Effect of Acute Coronary Syndrome-Probability on Diagnostic and Prognostic Performance of High-Sensitivity Cardiac Troponin

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List of abbreviations:

hs-cTn	=	high-sensitivity cardiac troponin
AMI	=	acute myocardial infarction
NSTEMI	=	non-ST-segment elevation myocardial infarction
ACS	=	acute coronary syndrome
VAS	=	visual analogue scale
ECG	=	electrocardiogram
LoD	=	limit of detection
AUC	=	area under the receiver-operating-characteristics curve
NPV	=	negative predictive value
PPV	=	positive predictive value
CI	=	confidence interval
IQR	=	interquartile range

ABSTRACT

Background: There is concern that high-sensitivity cardiac troponin (hs-cTn) may have low diagnostic accuracy in patients with low acute coronary syndrome (ACS)-probability.

Methods: We prospectively stratified patients presenting with acute chest discomfort to the emergency department (ED) into three groups according to their probability for ACS as assessed by the treating ED physician using a visual analogue scale (VAS): ≤10%, 11-79%, ≥80%, reviewing all information available at 90 minutes. hs-cTnT- and hs-cTnI-concentrations were determined in a blinded fashion. Two independent cardiologists adjudicated the final diagnosis.

Results: Among 3828 patients eligible for analysis, 1189 patients had low (≤10%) probability for ACS. The incidence of non-ST-segment elevation myocardial infarction (NSTEMI) increased from 1.3% to 12.2% and 54.8% in patients with low, intermediate and high ACS-probability, respectively. The positive predictive value of hs-cTnT and hs-cTnI was low in patients with low ACS-probability and increased with the incidence of NSTEMI, while the diagnostic accuracy of hs-cTnT and hs-cTnI for NSTEMI as quantified by the area under the curve (AUC) were very high and comparable among all three strata (e.g. AUC hs-cTnI 0.96 (95%CI 0.94-0.97); 0.87 (95%CI 0.85-0.89), and 0.89 (95%CI 0.87-0.92), respectively. Findings were validated using bootstrap analysis as an alternative methodology to define ACS-probability. Similarly, higher hs-cTnT/I concentrations independently predicted all-cause mortality within two years (e.g. hs-cTnT hazard ratio 1.39, 95%CI 1.27-1.52), irrespective of ACS-probability.

Conclusions: Diagnostic and prognostic accuracy and utility of hs-cTnT and hs-cTnI remain high in patients with acute chest discomfort and low ACS-probability.

Introduction

Patients with symptoms suggestive of acute myocardial infarction (AMI) account for about 10% of all emergency department (ED) consultations (1). Rapid identification of AMI as a life-threatening disorder is important for the early initiation of appropriate, evidence-based therapy (2–4). Electrocardiography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstones and complement clinical assessment (2–4). The introduction of sensitive and high-sensitivity cardiac troponin (hs-cTn) assays enabled precise measurement of cTn blood concentrations in the low-pathological and normal range (4), and more accurate diagnosis of non-ST-segment elevation myocardial infarction (NSTEMI) (5,6).

Cardiomyocyte damage as quantified by hs-cTn blood concentrations is not unique to NSTEMI, but also associated with other cardiac disorders including heart failure, tachyarrhythmias, left ventricular hypertrophy, hypertensive crises, cardiomyopathies, valvular heart disease, myocarditis, and even stable coronary artery disease (1,2). Moreover, hs-cTn allowed the detection of cardiomyocyte damage as a probable consequence of severe primarily non-cardiac disease such as severe sepsis, septic shock, stroke, and pulmonary embolism (1,2). Concern of misinterpretation of these hs-cTn elevations as NSTEMI and patient harm associated with therapies for NSTEMI such as anticoagulation and coronary angiography applied in these non-AMI patients has led some authors to recommend withholding cTn testing in patients with low probability for acute coronary syndrome (ACS) (7,8). In contrast, practice guidelines highlight that NSTEMI frequently presents with atypical symptoms e.g. in women and elderly patients, and mandate high scrutiny for NSTEMI, which means ECG and cTn testing also in patients with atypical symptoms (2). These divergent recommendations highlight major gaps in knowledge and as a result uncertainty in clinical practice regarding cTn testing in patients with low

probability for ACS.

Our aim was to address this inconsistency by directly comparing the diagnostic and prognostic accuracy of hs-cTnT and hs-cTnI among patients with low versus intermediate or high probability for ACS in patients presenting with any kind of acute chest discomfort to the ED.

Materials and Methods

The study design and population, as well as routine clinical assessment, adjudication of final diagnosis, and follow-up and clinical endpoints are described in the supplemental data.

Quantification of ACS-probability

ACS-probability was quantified using two complimentary methods. First, probability for ACS as the cause of the presenting symptom was quantified 90 minutes after presentation by the treating ED physician using a visual analogue scale (VAS, depicted in the supplemental data). At this time point, the ED physician had completed his/her clinical assessment including patient history, chest pain characteristics, detailed physical examination including vital signs and reviewed the ECG and the first local cTn measurement. We considered the levels of ≤10% as low, 11-79% as intermediate and ≥80% as high pre-test probability for ACS (9,10). Further details regarding the assessment of the ACS-probability of the ED-physician is given within the supplemental data. Second, to generate an alternative classification, we used bootstrap analysis to produce a predetermined prevalence different from the true prevalence of NSTEMI to simulate a low ACS-probability

setting. We 10'000 times randomly sampled 100 NSTEMI cases and 1900 non-NSTEMI cases to an incidence of NSTEMI of 2%.

