

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

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1 **List of abbreviations:**

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

14

1 ABSTRACT

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

5 **Methods:** We prospectively stratified patients presenting with acute chest discomfort
6 to the emergency department (ED) into three groups according to their probability for
7 ACS as assessed by the treating ED physician using a visual analogue scale (VAS):
8 $\leq 10\%$, 11-79%, $\geq 80\%$, reviewing all information available at 90 minutes. hs-cTnT-
9 and hs-cTnI-concentrations were determined in a blinded fashion. Two independent
10 cardiologists adjudicated the final diagnosis.

11 **Results:** Among 3828 patients eligible for analysis, 1189 patients had low ($\leq 10\%$)
12 probability for ACS. The incidence of non-ST-segment elevation myocardial infarction
13 (NSTEMI) increased from 1.3% to 12.2% and 54.8% in patients with low,
14 intermediate and high ACS-probability, respectively. The positive predictive value of
15 hs-cTnT and hs-cTnI was low in patients with low ACS-probability and increased with
16 the incidence of NSTEMI, while the diagnostic accuracy of hs-cTnT and hs-cTnI for
17 NSTEMI as quantified by the area under the curve (AUC) were very high and
18 comparable among all three strata (e.g. AUC hs-cTnI 0.96 (95%CI 0.94-0.97); 0.87
19 (95%CI 0.85-0.89), and 0.89 (95%CI 0.87-0.92), respectively. Findings were
20 validated using bootstrap analysis as an alternative methodology to define ACS-
21 probability. Similarly, higher hs-cTnT/I concentrations independently predicted all-
22 cause mortality within two years (e.g. hs-cTnT hazard ratio 1.39, 95%CI 1.27-1.52),
23 irrespective of ACS-probability.

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

1 **Introduction**

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

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

1 probability for ACS.

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

6

7 **Materials and Methods**

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

11

12 **Quantification of ACS-probability**

13 ACS-probability was quantified using two complimentary methods. First, probability
14 for ACS as the cause of the presenting symptom was quantified 90 minutes after
15 presentation by the treating ED physician using a visual analogue scale (VAS,
16 depicted in the supplemental data). At this time point, the ED physician had
17 completed his/her clinical assessment including patient history, chest pain
18 characteristics, detailed physical examination including vital signs and reviewed the
19 ECG and the first local cTn measurement. We considered the levels of $\leq 10\%$ as low,
20 11-79% as intermediate and $\geq 80\%$ as high pre-test probability for ACS (9,10).
21 Further details regarding the assessment of the ACS-probability of the ED-physician
22 is given within the supplemental data. Second, to generate an alternative
23 classification, we used bootstrap analysis to produce a predetermined prevalence
24 different from the true prevalence of NSTEMI to simulate a low ACS-probability

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

3

4 **Measurements of hs-cTnT and hs-cTnI**

5 Blood samples for determination of hs-cTnT and hs-cTnI were collected at
6 presentation and serially thereafter. After centrifugation, samples were frozen at -
7 80°C until assayed in a blinded fashion in a dedicated core laboratory. According to
8 the manufacturer, the hs-cTnT assay (Roche Elecsys 2010, Roche Diagnostics,
9 Rotkreuz, Switzerland) had a 99th percentile concentration of 14 ng/L with a
10 corresponding co-efficient of variation (CV) of 10% at 13 ng/L (4). Limit of blank (LoB)
11 and limit of detection (LoD) have been determined to be 3 ng/L and 5 ng/L. According
12 to the manufacturer, the hs-cTnI assay (ARCHITECT STAT, Abbott Laboratories, IL)
13 had a 99th percentile concentration of 26 ng/L with a corresponding co-efficient of
14 variation (CV) of <5% and a limit of detection (LoD) of 2 ng/L (11–13). Further, two
15 additional pre-commercial hs-cTnI assays and one s-cTnI assay were used. The
16 detailed information from the manufacturer for these assays is given in the
17 supplemental data.

