Clinical Validation of a Novel High-Sensitivity Cardiac Troponin I Assay for Early Diagnosis of Acute Myocardial Infarction

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Abstract (250 words)

Background: Clinical performance of the novel high-sensitivity cardiac troponin I (Siemens-hs-cTnI-Centaur) assay is unknown. We aimed to clinically validate the Siemens-hs-cTnI-Centaur assay and develop 0/1h- and 0/2h-algorithms.

Methods: We enrolled patients presenting to the emergency department with symptoms suggestive of acute myocardial infarction (AMI). Final diagnoses were centrally adjudicated by two independent cardiologists including all clinical information twice: first, using serial hs-cTnT (Roche-Elecsys, primary analysis) and second, using hs-cTnI (Abbott-Architect, secondary analysis) measurements in addition to the clinically applied (hs)-cTn. Siemens-hs-cTnI-Centaur was measured at presentation, at 1h, and 2h. Primary objective was a direct comparison of diagnostic accuracy, quantified by the area under the receiver-operating-characteristic curve (AUC), of Siemens-hs-cTnI-Centaur versus the two established hs-cTn assays (Roche-hs-cTnT-Elecsys, Abbott-hs-cTnI-Architect). Secondary objectives included the development of Siemens-hs-cTnI-Centaur specific 0/1h- and 0/2h-algorithms.

Results: AMI was the final diagnosis in 318/1755 (18%) patients (using Roche-hs-cTnT-Elecsys for adjudication). The AUC at presentation for Siemens-hs-cTnI-Centaur was 0.94 (95%CI, 0.92-0.96) and comparable to 0.95 (95%CI, 0.93-0.97) for Roche-hs-cTnT-Elecsys and 0.93 (95%CI, 0.90-0.96) for Abbott-hs-cTnI-Architect. Applying the derived Siemens-hs-cTnI-Centaur 0/1h-algorithm to the validation cohort, 46% of patients were ruled-out (sensitivity 99.1% [95%CI, 95.3-100]), and 18% of patients were ruled-in (specificity 94.1% [95%CI, 91.8-95.9]). The Siemens-hs-cTnI-Centaur 0/2h-algorithm ruled-out 55% of patients (sensitivity 100% [95%CI, 94.1-100]), and ruled-in 18% of patients (specificity 96.0% [95%CI, 93.1-97.9]). Findings were confirmed in the secondary analyses using serial measurements of Abbott-hs-cTnI-Architect for adjudication.

Conclusions: Diagnostic accuracy and clinical utility of the novel Siemens-hs-cTnI-Centaur assay are very high and comparable to the established hs-cTn assays.

Trial Registration: **ClinicalTrials.gov number, NCT00470587**

Abbreviations

ED – Emergency department

AMI – Acute myocardial infarction

ECG – Electrocardiography

cTn – Cardiac troponin

hs-cTn – High-sensitivity cardiac troponin

eGFR – Estimated glomerular filtration rate

NPV – Negative predictive value

CART – Classification and regression tree

PPV – Positive predictive value

IQR – Interquartile range

Introduction

Up to 10% of all emergency department (ED) consultations are by patients with symptoms suggestive of acute myocardial infarction (AMI).(1) Rapid identification of AMI as a life-threatening disorder is important for the early initiation of appropriate, evidence-based therapy.(2) Electrocardiography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstones and complement clinical assessment in the early rule-out or rule-in of AMI.(2–4)

The introduction of high-sensitivity cardiac troponin (hs-cTn) assays enabled reliable measurement of cTn concentrations in the reference interval,(5) and increased diagnostic accuracy for AMI at presentation.(6) Two hs-cTn assays, Roche-hs-cTnT-Elecsys and Abbott-hs-cTnI-Architect, have been extensively investigated in large diagnostic studies, including the successful derivation and validation of early 0/1h and 0/2h triage-algorithms.(3,7–20)

More recently, the novel hs-cTnI-Centaur assay was developed. It constitutes only the third hs-cTn assay to become available for clinical use. Before its possible implementation into routine clinical care, its performance in patients presenting with suspected AMI must be thoroughly examined. We therefore set out to compare its diagnostic accuracy with that of the two established hs-cTn assays, and derived and validated assay-specific 0/1h- and 0/2h-algorithms.

Materials and Methods

Study design and population

Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE), is an ongoing prospective international multicenter study with 12 centers in 5 countries aiming to advance the early diagnosis of AMI (ClinicalTrials.gov registry, number NCT00470587).(6,7,9,11,12,14,15,21–23; **Online Supplemental file)**.

Adjudication of the final diagnosis

Central adjudication of the final diagnosis was performed by two independent cardiologists applying the universal definition of AMI using two sets of data: first, all available medical records obtained during clinical care including cardiac imaging and second, study-specific assessments including serial hs-cTnT concentrations. In order to address the uncommon, but previously described phenomenon of discrepant results for hs-cTnT and hs-cTnI, we performed a second adjudication using serial hs-cTnI (rather than hs-cTnT) blood concentrations from study samples (**Online Supplemental)**.

