# Clinical Use of a New High-Sensitivity Cardiac Troponin I Assay in Patients with Suspected Myocardial Infarction

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## Abstract (250 words)

**Background:** We aimed to validate the clinical performance of the high-sensitivity cardiac troponin I (VITROS® Immunodiagnostic Products hs Troponin I [hs-cTnI-VITROS]) assay.

**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 including cardiac imaging twice: first, using serial hs-cTnT-Elecsys (primary analysis) and second, using hs-cTnI-Architect (secondary analysis) measurements in addition to the clinically used (hs)-cTn. Hs-cTnI-VITROS was measured at presentation and at 1h in a blinded fashion. Primary objective was direct comparison of diagnostic accuracy as quantified by the area under the receiver-operating-characteristic curve (AUC) of hs-cTnI-VITROS versus hs-cTnT-Elecsys and hs-cTnI-Architect, and in a subgroup also hs-cTnI-Centaur and hs-cTnI-Access. Secondary objectives included the derivation and validation of a hs-cTnI-VITROS-0/1h-algorithm.

**Results:** AMI was the adjudicated final diagnosis in 158/1231 (13%) patients. At presentation, the AUC for hs-cTnI-VITROS was 0.95 (95%CI, 0.93-0.96), for hs-cTnT-Elecsys 0.94 (95%CI, 0.92-0.95), and for hs-cTnI-Architect 0.92 (95%CI, 0.90-0.94). AUCs for hs-cTnI-Centaur and hs-cTnI-Access were 0.95 (95%CI, 0.94-0.97). Applying the derived hs-cTnI-VITROS-0/1h-algorithm (derivation cohort n=519) to the validation cohort (n=520), 53% of patients were ruled-out (sensitivity 100% [95%CI, 94.1-100]), and 14% of patients were ruled-in (specificity 95.6% [95%CI, 93.4-97.2]). Patients ruled-out by the 0/1h-algorithm had a survival rate of 99.8% at 30 days. Findings were confirmed in the secondary analyses using the adjudication including serial measurements of hs-cTnI-Architect.

**Conclusions:** The hs-cTnI-VITROS assay has at least comparable diagnostic accuracy to the currently best validated hs-cTnT and hs-cTnI assays.

**Clinical 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

PPV – Positive predictive value

IQR – Interquartile range

NPV – Negative predictive value

PPV – Positive predictive value

# Introduction

Patients with symptoms suggestive of an acute myocardial infarction (AMI) such as chest discomfort or angina pectoris account for about 10% of all emergency department (ED) consultations worldwide.(1) For early rule-out and rule-in of AMI, electrocardiography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstones and complement clinical assessment.(1–3)

Since high-sensitivity cardiac troponin (hs-cTn) assays were introduced, reliable measurement of cTn concentrations in the normal range became possible,(1,4–7) which increased diagnostic accuracy for AMI at presentation.(2,5,6,8,9) During the last decade, two hs-cTn assays have been extensively investigated in large diagnostic studies, including the successful derivation and validation of rapid 0/1h-algorithms.(3,7,10–22) These rapid triage algorithms are recommended with a class I recommendation in current clinical practice guidelines.(3)

Recently, the hs-cTnI-VITROS assay was developed. Before its possible implementation into routine clinical care, its performance in patients presenting with suspected AMI must be thoroughly examined. Here, we aimed to directly compare in a large multicenter diagnostic study the diagnostic accuracy of the hs-cTnI-VITROS assay with the two established hs-cTn assays (hs-cTnT-Elecsys and hs-cTnI-Architect) and two other new hs-cTnI assays (hs-cTnI-Centaur and hs-cTnI-Access). In addition, we sought to derive and validate an assay-specific 0/1h-algorithm using hs-cTnI-VITROS concentrations at ED presentation and absolute 1h-changes for the early triage of patients towards rule-out or rule-in of AMI.

# Materials and Methods

## Study design and population

Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) is an ongoing prospective international multicenter study with 12 centres in 5 countries aiming to advance the early diagnosis of AMI (ClinicalTrials.gov registry, number NCT00470587).(2,15,16,18,19,21–27) In order to best reflect the clinical application of hs-cTn, patients with ST-elevation myocardial infarction were excluded (**Online Supplemental file**).