18

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

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

22

23 **Statistical analysis**

24 Continuous variables are described as mean \pm SD or median with interquartile range
25 (IQR), categorical variables by numbers and percentages. Differences in baseline

1 characteristics between patients were assessed using the Mann-Whitney-U-test for
2 continuous variables and the Pearson Chi-square test for categorical variables.
3 Receiver-operating characteristics (ROC) curves were constructed to assess the
4 sensitivity and specificity throughout the concentrations of hs-cTnT and hs-cTnI at
5 presentation, at 1-h and 3-hour. Furthermore, ROC curves were constructed for early
6 absolute changes of hs-cTnT and hs-cTnI within 1-hour, alone and in combination
7 with hs-cTn concentrations at presentation. Logistic regression was used to combine
8 hs-cTn concentrations at presentation with early changes in hs-cTn concentrations.
9 Specificity, sensitivity, negative predictive value (NPV) and positive predictive value
10 (PPV) for predefined cut-off-levels were calculated. We calculated the bootstrapped
11 AUC and 95%-confidence intervals (CI) from the dataset simulating a low ACS-
12 probability defined as an NSTEMI incidence of 2%, calculated the AUC in each set
13 and then calculated the mean AUC. Univariate and multivariate Cox regression
14 analysis was used to calculate hazard ratios (HR) and 95%CI to reveal associations
15 between hs-cTnT, hs-cTnI and long-term mortality of patients. We calculated the
16 interaction p-value for the prognostic value of hs-cTn with levels of ACS-probability
17 for all-cause mortality using a binary logistic regression model. Kaplan Meier analysis
18 was performed using predefined cut-off-levels of hs-cTnT and hs-cTnI. All hypothesis
19 testing was two-tailed and p-values <0.05 were considered statistically significant.
20 Statistical analyses were performed using IBM SPSS Statistics for Windows, version
21 22.0 (SPSS Inc, Chicago, IL) and the R statistical package (R Foundation for
22 Statistical Computing, Vienna, Austria).

23

24 **Results**

25

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

9

10 **ACS-probability and incidence of NSTEMI**

11 Among 1189 patients who had low ($\leq 10\%$) probability for ACS, NSTEMI was the
12 adjudicated diagnosis in 15/1189 patients (1.3%). The incidence of NSTEMI in
13 patients with intermediate (11-79%) and high ($\geq 80\%$) probability for ACS was 12.2%
14 (243/1986) and 54.8% (358/653), respectively. The prevalence of predefined
15 alternative diagnoses including “unstable angina”, “cardiac symptoms of origin other
16 than coronary artery disease” and “non-cardiac chest pain” are listed in Tables 2SA
17 for the analyses of hs-cTnT and in Table 2SB for hs-cTnl.

18 Concentrations of hs-cTnT and hs-cTnl at presentation and during serial
19 sampling were significantly higher in patients with NSTEMI as compared to patients
20 with other final diagnoses among all three ACS-probability strata (Table S3A and
21 S3B). The proportion of patients with elevated hs-cTnT and hs-cTnl concentrations
22 obtained from the blinded study-specific samples taken at ED presentation across
23 the different ACS-probabilities (low/intermediate/high) were 151 (13%), 629 (32%),
24 and 463 (71%) patients with hs-cTnT ≥ 14 ng/l and 72 (7%), 292 (16%) and 336
25 (55%) patients with hs-cTnl ≥ 26 ng/l.