Measurements of hs-cTnT and hs-cTnI

Blood samples for determination of hs-cTnT and hs-cTnI were collected at presentation and serially thereafter. After centrifugation, samples were frozen at -80°C until assayed in a blinded fashion in a dedicated core laboratory. According to the manufacturer, the hs-cTnT assay (Roche Elecsys 2010, Roche Diagnostics, Rotkreuz, Switzerland) had a 99th percentile concentration of 14 ng/L with a corresponding co-efficient of variation (CV) of 10% at 13 ng/L (4). Limit of blank (LoB) and limit of detection (LoD) have been determined to be 3 ng/L and 5 ng/L. According to the manufacturer, the hs-cTnI assay (ARCHITECT STAT, Abbott Laboratories, IL) had a 99th percentile concentration of 26 ng/L with a corresponding co-efficient of variation (CV) of <5% and a limit of detection (LoD) of 2 ng/L (11–13). Further, two additional pre-commercial hs-cTnI assays and one s-cTnI assay were used. The detailed information from the manufacturer for these assays is given in the supplemental data.

The European Society of Cardiology (ESC) hs-cTn 0/1h-algorithm

The concept of the ESC 0/1h-algorithm is shown in Figure S2 and described in detail in the supplemental data.

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 were assessed using the Mann-Whitney-U-test for continuous variables and the Pearson Chi-square test for categorical variables. Receiver-operating characteristics (ROC) curves were constructed to assess the sensitivity and specificity throughout the concentrations of hs-cTnT and hs-cTnI at presentation, at 1-h and 3-hour. Furthermore, ROC curves were constructed for early absolute changes of hs-cTnT and hs-cTnI within 1-hour, alone and in combination with hs-cTn concentrations at presentation. Logistic regression was used to combine hs-cTn concentrations at presentation with early changes in hs-cTn concentrations. Specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV) for predefined cut-off-levels were calculated. We calculated the bootstrapped AUC and 95%-confidence intervals (CI) from the dataset simulating a low ACS-probability defined as an NSTEMI incidence of 2%, calculated the AUC in each set and then calculated the mean AUC. Univariate and multivariate Cox regression analysis was used to calculate hazard ratios (HR) and 95%CI to reveal associations between hs-cTnT, hs-cTnI and long-term mortality of patients. We calculated the interaction p-value for the prognostic value of hs-cTn with levels of ACS-probability for all-cause mortality using a binary logistic regression model. Kaplan Meier analysis was performed using predefined cut-off-levels of hs-cTnT and hs-cTnI. All hypothesis testing was two-tailed and p-values <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (SPSS Inc, Chicago, IL) and the R statistical package (R Foundation for Statistical Computing, Vienna, Austria).

Results

From April 2006 to August 2015, a total of 4323 patients were enrolled, of which 3'828 patients were eligible for analysis (Figure S1). Baseline characteristics of the study population for the analyses of hs-cTnT are shown in Table 1 and for hs-cTnI in Table S1. Patients with low probability for ACS (VAS ≤10%) were significantly younger, less often had cardiovascular risk factors, established cardiovascular disease, and cardiovascular medication. Median time from chest pain onset to ED presentation was 5 hours (interquartile range 2 to 12 hours). 983 patients (25.7%) presented within two hours of chest pain onset to the ED.

ACS-probability and incidence of NSTEMI

Among 1189 patients who had low (≤10%) probability for ACS, NSTEMI was the adjudicated diagnosis in 15/1189 patients (1.3%). The incidence of NSTEMI in patients with intermediate (11-79%) and high (≥80%) probability for ACS was 12.2% (243/1986) and 54.8% (358/653), respectively. The prevalence of predefined alternative diagnoses including "unstable angina", "cardiac symptoms of origin other than coronary artery disease" and "non-cardiac chest pain" are listed in Tables 2SA for the analyses of hs-cTnT and in Table 2SB for hs-cTnI.

Concentrations of hs-cTnT and hs-cTnI at presentation and during serial sampling were significantly higher in patients with NSTEMI as compared to patients with other final diagnoses among all three ACS-probability strata (Table S3A and S3B). The proportion of patients with elevated hs-cTnT and hs-cTnI concentrations obtained from the blinded study-specific samples taken at ED presentation across the different ACS-probabilities (low/intermediate/high) were 151 (13%), 629 (32%), and 463 (71%) patients with hs-cTnT ≥14 ng/l and 72 (7%), 292 (16%) and 336 (55%) patients with hs-cTnI ≥26 ng/l.

Diagnostic accuracy of ACS-probability

The diagnostic accuracy of the ACS-probability as quantified by the ED physician for an adjudicated diagnosis of ACS was 0.86 (95%CI 0.84-0.87). Diagnostic accuracy of hs-cTn for NSTEMI were very high and comparable among all three strata of ACS-probability for hs-cTnT (low: AUC 0.94; 95%CI 0.87-1.00), intermediate: 0.89; 95%CI 0.87-0.91, high: 0.90; 95%CI 0.87-0.92) and even higher in patients with low-ACS-probability for hs-cTnI (AUC 0.96; 95%CI 0.94-0.97) as compared to patients with intermediate (AUC 0.87; 95%CI 0.85-0.89, p<0.01) and high ACS-probability (AUC 0.89: 95%CI 0.87-0.92, p<0.01, Figure 1). These findings were consistent in all predefined subgroups (data not shown), for serial measurements of hs-cTnT and hs-cTnI (Table S4), and for two additional pre-commercial hs-cTnI assays and one s-cTnI assay (Table S5).