## Adjudicated final diagnosis

Adjudication of the final diagnosis was performed by two independent cardiologists at the core laboratory (University Hospital Basel) applying the Fourth Universal Definition of AMI using two sets of data: first, all available medical records obtained during clinical care including history, physical examination, results of laboratory testing including serial clinical hs-cTn concentrations, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, cardiac magnetic resonance imaging - pertaining to the patient from the time of ED presentation to 90-day follow up; second, study-specific assessments including detailed chest pain characteristics using 34 predefined criteria, serial hs-cTnT blood concentrations (primary analysis) obtained from study samples, and clinical follow-up by telephone and/or mail. In situations of disagreement about the diagnosis, all cases were reviewed and adjudicated in conjunction with a third cardiologist.

In order to address the uncommon, but previously described phenomenon of discrepant results for hs-cTnT and hs-cTnI and the corresponding underestimation of the true performance of hs-cTnI-based early algorithms using an adjudication based at least in part on serial hs-cTnT measurements (27,28), we performed a second adjudication using serial hs-cTnI-Architect (rather than hs-cTnT) blood concentrations from study samples for internal validation as a secondary analysis. Uniform 99$^{th}$ percentiles and not sex-specific ones were used for final

adjudication. In the case of missing serial samples of hs-cTnT-Elecsys (for primary adjudication) or hs-cTnI-Architect (for secondary adjudication), cTn concentrations that were measured as part of routine clinical care at the participating study sites were used for final adjudication. Local (hs)-cTn concentrations were used in conjunction with hs-cTn concentrations for final adjudication if both were available.

AMI was defined and hs-cTn interpreted as recommended in the current Fourth Universal Definition guidelines.(29) Further details are given in the **Online Supplemental**.

**Investigational hs-cTn measurements**

Blood samples for determination of hs-cTnI-VITROS were collected in serum tubes and measured in June/July 2018 for study purposes. For hs-cTnI-Architect and hs-cTnT-Elecsys, samples were collected in plasma or serum tubes. Additional samples were collected at 1h, 2h, 3h, and 6h after presentation. 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-VITROS assay (VITROS® Immunodiagnostic Products hs Troponin I assay, Ortho Clinical Diagnostics, Rochester, NY, USA) on the VITROS 3600 Immunodiagnostic System has an overall 99$^{th}$ percentile concentration of 11ng/L (female 9ng/L, male 12ng/L) for serum with a corresponding co-efficient of variation (CV) of <7% at the 99$^{th}$ percentile. The 99$^{th}$ percentile values were established on similar male and female population using very strict criteria for inclusion/exclusion, in accordance with IFCC Task Force(30). Limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) have been determined to be 0.19ng/L, 0.39ng/L, and 1.23ng/L. The hs-cTnT-Elecsys assay (measured on different analyzers throughout the course of the study, Roche Diagnostics, Rotkreuz, Switzerland) has a 99$^{th}$ percentile concentration of 14ng/L (women: 9ng/L, men: 16ng/L) with a corresponding CV of 10% at 13ng/L.(4) LoB and LoD have been determined to

be 3ng/L and 5ng/L.(4) The hs-cTnI-Architect assay (ARCHITECT STAT high-sensitivity troponin I, ARCHITECT, Abbott Laboratories, IL, USA) has a 99$^{th}$ percentile concentration of 26ng/L (women: 16ng/L, men: 34ng/L) with a corresponding CV of <5% and a LoD of 1.9ng/L.(31–33). Characteristics of the hs-cTnI-Centaur and hs-cTnI-Access assay are described in the **Online Supplemental**. Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease formula.(34)

**Derivation and validation of the hs-cTnI-VITROS 0/1h-algorithm**

We used the concept of the current hs-cTnT/I 0/1h-algorithms suggested by the European Society of Cardiology (ESC)(3) (**Supplemental Figure 1).** Target negative predictive value (NPV) was 99.5% and target positive predictive value (PPV) 70%. The hs-cTnI-VITROS 0/1h-algorithm was developed in a derivation sample of randomly (1:1 fashion) selected patients with available hs-cTnI-VITROS measurements at ED presentation and after 1h, and directly compared with the established ESC 0/1h-algorithms (**Online Supplemental**).

