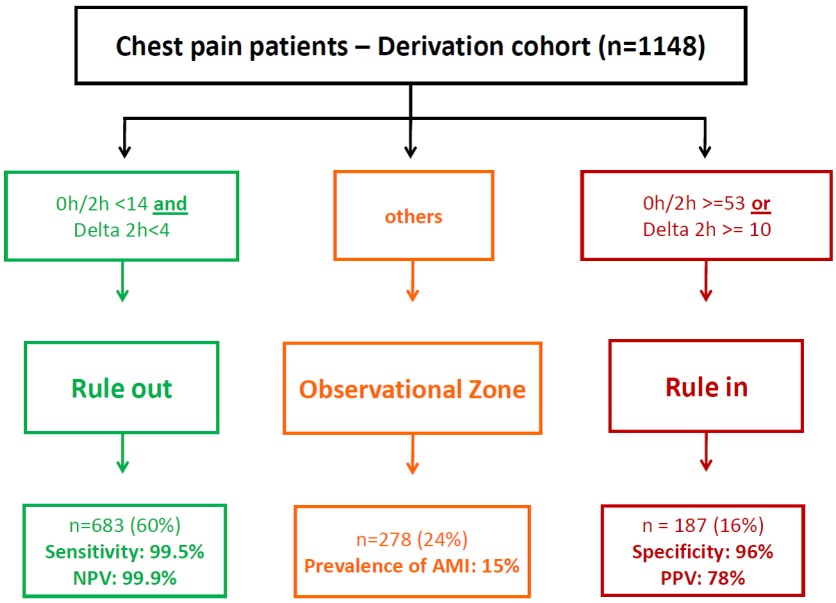
† ECG denotes electrocardiogram; numbers are presented as median (IQR) or numbers (%)

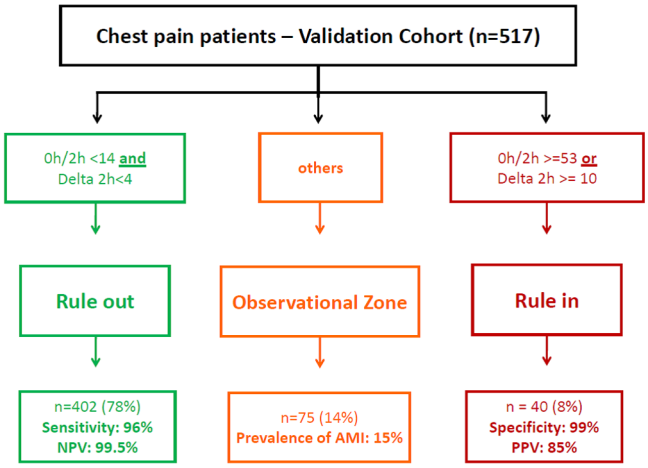
Table 2 **Baseline Characteristics of Patients in the Validation Cohort**

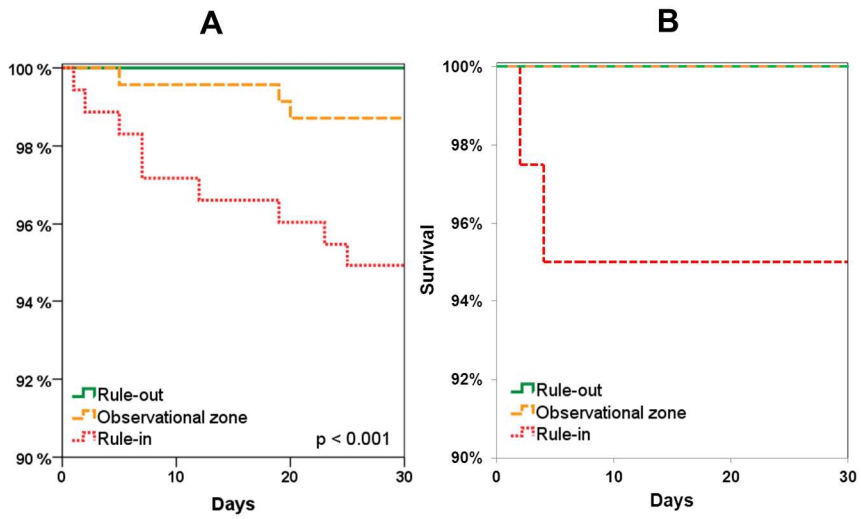
	All patients (n=517)	Acute MI (n=47)	Others (n=470)	P Value
Age – yr	54 (45 – 65)	69 (59-82)	53 (44 – 64)	< 0.001
Male gender – no. (%)	316 (61)	25 (53)	291 (62)	0.24
Risk factors – no. (%)				
Hypertension	268 (52)	32 (68)	236 (50)	0.02
Hypercholesterolemia	269 (52)	32 (68)	237 (50)	0.02
Diabetes	75 (15)	10 (21)	65 (14)	0.17
Current smoking	127 (25)	9 (19)	118 (25)	0.37
History of smoking	196 (38)	20 (43)	176 (37)	0.49
History – no. (%)				
Coronary artery disease	135 (26)	25 (53)	110 (23)	<0.001
Previous myocardial infarction	103 (20)	18 (38)	85 (18)	0.001
Previous revascularization	83 (16)	12 (26)	71 (15)	0.06
Peripheral artery disease	11 (2)	5 (11)	6 (1)	<0.001
Previous stroke	59 (11)	6 (13)	53 (11)	0.76
Creatinine clearance - (ml/min/m2)	77 (64-90)	83 (73-130)	76 (64-88)	0.002
ECG findings – no. (%)†				
ECG indicative of AMI	5 (1)	2 (4)	3 (1)	0.02
ECG indicative of ischemia not known to be old	20 (4)	10 (21)	10 (2)	<0.001
ECG indicative of ischemia known to be old	27 (5)	5 (11)	22 (5)	0.08
No significant ECG abnormalities	465 (90)	30 (64)	435 (93)	<0.001

