Two-hour algorithm for rapid triage of suspected acute myocardial infarction using a high-sensitivity cardiac troponin I assay

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Abstract

Background: We aimed to derive and externally validate a 0/2h-algorithm using the high-sensitivity cardiac troponin I (hs-cTnI)-Access assay.

Methods: We enrolled patients presenting to the emergency department with symptoms suggestive of acute myocardial infarction (AMI) in two prospective diagnostic studies using central adjudication. Two independent cardiologists adjudicated the final diagnosis including all available medical information including cardiac imaging. hs-cTnI-Access concentrations were measured at presentation and after 2h in a blinded fashion.

Results: AMI was the adjudicated final diagnosis in 164/1131 (14.5%) patients in the derivation cohort. Rule-out by the hs-cTnI-Access 0/2h-algorithm was defined as 0h-hs-cTnI-Access concentration <4ng/L in patients with an onset of chest pain >3h (direct rule-out), or a 0h-hs-cTnI-Access concentration <5ng/L and an absolute change within 2h <5ng/L in all other patients. Derived thresholds for rule-in were a 0h-hs-cTnI-Access concentration ≥50ng/L (direct rule-in), or an absolute change within 2h ≥20ng/L. In the derivation cohort, these cut-offs ruled-out 55% of patients with a negative predictive value (NPV) of 99.8% (95%CI, 99.3-100), sensitivity of 99.4% (95%CI 96.5-99.9) and ruled-in 30% of patients with a positive predictive value (PPV) of 73% (95%CI, 66.1-79). In the validation cohort, AMI was the adjudicated final diagnosis in 88/1280 (6.9%) patients. These cut-offs ruled-out 77.9% of patients with a NPV of 99.8% (95%CI, 99.3-100), sensitivity of 97.7% (95%CI 92.0-99.7) and ruled-in 5.8% of patients with a PPV of 77% (95%CI, 65.8-86) in the validation cohort.

Conclusions: Safety and efficacy of the I hs-cTnI-Access 0/2h-algorithm for triage towards rule-out or rule-in of AMI are very high.

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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

PPV – Positive predictive value

IQR – Interquartile range

Introduction

Patients with symptoms suggestive of an acute myocardial infarction (AMI) such as chest discomfort or angina pectoris, account for approximately 10% of all emergency department (ED) consultations worldwide(1) Early diagnosis of AMI is important for immediate initiation of appropriate, evidence-based therapy. For early rule-out and rule-in of AMI, electrocardiography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstones and complement clinical assessment.(2–4)

High-sensitivity cardiac troponin (hs-cTn) assays allow the precise measurement of cTn concentrations even in the normal range,(2) and have improved the diagnostic accuracy for AMI.(3,4) During the last decade, two hs-cTnT/I assays have been extensively investigated in large diagnostic studies, including the derivation and validation of safe and effective 0/1h-algorithms and 0/2h-algorithms(5–14) These rapid triage algorithms are recommended by the European Society of Cardiology (ESC) for routine clinical use with a class I recommendation. (7,15)

Recently, the new hs-cTnI-Access assay was developed.(16–18) Here, we aimed to follow the ESC recommendations to derive and externally validate an assay-specific 0/2h-algorithm. The algorithm incorporates hs-cTnI-Access concentrations at ED presentation and absolute 2h-changes for the very early triage of patients towards rule-out or rule-in of AMI.

Materials and Methods

Study design and population

We enrolled adult patients presenting to the ED with suspected AMI in large prospective multicenter diagnostic studies carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE), 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), was used as the derivation cohort.(3,19,20) Patients from two studies using similar inclusion and exclusion criteria were used as the external validation cohort: Accelerated Diagnostic Protocol to Assess patients with chest Pain symptoms using contemporary Troponins as the only biomarker (ADAPT) and Improved Assessment of Chest Pain Trial (IMPACT). ADAPT was a multicenter, diagnostic study enrolling patients between November 2007 and February 2011 in two study centers in Australia and New Zealand (16,21,22). Only the Australian data were available for this study. IMPACT was an intervention trial conducted at the same Australian site between February 2011 and March 2014.(23) Patients with ST segment elevation myocardial infarction (STEMI) have been excluded from analysis in all cohorts.

Clinical Assessment

In both the derivation and validation cohorts we included unselected patients presenting to the ED with acute chest discomfort. All patients underwent a clinical assessment that included standardized and detailed medical history incorporating assessment of chest pain characteristics, vital signs, physical examination, 12-lead electrocardiogram (ECG), continuous ECG rhythm monitoring, pulse oximetry, standard blood tests, and chest radiography and echocardiography if indicated.

Detailed methodical descriptions in both cohorts including study design, eligibility criteria and study population, adjudication of final diagnoses, follow-up and clinical endpoints are shown in the **online Supplement** including **online Supplemental Table 1**.

The authors designed the study, gathered, analyzed and reported the data according to the STARD guidelines for studies of diagnostic accuracy(24) (online **Supplemental Table 2**), vouched for the data and analysis, wrote the paper, and made the decision to submit it for publication. The sponsors had no role in the design of the study, the analysis of the data, the preparation of the manuscript, or the decision to submit the manuscript for publication.