**Statistical analysis**

Detailed information on the statistical analyses performed is given in the **Online Supplemental**. In brief, for the primary analysis, serial hs-cTnT concentrations were used as part of the study specific data set in the final adjudication. For the secondary analysis, serial hs-cTnI (Architect) concentrations were used as part of the study specific data set in the final adjudication. Receiver-operating characteristics (ROC) curves were constructed in all patients (n=1231), in early presenters (n=472) as well as in patients with available hs-cTn concentrations of all five assays (hs-cTnI-VITROS, hs-cTnT-Elecsys, hs-cTnI-Architect, hs-cTnI-Centaur, and hs-cTnI-Access) at presentation (n=703). Areas under the curves (AUC) were compared as recommended by DeLong et al.(35) or by *z*-statistic, as appropriate.

Survival during 30 days and 720 days of follow-up according to the classification provided by the hs-cTnI-VITROS 0/1h-algorithm was plotted in Kaplan-Meier curves and the

log-rank test was used to assess differences in survival between groups. Continuous variables are described as mean ± SD or median with interquartile range (IQR), categorical variables by numbers and percentages. Differences in baseline characteristics were assessed using the Mann-Whitney U test for continuous and the Pearson Chi-square test for categorical variables. 95% CI for proportions were calculated by bootstrapping with 1000 resamples. All hypothesis testing was two-tailed and p-values <0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (SPSS Inc, Chicago, IL) and MedCalc 17.6 (MedCalc Software, Ostend, Belgium).

# Results

## Characteristics of patients

From February 2011 to August 2015, 1231 patients eligible for this analysis were enrolled (**Supplemental Figure 2**). Thirty-eight 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 in **Supplemental Table 2**.

## Adjudicated final diagnosis

The adjudicated final diagnosis was AMI in 158/1231 patients (13%), unstable angina in 109/1231 (9%), cardiac symptoms of origin other than coronary artery disease (CAD) such as tachyarrhythmia, Takotsubo cardiomyopathy, heart failure or myocarditis in 203/1231 (16%), non-cardiac symptoms in 721/1231 (59%), and symptoms of unknown origin with normal concentrations of hs-cTn in 40/1231 (3%). Final diagnoses according to the second final adjudication including hs-cTnI-Architect were similar (**Online Supplemental**).

## Concentrations of hs-cTnI-VITROS at presentation according to final diagnoses

Concentrations of hs-cTnI-VITROS at ED presentation were higher in patients with AMI as compared to patients with other final diagnoses ($p<0.001$). Median concentrations of hs-cTnI-VITROS in patients with AMI were 74ng/L (IQR, 22-226), with unstable angina 3.0ng/L (IQR, 1.4-6.4), with cardiac, but not CAD 3.9ng/L (IQR, 1.5-9.0), with non-cardiac disease 1.0ng/L (IQR, 0.6-2.2), and with symptoms of unknown origin with normal concentrations of hs-cTn 2.0ng/L (IQR, 1.3-2.7; **Figure 1**). Similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI-Architect (**Supplemental Figure 3**).

## Diagnostic accuracy for AMI

The diagnostic accuracy of measurements obtained at presentation, as quantified by AUCs, for hs-cTnI-VITROS was 0.95 (95%CI, 0.93-0.96) versus 0.94 (95%CI, 0.92-0.95) for hs-cTnT-

Elecsys and 0.92 (95%CI, 0.90-0.94) for hs-cTnI-Architect (**Figure 2A**). In the analysis of patients with all five hs-cTnT/I assays, the AUC for hs-cTnI-VITROS was 0.95 (95%CI, 0.93-0.97), for hs-cTnT-Elecsys 0.94 (95%CI, 0.92-0.96), for hs-cTnI-Architect 0.90 (95%CI, 0.87-0.93), for hs-cTnI-Centaur 0.95 (95%CI, 0.94-0.97), and for hs-cTnI-Access 0.95 (95%CI, 0.94-0.97) (**Figure 2B**). AUCs for serial sampling of hs-cTnI-VITROS are shown in **Table 2**. Similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI-Architect (**Supplemental Figure 4A+B**).

**Subgroup analyses according to time since chest pain onset and sex**

Diagnostic accuracy at presentation was also high in all predefined subgroups (**Online Supplemental and Supplemental Table 3**). AUCs in early presenters (within 3h after chest pain onset, 472/1231, 38%) remained very high irrespective of primary or secondary final adjudication (**Supplemental Figure 4C+D**).