Numbers are presented as median (IQR) or numbers (%)
† ECG denotes electrocardiogram; classification of the ECG was performed as suggested by Forest[39]









Clinical Significance

- A simple algorithm incorporating hs-cTnT baseline values and absolute changes over two hours allowed a safe rule-out or accurate rule-in of Acute Myocardial Infarction.
- Use of this algorithm may obviate the need for prolonged monitoring and serial blood sampling in 8 out of 10 unselected patients with acute chest pain and significantly shortens the time for triage in the emergency room.
- 30-days survival was 100% in patients ruled-out for AMI

Online supplemental Appendix

Supplemental Methods

Adjudication of the final diagnosis

Acute myocardial infarction was defined and cTnT levels interpreted as recommended in current guidelines.[1-4] In brief, acute myocardial infarction was diagnosed when there was evidence of myocardial necrosis with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Patients with acute myocardial infarction were further subdivided into acute myocardial infarction type 1 (primary coronary events) and acute myocardial infarction type 2 (ischemia due to increased demand or decreased supply, for example tachyarrhythmias or hypertensive crisis).[1,2]

For the derivation cohort, adjudication of final diagnoses was performed centrally in the core lab (University Hospital Basel) for all patients incorporating levels of hs-cTnT (see test characteristics above). More specifically, two independent cardiologists not directly involved in patient care reviewed all available medical records (including patient history, physical examination, results of laboratory testing including hs-cTnT levels, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, discharge summary) pertaining to the patient from the time of ED presentation to 60-day follow-up. Late samples were available for adjudication of final diagnosis in all patients of the derivation cohort. In general, serial sampling was performed until at least 6h after presentation to the ED (90% of the patients). In 10% of patients with a low pretest probability for an acute myocardial infarction, serial sampling was stopped, at the discretion of the attending physician and in accordance with current ESC guidelines

once a second sample 3 hours after presentation to the ED was again negative.[5]

In situations of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third cardiologist. While discharge diagnoses often were correct and in agreement with the final adjudicated diagnosis, there were also cases where those diagnoses needed to be revised, most often because more information became available from medical testing during early follow-up, and more rarely, because the discharge diagnosis was not in agreement with the Universal Definition of acute myocardial infarction.

The 99th percentile (14 ng/l) was used as cut-off for myocardial necrosis. Absolute cTn changes were used to determine significant changes based on the diagnostic superiority of absolute over relative changes.[6] Based on studies of the biological variation of cTn[7,8] as well as on data from previous chest pain cohort studies,[9,10] a significant absolute change was defined as a rise or fall of at least 10 ng/l within six hours, or, in an assumption of linearity, as an absolute change of 6ng/l within three hours, 4ng/l within two hours or 2ng/l within one hour. Alternative if discordant findings occurred, the longest time interval available was required to fulfill the change criteria. Predefined alternative diagnoses included “unstable angina” (UA), “Cardiac symptoms of origin other than coronary artery disease” and “non-cardiac chest pain”.