Investigational hs-cTn measurements

Blood samples for determination of hs-cTnI-Access, hs-cTnI-Architect and hs-cTnT-Elecsys were collected into tubes containing lithium heparin plasma or serum, respectively. Additional samples were collected at 1, 2, 3, and 6h after presentation in the derivation cohort and after 2 and/or 6 to 12h in the validation cohort. Serial sampling was discontinued when a patient was discharged 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.

The hs-cTnI-Access assay (ACCESS hs-cTnI, Beckman Coulter) is a paramagnetic particle, chemiluminescent immunoassay for high sensitivity quantitative determination of cTnI concentrations in human serum and plasma using the Access Immunoassay Systems.(15–17) The hs-cTnI-Access assay has an overall 99th percentile concentration of 18ng/L (women: 12ng/L, men: 20ng/L) with a corresponding co-efficient of variation (CV) of <10%. Limit of blank (LoB) and limit of detection (LoD) have been determined to be 1.7ng/L and 2.3ng/L.

The hs-cTnT-Elecsys assay (Elecsys 2010 high-sensitivity troponin T, Roche Diagnostics) has a 99th percentile concentration of 14ng/L with a corresponding CV of 10% at 13ng/L.(2) LoB and LoD have been determined to be 3ng/L and 5ng/L.(2) The hs-cTnI-Architect assay (ARCHITECT STAT high-sensitivity troponin I, Abbott Laboratories) has a 99th percentile concentration of 26ng/L with a corresponding CV of <5% and a LoD of 1.9ng/L.(25–27) Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease formula.(28)

Reference Standard: Adjudicated Final Diagnosis

AMI was defined and cTn concentrations interpreted as recommended in current guidelines.(29–31) In brief, AMI was diagnosed when there was evidence of myocardial necrosis with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Patients with AMI were further subdivided into type 1 AMI (primary coronary events) and type 2 AMI (ischemia due to increased demand or decreased supply, for example tachyarrhythmia or hypertensive crisis).(29,32) In APACE the adjudication of final diagnoses was performed centrally in the core lab (University Hospital Basel) for all patients incorporating concentrations of (hs)-cTn. More specifically, two independent cardiologists not directly involved in patient care reviewed all available medical records (including patient history, physical examination, results of laboratory testing including hs-cTnT concentrations, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, discharge summary) pertaining to the patient from the time of ED presentation to 90-day follow-up (APACE) and to 30-day follow-up (ADAPT and Impact). Detailed information about adjudication of final diagnoses are shown in the **online Supplement**.

Derivation and validation of the hs-cTnI-Access 0/2h-algorithm

We combined hs-cTnI-Access concentrations at ED presentation and absolute 2h-changes to achieve predefined performance characteristics using the same methodology as applied in the derivation of the established hs-cTnT/I 0/2h-algorithms (14,15,32,33) (**online Supplemental Figure 1).** Derived thresholds for rule-out were selected to allow for a minimal sensitivity and negative predictive value (NPV) of 99.5% and sensitivity of 99.0%. Derived thresholds for rule-in were obtained based on a classification and regression tree (CART) analysis targeting a minimal positive predictive value (PPV) of 70%. 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. A more detailed explanation for derivation and validation of the algorithm is given within the online supplement.

The hs-cTnI-Access 0/2h-algorithm was developed in the derivation cohort in all patients with available hs-cTnI-Access measurements at ED presentation and after 2h. The algorithm was then externally validated in the validation cohort, and directly compared with the established 0/2h-algorithms.

Follow-up and statistical analysis

Clinical follow-up and statistical analysis are described in detail in the **Online Supplement**.

Results

Characteristics of patients and final adjudicated diagnosis

Patient flow for eligible patients for this analysis within the derivation and validation cohort is shown in **online Supplemental Figure 1A and 1B**. Baseline characteristics of the patients in the derivation cohort (n=1131) and the validation cohort (n=1280) are shown in **Tables 1 and 2**. Thirty-nine percent and 81% of patients presented to the ED within the first three hours after chest pain onset in both cohorts, respectively. The adjudicated final diagnosis in the derivation cohort was AMI in 164/1131 patients (14.5%), and in 88/1280 patients (6.9%) in the validation cohort.

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

Concentrations of hs-cTnI at presentation and after 2 hours were significantly higher in patients with AMI compared to those with other final diagnoses (**online Supplemental Figure 3A and 3B and Supplemental Figure 4**).

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

Derived thresholds for rule-out of AMI were defined as either a hs-cTnI-Access concentration at presentation <4ng/L in patients with an onset of chest pain >3h (direct rule-out) or as a hs-cTnI-Access concentration at presentation <5ng/L and an absolute change within 2h <5ng/L in all other patients (**online Supplemental Figure 2**). Derived thresholds for rule-in of AMI were defined as either a hs-cTnI-Access concentration at presentation ≥50ng/L (direct rule-in) or an absolute change within 2h ≥20ng/L. Patients fulfilling neither of the above criteria for rule-out or for rule-in were classified as observe. The hs-cTnI-Access 0/2h-algorithm classified 620 (55%) patients as rule-out, 333 (29%) as rule-in and 178 (16%) patients to observe (**Figure 1A**). The algorithm achieved a NPV of 99.8% (95%CI, 99.1-100) and a sensitivity of 99.4% (95%CI, 96.5-99.9) for rule-out (**Table 3**). PPV and specificity for rule-in were 73% (95%CI, 66.1-79.0) and 95% (95%CI, 93.5-96.2), respectively. Overall, the hs-cTnI-Access 0/2h-

algorithm allowed a definite triage (either rule-out or rule-in) in 798/1131 patients (71%).

