Combining High Sensitivity Cardiac Troponin I and Cardiac Troponin T in the Early Diagnosis of Acute Myocardial Infarction

Running Title: *van der Linden and Wildi, et al.; Combining Troponin T and I for*

Diagnosis of AMI

Noreen van der Linden, MD, PhD & Karin Wildi, MD, et al.

The full author list is available on page 20.

Address for Correspondence:
Christian Mueller, MD
Department of Cardiology and Cardiovascular Research Institute Basel (CRIB)
University Hospital Basel, Petersgraben 4
CH-4031 Basel, Switzerland
Tel.: +41 61 328 65 49
Fax: +41 61 265 53 53
Email: Christian.Mueller@usb.ch

Abstract

Background—Combining two signals of cardiomyocyte injury, cardiac troponin I (cTnI) and T (cTnT), might overcome some individual pathophysiological and analytical limitations and thereby increase diagnostic accuracy for acute myocardial infarction (AMI) with a single blood draw. We aimed to evaluate the diagnostic performance of combinations of high sensitivity (hs) cTnI and hs-cTnT for the early diagnosis of AMI.

Methods—The diagnostic performance of combining hs-cTnI (Architect, Abbott) and hs-cTnT (Elecsys, Roche) concentrations (sum, product, ratio and a combination algorithm) obtained at the time of presentation was evaluated in a large multicenter diagnostic study of patients with suspected AMI. The optimal rule out and rule in thresholds were externally validated in a second large multicenter diagnostic study. The proportion of patients eligible for early rule out was compared with the ESC 0/1 and 0/3 hour algorithms.

Results—Combining hs-cTnI and hs-cTnT concentrations did not consistently increase overall diagnostic accuracy as compared with the individual isoforms. However, the combination improved the proportion of patients meeting criteria for very early rule-out. With the ESC 2015 guideline recommended algorithms and cut-offs, the proportion meeting rule out criteria after the baseline blood sampling was limited (6-24%) and assay dependent. Application of optimized cut-off values using the sum (9 ng/L) and product (18 ng^2/L^2) of hs-cTnI and hs-cTnT concentrations led to an increase in the proportion ruled-out after a single blood draw to 34-41% in the original (sum: negative predictive value (NPV) 100% (95%CI: 99.5-100%); product: NPV 100% (95%CI: 99.5-100%) and in the validation cohort (sum: NPV 99.6% (95%CI: 99.0-99.9%); product: NPV 99.4% (95%CI: 98.8-99.8%). The use of a combination algorithm (hs-cTnI <4 ng/L and hs-cTnT <9 ng/L) showed comparable results for rule out (40-43% ruled out; NPV original cohort 99.9% (95%CI: 99.2-100%); NPV validation cohort 99.5% (95%CI: 98.9-99.8%)) and rule-in (PPV original cohort 74.4% (95%Cl 69.6-78.8%); PPV validation cohort 84.0% (95%Cl 79.7-87.6%)).

Conclusions—New strategies combining hs-cTnI and hs-cTnT concentrations may significantly increase the number of patients eligible for very early and safe rule-out, but do not seem helpful for the rule-in of AMI.

Clinical Trial Registration—APACE URL: www.clinicaltrial.gov, Unique Identifier: NCT00470587; ADAPT URL: www.anzctr.org.au, Unique Identifier: ACTRN12611001069943

Key Words: High-sensitivity cardiac troponin; combination of assays for diagnosis; acute myocardial infarction; early rule-out, early rule-in

Clinical Perspective

What is new?

- Measuring both cardiac troponin T (hs-cTnT) and cardiac troponin I (hs-cTnI) for the diagnosis of acute myocardial infarction does not consistently increase overall diagnostic accuracy as compared with measurement of the individual troponins.

- Using a combination of cardiac troponin T and cardiac troponin I concentrations, both obtained at a single blood draw at presentation, leads to a substantial increase in the proportion of patients in whom an acute myocardial infarction can be safely excluded.

- In contrast, the combination of cardiac troponin T and cardiac troponin I does not improve the determination of patients with acute myocardial infarction.

What are the clinical implications?

- Combining cardiac troponin T and cardiac troponin I may contribute to a clinically relevant 3-to-6-fold increase in the number of rule-outs after a single blood draw at presentation compared to the current ESC 0/3 hour algorithms.

- The increased rule-out of myocardial infarction at presentation may reduce the number of patients that have to wait for a consecutive cardiac troponin measurement, and may therefore favorable impact resource use and overcrowding in the emergency department.

Introduction

Approximately 10% of all patients seeking medical attention at the emergency department (ED) report chest discomfort, a complaint that reflects many potential etiologies including acute myocardial infarction (AMI) [1]. Rapid identification of patients with AMI is of profound clinical importance for fast initiation of medical treatment and management [2]. In addition, rapid rule-out of patients without AMI can overcome prolonged patient anxiety, unnecessary resource use and overcrowding in the ED [3–7]. Despite major improvements in diagnostic accuracy due to the introduction of high-sensitivity cardiac troponin (hs-cTn) assays and data-driven optimized diagnostic algorithms, rapid, accurate and safe rule-out based on a single measurement of hs-cTn is still possible only in a minority of patients [2,3,8,9].