26

1 **Diagnostic accuracy of ACS-probability**

2 The diagnostic accuracy of the ACS-probability as quantified by the ED physician for
3 an adjudicated diagnosis of ACS was 0.86 (95%CI 0.84-0.87). Diagnostic accuracy
4 of hs-cTn for NSTEMI were very high and comparable among all three strata of ACS-
5 probability for hs-cTnT (low: AUC 0.94; 95%CI 0.87-1.00), intermediate: 0.89; 95%CI
6 0.87-0.91, high: 0.90; 95%CI 0.87-0.92) and even higher in patients with low-ACS-
7 probability for hs-cTnI (AUC 0.96; 95%CI 0.94-0.97) as compared to patients with
8 intermediate (AUC 0.87; 95%CI 0.85-0.89, $p<0.01$) and high ACS-probability (AUC
9 0.89; 95%CI 0.87-0.92, $p<0.01$, Figure 1). These findings were consistent in all
10 predefined subgroups (data not shown), for serial measurements of hs-cTnT and hs-
11 cTnI (Table S4), and for two additional pre-commercial hs-cTnI assays and one s-
12 cTnI assay (Table S5).

13 The specificity for NSTEMI of the 99th-percentiles or 52ng/l as possible rule-in
14 cut-off values for hs-cTnT and hs-cTnI was high. Increasing ACS-probability was
15 associated with a decrease in specificity for both hs-cTnT and hs-cTnI (Table 2A-B).
16 The PPV, which in contrast to specificity is depending on NSTEMI incidence, was low
17 in patients with low ACS-probability and increased with increasing ACS-probability for
18 both hs-cTnT and hs-cTnI. The distribution of final diagnoses in patients with hs-cTnT
19 ≥ 14 ng/L and patients with hs-cTnT ≥ 52 ng/L within each of the three ACS-probability
20 strata is shown in Figure 2.

21 Sensitivity and NPV were very high and comparable among all three strata
22 using the LOD as a possible rule-out cut-off value for hs-cTnT and hs-cTnI. Using the
23 99th percentiles recommended by the manufacturers, sensitivity and NPV were lower
24 with hs-cTnI as compared to that obtained for hs-cTnT.

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

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

5 Combination of hs-cTn concentrations at presentation with early absolute
6 changes was again very high in the low ACS-probability subgroup and comparable
7 among all three strata: low ACS-probability hs-cTnT AUC 0.98 (95%CI 0.96-0.99),
8 hs-cTnI AUC 0.94 (95%CI, 0.91-0.97); intermediate ACS-probability hs-cTnT AUC
9 0.94; (95%CI 0.93-0.95), hs-cTnI AUC 0.91 (95%CI 0.89-0.93); high ACS-probability
10 hs-cTnT AUC 0.93 (95%CI 0.90-0.96), hs-cTnI AUC 0.89 (95%CI 0.86-0.92).

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

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

18

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

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

1 2.16 (95%CI 1.51-3.09)) as compared to intermediate (HR 1.46 (95%CI 1.24-1.72))
2 and high ACS-probability (HR 1.30 (95%CI 1.12-1.50); interaction p-value <0.01).
3 Similar findings were obtained for hs-cTnI (Figure 4).

4

5 **Discussion**

6

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

1 accuracy for AMI (14), and hs-cTnI seems to have even higher analytical sensitivity
2 as compared to hs-cTnT (11). The most likely explanation for this finding therefore is
3 the biological non-equivalence of 26 ng/L for hs-cTnI versus 14ng/L for hs-cTnT as
4 previously documented in two large studies (15,16). The biological equivalent hs-cTnI
5 concentration corresponding to the 99th percentile for hs-cTnT was about half the
6 approved 99th percentile for hs-cTnI in these studies (15,16). This major discrepancy
7 in the currently recommended 99th-percentiles became also evident in this dataset:
8 32.5% of patients had a hs-cTnT concentration ≥ 14 ng/L, whereas only 19.7% had a
9 hs-cTnI concentration ≥ 26 ng/L. Thus, the 99th percentile variability between assays
10 is substantial (17). To overcome the poor consistency in the composition of
11 individuals enrolled for determining the 99th percentile, future studies comparing all
12 contemporary sensitive and hs-assays within the same reference or disease
13 population are warranted. Defining what constitutes the appropriate reference
14 population is a topic of debate (18). Fourth, and perhaps of most importance, the
15 diagnostic accuracy for hs-cTnT/I to diagnose NSTEMI in patients with acute chest
16 discomfort and low ACS likelihood was very high (AUC 0.94 and 0.96) and
17 comparable to that in patients with intermediate or high likelihood for ACS. Fifth,
18 diagnostic accuracies for NSTEMI provided by early absolute changes of hs-cTn
19 within 1-hour, alone or in combination with hs-cTn concentrations at presentation,
20 provided very high and similar diagnostic accuracy in patients with low ACS-
21 probability as compared to the other strata. Sixth, the overall diagnostic performance
22 of the ESC 0/1h-algorihm was very good among all ACS-probability strata, confirming
23 the safety and efficacy of this approach also in patients with low ACS-probability.
24 Seventh, hs-cTn was an independent predictor of all-cause mortality irrespective of
25 the ACS-probability.