Investigational hs-cTn measurements

For determination of Siemens-hs-cTnI-Centaur we used blood samples collected into serum containers and for determination of Abbott-hs-cTnI-Architect and Roche-hs-cTnT-Elecsys we used blood samples collected into serum containers or lithium heparin plasma containers, respectively. Study-specific blood draws were performed immediately after informed consent had been obtained at ED presentation and additionally at 1, 2, 3, and 6h. Serial sampling was discontinued when a patient was released or transferred to the catheter laboratory for acute treatment. 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-cTnI-Centaur assay (ADVIA Centaur TNIH, Siemens Healthcare) has a population 99th percentile concentration (both sexes) of 47ng/L with a

corresponding co-efficient of variation (CV) of <5%. 99th percentiles for men and women are 58ng/L and 39ng/L, respectively. Limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) have been determined to be 0.9ng/L, 2.2ng/L, and 2.5ng/L. The assay is a dual-capture sandwich immunoassay using magnetic latex particles and a proprietary acridinium ester for chemiluminescence detection. The detection reagent is a recombinant sheep Fab antibody covalently linked to a tri-sulfo propyl acridinium ester (TSPAE)-BSA conjugate. TSPAE is a new generation of high-yield acridinium esters developed for enhanced chemiluminescent detection. Simultaneous addition of solid-phase reagent and detection reagent to the sample forms a classic sandwich immune complex, which is subsequently washed. Chemiluminescence is initiated and measured. Relative light units are directly proportional to the cTnI concentration. The time to first result is 18 minutes. The assay meets the current International Federation of Clinical Chemistry (IFCC) recommendations for hs-cTn assays.(24,25)

The hs-cTnT-Elecsys assay (Elecsys 2010, Roche Diagnostics) has a 99th percentile concentration of 14ng/L with a corresponding CV of 10% at 13ng/L.(5) LoB and LoD have been determined to be 3ng/L and 5ng/L.(5) The hs-cTnI-Architect assay (ARCHITECT STAT high-sensitivity troponin I, Abbott Laboratories) has a 99th percentile concentration of 26.2ng/L with a corresponding CV of <5% and a LoD of 1.9ng/L.(26–28) Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease formula.(29)

Derivation of the hs-cTnI-Centaur 0/1h-algorithm

The hs-cTnI-Centaur 0/1h-algorithm was developed in a derivation sample of randomly (1:1 fashion) selected patients with available hs-cTnI-Centaur measurements at baseline and after 1h) according to the central adjudication by two independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnT-Elecsys concentrations (primary

adjudication). The 0/1h-algorithm incorporates hs-cTnI-Centaur concentrations at presentation and absolute hs-cTnI-Centaur changes within 1h (*hs-cTnI-Centaur$_{1h}$ – hs-cTnI-Centaur$_{0h}$*) as well as time since chest pain onset in order to reflect the concept of the current hs-cTn 0/1h-algorithms suggested by the ESC(3) (**Supplemental Figure 4**). Selection of these parameters was based on the very high diagnostic accuracy of the combination of blood concentrations at presentation with absolute changes for rule-out and rule-in of AMI.(7,8,11,12,14,15,21,30,31) Optimal thresholds for rule-out were selected to allow for a minimal sensitivity and negative predictive value (NPV) of 99% and were independent from the assay package insert specified thresholds. Optimal thresholds for rule-in were obtained based on a classification and regression tree (CART) analysis targeting a minimal positive predictive value (PPV) of 70%.(32,33) Nodes in the CART tree were constrained to have a minimal number of cases of 20 in parent and child nodes. If a predefined target performance was missed in the derivation sample using the CART-derived thresholds, thresholds were changed stepwise until the predefined performance was fulfilled.

Derivation of the hs-cTnI-Centaur 0/2h-algorithm

The hs-cTnI-Centaur 0/2h-algorithm was developed in a derivation sample randomly (2:1 fashion in order to compensate for the slightly lower number of patients with available 2h versus 1h samples and to ensure a sufficient number of patients in the derivation cohort) selected of patients with available hs-cTnI-Centaur measurements at ED presentation and after 2h (**Online Supplemental file**) according to the central adjudication by two independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnT-Elecsys concentrations (primary adjudication).

Validation of the hs-cTnI-Centaur 0/1h- and 0/2-algorithm

The algorithms developed in the derivation samples were tested for their diagnostic accuracy in internal validation samples consisting of the remaining subjects. The optimal decision values

derived in the derivation sample were rounded to give whole values in ng/L. The first validation was done according to the central adjudication by two independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnT-Elecsys concentrations. The secondary validation was done according to the central adjudication by two independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnI-Architect concentrations.

Follow-up and clinical endpoints

Clinical follow-up is described in detail in the **Online Supplemental file**. The co-primary prognostic endpoints were overall survival after 30 days and two years. The secondary prognostic endpoint was major adverse cardiac events (MACE) defined as the composite of all-cause mortality, AMI, cardiogenic shock, ventricular tachyarrhythmias, or higher-degree atrioventricular block at 30-days.