**Derivation of the hs-cTnI-VITROS 0/1h-algorithm**

Optimal thresholds for rule-out of AMI were defined in the derivation cohort (n=519) as either an hs-cTnI-VITROS concentration at presentation <1ng/L in patients with an onset of chest pain >3h (direct rule-out) or as an hs-cTnI-VITROS concentration at presentation <2ng/L *and* an absolute change within 1h <1ng/L for all patients (irrespective of time since chest pain onset). Optimal cut-off criteria for rule-in of AMI were defined as either an hs-cTnI-VITROS concentration at presentation ≥40ng/L (direct rule-in) *or* an absolute change within 1h ≥4ng/L. Patients fulfilling neither of the above criteria for rule-out or for rule-in were classified as observe. The diagnostic performance of the hs-cTnI-VITROS 0/1h-algorithm in the derivation cohort is shown in **Figure 3A,** and **Supplemental Figure 5A**. Direct rule-out based on a single hs-cTnI-VITROS concentration at presentation was feasible in 101/519 patients (19%). One patient with AMI was missed out of 519 patients with suspected AMI in the derivation cohort (**Supplemental Table 4** for detailed patient characteristics). Overall, the hs-cTnI-VITROS

0/1h-algorithm allowed a definite triage after 1h in 342/519 patients (66%; either rule-out or rule-in).

**Validation of the hs-cTnI-VITROS 0/1h-algorithm**

Applying the derived optimal cut-off criteria to the internal validation cohort, 275/520 patients (53%) could be classified as rule-out with a corresponding NPV of 100% (95%CI, 98.6-100) and a sensitivity of 100% (95%CI, 94.1-100; **Figure 3B** and **Supplemental Figure 5B**). Direct rule-out based on a single hs-cTnI-VITROS concentration at presentation was feasible in 96/520 patients (18%). The 0/1h-algorithm classified 74/520 patients (14%) as rule-in with a corresponding PPV of 73.0% (95%CI, 61.9-81.8) and a specificity of 95.6% (95%CI, 93.4-97.2). Direct rule-in based on a single hs-cTnI-VITROS concentration at presentation was feasible in 55/520 patients (11%). Overall, the hs-cTnI-VITROS 0/1h-algorithm allowed a definite diagnosis after 1h in 349/520 patients (67%; either rule-out or rule-in). The remaining 171/520 patients (33%) were classified as observe with an AMI prevalence of 4%. Similar findings emerged when assessing the diagnostic performance of the hs-cTnI-VITROS 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-VITROS 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-VITROS 0/1h-algorithm was similar to that of the hs-cTnT-Elecsys 0/1h-algorithm and the hs-cTnI-Architect 0/1h-algorithm (**Online Supplemental and Supplemental Figure 7+8).** The efficacy for direct rule-out or rule-in based on the 0h-sample was 29% (95%CI, 26-32) for the hs-cTnI-VITROS 0/1h-algorithm compared to 26% (95%CI, 23-29) for hs-cTnT-Elecsys and 22% (95%CI, 20-25) for hs-cTnI-Architect. Detailed performance characteristics are shown in **Supplemental Table 5**.

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

Median follow-up time was 399 days (IQR, 321-744) with 5 deaths (3 cardiovascular) occurring within 30 days and 36 deaths (20 cardiovascular) within 2 years. Cumulative 30-days survival rates were 99.8% (1 event), 99.7% (1 event) and 98.0% (3 events; log-rank, p=0.015) in the rule-out, observe and rule-in group, respectively. At 2 years, cumulative survival rates were 98.7 (4 events), 91.5% (18 events) and 86.9% (14 events), respectively (log-rank, p<0.001; **Figure 4**).

**MACE-free survival within 30 days**

MACE-free survival (including the index event) was 99.4% (3 events) within 30 days in patients triaged towards rule-out, 93.1% (24 events) in patients triaged towards observe, and 26.5% (111 events) in patients triaged towards rule-in by the hs-cTnI-VITROS 0/1h-algorithm (log-rank, p<0.001).