The specificity for NSTEMI of the 99th-percentiles or 52ng/l as possible rule-in cut-off values for hs-cTnT and hs-cTnI was high. Increasing ACS-probability was associated with a decrease in specificity for both hs-cTnT and hs-cTnI (Table 2A-B). The PPV, which in contrast to specificity is depending on NSTEMI incidence, was low in patients with low ACS-probability and increased with increasing ACS-probability for both hs-cTnT and hs-cTnI. The distribution of final diagnoses in patients with hs-cTnT ≥14ng/L and patients with hs-cTnT ≥52ng/L within each of the three ACS-probability strata is shown in Figure 2.

Sensitivity and NPV were very high and comparable among all three strata using the LOD as a possible rule-out cut-off value for hs-cTnT and hs-cTnI. Using the 99th percentiles recommended by the manufacturers, sensitivity and NPV were lower with hs-cTnI as compared to that obtained for hs-cTnT.

Additional samples after 1-hour of hs-cTnT were available in 3123/3828 patients and of hs-cTnI in 2828/3548 patients. The diagnostic accuracy of absolute

hs-cTn changes for the diagnosis of NSTEMI in patients with low ACS-probability was very high after 1 hour for hs-cTnT (AUC, 0.91; 95%CI 0.79-1.00) and for hs-cTnI (AUC, 0.93; 95%CI 0.88-0.99) and was at least comparable to that in patients with intermediate or high likelihood of ACS (Table S4).

Combination of hs-cTn concentrations at presentation with early absolute changes was again very high in the low ACS-probability subgroup and comparable among all three strata: low ACS-probability hs-cTnT AUC 0.98 (95%CI 0.96-0.99), hs-cTnI AUC 0.94 (95%CI, 0.91-0.97); intermediate ACS-probability hs-cTnT AUC 0.94; (95%CI 0.93-0.95), hs-cTnI AUC 0.91 (95%CI 0.89-0.93); high ACS-probability hs-cTnT AUC 0.93 (95%CI 0.90-0.96), hs-cTnI AUC 0.89 (95%CI 0.86-0.92).

In the bootstrap model with an NSTEMI incidence of 2%, diagnostic accuracy was very high for hs-cTnT (AUC, 0.93; 95%CI 0.89-0.96) and very high for hs-cTnI (AUC, 0.92; 95%CI 0.89-0.95). PPV was low and specificity high (Table S6).

The diagnostic performance of the ESC 0/1h-algorithm among the three different ACS-probability strata using hs-cTnT and hs-cTnI overall was very good (Figure 3, Table S7). Similar results were obtained when analyzing the subgroup of patients presenting very early (within 2h from chest pain onset, Table S8A-B).

Outcome of patients according to likelihood level of ACS and cardiac troponin

Patients with a low likelihood of ACS and a hs-cTnT level < 5 ng/L or a hs-cTnI <2 ng/L (LOD) had an excellent prognosis with 0 deaths at 720 days. In patients with VAS ≤10% and hs-cTn above the 99th percentile (≥14 ng/L respectively ≥26 ng/L) 17 deaths (11.3%) and correspondingly 9 deaths (12.5%) occurred at 720 days follow-up. hs-cTnT was a strong predictor of death independent of age, gender and renal function (HR 1.39, 95%CI 1.27-1.52, p< 0.001). hs-cTnT was an even stronger predictor of all-cause mortality in patients with low ACS-probability (hazard ratio (HR)

2.16 (95%CI 1.51-3.09)) as compared to intermediate (HR 1.46 (95%CI 1.24-1.72)) and high ACS-probability (HR 1.30 (95%CI 1.12-1.50); interaction p-value <0.01). Similar findings were obtained for hs-cTnI (Figure 4).

Discussion

In this multicenter diagnostic study, we directly compared the diagnostic and prognostic accuracy of hs-cTnT and hs-cTnI among patients with low versus intermediate or high ACS-probability. We report seven major findings: First, in patients with low ACS-probability the prevalence of NSTEMI is low, resulting in a low PPV for hs-cTnT and hs-cTnI. Accordingly, the majority of patients with low ACS-probability and elevated hs-cTnT/I blood concentrations will be found to have diagnoses other than NSTEMI. However, in patients with low ACS-probability, concentrations of hs-cTnT and hs-cTnI were significantly higher in patients with NSTEMI as compared to patients with other final diagnoses. The specificity of hs-cTnT/I remained high at about 90% in patients with low ACS-probability when using the 99th percentiles and further increased when using higher cut-off values. Thus, the higher the hs-cTnT/I blood concentrations, the higher is the likelihood for NSTEMI also in patients with low ACS-probability. Second, with increasing ACS-probability NSTEMI prevalence and the PPV of hs-cTnT and hs-cTnI increased. In contrast, specificity for NSTEMI decreased with increasing ACS-probability. Third, sensitivity and NPV were very high and comparable among all three strata using the LOD as a possible rule-out cut-off value for hs-cTnT and hs-cTnI. Using the 99th percentiles currently recommended by the manufacturers (14ng/L for hs-cTnT and 26 ng/L for hs-cTnI), sensitivity and NPV were lower with hs-cTnI as compared to hs-cTnT. At first glance, this is surprising as both assays seem to have comparable diagnostic