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

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

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

14 The implementation of the kinetics of the marker could provide some
15 reassurance regarding the widespread concern of too many false-positive results by
16 ordering hs-cTn in patients with low likelihood of ACS. Serial measurements of hs-
17 cTnT-levels at 1-hour were available in 969/1189 patients with low ACS-probability.
18 3.6% (35/969) patients showed a relevant rise of ≥ 5 ng/L in 1-hour, identifying 10/13
19 patients with the final diagnosis of an NSTEMI even though the ACS-probability for
20 initially was considered to be $\leq 10\%$. Serial measurements of hs-cTn allow a better
21 discrimination of ischemia-induced cardiac injury from cardiomyocyte damage by
22 other cardiac disorders by a noninvasive, widely available test. Findings were
23 confirmed by the ESC 0/1h-algorithm, which is based on the integrated use of hs-cTn
24 concentrations at presentation and their absolute changes during serial sampling.

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

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

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

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

1 this study. Third, even by experienced cardiologists applying current guideline
2 recommendations (2,4,13,26), NSTEMI could not be reliably excluded in a small
3 number of patients (2.5%, 108/4'232), although further clinical course did not reveal
4 additional information indicating the diagnosis of AMI. Therefore, this subgroup had
5 to be excluded from analysis. Fourth, we did not use sex-specific cut-off values in this
6 analysis and thus cannot help broaden the scientific basis for the ongoing discussion
7 regarding sex-specific cut-off values in the diagnosis of AMI (40). Fifth, we used the
8 99th percentile as recommended by the manufacturers, calculated from two separate
9 reference populations (hs-cTnT in 533 apparently healthy European subjects; hs-
10 cTnI in 449 apparently healthy US subjects) and not a 99th percentile derived from a
11 similar single reference set (4,11,12). Sixth, we cannot generalize these findings to
12 patients with terminal kidney failure requiring dialysis, since they were excluded from
13 this study.

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

21

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20 **Conflict of interest**

21 The authors designed the study, gathered and analyzed the data, vouch for the data

22 and analysis, wrote the paper, and decided to publish. Drs. Badertscher,

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1 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

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2 Numbers are presented as median [IQR] or numbers (%). VAS = visual analogue
3 scale; ECG = electrocardiogram; BMI = body mass index; MI = myocardial infarction;
4 hs-cTnT = high sensitive cardiac troponin T; ASA = Acetylsalicylic acid; ACE =
5 angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker.
6 *p-Value was calculated for differences in baseline characteristics between patients
7 with VAS \leq 10% and patients with VAS 11-79% and VAS \geq 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 VAS ≤ 10%	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)
	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 VAS 11-79%	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)
	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 VAS ≥ 80	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)
	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 VAS ≤ 10%	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)
	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 VAS 11-79%	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)
	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 ≥ 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 (≥ 14 ng/L, top) and for patients with hs-cTnT above ≥ 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).