# Discussion

This large multicentre study was performed to assess the diagnostic performance and clinical utility of the hs-cTnI-VITROS assay for the early diagnosis of AMI. We report **seven** major findings:

**First,** the diagnostic accuracy of hs-cTnI-VITROS was high for concentrations obtained at ED presentation as well as absolute 1h-, 2h-, and 3h-changes and their combinations with an AUC ranging from 0.95 to 0.97. **Second,** overall the diagnostic accuracy of hs-cTnI-VITROS was comparable to that provided by hs-cTnT-Elecsys and hs-cTnI-Architect (the currently most used). In addition, diagnostic accuracy was similar to that provided by two other recently developed assays: hs-cTnI-Centaur and hs-cTnI-Access. This indicates that newer generations of hs-cTnI assays seem to have at least comparable diagnostic accuracies than established hs-cTn assays. Findings were consistent in the primary analysis (including hs-cTnT in the adjudication) and secondary analysis (including hs-cTnI-Architect in the adjudication). Similarly, findings were consistent in the overall population, as well as in early presenters. **Third,** the application of the derived 0/1h-algorithm for hs-cTnI-VITROS, defined by concentrations at presentation and its absolute change within 1h, in the independent validation cohort resulted in high safety in the rule-out zone with a NPV of 100% and a sensitivity of 100%, as well as a high PPV of 73% in the rule-in zone for AMI. 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 1200 patients enrolled, the hs-cTnI-VITROS 0/1h-algorithm only triaged one AMI patient incorrectly. **Fourth**, overall, the performance of the 0/1h-algorithm for hs-cTnI-VITROS was similar 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.(3,15,22,36) Of note, the hs-cTnI-VITROS 0/1h-algorithm allowed to directly triage 29% (95%CI, 26-32) of patients at presentation towards either rule-out or rule-in based on a

single hs-cTnI-VITROS concentration without the need for serial hs-cTnI sampling. This was at least comparable to the proportions triaged by the hs-cTnT-Elecsys 0/1h-algorithm (26%; 95%CI, 23-29) and the hs-cTnI-Architect 0/1h-algorithm (22%; 95%CI, 20-25). **Fifth**, the overall efficacy of the new hs-cTnI-VITROS 0/1h-algorithm was high by assigning about 67% of consecutive patients to either rule-out or rule-in within 1h, and only about one third of patients remaining in the observe zone. **Sixth,** these findings were internally validated using a second adjudication including serial hs-cTnI concentrations. Thereby, the strategy of central adjudication which included another hs-cTnI assay (Architect) and which was applied in this large diagnostic study of patients presenting with suspected AMI seems to be stringent and robust and it was used previously.(6) By adding a secondary analysis that included hs-cTnI (rather than hs-cTnT as in the primary analysis) in addition to the clinical and imaging information available for the adjudication of the final diagnosis, the generalizability of our findings was further increased. **Seventh,** overall survival in patients assigned to the rule-out zone by the 0/1h-algorithm was 99.8% after 30 days and 98.7% 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.

These findings may have important clinical implications, as they will allow a substantial number of additional institutions, those currently working with Ortho Clinical Diagnostics VITROS Systems, to introduce hs-cTnI testing into their clinical management of patients with suspected AMI. Adoption of current clinical practice guideline recommendations without the logistic challenges and costs of introducing an additional analyzer exclusively for the measurement of hs-cTnT/I will be a major benefit.(3,17,37)

It is a matter of debate, whether the slightly higher diagnostic accuracy of hs-cTnI-VITROS versus the hs-cTnT-Elecsys (ΔAUC 0.01) and hs-cTnI-Architect (ΔAUC 0.03) is also of clinical significance. Arguments in favor include the fact that for such a common, dangerous, and well-treatable disorder as AMI, even small differences in diagnostic accuracy may translate

into benefits for an institution and/or the population at large. Arguments against include the fact that overall the diagnostic performance of the hs-cTnI-VITROS 0/1h-algorithm was similar, and not superior, to the 0/1h-algorithms of the two established hs-cTnT/I-assays.