accuracy for AMI (14), and hs-cTnI seems to have even higher analytical sensitivity as compared to hs-cTnT (11). The most likely explanation for this finding therefore is the biological non-equivalence of 26 ng/L for hs-cTnI versus 14ng/L for hs-cTnT as previously documented in two large studies (15,16). The biological equivalent hs-cTnI concentration corresponding to the 99th percentile for hs-cTnT was about half the approved 99th percentile for hs-cTnI in these studies (15,16). This major discrepancy in the currently recommended 99th-percentiles became also evident in this dataset: 32.5% of patients had a hs-cTnT concentration ≥14 ng/L, whereas only 19.7% had a hs-cTnI concentration ≥26 ng/L. Thus, the 99th percentile variability between assays is substantial (17). To overcome the poor consistency in the composition of individuals enrolled for determining the 99th percentile, future studies comparing all contemporary sensitive and hs-assays within the same reference or disease population are warranted. Defining what constitutes the appropriate reference population is a topic of debate (18). Fourth, and perhaps of most importance, the diagnostic accuracy for hs-cTnT/I to diagnose NSTEMI in patients with acute chest discomfort and low ACS likelihood was very high (AUC 0.94 and 0.96) and comparable to that in patients with intermediate or high likelihood for ACS. Fifth, diagnostic accuracies for NSTEMI provided by early absolute changes of hs-cTn within 1-hour, alone or in combination with hs-cTn concentrations at presentation, provided very high and similar diagnostic accuracy in patients with low ACS-probability as compared to the other strata. Sixth, the overall diagnostic performance of the ESC 0/1h-algorihm was very good among all ACS-probability strata, confirming the safety and efficacy of this approach also in patients with low ACS-probability. Seventh, hs-cTn was an independent predictor of all-cause mortality irrespective of the ACS-probability.

These findings corroborate and extend previous studies indicating the most appropriate clinical use of hs-cTnT and hs-cTn in patients with low ACS-probability(3,5,6,14,19–25). In addition, these findings support the diagnostic use of hs-cTnT/I as a quantitative, and not as a dichotomous variable (“troponin-negative” and “troponin-positive”) (3,5,6,14,19–21). The proportion of patients who have NSTEMI rises with increasing blood concentrations of hs-cTn as well as with increasing absolute changes within serial measurements (6,14,16,19,21–23,26). Overall, the diagnostic performance of hs-cTnT and hs-cTnI in patients with low ACS-probability supports current guideline recommendations that both the ECG and cTn must complement clinical assessment in all patients presenting with acute chest discomfort to the ED, also in patients with low ACS-probability.[2,3]

To the best of our knowledge, this is the first prospective analysis explicitly assessing the role of hs-cTn-testing in patients with quantified low ACS-probability for ACS. Previous research focused predominantly on the evaluation of elevated cTn concentrations in unselected patients (22–25). In addition, these studies were either performed retrospectively (22,23,25) or in hospitalized patients only (24). In a retrospective analysis with 4’928 unselected patients that had cTnI testing as part of their ED-evaluation for various presenting symptoms and settings only 1.8% had a final diagnosis of Type I AMI (22). Similar to our findings Yiadom et al. (22) found that patients with high initial cTn concentrations had a much higher incidence of Type I NSTEMI and that sensitivity and specificity of s-cTn increased with serial testing. In contrast, a recent retrospective analysis (23) reported low specificity for hs-cTnT to diagnose NSTEMI when analyzing ED patients irrespective of symptoms and including patients with acute heart failure and patients with documented pulmonary embolism.

Many EDs use standard operating procedures (SOPs) for the initial assessments of patients presenting with common key symptoms such as acute chest discomfort, acute abdominal pain, or acute dyspnea. Our findings have major clinical implications since they clearly support the incorporation of hs-cTn testing, besides the immediate recording of an ECG, into the SOP for the assessment of patients presenting with acute chest discomfort to the ED. In contrast, cTn testing should not be part of the initial SOP with other presenting symptoms, but rather added once the evaluating physician suspects an AMI (27). It is very important to highlight, that our findings are specific for the ED-setting for patients presenting with any kind of chest discomfort, including “pressure”, “stinging”, “burning” or “pulling” and do not apply to patients in the ED without any chest discomfort, e.g. patients with a stroke (28,29). Further, our results do not apply to other settings, in which hs-cTn may be obtained, e.g. critical ill patients in the intensive care unit (30,31).

The implementation of the kinetics of the marker could provide some reassurance regarding the widespread concern of too many false-positive results by ordering hs-cTn in patients with low likelihood of ACS. Serial measurements of hs-cTnT-levels at 1-hour were available in 969/1189 patients with low ACS-probability. 3.6% (35/969) patients showed a relevant rise of ≥ 5 ng/L in 1-hour, identifying 10/13 patients with the final diagnosis of an NSTEMI even though the ACS-probability for initially was considered to be ≤10%. Serial measurements of hs-cTn allow a better discrimination of ischemia-induced cardiac injury from cardiomyocyte damage by other cardiac disorders by a noninvasive, widely available test. Findings were confirmed by the ESC 0/1h-algorithm, which is based on the integrated use of hs-cTn concentrations at presentation and their absolute changes during serial sampling.