Statistical analysis

For the primary analysis, serial hs-cTnT-Elecsys concentrations were used for final adjudication. For the secondary analysis, serial hs-cTnI-Architect concentrations were used for final adjudication. Receiver-operating characteristics (ROC) curves were constructed to assess the sensitivity and specificity throughout the hs-cTn-concentrations to compare the ability to diagnose AMI. Subgroup analyses were performed in patients presenting to the ED very soon (≤2h), soon (≤3h) and late (>3h) after chest pain onset/maximum as well as in women and men. We further included analysis using sex-specific cut-offs and investigated the performance of the ESC 0/3h-algorithm. Biological equivalent concentrations were determined by plotting log-transformed hs-cTnI-Centaur and hs-cTnI-Architect or hs-cTnT-Elecsys concentrations from the same sample.(22) The areas under the ROC curves (AUC) were compared as recommended by DeLong et al.(34) or by z-statistic, as appropriate (**Online Supplemental file).**

Safety was assessed as the NPV and the sensitivity for AMI for rule-out, accuracy for rule-in as the PPV and specificity for AMI, and efficacy was quantified as the percentage of patients triaged towards rule-out or rule-in for AMI within 1h or 2h.

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (SPSS Inc) and MedCalc 17.6 (MedCalc Software).

Results

Characteristics of patients

From April 2006 to February 2013, 1755 patients eligible for this analysis were enrolled (**Supplemental Figure 1**). Thirty-four percent of patients presented to the ED within the first three hours after chest pain onset. Baseline characteristics of all patients are shown in **Table 1** and of patients in the derivation and validation cohorts are shown in **Supplemental Table 2**.

Adjudicated final diagnosis

The adjudicated final diagnosis was AMI in 318/1755 patients (18%), unstable angina in 156/1755 (9%), cardiac symptoms of origin other than coronary artery disease such as tachyarrhythmia, Tako-Tsubo cardiomyopathy, heart failure or myocarditis in 238/1755 (14%), non-cardiac symptoms in 968/1755 (55%), and unknown in 75/1755 (4%). Final diagnoses according to the second final adjudication including hs-cTnI (Architect) were similar (**Online Supplemental file**).

Concentrations of hs-cTnI-Centaur at presentation according to final diagnoses

Concentrations of hs-cTnI-Centaur at ED presentation were significantly higher in patients with AMI as compared to patients with other final diagnoses (P<0.001). Median concentrations of hs-cTnI-Centaur in patients with AMI were 235ng/L (IQR, 39-1018), with unstable angina 8.5ng/L (IQR, 5.0-17), with cardiac, but not coronary disease 12ng/L (IQR, 4.7-36), with non-cardiac disease 4ng/L (IQR, 2.4-7.6), and with unknown diagnosis 4.2ng/L (IQR, 2.8-7.0; **Figure 1**). Similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI (Architect; **Supplemental Figure 2)**.

Diagnostic accuracy for AMI

The diagnostic accuracy of measurements obtained at presentation, as quantified by AUCs, for the hs-cTnI-Centaur assay was 0.94 (95%CI, 0.92-0.96) and comparable to hs-cTnT-Elecsys

0.95 (95%CI, 0.93-0.97), and hs-cTnI-Architect 0.93 (95%CI, 0.90-0.96), respectively (*P*=0.370 and *P*=0.780 for direct comparisons; **Figure 2A**). For hs-cTnI-Centaur, the AUCs for concentrations at 1h, 2h, and 3h were 0.95 (95%CI, 0.93-0.96), 0.95 (95%CI, 0.94-0.97), and 0.97 (95%CI, 0.95-0.99), respectively (**Supplemental Table 3A**). Similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI (Architect; **Supplemental Figure 3A)**. The diagnostic performances of uniform and sex-specific cut-offs are summarized in **Table 2B** and detailed information given in the **Online Supplemental Results**.

Subgroup analyses according to time since chest pain onset and sex

Diagnostic accuracy at presentation was also similar in the predefined subgroups (**Online Supplemental Results, Supplemental Table 3B, Figure 2B).** Again, similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI (Architect; **Supplemental Figure 3B)**.

Hs-cTnI-Centaur 0/1h-algorithm

The diagnostic performance of the hs-cTnI-Centaur 0/1h-algorithm in the derivation cohort is shown in **Figure 3A,** and **Supplemental Figure 5A**.

Validation of the hs-cTnI-Centaur 0/1h-algorithm

Applying the derived optimal cut-off levels to the internal validation cohort, 313/675 patients (46%) could be classified as rule-out with a corresponding NPV of 99.7% (95%CI, 97.8-100) and a sensitivity of 99.1% (95%CI, 95.3-100; **Figure 3B** and **Supplemental Figure 5B**). Direct rule-out based on a single hs-cTnI-Centaur concentration at presentation was feasible in 111/675 patients (16%). One patient with AMI was missed out of 675 patients with suspected AMI in the validation sample (**Supplemental Table 4** for detailed patient characteristics). The 0/1h-algorithm classified 120/675 patients (18%) as rule-in with a corresponding PPV of 72.5% (95%CI, 63.6-80.3) and a specificity of 94.1% (95%CI, 91.8-95.9). Direct rule-in based on a

single hs-cTnI-Centaur concentration at presentation was feasible in 79/675 patients (12%). Overall, the hs-cTnI-Centaur 0/1h-algorithm allowed a definite diagnosis after 1h in 433/675 patients (64%; either rule-out or rule-in). The remaining 242/675 patients (36%) were classified to observe with an AMI prevalence of 11% (95%CI, 8-15). Similar findings emerged when assessing the diagnostic performance of the hs-cTnI-Centaur 0/1h-algorithm in the validation cohort using the second final adjudication including hs-cTnI (Architect, **Supplemental Figure 6**).