External validation of the hs-cTnI-Access 0/2h-algorithm

Applying the derived cut-off criteria to the independent validation cohort, 997/1280 patients (77.9%) could be classified as rule-out with a corresponding NPV of 99.8% (95%CI, 99.3-100) and sensitivity of 97.7% (95%CI, 92.0-99.7; **Figure 1B, Table 3**). The 0/2h-algorithm classified 74/1280 patients (5.8%) as rule-in with a corresponding PPV of 77.0% (95%CI, 65.8-86.0) and a specificity of 98.6% (95%CI, 97.7-99.2). Overall, the hs-cTnI-Access 0/2h-algorithm allowed to triage (either rule-out or rule-in) 1071/1280 patients (84%).

Direct comparison with established 0/2h-algorithms

Overall, the diagnostic performance of the hs-cTnI-Access 0/2h-algorithm was similar to that of the hs-cTnT-Elecsys 0/2h-algorithm and the hs-cTnI-Architect 0/2h-algorithm within the derivation and the validation cohorts. (**online Supplemental Figure 5A and 5B).**

Performance of the hs-cTnI-Access 0/2h-algorithm in predefined subgroups

The performance of the hs-cTnI-Access 0/2h- algorithm in five predefined subgroups including early presenters was very good and comparable to that in the overall cohort (**online Supplemental Figure 6A and 6B**).

Prognostic performance of the hs-cTnI-Access 0/2h-algorithm

Within the derivation cohort median follow-up time was 735 days (IQR, 410-772) with 9 deaths occurring within 30 days and 60 deaths within two years. Cumulative 30-day survival rates were 99.7%, 98.5% and 97.1% (standard error 0.2, 0.7 and 1.2 respectively; log-rank, *P*=0.001) in the rule-out, observe and rule-in group,

respectively. At 2 years, cumulative survival rates were 98.2%, 91.1% and 89.6%, within the rule-out, rule-in and observe group, respectively (standard error 0.6, 1,9 and 2.3, respectively; log-rank, *P*<0.001; **Figure 2A**).

Within the validation cohort the median follow-up time was 365 days (IQR, 365-365) with 2 deaths occurring within 30 days and 13 deaths within one year. Cumulative 30-day survival rates were 100%, 99.4% and 98.2% (standard error 0, 0.6 and 0.2, respectively log-rank, *P*=0.005) in the rule-out, observe and rule-in group, respectively. After one-year, cumulative survival rates were 99.9%, 95.2% and 92.9% within the rule-out, observe, and rule-in group, respectively (standard error 0.1, 1.6 and 3.4, respectively; log-rank, *P*<0.001; **Figure 2B**).

Discussion

We derived and validated a 2h-algorithm for the hs-cTnI-Access assay in three large, well-characterized prospective diagnostic cohorts using central adjudication of AMI. Institutions using this assay will be able to apply this attractive rapid protocol to triage a high volume of patients presenting to ED's with symptoms suggestive of AMI.(13,14) We report **six** major findings:

First, the derived hs-cTnI-Access 0/2h-algorithm provided a very high (>99.5%) NPV in both the derivation and validation cohorts, while sensitivity was slightly lower in the validation cohort (97.7%) as compared to the derivation cohort (99.4%). The high safety of this approach is further highlighted by the fact that both type 1 and type 2 AMI were included in this analysis and that among more than 2400 patients enrolled, the hs-cTnI-Access 0/2h-algorithm incorrectly triaged only one patient with type 1 AMI. Still, as the point estimate for sensitivity in patients triaged towards rule-out was lower than aimed for, further studies with an even higher number of patients with AMI are

required. **Second**, the PPV and specificity for AMI of patients triaged towards rule-in was high enough (>70% and >95%, respectively) to justify early coronary angiography and admission to a monitored unit, particularly as most non-AMI patients in the rule-in group still have conditions that require coronary angiography for diagnostic purposes including myocarditis and takotsubo syndrome. **Third**, the overall efficacy of the hs-cTnI-Access 0/2h-algorithm was very high by assigning more than 70% of patients to either rule-out or rule-in, with less than 30% of patients remaining in the observe zone. **Fourth,** overall, the performance of the 0/2h-algorithm for hs-cTnI-Access was comparable to that of the established 0/2h-algorithms for hs-cTnT-Elecsys and hs-cTnI-Architect, and also similar to their performance in previous studies.(33)(14)(16) **Fifth**, the performance of the hs-cTnI-Access 0/2h-algorithm was also very good in five predefined subgroups including early presenters. **Sixth,** survival in patients triaged towards rule-out by the 0/2h-algorithm was very high in both cohorts, further underscoring the convenient and safety of early discharge from the ED for most patients classified as rule-out, with further outpatient management as clinically appropriate.