Previous studies deriving and validating the ESC 0/1h-algorithm allowed as a variability for the 1-hour sample a period of +/-30 minutes. This rather liberal time

frame was intentionally chosen to reflect the challenge to adhere to a stricter phlebotomy collection timing in daily clinical practice. Accordingly, most institutions applying the ESC 0/1h-algorithm clinically should be able to do the 1h-sample in the same 30-90min time window as done in the initial studies (20,32–35).

hs-cTn was an independent predictor of all-cause mortality across all ACS-probability groups. This finding is in accordance to previous observations made in studies investigating the prognostic value of cTn in various other settings(36–38) and highlights that cardiomyocyte injury irrespective of its exact pathophysiological mechanism portend a worse prognosis (39). Therefore, beyond its diagnostic utility in the detection of NSTEMI, hs-cTn measurements provide a simple method to quantify the risk of death and thereby help in the delineation of a personalized management plan.

Some limitations merit consideration when interpreting the findings of this study. First, in one of the two methods used to quantify ACS-probability, the treating physician was aware of the first clinical cTn measurement. While the ED physician was at all times blinded to the actual hs-cTnT and hs-cTnI concentrations used in this analysis, knowledge of the first clinical cTn concentrations likely introduced an unavoidable classification bias regarding the stratification of the likelihood levels for ACS. It is therefore very reassuring that our findings regarding diagnostic accuracy were confirmed using the alternative bootstrap simulation method. Furthermore, we assessed the AUC for serial measurements of both assays (hs-cTnT and hs-cTn), after 1 and 3 hours. The diagnostic accuracy of hs-cTn for patients with low likelihood of ACS increased for later sampling-points and was at least comparable to patients with intermediate or high likelihood of ACS. Second, this was a secondary analysis from a large ongoing multicenter study designed to improve the early diagnosis of AMI. As such, no specific power analysis was performed to justify the sample size for

this study. Third, even by experienced cardiologists applying current guideline recommendations (2,4,13,26), NSTEMI could not be reliably excluded in a small number of patients (2.5%, 108/4'232), although further clinical course did not reveal additional information indicating the diagnosis of AMI. Therefore, this subgroup had to be excluded from analysis. Fourth, we did not use sex-specific cut-off values in this analysis and thus cannot help broaden the scientific basis for the ongoing discussion regarding sex-specific cut-off values in the diagnosis of AMI (40). Fifth, we used the 99th percentile as recommended by the manufacturers, calculated from two separate reference populations (hs-cTnT in 533 apparently healthy European subjects; hs-cTnI in 449 apparently healthy US subjects) and not a 99th percentile derived from a similar single reference set (4,11,12). Sixth, we cannot generalize these findings to patients with terminal kidney failure requiring dialysis, since they were excluded from this study.

In conclusion, diagnostic and prognostic accuracy and utility of hs-cTnT/I remain very high in patients with acute chest discomfort and low ACS-probability for ACS when appropriately applied as a quantitative marker. The higher the hs-cTnT/I blood concentrations, the higher is the likelihood for NSTEMI also in patients with low ACS-probability. As the PPV remains low, the majority of patients with low ACS-probability and elevated hs-cTnT/I blood concentrations will be found to have diagnoses other than NSTEMI.

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Conflict of interest

The authors designed the study, gathered and analyzed the data, vouch for the data and analysis, wrote the paper, and decided to publish. Drs. Badertscher, Boeddinghaus 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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References

1. Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. Eur Heart J. 2007;28:2525–38.
2. Roffi M, Patrono C, Collet J-P, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J. 2016;37:267–315.
3. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J. 2012;33:2252–7.
4. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus H a. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin Chem. 2010;56:254–61.
5. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early Diagnosis of Myocardial Infarction with Sensitive Cardiac Troponin Assays. N Engl J Med. 2009;361:858–67.
6. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, et al. Sensitive Troponin I Assay in Early Diagnosis of Acute Myocardial Infarction. N Engl J Med. 2009;361:868–77.
7. Baxi S, Lakin J, Stapleton S, Redberg R. High-sensitivity troponin: elevated without infarction, is the horse out of the barn? Emerg Med J. 2014;31:354–5.
8. Jarolim P. High sensitivity cardiac troponin assays in the clinical laboratories. Clin. Chem. Lab. Med. 2015. page 635–52.
9. Amsterdam EA, Kirk JD, Bluemke DA, Diercks D, Farkouh ME, Garvey JL, et al. Testing of low-risk patients presenting to the emergency department with

chest pain: a scientific statement from the American Heart Association. Circulation. 2010;122:1756–76.

10. Farkouh ME, Aneja A, Reeder GS, Smars PA, Bansilal S, Lennon RJ, et al. Clinical Risk Stratification in the Emergency Department Predicts Long-Term Cardiovascular Outcomes in a Population- Based Cohort Presenting with Acute Chest Pain: Primary Results of the Olmsted County Chest Pain Study. Medicine (Baltimore). 2009;88:307–13.

11. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. Clin Chem. 2012;58:1574–81.

12. Koerbin G, Tate J, Potter JM, Cavanaugh J, Glasgow N, Hickman PE. Characterisation of a highly sensitive troponin I assay and its application to a cardio-healthy population. Clin Chem Lab Med. 2012;50:871–8.

13. Krintus M, Kozinski M, Boudry P, Capell NE, Köller U, Lackner K, et al. European multicenter analytical evaluation of the Abbott ARCHITECT STAT high sensitive troponin I immunoassay. Clin Chem Lab Med. 2014;52:1657–65.

14. Rubini Gimenez M, Twerenbold R, Reichlin T, Wildi K, Haaf P, Schaefer M, et al. Direct comparison of high-sensitivity-cardiac troponin I vs. T for the early diagnosis of acute myocardial infarction. Eur Heart J. 2014;35:2303–11.