Direct comparison of the hs-cTnI-Centaur 0/1h-algorithm with the ESC 0/1h-algorithms using hs-cTnT-Elecsys and hs-cTnI-Architect

Overall, the diagnostic performance of the hs-cTnI-Centaur 0/1h-algorithm was similar to that of the hs-cTnT-Elecsys 0/1h-algorithm and the hs-cTnI-Architect 0/1h-algorithm (**Supplemental Figure 7+8).**

Hs-cTnI-Centaur 0/2h-algorithm

Optimal thresholds for rule-out and rule-in are shown in **Figure 3C**.

Validation of the hs-cTnI-Centaur 0/2h-algorithm

Applying the derived optimal thresholds to the internal validation cohort, 200/361 patients (55%) could be classified as rule-out with a corresponding NPV of 100% and a sensitivity of 100% (95%CI, 94.1-100; **Figure 3D**, **Supplemental Figure 9)**.

Prognostic performance of the hs-cTnI-Centaur 0/1h-algorithm

Median follow-up time was 772 days (IQR, 734-915) with 13 deaths occurring within 30 days and 99 deaths within two years. Cumulative 30-days survival rates were 100%, 98.6% and 97.5% (log-rank, P=0.002) in the rule-out, observe and rule-in group, respectively. At 2 years, cumulative survival rates were 98.4%, 89.2% and 85.1%, respectively (log-rank, P<0.001;

Figure 4). Similar findings emerged regarding MACE-free survival including the index event (**Supplemental Figure 10**).

Biological equivalent concentrations

Biological equivalent concentrations for hs-cTnI-Centaur of hs-cTnI-Architect and hs-cTnT-Elecsys are shown in **Supplemental Figure 11**.

Discussion

This large multicenter study was performed to validate the diagnostic performance and clinical utility of the novel hs-cTnI-Centaur assay for the early diagnosis of AMI. We report nine major findings:

First, the diagnostic accuracy of hs-cTnI-Centaur was very high for concentrations obtained at ED presentation as well as 1h- and 2h-changes and their combinations with an AUC ranging from 0.94 to 0.97. **Second,** the diagnostic accuracy of hs-cTnI-Centaur was comparable to the two hs-cTn assays already in clinical use: hs-cTnT-Elecsys and hs-cTnI-Architect. This finding was consistent in the overall population as well as in early presenters. **Third,** as compared to the uniform cut-offs, sex-specific cut-offs provided slightly higher sensitivity and NPV, but lower specificity and PPV in women, and slightly lower sensitivity and NPV, but higher specificity and PPV in men. Whereas the use of the sex-specific 99th percentile in women seems reasonable for classification of women at low risk, the use in men seems to be associated with potential harm. **Fourth,** the application of the derived 0/1h-algorithm for hs-cTnI-Centaur, defined by concentrations at presentation and its absolute change within 1h, in the internal validation cohort resulted in very high safety in the rule-out zone with a NPV of 99.7% and a sensitivity of 99.1%, as well as a high PPV in the rule-in zone for AMI. Only two patients were missed, both with falling hs-cTn concentrations possibly due to late presentation to the ED. **Fifth**, overall, the performance of the 0/1h-algorithm for hs-cTnI-Centaur was comparable to that of the established 0/1h-algorithms for hs-cTnT-Elecsys and hs-cTnI-Architect, and also similar to their performance in previous studies.(3,7,21) In contrast to the established 0/1h-algorithms, the novel hs-cTnI-Centaur 0/1h-algorithm uses a slightly higher cut-off (3ng/L) for direct rule-out instead of its LoD (2ng/L). This resulted in a greater proportion of patients ruled-out than using the LoD (16% vs. 7%; P<0.001). As with most other early rule-out algorithms, the cut-offs for rule-out of AMI of the 0/1h-algorithm and the 0/2h-algorithm for hs-cTnI-

Centaur are very low and therefore in a range where the assay has suboptimal precision. This aspect highlights the need for further independent validation studies. As it is unknown to what extent the analytical qualification as a hs-cTnI assay correlate with the diagnostic accuracy for AMI as quantified by the AUC and the clinical utility as quantified by the performance of the 0/1h- and 0/2h-algorithms, it was mandatory to prospectively evaluate them in this large diagnostic study. **Sixth**, the application of the derived 0/2h-algorithm for the hs-cTnI-Centaur, defined by concentrations at presentation and its absolute change within 2h, in the internal validation cohort resulted also in a very high NPV and sensitivity of 100%, and high PPV for AMI. **Seventh,** the overall efficacy of the novel hs-cTnI-Centaur 0/1h- and 0/2h-algorithms were high by assigning about 70% of patients to either rule-out or rule-in within 1h or 2h (rule-out efficacy was even higher for the 0/2h-algorithm), with only about 30% of patients remaining in the observe zone. Of note, more than one fourth (28%) of all patients were either directly ruled-out or ruled-in for AMI at presentation based on a single hs-cTnI-Centaur concentration without the need for serial hs-cTnI sampling. **Eighth,** these findings were internally validated when using as an additional reference standard the second adjudication including serial hs-cTnI concentrations. By the use of a reference standard including hs-cTnI in addition to a reference standard including hs-cTnT, this large diagnostic study of patients presenting with suspected AMI overcame the small but inherent verification bias of previous studies that used only one (hs)-cTn assay as part of the reference standard(7,11,19,23,30). Using the second adjudication resulted in final diagnoses that slightly differed from those using the primary adjudication. This can be explained by the differences among both assays, e.g. by the fact that 99th percentiles are not biological equivalent to each other. This methodological detail further increases the generalizability of our findings.