These findings corroborate and extend previous pilot studies with hs-cTnI-Access,(15–17) and may have important clinical implications, as they will allow institutions utilizing the Beckman Coulter platform, to introduce the hs-cTnI-Access 0/2h-algorithm for management of patients with suspected AMI. For some sites, adoption of clinical practice guidelines without the logistic challenges and costs of introducing additional analyzers will be a major benefit. (29,30,32)

Local institution and physician preferences, as well as patient flow characteristics, will determine whether performing the second hs-cTn measurement at 1h (for the 0/1h-algorithm) or at 2h (for the 0/2h-algorithm) is preferable. Overall, the performance characteristics of the new hs-cTnI-Access 0/2h-algorithm were comparable to that of

315 the recently developed hs-cTnI-Access 0/1h-algorithm, which allowed triage of 60% of
316 patients towards rule-out with a sensitivity of 98.9% and 15% of patients towards ruled
317 in with a specificity of 95.9% in the respective validation cohort.(18) Greenslade and
318 colleagues reported a high sensitivity of 99% and NPV of 99.8% with 34% ruled-out
319 using a single cut-off strategy with <2 ng/L (LOD strategy) for the hs-cTnI Access
320 Assay. A cutoff of <6 ng/L enabled 78.8% of patients to be ruled out on presentation,
321 with a sensitivity of 93.9% and a NPV of 99.5%(16) The present study used a
322 combination of 0h and 2h hs-cTnI concentrations. A cut-off of 4 ng/L at presentation
323 together with a chest pain onset of >3h revealed the best performance for direct rule-
324 out. Simplicity and higher efficacy may favor these hs-cTn-only algorithms versus other
325 well-validated algorithms also including formal risk scores. (18,21,34–37) The present
326 findings extend and corroborate previous work with other hs-cTnT/I assays.
327 (7,11,38,39) Accordingly, the same concepts and caveats apply to the most
328 appropriate clinical use of any of the hs-cTnT/I assays and their respective 0/1h or
329 0/2h-algorithms in the early diagnosis of AMI. (7,13,14,35) First, these algorithms
330 should only be applied after ST elevation MI has been ruled-out by the ECG performed
331 at presentation. Second, although the hs-cTnI-Access 0/2h-algorithm had a very high
332 NPV for AMI, the algorithm should always be used in conjunction with all other clinical
333 information, including a detailed assessment of chest pain characteristics, physical
334 examination, and the ECG. Additional measurements of hs-cTnI (for example at 3h)
335 are advised whenever the patient is in the observe group, remains symptomatic, or
336 where clinical judgment still argues in favor of AMI. These will help to detect the rare
337 but existing phenomenon of delayed release of hs-cTn into the circulation, particularly
338 in early presenters.(32) It will also help to detect uncommon but possible errors in the
339 handling of the clinical blood samples. Third, not all patients triaged towards rule-out
340 of AMI are appropriate candidates for early discharge from the ED. Fourth, patients

triaged towards rule-in AMI in general are candidates for consideration of 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 may still benefit from coronary angiography for diagnostic and possible therapeutic purposes as common differential diagnoses including takotsubo syndrome, myocarditis, and unstable angina.(32)

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 this 0/2h-algorithm 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 practice setting) for AMI, as well as in the inherently challenging group of critically ill patients. **Second,** the data presented were obtained from prospective observational diagnostic studies. Prospective studies applying the diagnostic algorithm in clinical decision-making are warranted. **Third,** not all patients with acute chest pain had a second set of laboratory measurements at 2h and later. The most common reasons for missing blood samples were logistics issues in the ED that precluded blood draw around the 2h-window. This limitation is inherent to studies enrolling consecutive patients and is very unlikely to have affected the main findings of the present study. Additionally, for the reference standard, not all patients had measurements of hs-cTn at 3-6h after presentation. In all remaining patients for adjudication of final diagnoses the ESC hs-cTnT 0/1h algorithm has been used. **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.(30) This invariably would have led to an underestimation of the true diagnostic accuracy of the 0/2h-algorithm. **Fifth**, although all laboratory procedures were performed according to stringent standardized

operating procedures, human error in the handling of the study specific blood samples may have occurred in a small number of samples leading incorrect to results pertaining to the individual patient. This again would have led to an underestimation of the true diagnostic accuracy of the 0/2h-algorithm. In fact, this error might well have occurred in all three AMI patients presumably missed by the 0/2h-algorithm as not only hs-cTnI-Access, but all hs-cTnT/I concentrations measured from the study specific blood samples were in the low normal range. **Sixth,** our findings are specific to the hs-cTnI-Access assay. The derived 0/2h-algorithm cannot be generalized to other hs-cTnI assays. **Seventh,** we cannot generalize our findings to patients with terminal kidney failure requiring dialysis, since they were excluded in the derivation cohort.