For the validation cohort, final diagnoses were adjudicated independently by one of two cardiologists. All adjudicated endpoints of acute coronary syndrome and 10% of non-acute coronary syndrome endpoints were adjudicated by both cardiologists to ensure agreement by consensus. Consensus was achieved for all endpoints. Late samples were

available for adjudication of final diagnosis in all patients of the validation cohort, which means that serial sampling was performed until at least 6h after presentation to the ED in all patients. Levels of the DxI Access Accu cTnI assay (Beckman Coulter, Chaska, Minnesota) were used for adjudication. This assay has a LoD of 0.01µg/l, a 99th percentile of 0.04µg/l and a 10% CV of 0.06µg/l. The 99th percentile (0.04µg/l) was used as cut-off for myocardial necrosis. A delta of ≥20%was used to detect a rising or falling pattern. The cTnI results from blood draws at presentation, and after 6 to 12 h (i.e. from routine care) were used for determination of necrosis.

Supplemental Results

Further characterization of patients classified into the observation zone

In the derivation cohort, 278 patients (24%) of patients did not fulfill neither the criteria for rule-out nor for rule-in and were classified in the so called “observational zone group”. Finally 41 of them (15%) were diagnosed with an acute myocardial infarction.

In the validation cohort, 75 (14%) patients did not fulfill neither the criteria for rule-out nor for rule-in and were classified in the so called “observational zone group”. Finally 11 of them (15%) were diagnosed with an acute myocardial infarction.

Diagnostic accuracy of the hs-cTnT algorithm in early vs. late presenters

In the derivation cohort, 26% of the patients presented very early (≤2h) after the onset of symptoms. In this very early presenters group, 61% were assigned to the rule-out group, 22% in the observational group and 18% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 100% and 100% and a specificity and PPV for rule-in of 96% and 83%. 55% of patients presented early (<6h after the onset of symptoms). In this early presenters group, 59% were assigned to the rule-out group, 24% in the observational group and 17% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 100% and 100% and a specificity and PPV for rule-in of 96% and 80%. In the late presenters group (>6h after presentation), 61% were assigned to the rule-out group, 24% in the observational group and 16% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 99% and 99.7% and a specificity and PPV for rule-in of 95% and 74%.

In the validation cohort, 47% of patients presented very early (≤2h) after the onset of symptoms. In this very early presenters group, 78% were assigned to the rule-out group, 14% in the observational group and 8% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 100% and 100% and a specificity and PPV for rule-in of 99.6% and 95%.

82% of patients presented early (<6h after the onset of symptoms). In this early presenters group, 77% were assigned to the rule-out group, 15% in the observational group and 8% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 97% and 99.7% and a specificity and PPV for rule-in of 99% and 85%. In the late presenters group (>6h after onset of symptoms) , 78% were assigned to the rule-out group, 14% in the observational group and 8% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 92% and 99% and a specificity and PPV for rule-in of 99% and 86%.

Diagnostic accuracy of the hs-cTnT algorithm in patients with a normal hs-cTnT level at presentation

With regards to hs-cTnT values at the time of inclusion, 67% of patients in the derivation cohort presented with hs-cTnT values below the 99th percentile. Of those, 89% were assigned to the rule-out group, 7% in the observational group and 4% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 97% and 99% and a specificity and PPV for rule-in of 99% and 64%.

In the validation cohort, 80% of patients presented with hs-cTnT values below the 99th percentile. Of those, 98% were assigned to the rule-out group, 2% in the observational group and 1% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 60% and 99.5% and a specificity and PPV for rule-in of 100% and 100%.

Diagnostic accuracy of the hs-cTnT algorithm according to the TIMI risk score

In the derivation cohort, 48% of patients had a TIMI risk score ≤1 and 52% had a TIMI risk score >1. In the group with a score ≤1, 87% were assigned to the rule-out group, 9% in the observational group and 5% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 100% and 100% and a specificity and PPV for rule-in of 97% and 40%. In the group with a score >1, 35% were assigned to the rule-out group, 38% in the observational group and 27% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 99% and 99.5% and a specificity and PPV for rule-in of 94% and 83%.

In the validation cohort, 58% of patients had a TIMI risk score ≤1 and 42% had a TIMI risk score >1. In the group with a score ≤1, 96% were assigned to the rule-out group, 2% in the observational group and 2% in the rule-in group. For the diagnosis of AMI, this resulted in a sensitivity and NPV for rule-out of 88% and 99.7% and a specificity and PPV for rule-in of 99.7% and 86%. In the group with a score >1, 52% were assigned to the rule-out group, 32% in the observational group and 15% in the rule-in group. For the diagnosis

of AMI, this resulted in a sensitivity and NPV for rule-out of 97% and 99.1% and a specificity and PPV for rule-in of 97% and 85%.