Ninth, survival in patients assigned to the rule-out zone by the 0/1h-algorithm was 100% after 30 days and 98.4% after two years, further underscoring the safety of early discharge from the ED for most patients classified as rule-out, with further outpatient management as clinically

appropriate. Similarly, MACE-free survival within 30 days in patients triaged towards ruled-out was very high at 99.4%. Of note, the rather high rate of all-cause mortality during follow-up and MACE within 30 days of observe patients can be at least in part explained by the high incidence of chronic diseases such as chronic heart failure which are directly associated with high rates of both overall mortality and MACE.

The findings of the present study have enormous clinical implications as they will allow a substantial number of additional institutions to clinically introduce hs-cTn testing into their management of patients with suspected AMI and, thereby, to adopt current clinical practice guideline recommendations without the logistic challenges and costs of introducing an additional analyzer exclusively for the measurement of hs-cTn.(2–4,10) These findings also extend and corroborate previous work with the two other hs-cTn assays.(3,7,19,30) Accordingly, the same concept and caveats apply to the most appropriate clinical use of any of the three hs-cTn assays and their respective 0/1h- and 0/2h-algorithms in the early diagnosis of AMI.(3,7,11,15,19,30) First, these algorithms should only be applied after STEMI has been ruled-out by the ECG performed at presentation. Second, although the hs-cTnI-Centaur 0/1h- and 0/2h-algorithm had a very high NPV and sensitivity for AMI, they should always be used in conjunction with all other clinical information including a detailed assessment of chest pain characteristics, physical examination, and the ECG. Additional measurements of hs-cTnI at e.g. 3h are advised whenever the patient remains symptomatic or clinical judgment still argues in favor of AMI. These will help to detect the rare but existing phenomenon of delayed release of hs-cTn into the circulation, particularly in early presenters.(3) It will also help detect rare but possible errors in the handling of the clinical blood samples, e.g. blood sample of a patient without AMI (and normal hs-cTnI concentrations) erroneously attributed to a patient with AMI. Third, not all patients triaged towards rule-out of AMI are appropriate candidates for early discharge from the ED. Fourth, patients triaged towards rule-in in general are candidates for early coronary angiography. About 75% of patients triaged towards rule-in will be found to

have AMI. Most of the remaining patients in the rule-in zone will still benefit from coronary angiography for diagnostic and possible therapeutic purposes as they will be found to have Tako-Tsubo cardiomyopathy, myocarditis, and unstable angina.(3)

Some limitations merit consideration when interpreting these findings. **First,** this study was conducted in ED patients with symptoms suggestive of AMI. Further studies are required to quantify the utility of rule-out and rule-in strategies in patients with either a higher pre-test probability (e.g., in a coronary care unit setting) or in patients with a lower pre-test probability (e.g., in a general practitioner setting) for AMI, as well as in the inherently challenging group of critically ill patients. **Second,** the data presented were obtained from a prospective diagnostic study. Studies applying the diagnostic algorithms prospectively for clinical decision-making are warranted. **Third,** not all patients with acute chest pain had a second set of laboratory measurements at 1h and later. The most common reasons for missing blood samples were logistic issues in the ED that precluded blood draw around the 1h-window. This limitation is inherent to studies enrolling consecutive patients and is very unlikely to have affected the main findings of the present study. **Fourth,** although we used the most stringent methodology to adjudicate the presence or absence of AMI including central adjudication by experienced cardiologists, we still may have misclassified a small number of patients.(4) **Fifth,** our findings are specific to the hs-cTnI-Centaur assay. The derived 0/1h- and 0/2h-algorithm cannot be generalized to other hs-cTnI assays. **Sixth,** we cannot generalize our findings to patients with terminal kidney failure requiring dialysis, since they were excluded from this study. Finally, we acknowledge that using hs-cTnT , an assay that is different from hs-cTnI assays, as the gold standard assay for the primary validation may not be the ideal way to validate a hs-cTnI assay. However, we addressed this potential limitation by use of a secondary adjudication including serial hs-cTnI concentrations.

In conclusion, the diagnostic accuracy of the novel hs-cTnI-Centaur assay for AMI is very high and comparable to both well-established hs-cTn assays: hs-cTnT-Elecsys and hs-

cTnI-Architect. Simple algorithms incorporating hs-cTnI-Centaur concentrations at presentation and absolute changes within the first 1h or 2h, allow triage towards safe rule-out and accurate rule-in of AMI within 1h or 2h in the majority of patients presenting with chest pain to the ED.