In conclusion, using a simple algorithm incorporating hs-cTnI values at presentation and absolute changes within the first 2 hours, a safe rule-out or accurate rule-in of AMI could be performed in the vast majority of patients presenting with chest pain. The use of this algorithm seems to be safe and highly efficacious. It may substantially shorten the time needed for rule-out and rule-in of AMI. About one quarter of chest pain patients will remain in the observe zone and continue to require more prolonged monitoring and serial hs-cTnI testing at 3-6h. Further prospective studies are inevitable to validate these findings

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References

1. Twerenbold R, Boeddinghaus J, Nestelberger T, Wildi K, Rubini Gimenez M, Badertscher P, et al. Clinical Use of High-Sensitivity Cardiac Troponin in Patients With Suspected Myocardial Infarction. J Am Coll Cardiol 2017;70:996–1012.
2. 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.
3. 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.
4. 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.
5. Neumann JT, Twerenbold R, Ojeda F, Sörensen NA, Chapman AR, Shah AS V, et al. Application of High-Sensitivity Troponin in Suspected Myocardial Infarction. N Engl J Med 2019;380:2529–40.
6. Mair J, Lindahl B, Hammarsten O, Müller C, Giannitsis E, Huber K, et al. How is cardiac troponin released from injured myocardium? Eur Heart J Acute Cardiovasc Care 2018;7:553–60.
7. 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.
8. Neumann JT, Sörensen NA, Schwemer T, Ojeda F, Bourry R, Sciacca V, et al. Diagnosis of Myocardial Infarction Using a High-Sensitivity Troponin I 1-Hour

Algorithm. JAMA Cardiol 2016;1:397–404.

9. Shah AS V, Anand A, Sandoval Y, Lee KK, Smith SW, Adamson PD, et al. High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study. Lancet 2015;386:2481–8.

10. Twerenbold R, Badertscher P, Boeddinghaus J, Nestelberger T, Wildi K, Rubini Gimenez M, et al. Effect of the FDA Regulatory Approach on the 0/1-h Algorithm for Rapid Diagnosis of MI. J Am Coll Cardiol 2017;70:1532–4.

11. Boeddinghaus J, Twerenbold R, Nestelberger T, Badertscher P, Wildi K, Puelacher C, et al. Clinical Validation of a Novel High-Sensitivity Cardiac Troponin I Assay for Early Diagnosis of Acute Myocardial Infarction. Clin Chem 2018;64:1347–60.

12. 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.e4.

13. Boeddinghaus J, Reichlin T, Cullen L, Greenslade JH, Parsonage WA, Hammett C, et al. Two-Hour Algorithm for Triage toward Rule-Out and Rule-In of Acute Myocardial Infarction by Use of High-Sensitivity Cardiac Troponin I. Clin Chem 2016;62:494–504.

14. 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.e4.

15. Pretorius CJ, Tate JR, Wilgen U, Cullen L, Ungerer JPJ. A critical evaluation of the Beckman Coulter Access hsTnI : Analytical performance, reference interval and concordance. Clin Biochem 2018;55:49–55.

16. Greenslade J, Cho E, Van Hise C, Hawkins T, Parsonage W, Ungerer J, et al. Evaluating Rapid Rule-out of Acute Myocardial Infarction Using a High-Sensitivity Cardiac Troponin I Assay at Presentation. Clin Chem 2018;64:820–9.
17. Kavsak PA, Malinowski P, Roy C, Clark L, Lamers S. Assessing matrix, interferences and comparability between the Abbott Diagnostics and the Beckman Coulter high-sensitivity cardiac troponin I assays. Clin Chem Lab Med 2018;56:1176–81.
18. Boeddinghaus J, Nestelberger T, Twerenbold R, Koechlin L, Meier M, Troester V, et al. High-Sensitivity Cardiac Troponin I Assay for Early Diagnosis of Acute Myocardial Infarction. Clin Chem 2019;65:893–904.
19. Nestelberger T, Boeddinghaus J, Badertscher P, Twerenbold R, Wildi K, Breitenbücher D, et al. Effect of Definition on Incidence and Prognosis of Type 2 Myocardial Infarction. J Am Coll Cardiol 2017;70.
20. Badertscher P, Boeddinghaus J, Twerenbold R, Nestelberger T, Wildi K, Wussler D, et al. Direct Comparison of the 0/1h and 0/3h Algorithms for Early Rule-Out of Acute Myocardial Infarction. Circulation 2018;137:2536–8.
21. Than M, Cullen L, Aldous S, Parsonage W a., Reid CM, Greenslade J, et al 2-Hour accelerated diagnostic protocol to assess patients with chest pain symptoms using contemporary troponins as the only biomarker: The ADAPT trial. J Am Coll Cardiol; 2012;59:2091–8.
22. Greenslade JH, Carlton EW, Van Hise C, Cho E, Hawkins T, Parsonage WA, et al. Diagnostic Accuracy of a New High-Sensitivity Troponin I Assay and Five Accelerated Diagnostic Pathways for Ruling Out Acute Myocardial Infarction and Acute Coronary Syndrome. Ann Emerg Med 2018;71:439-451.e3.
23. Cullen L, Greenslade JH, Hawkins T, Hammett C, O'Kane S, Ryan K, et al.