Our findings also extend and corroborate previous work with other hs-cTnT/I assays.(5,6,13,15,36) Accordingly, the same concept and caveats apply to the most appropriate clinical use of any of the hs-cTnT/I assays and their respective 0/1h-algorithms in the early diagnosis of AMI.(3,5,13,15,18,22,36) First, these algorithms should only be applied after STEMI has been ruled-out by the ECG performed at presentation. Second, although the hs-cTnI-VITROS 0/1h-algorithm had a high NPV and sensitivity for AMI, per guidelines, troponin results and validated algorithms should always be used in conjunction with all other clinical information including a detailed assessment of chest pain characteristics, physical examination, and the ECG.(3) 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 cTn into the circulation, which could occur in early presenters.(3) It will also help to detect rare but possible errors in the handling of the clinical blood samples. Third, not all patients triaged towards rule-out of AMI are appropriate candidates for early discharge from the ED as they may have other diagnoses such as pneumonia that sometimes require hospitalization. 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 Takotsubo cardiomyopathy, myocarditis, and unstable angina.(3) Fifth, like for all other immunoassays, rare cases with “false-negative” or “false-positive” results due to heterophilic antibodies(8,30) or macrotroponin(38) have been described for previous generation (hs)-cTnI assays and should be considered whenever hs-cTn results seem to contradict the clinical picture.

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.(39,40) **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 unlikely to have affected the main findings of the present study. **Fourth,** although we used a 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. This invariably would have led to an underestimation of the true diagnostic accuracy of the new 0/1h-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 to incorrect results pertaining to the individual patient. This again invariably would have led to an underestimation of the true diagnostic accuracy of the new hs-cTnI-VITROS 0/1h-algorithm. In fact, this error might well have occurred in the single AMI patient presumable missed by both the hs-cTnI-VITROS and the hs-cTnT-Elecsys 0/1h-algorithm, as not only hs-cTnI-VITROS, but all hs-cTnT/I concentrations measured from the study specific blood samples were in the low normal range or without significant changes. **Sixth,** we cannot generalize our findings to patients with terminal kidney failure requiring dialysis, since they were excluded from this study. **Seventh**, further studies assessing analytical

performance data including lot-to-lot variation are necessary to better characterize the hs-cTnI-VITROS assay.

In conclusion, the diagnostic accuracy of the hs-cTnI-VITROS assay for AMI is high and at least comparable to well-established and other new hs-cTnT/I assays. A simple algorithm incorporating hs-cTnI-VITROS concentrations at presentation and absolute changes within the first 1h, allows triaging towards safe rule-out and accurate rule-in of AMI 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, Nestelberger, Twerenbold, Koechlin, Badertscher, Rubini Gimenez, Wildi, Puelacher, Reichlin, and Mueller had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and approved the manuscript. The sponsors had no role in designing or conducting the study and no role in gathering or analyzing the data or writing the manuscript. The manuscript and its contents have not been published previously and are not being considered for publications elsewhere in whole or in part in any language, including publicly accessible web sites or e-print servers.

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| Table 1 | Baseline Characteristics of the Patients | | | |
|---|---|---|---|---|
| | All patients (n=1231) | AMI (n=158) | No AMI (n=1073) | p-Value |
| Age – yr | 60 (48-74) | 75 (62-81) | 58 (47-72) | <0.001 |
| Female gender – no. (%) | 420 (34) | 41 (26) | 379 (35) | 0.02 |
| Early presenters (within 3h after cpo) | 472 (38%) | 65 (41%) | 407 (38%) | 0.49 |
| Risk factors – no. (%) | | | | |
| Hypertension | 720 (58) | 119 (75) | 601 (56) | <0.001 |
| Hypercholesterolemia | 566 (46) | 110 (70) | 456 (42) | <0.001 |
| Diabetes | 200 (16) | 42 (27) | 158 (15) | <0.001 |
| Current smoking | 301 (24) | 31 (20) | 270 (25) | 0.13 |
| History of smoking | 486 (39) | 78 (49) | 408 (38) | 0.007 |
| History – no. (%) | | | | |
| Coronary artery disease | 374 (30) | 72 (46) | 302 (28) | <0.001 |
| Previous MI | 284 (23) | 65 (41) | 219 (20) | <0.001 |
| Previous revascularization | 332 (27) | 67 (42) | 265 (25) | <0.001 |
| Peripheral artery disease | 53 (4) | 18 (11) | 35 (3) | <0.001 |
| Previous stroke | 66 (5) | 15 (9) | 51 (5) | 0.01 |
| ECG findings – no. (%) | | | | |
| Left bundle branch block | 41 (3) | 13 (8) | 28 (3) | <0.001 |
| ST-segment depression | 75 (6) | 34 (22) | 41 (4) | <0.001 |
| T-wave inversion | 81 (7) | 21 (13) | 60 (6) | <0.001 |
| No significant ECG abnormalities | 1003 (81) | 86 (54) | 917 (85) | <0.001 |
| Body mass index ($kg/m^2$) | 26 (24-30) | 27 (24-29) | 26 (24-30) | 0.90 |
| Laboratory findings | | | | |
| Creatinine clearance, $mL/min/m^2$ | 85 (70-100) | 75 (61-91) | 86 (71-102) | <0.001 |
| Chronic medication – no. (%) | | | | |
| Aspirin | 426 (35) | 87 (55) | 339 (32) | <0.001 |
| Vitamin K antagonists | 160 (13) | 24 (15) | 136 (13) | 0.38 |
| Beta blockers | 406 (33) | 67 (42) | 339 (32) | 0.007 |
| Statins | 418 (34) | 77 (49) | 341 (32) | <0.001 |
| ACEIs/ARBs | 493 (40) | 89 (56) | 404 (38) | <0.001 |
| Calcium antagonists | 192 (16) | 33 (21) | 159 (15) | 0.05 |
| Nitrates | 101 (8) | 22 (14) | 79 (7) | 0.005 |