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Disclosures

The authors designed the study, gathered and analyzed the data, vouched for the data and analysis, wrote the paper, and decided to publish. Drs. Boeddinghaus, Twerenbold, Nestelberger, Badertscher, Rubini Gimenez, Wildi, Puelacher, 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

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727 **Tables**

Table 1	Baseline Characteristics of the Patients			
	All patients (n=1755)	AMI (n=318)	No AMI (n=1437)	*P*-Value
Age – years	62 (49-75)	72 (59-80)	60 (47-73)	<0.001
Male gender – no. (%)	1216 (69)	231 (73)	985 (69)	0.15
Time from cpo to first blood draw - hours	5 (3-10)	5 (3-11)	5 (3-10)	0.41
Early presenters (within 3h after cpo)	603 (34%)	106 (33%)	497 (35%)	0.67
Risk factors – no. (%)				
Hypertension	1087 (62)	255 (80)	832 (58)	<0.001
Hypercholesterolemia	876 (50)	219 (69)	657 (46)	<0.001
Diabetes	317 (18)	84 (27)	233 (16)	<0.001
Current smoking	442 (25)	77 (24)	365 (25)	0.69
History of smoking	658 (38)	131 (42)	527 (37)	0.11
History – no. (%)				
Coronary artery disease	619 (35)	160 (50)	459 (32)	<0.001
Previous MI	409 (23)	107 (34)	302 (21)	<0.001
Previous revascularization	479 (27)	116 (37)	363 (25)	<0.001
Peripheral artery disease	116 (7)	48 (15)	68 (5)	<0.001
Previous stroke	89 (5)	24 (8)	65 (5)	0.03
ECG findings – no. (%)				
Left bundle branch block	55 (3)	15 (5)	40 (3)	0.07
ST-segment depression	156 (9)	88 (28)	68 (5)	<0.001
T-wave inversion	152 (9)	39 (12)	113 (8)	0.01
No significant ECG abnormalities	1358 (77)	169 (53)	1189 (83)	<0.001
Body mass index (kg/m^2)	27 (24-30)	26 (24-29)	27 (24-30)	0.47
Laboratory findings				
Creatinine clearance, $mL/min/m^2$	84 (68-100)	72 (54-89)	86 (70-102)	<0.001
Chronic medication – no. (%)				
Aspirin	638 (36)	155 (49)	483 (34)	<0.001
Vitamin K antagonists	143 (8)	31 (10)	112 (8)	0.25
B-blockers	595 (34)	137 (43)	458 (32)	<0.001
Statins	613 (35)	140 (44)	473 (33)	<0.001
ACEIs/ARBs	656 (37)	167 (53)	489 (34)	<0.001
Calcium antagonists	236 (13)	59 (19)	177 (12)	0.003
Nitrates	200 (11)	65 (20)	135 (9)	<0.001

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729 Numbers are presented as median (IQR) or numbers (%). CPO denotes chest pain onset; AMI
730 denotes acute myocardial infarction; ECG denotes electrocardiogram; ACEIs denotes
731 angiotensin-converting-enzyme inhibitors. ARBs denotes angiotensin receptor blockers.

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Table 2A	**Performance of the hs-cTnI-Centaur 0/1h-algorithm in the validation cohort (n=675) according to time since cpo**				
Time since cpo until first study blood draw	**Direct rule-out** 0h<3ng/L (if cpo>3h)	**Overall rule-out** Direct rule-out or 0h<6ng/L AND 1h-delta <3ng/L	**Observe zone**	**Direct rule-in** 0h≥120ng/L	**Overall rule-in** 0h≥120ng/L OR 1h-delta ≥12ng/L
≤1h (n=38/675)	n/a	19/38 (50%) Sens.: 100% (61.0-100) NPV: 100% (83.2-100)	11/38 (29%) NSTEMI: 27%	3/38 (8%) Spec.: 96.9% (84.3-99.4) PPV: 66.7% (20.8-93.9)	8/38 (21%) Spec.: 84.4% (68.2-93.1) PPV: 37.5% (13.7-69.4)
≤2h (n=171/675)	n/a	79/171 (46%) Sens.: 100% (89.6-100) NPV: 100% (95.4-100)	56/171 (33%) NSTEMI: 14%	17/171 (10%) Spec.: 97.8% (93.8-99.3) PPV: 82.4% (59.0-93.8)	36/171 (21%) Spec.: 92.0% (86.3-95.5) PPV: 69.4% (53.1-82.0)
≤3h (n=252/675)	n/a	117/252 (46%) Sens.: 100% (92.3-100) NPV: 100% (96.8-100)	83/252 (33%) NSTEMI: 13%	28/252 (11%) Spec.: 97.1% (93.8-98.7) PPV: 78.6% (60.5-89.8)	52/252 (21%) Spec.: 91.7% (87.2-94.8) PPV: 67.3% (53.8-78.5)
≤4h (n=325/675)	16/325 (5%) Sens.: 100% (93.5-100) NPV: 100% (80.6-100)	151/325 (47%) Sens.: 100% (93.5-100) NPV: 100% (97.5-100)	112/352 (34%) NSTEMI: 11%	33/325 (10%) Spec.: 97.4% (94.7-98.7) PPV: 78.8% (62.2-89.3)	62/325 (19%) Spec.: 93.0% (89.3-95.4) PPV: 69.4% (57.0-79.4)
≤5h (n=380/675)	26/380 (7%) Sens.: 100% (94.2-100) NPV: 100% (87.1-100)	176/380 (46%) Sens.: 100% (94.2-100) NPV: 100% (97.9-100)	134/380 (35%) NSTEMI: 10%	40/380 (11%) Spec.: 97.2% (94.7-98.5) PPV: 77.5% (62.5-87.7)	70/380 (19%) Spec.: 93.4% (90.1-95.6) PPV: 70.0% (58.5-79.5)
≤6h (n=427/675)	37/427 (9%) Sens.: 100% (94.8-100) NPV: 100% (90.6-100)	196/427 (46%) Sens.: 98.6% (92.3-99.7) NPV: 99.5% (97.2-99.9)	151/427 (35%) NSTEMI: 9%	44/427 (10%) Spec.: 97.2% (94.9-98.5) PPV: 77.3% (63.0-87.2)	80/427 (19%) Spec.: 93.0% (89.9-95.2) PPV: 68.8% (57.9-77.8)
	Performance of the hs-cTnI-Centaur 0/2h-algorithm in the validation cohort (n=361) according to time since cpo				
Time since cpo until first study blood draw	**Direct rule-out** 0h<3ng/L (if cpo>3h)	**Overall rule-out** Direct rule-out or 0h<8ng/L AND 1h-delta <7ng/L	**Observe zone**	**Direct rule-in** 0h≥120ng/L	**Overall rule-in** 0h≥120ng/L OR 1h-delta ≥20ng/L