15. Kimenai DM, Martens RJH, Kooman JP, Stehouwer CDA, Tan FES, Schaper NC, et al. Troponin I and T in relation to cardiac injury detected with electrocardiography in a population-based cohort - The Maastricht Study. Sci Rep. 2017;7:6610.

16. Wildi K, Gimenez MR, Twerenbold R, Reichlin T, Jaeger C, Heinzelmann A, et al. Misdiagnosis of Myocardial Infarction Related to Limitations of the Current Regulatory Approach to Define Clinical Decision Values for Cardiac Troponin.

Circulation. 2015;131:2032–40.

17. Apple FS, Sandoval Y, Jaffe AS, Ordonez-Llanos J. Cardiac troponin assays: Guide to understanding analytical characteristics and their impact on clinical care. Clin Chem. 2017;63:73–81.

18. Apple FS, Jaffe AS, Collinson P, Mockel M, Ordonez-Llanos J, Lindahl B, et al. IFCC educational materials on selected analytical and clinical applications of high sensitivity cardiac troponin assays. Clin Biochem. 2015;48:201–3.

19. Keller T, Zeller T, Ojeda F, Tzikas S, Lillpopp L, Sinning C, et al. Serial Changes in Highly Sensitive Troponin I Assay and Early Diagnosis of Myocardial Infarction. JAMA. 2011;306:2684.

20. Reichlin T, Schindler C, Drexler B, Twerenbold R, Reiter M, Zellweger C, et al. One-hour rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. Arch Intern Med. 2012;172:1211–8.

21. Haaf P, Drexler B, Reichlin T, Twerenbold R, Reiter M, Meissner J, et al. High Sensitivity Cardiac Troponin in the Distinction of Acute Myocardial Infarction from Acute Cardiac Non-Coronary Artery Disease. Circulation. 2012;126:31–40.

22. Yiadom MY, Jarolim P, Jenkins C, Melanson SEF, Conrad M, Kosowsky JM. Diagnostic implications of an elevated troponin in the emergency department. Dis Markers. 2015;2015.

23. Haider DG, Klemenz T, Fiedler GM, Nakas CT, Exadaktylos AK, Leichtle AB. High sensitive cardiac troponin T: Testing the test. Int J Cardiol. 2016;228:779–83.

24. Javed U, Aftab W, Ambrose JA, Wessel RJ, Mouanoutoua M, Huang G, et al. Frequency of Elevated Troponin I and Diagnosis of Acute Myocardial Infarction. Am J Cardiol. 2009;104:9–13.

25. Alcalai R, Planer D, Culhaoglu A, Osman A, Pollak A, Lotan C. Acute coronary syndrome vs nonspecific troponin elevation: clinical predictors and survival analysis. Arch Intern Med. 2007;167:276–81.
26. Reichlin T, Irfan A, Twerenbold R, Reiter M, Hochholzer W, Burkhalter H, et al. Utility of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. Circulation. 2011;124:136–45.
27. Makam AN, Nguyen OK. Use of cardiac biomarker testing in the emergency department. JAMA Intern Med. 2015;175:67–75.
28. Scheitz JF, Endres M, Mochmann HC, Audebert HJ, Nolte CH. Frequency, determinants and outcome of elevated troponin in acute ischemic stroke patients. Int J Cardiol. 2012;157:239–42.
29. Hjalmarsson C, Bokemark L, Fredriksson S, Antonsson J, Shadman A, Andersson B. Can prolonged QTc and cTNT level predict the acute and long-term prognosis of stroke? Int J Cardiol. 2012;155:414–7.
30. Lim W, Qushmaq I, Devereaux PJ, Heels-Ansdell D, Lauzier F, Ismaila AS, et al. Elevated cardiac troponin measurements in critically ill patients. Arch Intern Med. 2006;166:2446–54.
31. Guest TM, Ramanathan A V., Tuteur PG, Schechtman KB, Ladenson JH, Jaffe AS. Myocardial Injury in Critically Ill Patients. JAMA. 1995;273:1945.
32. Reichlin T, Irfan A, Twerenbold R, Reiter M, Hochholzer W, Burkhalter H, et al. Utility of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. Circulation. 2011;124:136–45.
33. Reichlin T, Cullen L, Parsonage W a, Greenslade J, Twerenbold R, Moehring B, et al. Two-hour algorithm for triage toward rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. Am J Med. 2015;128:369–79.

34. Rubini Gimenez M, Twerenbold R, Jaeger C, Schindler C, Puelacher C, Wildi K, et al. One-hour Rule-in and Rule-out of Acute Myocardial Infarction Using High-sensitivity Cardiac Troponin I. Am J Med. 2015;128:861–70.
35. Mueller C, Giannitsis E, Christ M, Ordóñez-Llanos J, DeFilippi C, McCord J, et al. Multicenter Evaluation of a 0-Hour/1-Hour Algorithm in the Diagnosis of Myocardial Infarction With High-Sensitivity Cardiac Troponin T. Ann Emerg Med. 2016;68:76–87.
36. Eggers KM, Lagerqvist B, Venge P, Wallentin L, Lindahl B. Persistent cardiac troponin I elevation in stabilized patients after an episode of acute coronary syndrome predicts long-term mortality. Circulation. 2007;116:1907–14.
37. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, et al. A Sensitive Cardiac Troponin T Assay in Stable Coronary Artery Disease. N Engl J Med. 2009;361:2538–47.
38. Twerenbold R, Wildi K, Jaeger C, Gimenez MR, Reiter M, Reichlin T, et al. Optimal Cutoff Levels of More Sensitive Cardiac Troponin Assays for the Early Diagnosis of Myocardial Infarction in Patients With Renal Dysfunction. Circulation. 2015;131:2041–50.
39. Hollander JE, Than M, Mueller C. State-of-the-Art Evaluation of Emergency Department Patients Presenting With Potential Acute Coronary Syndromes. Circulation. 2016;134:547–64.
40. Rubini Giménez M, Twerenbold R, Boeddinghaus J, Nestelberger T, Puelacher C, Hillinger P, et al. Clinical Effect of Sex-Specific Cutoff Values of High-Sensitivity Cardiac Troponin T in Suspected Myocardial Infarction. JAMA Cardiol. 2016;1:912.