552 Improved Assessment of Chest pain Trial (IMPACT): assessing patients with
553 possible acute coronary syndromes. Med J Aust 2017;207:195–200.
554 24. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al.
555 STARD 2015: An Updated List of Essential Items for Reporting Diagnostic
556 Accuracy Studies. Clin Chem 2015;61:1446–52.
557 25. Koerbin G, Tate J, Potter JM, Cavanaugh J, Glasgow N, Hickman PE.
558 Characterisation of a highly sensitive troponin I assay and its application to a
559 cardio-healthy population. Clin Chem Lab Med 2012;50:871–8.
560 26. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T
561 assay 99th percentile values from a common presumably healthy population.
562 Clin Chem 2012;58:1574–81.
563 27. Krintus M, Kozinski M, Boudry P, Capell NE, Köller U, Lackner K, et al.
564 European multicenter analytical evaluation of the Abbott ARCHITECT STAT
565 high sensitive troponin I immunoassay. Clin Chem Lab Med 2014;52:1657–65.
566 28. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al.
567 Using Standardized Serum Creatinine Values in the Modification of Diet in
568 Renal Disease Study Equation for Estimating Glomerular Filtration Rate. Ann
569 Intern Med 2006;145:247.
570 29. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al.
571 Fourth universal definition of myocardial infarction (2018). Eur Heart J
572 2019;40:237-69
573 30. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al.
574 How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J
575 2012;33:2252–7.
576 31. Thygesen K, Mair J, Katus H, Plebani M, Venge P, Collinson P, et al.
577 Recommendations for the use of cardiac troponin measurement in acute

cardiac care. Eur Heart J 2010;31:2197-204.

32. 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.

33. Boeddinghaus J, Reichlin T, Cullen L, Greenslade JH, Parsonage WA, Hammett C, et al. Two-Hour Algorithm for Triage toward Rule-Out and Rule-In of Acute Myocardial Infarction by Use of High-Sensitivity Cardiac Troponin I. Clin Chem 2016;62:494–504.

34. Cullen L, Mueller C, Parsonage W a., Wildi K, Greenslade JH, Twerenbold R, et al. Validation of high-sensitivity troponin I in a 2-hour diagnostic strategy to assess 30-day outcomes in emergency department patients with possible acute coronary syndrome. J Am Coll Cardiol 2013;62:1242–9.

35. Wildi K, Cullen L, Twerenbold R, Greenslade JH, Parsonage W, Boeddinghaus J, et al. Direct comparison of 2 rule-out strategies for acute myocardial infarction: 2-h accelerated diagnostic protocol vs 2-h algorithm. Clin Chem 2017;63:1227–36.

36. Chapman AR, Anand A, Boeddinghaus J, Ferry A V., Sandeman D, Adamson PD, et al. Comparison of the efficacy and safety of early rule-out pathways for acute myocardial infarction. Circulation 2017;135:1586–96.

37. Morawiec B, Boeddinghaus J, Wussler D, Badertscher P, Koechlin L, Metry F, et al. Modified HEART Score and High-Sensitivity Cardiac Troponin in Patients With Suspected Acute Myocardial Infarction. J Am Coll Cardiol 2019;73:873–5.

38. Twerenbold R, Neumann JT, Sörensen NA, Ojeda F, Karakas M, Boeddinghaus J, et al. Prospective Validation of the 0/1-h Algorithm for Early Diagnosis of Myocardial Infarction. J Am Coll Cardiol 2018;72:620–32.

39. Reichlin T, Twerenbold R, Wildi K, Rubini Gimenez M, Bergsma N, Haaf P, et al. Prospective validation of a 1-hour algorithm to rule-out and rule-in acute myocardial infarction using a high-sensitivity cardiac troponin T assay. CMAJ 2015;187:E243-52.

Table 1 Baseline characteristics derivation cohort			
	All patients (n=1131)	AMI (n=164)	No AMI (n=967)
Age – y	61 (49-74)	71 (60-81)	59 (47-72)
Female gender	359 (32)	43 (26)	316 (33)
Time since cpo – hours	5 (2-12)	4 (2-12)	5 (2-12)
Early presenters (within 3h after CPO)	439 (39%)	72 (44%)	367 (38%)
Risk factors			
Hypertension	689 (61)	117 (71)	572 (59)
Hypercholesterolemia	580 (51)	116 (71)	464 (48)
Diabetes	199 (18)	45 (28)	154 (16)
Current smoking	279 (25)	39 (24)	240 (25)
History of smoking	432 (38)	76 (46)	356 (37)
History			
Coronary artery disease	386 (34)	73 (45)	313 (32)
Previous MI	281 (25)	59 (36)	222 (23)
Previous revascularization	329 (29)	63 (38)	266 (28)
Peripheral artery disease	62 (5.5)	24 (15)	38 (3.9)
Previous stroke	78 (6.9)	13 (7.9)	65 (6.7)
ECG findings			
Left bundle branch block	35 (3.1)	4 (2.4)	31 (3.3)
ST-segment depression	78 (6.9)	33 (20)	45 (4.7)
T-wave inversion	86 (7.6)	23 (14)	63 (6.5)
No significant ECG abnormalities	912 (81)	100 (61)	812 (84)
Body mass index – kg/m^2	27 (24-30)	26 (24-29)	27 (24-30)
Laboratory findings			
Creatinine clearance, $mL/min/m^2$	84 (70-100)	76 (60-94)	85 (71-101)
Chronic medication			
Aspirin	400 (35)	79 (48)	321 (33)
Vitamin K antagonists	135 (12)	25 (15)	110 (11)
B-blockers	388 (34)	64 (39)	324 (34)
Statins	431 (38)	76 (46)	355 (37)
ACEIs/ARBs	450 (40)	84 (51)	366 (38)
Calcium antagonists	185 (16)	40 (24)	145 (15)
Nitrates	132 (12)	34 (21)	98 (10)

622

623 Numbers are presented as numbers (%) or medians (IQR). CPO denotes chest pain
624 onset; AMI denotes acute myocardial infarction; ECG denotes electrocardiogram;
625 ACEIs denotes angiotensin-converting-enzyme inhibitors. ARBs denotes angiotensin
626 receptor blockers.