≤1h (n=24/361)	n/a	16/24 (67%) Sens.: 100% (43.9-100) NPV: 100% (80.6-100)	3/24 (12%) NSTEMI: 0%	2/24 (8%) Spec.: 100% (84.5-100) PPV: 100% (34.2-100)	5/24 (21%) Spec.: 90.5% (71.1-97.3) PPV: 60.0% (23.1-88.2)
≤2h (n=87/361)	n/a	48/87 (55%) Sens.: 100% (79.6-100) NPV: 100% (92.6-100)	23/87 (26%) NSTEMI: 9%	8/87 (9%) Spec.: 100% (94.9-100) PPV: 100% (67.6-100)	16/87 (18%) Spec.: 95.8% (88.5-98.6) PPV: 81.3% (57.0-93.4)
≤3h (n=133/361)	n/a	75/133 (56%) Sens.: 100% (72.2-100) NPV: 100% (95.1-100)	36/133 (27%) NSTEMI: 14%	13/133 (10%) Spec.: 100% (96.6-100) PPV: 100% (77.2-100)	22/133 (17%) Spec.: 96.4% (91.0-98.6) PPV: 81.8% (61.5-92.7)
≤4h (n=175/361)	11/175 (6%) Sens.: 100% (87.9-100) NPV: 100% (74.1-100)	102/175 (58%) Sens.: 100% (87.9-100) NPV: 100% (96.4-100)	45/175 (26%) NSTEMI: 13%	17/175 (10%) Spec.: 99.3% (96.2-99.9) PPV: 94.1% (73.0-99.0)	28/175 (16%) Spec.: 95.9% (91.4-98.1) PPV: 78.6% (60.5-89.8)
≤5h (n=204/361)	18/204 (9%) Sens.: 100% (89.3-100) NPV: 100% (82.4-100)	115/204 (56%) Sens.: 100% (89.3-100) NPV: 100% (96.8-100)	58/204 (28%) NSTEMI: 16%	20/204 (10%) Spec.: 98.3% (95.0-99.4) PPV: 85.0% (64.0-94.8)	31/204 (15%) Spec.: 95.3% (91.1-97.6) PPV: 74.2% (56.8-86.3)
≤6h (n=229/361)	23/229 (10%) Sens.: 100% (89.9-100) NPV: 100% (85.7-100)	130/229 (57%) Sens.: 100% (89.8-100) NPV: 100% (97.1-100)	64/229 (28%) NSTEMI: 14%	23/229 (10%) Spec.: 97.9% (94.8-99.2) PPV: 82.6% (62.9-93.0)	35/229 (15%) Spec.: 94.9% (90.8-97.2) PPV: 71.4% (54.9-83.7)

CPO denotes chest pain onset; NPV denotes negative predictive value; PPV denotes positive predictive value. Sens. denotes sensitivity; Spec. denotes specificity. n/a denotes not available