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Tables

Table 1	Baseline characteristics of the patients			
	VAS ≤ 10% (n=1'189)	VAS 11-79% (n=1'986)	VAS ≥ 80 (n=653)	p-Value*
Age – y	52 [40, 64]	63 [51, 75]	70 [60, 79]	<0.001
Male gender – no. (%)	765 (64)	1324 (67)	494 (76)	0.006
Early presenters (≤ 2 hours within chest pain onset) – no. (%)	299 (25%)	541 (28%)	143 (22%)	0.021
Time from chest pain onset to first study blood draw, hours	5 (2, 14)	5 (2, 11)	5 (3, 12)	0.012
Risk factors – no. (%)				
Hypertension	502 (42)	1'318 (66)	529 (81)	<0.001
Hypercholesterolemia	370 (31)	1'041 (52)	457 (70)	<0.001
Diabetes	135 (11)	354 (18)	167 (26)	<0.001
Current smoking	349 (29)	477 (24)	141 (22)	<0.001
History of smoking	383 (32)	739 (37)	288 (44)	<0.001
History – no. (%)				
Coronary artery disease	201 (17)	736 (37)	341 (52)	<0.001
Previous MI	147 (12)	512 (26)	242 (37)	<0.001
Previous revascularization	170 (14)	605 (31)	275 (42)	<0.001
Peripheral artery disease	31 (3)	114 (6)	67 (10)	<0.001
Previous stroke	49 (4)	111 (6)	47 (7)	0.022
ECG findings – no. (%)				
Left bundle branch block	24 (2)	72 (4)	39 (6)	0.001
ST-segment elevation	17 (1)	44 (2)	19 (3)	0.073
ST-segment depression	38 (3)	136 (7)	138 (21)	<0.001
T-wave inversion	53 (4)	148 (7)	85 (13)	<0.001
No significant ECG abnormalities	1057 (89)	1586 (80)	372 (57)	<0.001
Body mass index (kg/m^2)	26 [23, 29]	27 [24, 30]	27 [24, 29]	<0.001
Laboratory findings				
Creatinine clearance, mL/min/m^2	91 [78, 105]	83 [67, 99]	77 [61, 94]	<0.001
Chronic medication				
ASA	229 (19)	776 (39)	376 (58)	<0.001
Vitamin K antagonists	98 (8)	222 (11)	62 (10)	0.019
B-blockers	248 (21)	773 (39)	296 (45)	<0.001
Statins	233 (20)	773 (39)	341 (52)	<0.001
ACEIs/ARBs	306 (26)	841 (42)	347 (53)	<0.001

Calcium antagonists	107 (9)	323 (16)	141 (22)	<0.001
Nitrates	51 (4)	217 (11)	139 (21)	<0.001

Numbers are presented as median [IQR] or numbers (%). VAS = visual analogue scale; ECG = electrocardiogram; BMI = body mass index; MI = myocardial infarction; hs-cTnT = high sensitive cardiac troponin T; ASA = Acetylsalicylic acid; ACE = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker.

*p-Value was calculated for differences in baseline characteristics between patients with VAS ≤ 10% and patients with VAS 11-79% and VAS ≥ 80 combined.