Table 2 Baseline characteristics validation cohort			
	All patients (n=1280)	AMI (n=88)	No AMI (n=1192)
Age – y	51 (43-62)	62 (53-75)	51 (43-61)
Male sex	769 (60.1%)	59 (67.0%)	710 (59.6%)
Median Time since cpo – hours	2.1 (1.2-4.2)	2.1 (1.1-4.2)	2.1 (1.2-4.2)
Early presenters (within 3h after CPO)	1035 (80.9%)	72 (81.8%)	963 (80.8%)
Risk factors			
Hypertension	558 (43.6%)	49 (55.7%)	509 (42.7%)
Hypercholesterolemia	542 (42.3%)	49 (55.7%)	493 (41.4%)
Diabetes	164 (12.8%)	18 (20.5%)	146 (12.3%)
Current smoking	354 (27.7%)	22 (25.0%)	332 (27.9%)
History of smoking	434 (33.9%)	38 (43.2%)	396 (33.2%)
History			
Coronary artery disease	221 (17.3%)	36 (40.9%)	185 (15.5%)
Previous MI	183 (14.3%)	30 (34.1%)	153 (12.8%)
Previous revascularization	159 (12.4%)	24 (27.3%)	135 (11.3%)
Peripheral artery disease	18 (1.4%)	7 (8.0%)	11 (0.9%)
Previous stroke	78 (6.1%)	9 (10.2%)	69 (5.8%)
ECG findings			
Left bundle branch block	20 (1.6%)	4 (4.6%)	16 (1.3%)
New Ischaemia on ECG	37 (2.9%)	17 (19.5%)	20 (1.7%)
ECG normal or not diagnostic of ischaemia	1059 (82.9%)	50 (57.5%)	1009 (84.7%)
Body mass index – kg/m^2	28.3 (25.0-32.8)	28.0 (23.5-31.9)	28.3 (25.0-32.9)
Laboratory findings			
eGFR	92 (78-106)	79 (56-98)	93 (79-107)
Chronic medication			
Aspirin	264 (20.6%)	30 (34.1%)	234 (19.6%)
Warfarin	51 (4.0%)	6 (6.8%)	45 (3.8%)
B-blockers	210 (16.4%)	29 (33.0%)	181 (15.2%)
Statins	322 (25.2%)	34 (38.6%)	288 (24.2%)
ACE Inhibitors	191 (14.9%)	16 (18.2%)	175 (14.7%)
Calcium antagonists	101 (7.9%)	11 (12.5%)	90 (7.6%)
Nitrates	89 (7.0%)	15 (17.1%)	74 (6.2%)

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

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Table 3 – Patients with an Adjudicated Diagnosis of AMI missed by the hs-cTnI-Access 0/2h-algorithm in both cohorts

Age	Sex	Time from CPO to first study blood draw, h	Time from CPO/Peak to presentation, h	History of CAD	hs-cTnT Elecsys# (ng/L; peak value underlined) 99th percentile 14ng/L hs-cTnT Architect (ng/L; peak value underlined) 99th percentile 26.2ng/L				hs-cTnI Access (ng/L; peak value underlined) Accu cTnI# (ng/L; peak value underlined) 99th percentile 40ng/L				ST-depression	T-inversion	Clinical discharge diagnosis	PCI performed	CABG performed
					0h	1h	2h	4-14h	0h	1h	2h	4-14h					
79*	female	1	1	Yes	18 3.9	19 5.8	22 7	<u>24</u> -	2.6 -	3.3 -	<u>4.2</u> -	- -	Yes	No	Arrhythmia	No	No
64+	Male	9	9	Yes	4.7 1.3	- -	<u>4.9</u> 1.9	- -	2.1 110	- -	<u>2.5</u> 130	- <u>130</u>	No	No	Arrhythmia	No	No
63+	Male	2	2	No	- 2.3	- -	- <u>5</u>	- -	3.2 90	- -	5.4 92	- <u>100</u>	No	No	T1 NSTEMI	Yes	No

633 *missed in hs-cTnI 0/1h-algorithm derivation cohort; +missed in hs-cTnI 0/1h-algorithm validation cohort
634 CPO denotes chest pain onset; CAD denotes coronary artery disease; CABG denotes coronary artery bypass grafting; PCI denotes
635 percutaneous coronary intervention

636 #hs-cTnT in the derivation cohort and Accu TnI in the validation cohort were measured as part of routine clinical practice onsite at the
637 time of patient presentation. All other hs-cTnT/I measurements were performed from study specific samples at a later time point after a
638 freeze/thaw cycle.
639