Supplemental Table 1	Baseline Characteristics of the Patients in the Derivation Cohort and Patients with Missing Samples		
	Study patients (n=1148)	**Patients with missing samples (n=964)**	**P Value**
Final diagnosis of AMI	187 (16)	193 (20)	0.03
Age – yr	62 (51 – 74)	63 (49 – 76)	0.99
Male gender – no. (%)	795 (69)	652 (68)	0.43
Risk factors – no. (%)			
Hypertension	741 (65)	612 (64)	0.61
Hypercholesterolemia	538(47)	423 (44)	0.17
Diabetes	215 (19)	162 (17)	0.28
Current smoking	292 (25)	245 (26)	0.96
History of smoking	427 (37)	330 (34)	0.18
History – no. (%)			
Coronary artery disease	412 (36)	336 (35)	0.62
Previous myocardial infarction	280 (24)	234 (24)	0.95
Previous revascularization	325 (28)	270 (28)	0.88
Peripheral artery disease	78 (7)	58 (6)	0.47
Previous stroke	57 (5)	56 (6)	0.39
Creatinine clearance - (ml/min/m2)	91 (73 – 108)	88 (70 – 106)	0.13
ECG findings – no. (%)†			
Left bundle branch block	38 (3)	26 (3)	0.41
ST-segment elevation	20 (2)	20 (2)	0.58
ST-segment depression	99 (9)	111 (12)	0.03
T-wave inversion	88 (8)	97 (10)	0.05
No significant ECG abnormalities	903 (79)	710 (74)	0.01

† ECG denotes electrocardiogram; numbers are presented as median (IQR) or numbers (%)

Supplemental Table 2 **Baseline Characteristics of Patients in the Validation Cohort and Patients with Missing Samples**

	Study patients (n=517)	Patients with missing samples (n=159)	Patients who presented >12 h after pain onset (n=279)	P Value
Final diagnosis of AMI	47 (9)	19 (12)	30 (11)	0.52
Age – yr	54 (45 – 65)	52 (44-61)	54 (42-63)	0.11
Male gender – no. (%)	316 (61)	91 (57)	166 (59)	0.67
Risk factors – no. (%)				
Hypertension	268 (52)	80 (50)	129 (46)	0.32
Hypercholesterolemia	269 (52)	76 (48)	138 (50)	0.61
Diabetes	75 (15)	16 (10)	41 (15)	0.32
Current smoking	127 (25)	41 (26)	79 (28)	0.51
History of smoking	196 (38)	46 (29)	92 (33)	0.08
History – no. (%)				
Coronary artery disease	135 (26)	40 (25)	60 (22)	0.35
Previous myocardial infarction	103 (20)	33 (21)	44 (16)	0.29
Previous revascularization	83 (16)	27 (17)	40 (14)	0.73
Peripheral artery disease	11 (2)	6 (4)	7 (3)	0.51
Previous stroke	59 (11)	13 (8)	22 (8)	0.21
Creatinine clearance - (ml/min/m2)	77 (64-90)	73 (64-86)	76 (65-87)	0.45
ECG findings – no. (%)†				
ECG indicative of AMI	5 (1)	5 (3)	5 (2)	0.15
ECG indicative of ischemia not known to be old	20 (4)	6 (4)	18 (6)	0.60
ECG indicative of ischemia known to be old	27 (5)	9 (6)	12 (4)	0.77
No significant ECG abnormalities	465 (90)	137 (86)	244 (87)	0.33

Numbers are presented as median (IQR) or numbers (%) † ECG denotes electrocardiogram; classification of the ECG was performed as suggested by Forest[11]

Suppl. Table 3	**Characteristics of patients with AMI missed by the two hour hs-cTnT algorithm**							
Patient	**Age (yr)**	**Gender**	**History of CAD**	**Previous AMI**	**Time since onset or peak of symptoms**	**ECG findings**	**cTn max value**	**Findings from coronary angiography**
# 1	71	male	yes	yes	12 h	No evidence of ischemia	22 ng/l (Roche hs-cTnT)	Severe native vessel and vein graft disease. Left circumflex and vein graft stented.
# 2	64	Male	Yes	No	8.5 h	Non-specific ST-segment changes	100 ng/l (Beckman Coulter cTnI)	Patient had echocardiogram showing no regional wall motion abnormality indicative of AMI. Endpoint listed as paroxysmal AF
#3	64	Female	Yes	No	2.5	Normal	300 ng/l (Beckman Coulter cTnI)	Moderate coronary artery disease, no intervention performed.

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