Table 2A		Diagnostic performance for NSTEMI of predefined cutoff-levels of hs-cTnT among all three strata			
		Specificity, % (95%CI)	**PPV, % (95%CI)**	**Sensitivity, % (95%CI)**	**NPV, % (95%CI)**
Low likelihood	hs-cTnT ≥ 5 ng/L	0.41 (0.38-0.44)	0.02 (0.01-0.03)	1.00 (0.70-1.00)	1.00 (0.99-1.00)
VAS ≤ 10%	hs-cTnT ≥ 14 ng/L	0.88 (0.86-0.90)	0.09 (0.05-0.15)	0.93 (0.68-1.00)	1.00 (0.99-1.00)
	hs-cTnT ≥ 52 ng/L	0.99 (0.98-0.99)	0.26 (0.10-0.48)	0.40 (0.16-0.68)	0.99 (0.99-1.00)
Intermediate likelihood	hs-cTnT ≥ 5 ng/L	0.29 (0.27-0.31)	0.16 (0.14-0.18)	0.99 (0.97-1.00)	1.00 (0.99-1.00)
VAS 11-79%	hs-cTnT ≥ 14 ng/L	0.76 (0.74-0.78)	0.33 (0.30-0.37)	0.86 (0.81-0.90)	0.98 (0.97-0.98)
	hs-cTnT ≥ 52 ng/L	0.97 (0.96-0.98)	0.63 (0.55-0.71)	0.36 (0.30-0.43)	0.92 (0.90-0.93)
High likelihood	hs-cTnT ≥ 5 ng/L	0.14 (0.10-0.18)	0.58 (0.54-0.62)	1.00 (0.98-1.00)	0.98 (0.87-1.00)
VAS ≥ 80	hs-cTnT ≥ 14 ng/L	0.58 (0.52-0.64)	0.73 (0.69-0.77)	0.95 (0.92-0.97)	0.90 (0.85-0.94)
	hs-cTnT ≥ 52 ng/L	0.93 (0.89-0.95)	0.92 (0.87-0.95)	0.66 (0.61-0.71)	0.69 (0.64-0.74)
Table 2B		**Diagnostic performance for NSTEMI of predefined cutoff-levels of hs-cTnI among all three strata**			
		Specificity, % (95%CI)	**PPV, % (95%CI)**	**Sensitivity, % (95%CI)**	**NPV, % (95%CI)**
Low likelihood	hs-cTnI ≥ 2 ng/L	0.30 (0.27-0.32)	0.02 (0.01-0.03)	1.00 (0.68-1.00)	1.00 (0.98-1.00)
VAS ≤ 10%	hs-cTnI ≥ 26 ng/L	0.94 (0.93-0.96)	0.15 (0.08-0.26)	0.79 (0.49-0.95)	1.00 (0.99-1.00)
	hs-cTnI ≥ 52 ng/L	0.96 (0.95-0.97)	0.10 (0.03-0.22)	0.36 (0.13-0.65)	0.99 (0.98-1.00)
Intermediate likelihood	hs-cTnI ≥ 2 ng/L	0.14 (0.13-0.16)	0.14 (0.12-0.16)	1.00 (0.98-1.00)	1.00 (0.98-1.00)
VAS 11-79%	hs-cTnI ≥ 26 ng/L	0.90 (0.88-0.91)	0.44 (0.38-0.50)	0.56 (0.49-0.62)	0.94 (0.92-0.95)
	hs-cTnI ≥ 52 ng/L	0.93 (0.92-0.95)	0.48 (0.41-0.55)	0.43 (0.36-0.49)	0.92 (0.91-0.93)
High likelihood	hs-cTnI ≥ 2 ng/L	0.06 (0.04-0.10)	0.57 (0.52-0.61)	1.00 (0.98-1.00)	1.00 (0.73-1.00)

VAS ≥ 80	hs-cTnI ≥ 26 ng/L	0.82 (0.77-0.86)	0.85 (0.81-0.89)	0.86 (0.82-0.89)	0.83 (0.78-0.87)
	hs-cTnI ≥ 52 ng/L	0.87 (0.82-0.91)	0.88 (0.84-0.91)	0.78 (0.73-0.82)	0.76 (0.71-0.81)

NSTEMI = non-ST-segment elevation myocardial infarction, PPV = positive predictive value, NPV = negative predictive value, VAS = visual analogue scale, hs-cTn = high sensitive Troponin.

Figure Legends

Figure 1: **Receiver-operating characteristics (ROC) curves for high-sensitivity cardiac troponin (hs-cTn) T (left) and hs-cTnI (right) at presentation**

Receiver-operating characteristics (ROC) curves for the diagnostic performance of high-sensitivity cardiac troponin (hs-cTn) T (left) and hs-cTnI at presentation (right) to diagnose non-ST-segment elevation myocardial infarction (NSTEMI). Predefined cut-off levels are highlighted within the ROC Curves to demonstrate high specificity across different ACS-probability levels, for example, patients with hs-cTnT $\geq$ 52 ng/L have a specificity $\approx$ 90% in all three ACS-probability levels.

Figure 2: **Pie charts for distribution of final diagnoses according acute coronary syndrome (ACS)-probability and elevated hs-cTnT concentrations**

Distribution of final diagnoses in patients stratified according to acute coronary syndrome (ACS)-probability and high-sensitivity cardiac troponin T (hs-cTnT) levels. Shown for patients with hs-cTnT at presentation above the 99th percentile ($\geq$ 14 ng/L, top) and for patients with hs-cTnT above $\geq$ 52 ng/L (bottom). CAD = coronary artery disease.

Figure 3: **Diagnostic performance of the European Society of Cardiology (ESC) hs-cTnT 0/1h-algorithm in patients with low, intermediate and high acute coronary syndrome (ACS)-probability**

Diagnostic performance of the European Society of Cardiology (ESC) hs-cTnT 0/1h-algorithm for triage towards rule-out, observe, and rule-in of non-ST-segment elevation myocardial infarction (NSTEMI) in patients stratified according to acute coronary syndrome (ACS)-probability into low, intermediate, and high (*) if chest pain onset >3h; VAS = Visual analogue scale; NPV = Negative predictive value; PPV = Positive predictive value.

Figure 4: **Kaplan-Meier curves for the cumulative survival according to the ACS-probability group and displayed for different hs-cTnT (A) and hs-cTnI (B) levels**

Kaplan-Meier curves displaying survival during long-term follow-up (720 days) according to the ACS-probability group. On the top row (A) the green line displays high-sensitivity cardiac troponin (hs-cTnT) levels < 5 ng/L, the blue line hs-cTnT-levels ≥ 5 to < 14 ng/L and the red line hs-cTnT levels ≥ 14 ng/L. Hs-cTnI levels are displayed in a similar fashion in the bottom row (B).