Figure Legends

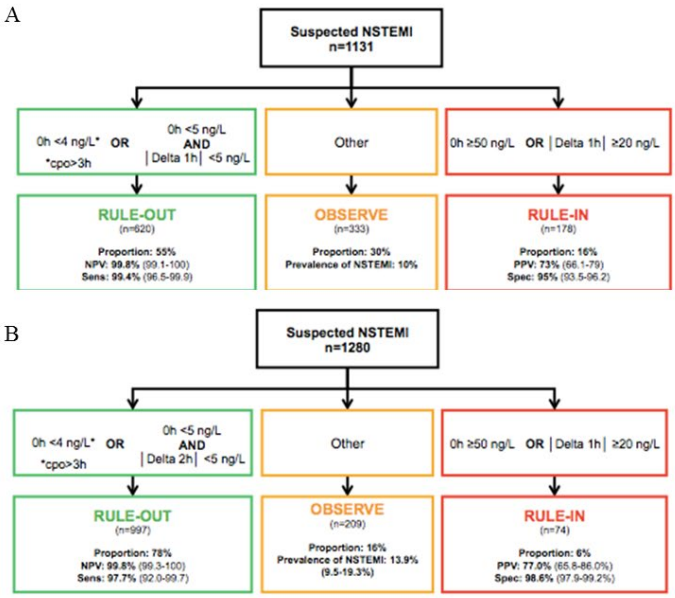

Figure 1 **Performance of the high-sensitivity cardiac troponin I Access 0/2h-algorithm in the A) derivation and B) validation cohorts**

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

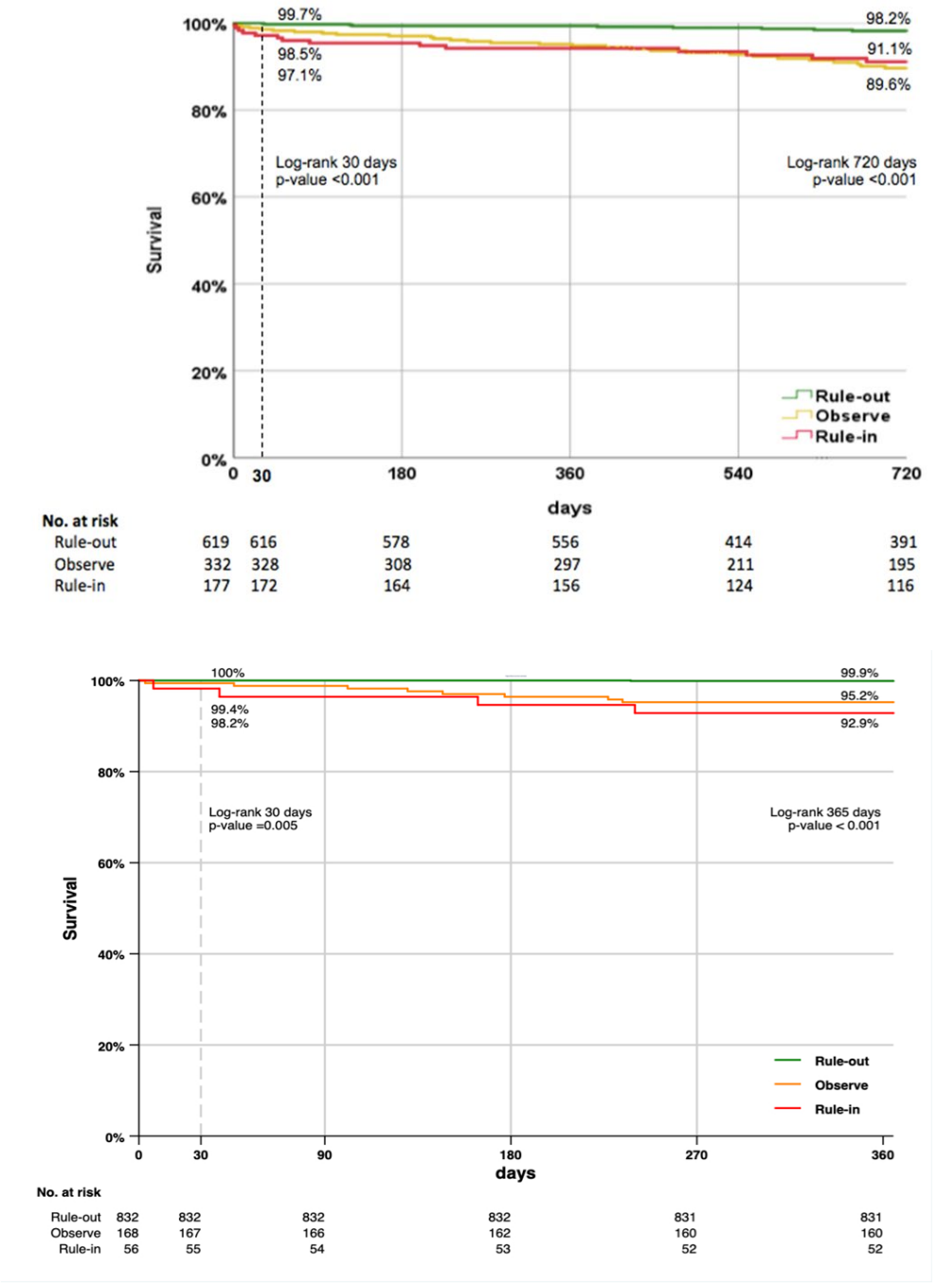

Figure 2 **Short-term and long-term Kaplan-Meier survival curves of patients classified according to the high-sensitivity cardiac troponin I Access 0/2h-algorithm for A) derivation and B) validation cohorts**