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

| Table 2 | Diagnostic Accuracy of High-Sensitivity Cardiac Troponin I (VITROS) for Single Concentrations, Absolute Changes and their Combination During Serial Sampling - ROC AUC (95%CI) |
|---|---|
| Hs-cTnI at presentation (n=1231) | 0.95 (0.93-0.96) |
| Hs-cTnI after 1 hour (n=1039) | 0.96 (0.95-0.98) |
| Hs-cTnI after 2 hours (n=869) | 0.97 (0.96-0.99) |
| Hs-cTnI after 3 hours (n=442) | 0.95 (0.91-0.99) |
| Hs-cTnI 1h-delta (n=1039) | 0.97 (0.95-0.98) |
| Hs-cTnI 2h-delta (n=869) | 0.96 (0.93-0.98) |
| Hs-cTnI 3h-delta (n=442) | 0.97 (0.95-0.99) |
| Hs-cTnI at presentation and 1h-delta (n=1039) | 0.97 (0.96-0.98) |
| Hs-cTnI at presentation and 2h-delta (n=869) | 0.97 (0.96-0.99) |
| Hs-cTnI at presentation and 3h-delta (n=442) | 0.97 (0.95-0.99) |

ROC AUC denotes area under the receiver-operating-characteristic curve. Delta values refer to the absolute (unsigned) change between the level of hs-cTnI at baseline and after 1h, 2h or 3h, respectively. There was no selection based on left over samples. Missing blood draws during the course in the emergency department was the only reason for missing hs-cTnI-VITROS concentrations at later time points. Hs-cTnI denotes high-sensitivity cardiac troponin I; ROC denotes receiver operating characteristic curve; AUC denotes area under the curve.

## Figure Legends

**Figure 1** **Boxplots showing Concentrations of hs-cTnI-VITROS at Presentation according to the Final Diagnoses including hs-cTnT-Elecsys**

Boxes represent medians and interquartile ranges (IQRs), while whiskers display the smallest and the largest non-outliers. AMI denotes acute myocardial infarction; hs-cTnI denotes high-sensitivity cardiac troponin I; UA denotes unstable angina.

**Figure 2** **Diagnostic Accuracy of High-Sensitivity Cardiac Troponin Assays at Presentation for the Diagnosis of Acute Myocardial Infarction according to the Final Diagnoses including hs-cTnT-Elecsys**

Receiver operating characteristic (ROC) curves describing the diagnostic performance at presentation of (A) the three high-sensitivity assays in all patients and of (B) the five high-sensitivity assays in patients with available concentrations at presentation for the diagnosis of acute myocardial infarction.

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

(A) Performance of the hs-cTnI-VITROS 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 4** **Short-term and Long-term Survival of Patients classified according to the High-Sensitivity Cardiac Troponin I VITROS 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 VITROS 0/1h-algorithm. No. denotes number.