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Table 2b	Diagnostic Performance of Uniform and Sex-specific Cut-Offs							
	Final Adjudication including hs-cTnT-Elecsys				Final Adjudication including hs-cTnI-Architect			
Hs-cTnI-Centaur	**Sensitivity**	**NPV**	**Specificity**	**PPV**	**Sensitivity**	**NPV**	**Specificity**	**PPV**
Uniform 99th perc 47ng/L.	70.4 (65.2-75.2)	93.4 (92.0-94.6)	93.3 (91.9-94.5)	70.0 (64.8-74.8)	75.5 (70.3-80.0)	94.9 (93.6-95.9)	93.5 (92.1-94.6)	70.3 (65.1-75.1)
Men 99th perc. 58ng/L	65.2 (58.9-71.1)	92.1 (90.3-93.6)	94.6 (93.0-95.9)	73.9 (67.4-79.5)	70.8 (64.3-76.5)	93.9 (92.2-95.2)	94.7 (93.2-95.9)	73.9 (67.4-79.5)
Women 99th perc. 39ng/L	77.3 (67.5-84.8)	95.4 (93.0-97.0)	92.0 (89.1-94.2)	65.4 (55.8-73.8)	80.2 (70.6-87.3)	96.1 (93.8-97.5)	92.3 (89.4-94.4)	66.3 (56.8-74.7)
Hs-cTnI-Architect								
Uniform 99th perc. 26.2ng/L	77.7 (73.3-81.6)	93.9 (92.6-95.1)	92.5 (91.1-93.8)	68.4 (63.3-73.2)	78.9 (73.9-83.1)	95.5 (94.3-96.5)	92.9 (91.4-94.1)	69.3 (64.2-74.0)
Men 99th perc. 34.2ng/L	67.0 (60.6-72.7)	92.4 (90.6-93.9)	94.3 (92.7-95.6)	73.3 (67.0-78.9)	73.1 (66.8-78.6)	94.3 (92.7-95.6)	94.5 (92.9-95.8)	73.8 (67.5-79.3)
Women 99th perc. 15.6ng/L	84.1 (75.0-90.3)	96.6 (94.3-97.9)	87.6 (84.2-90.3)	56.9 (48.3-65.1)	88.4 (79.9-93.6)	97.5 (95.5-98.7)	88.1 (84.7-90.7)	58.5 (49.9-66.6)
Hs-cTnT-Elecsys								
Uniform 99th perc 14ng/L	94.3 (91.2-96.4)	98.4 97.5-99.0)	78.1 (75.9-80.2)	48.9 (44.9-52.8)	93.3 (89.9-95.6)	98.2 (97.3-98.9)	76.9 (74.7-79.0)	45.3 (41.4.49.2)
Men 99th perc. 15.5ng/L	91.3 (87.0-94.3)	97.5 (96.2-98.4)	80.6 (78.0-83.0)	52.4 (47.5-57.2)	89.6 (84.8-93.0)	97.3 (95.9-98.2)	79.0 (76.4-81.4)	47.4 (42.5-52.3)
Women 99th perc. 9ng/L	98.9 (93.8-99.8)	99.6 (97.9-99.9)	58.9 (54.3-63.3)	32.0 (26.7-37.7)	97.7 (91.9-99.4)	99.2 (97.3-99.8)	58.4 (53.8-62.9)	30.9 (25.7-36.6)

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740 NPV denotes negative predictive value; PPV denotes positive predictive value; perc. denotes percentile.

Figure Legends

Figure 1 **Boxplots showing Concentrations of Hs-cTnI-Centaur at Presentation according to the Final Diagnosis**

Boxes represent medians and interquartile ranges (IQRs), while whiskers display the smallest and the largest non-outliers. Rings display outliers further than 1.5 IQRs and boxes display outliers further than 3 IQRs from the respective end of the box. AMI denotes acute myocardial infarction; hs-cTnI denotes high-sensitivity cardiac troponin I; UA denotes unstable angina.

Figure 2A **Diagnostic Accuracy of High-Sensitivity Cardiac Troponin Assays at Presentation for the Diagnosis of Acute Myocardial Infarction**

Receiver operating characteristic (ROC) curves describing the diagnostic performance of the three high-sensitivity cardiac troponin assays at presentation for the diagnosis of acute myocardial infarction.

Figure 2B **Diagnostic Accuracy of High-Sensitivity Cardiac Troponin Assays at Presentation for the Diagnosis of Acute Myocardial Infarction in Early Presenters**

Receiver operating characteristic (ROC) curves describing the diagnostic performance of the three high-sensitivity cardiac troponin assays at presentation for the diagnosis of acute myocardial infarction in patients presenting to the emergency department within three hours after chest pain onset.

Figure 3 A+B **Performance of the High-Sensitivity Cardiac Troponin I Centaur 0/1h-algorithm in the Derivation and Validation Cohort**

(A) Performance of the hs-cTnI-Centaur 0/1h-algorithm in the derivation cohort and (B) validation cohort. ⎪Delta 1h⎪ denotes absolute (unsigned) change of high-sensitivity cardiac troponin I within 1 hour; NSTEMI denotes non-ST-elevation myocardial infarction; NPV denotes negative predictive value; Sens. denotes sensitivity; PPV denotes positive predictive value; Spec. denotes specificity. *if chest pain onset >3h before presentation to the emergency department.

Figure 3 C+D **Performance of the High-Sensitivity Cardiac Troponin I Centaur 0/2h-algorithm in the Derivation and Validation Cohort**

(C) Performance of the hs-cTnI-Centaur 0/2h-algorithm in the derivation cohort and (D) validation cohort. ⎪Delta 2h⎪ denotes absolute (unsigned) change of high-sensitivity cardiac troponin I within 2 hours; NSTEMI denotes non-ST-elevation myocardial infarction; NPV denotes negative predictive value; Sens. denotes sensitivity; PPV denotes positive predictive value; Spec. denotes specificity. *if chest pain onset >3h before presentation to the emergency department.

Figure 4 **Short-term and Long-term Survival of Patients classified according to the High-sensitivity Cardiac Troponin I Centaur 0/1h-algorithm**

Kaplan-Meier curves depicting overall survival within 30 days and 720 days according to classification of the high-sensitivity cardiac troponin I Centaur 0/1h-algorithm.