Current guidelines recommend measurement of one of the cardiac specific isoforms of the cardiac troponin (cTn) complex: cTnI or cTnT [2,10]. The development of high-sensitivity methods for the measurements of cTnT and cTnI concentrations has allowed the delineation of pathophysiological and analytical differences between cTnT and cTnI. First, hs-cTnT plasma concentrations exhibit a diurnal rhythm, while (hs)-cTnI does not [11]. Second, hs-cTnT concentrations seem to be a stronger predictor of death as compared with hs-cTnI concentrations[12]. Third, cTnI seems to be released from injured cardiomyocyte slightly earlier and possibly by less intense injury as compared with cTnT [12]. Fourth, the association with renal dysfunction is stronger for cTnT clearance than for cTnI [13]. Fifth, hemolysis, which is common in blood samples taken in the ED, seems to increase cTnI concentrations, but decrease cTnT concentrations [14]. Sixth, while analytically false positive results overall seem rare with both hs-cTnT and hs-cTnI, they can be triggered by the re-expression of embryonic cTnT in the skeletal muscle of patients with neuromuscular disorders for hs-cTnT and heterophilic antibodies to cTnI

4

for hs-cTnI [15]. Combining two signals of cardiomyocyte damage, hs-cTnT and hs-cTnI, might overcome some individual pathophysiological and analytical limitations and thereby increase diagnostic accuracy for AMI with a single blood draw [11,16,17]. Despite differences in biochemical characteristics and release kinetics [18,19], a recent direct comparison between hs-cTnI and hs-cTnT showed similar, high diagnostic accuracy for AMI emphasizing the similarities between both isoforms [12]. Based on the observation of an imperfect correlation between blood concentrations of cTnT and cTnI in chronic and acute disorders [20,21], and in analogy to the quantification of renal function using creatinine and cystatin C, where the combination of two parameters associated with the same pathophysiological process but influenced by distinct factors lead to a more precise and accurate indicator [22], we hypothesize that combining hs-cTnI and hs-cTnT concentrations will overcome independent pathophysiological, pre-analytical and analytical differences of the individual molecules, and might therefore have higher diagnostic accuracy for AMI than either hs-cTnI or hs-cTnT alone. This hypothesis was tested in two large prospective multicenter diagnostic studies.

Methods

The data and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. The analytic methods will be available upon request.

Patients and setting

The combination of hs-cTnI and hs-cTnT for the diagnosis of AMI was investigated in two diagnostic cohorts; The primary cohort was the Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) study [3,12,23,24], and the secondary (external validation) cohort

was the New Zealand-Australia combined data from the multicentre 2-Hour Accelerated

Diagnostic Protocol to Assess Patients Witch Chest Pain Symptoms Using Contemporary

Troponins as the Only Biomarker (ADAPT) study [25], the ADAPT-RCT, and the Emergency

Department Chest Pain Score (EDACS)-RCT [26,27]. For convenience we will refer to this

combined cohort as the ADAPT cohort.

APACE is an ongoing prospective international multicenter diagnostic study that enrolls

patients presenting to the ED with acute chest discomfort with an onset of peak within the last 12

hours. Patients are enrolled regardless of their renal function. Only patients with terminal kidney

failure on chronic dialysis are excluded. This analysis contains data of patients enrolled between

April 2006 and May 2013 who had a final diagnosis adjudicated by two independent

cardiologists (n=3029). For this analysis, patients were excluded if hs-cTnI or hs-cTnT blood

concentrations at presentation were not available (n=661), if the final adjudicated diagnosis was

ST-elevation myocardial infarction (STEMI) (n=74), or if the final diagnosis remained unclear

after adjudication and at least one (hs)-cTn level was elevated (possibly indicating the presence

of AMI) (n=69).

In the ADAPT cohort, patients with at least 5 min of symptoms consistent with acute

coronary syndrome [28], but without ST-segment elevation, were enrolled at two EDs in Brisbane,

Australia and Christchurch, New Zealand between November 2007 and July 2014.

Both studies were carried out according to the principles of the Declaration of Helsinki,

approved by the local ethics committees, and registered at clinicaltrial.gov (APACE:

NCT00470587) or at the Australia-New Zealand Clinical Trials Registry (ADAPT:

ACTRN12611001069943, ADAPT-RCT: ACTRN12610000766011, EDACS-RCT:

ACTRN12613000745741). Written informed consent was obtained from all patients.

Routine clinical assessment

In both cohorts, patients underwent routine clinical assessment that included medical history, physical examination, standard blood tests including serial measurements of local (hs)-cTn, 12-lead ECG, chest radiography, continuous ECG rhythm monitoring and pulse oximetry. Management of patients was left to the discretion of the attending physician.

Adjudicated final diagnosis

In the APACE cohort, adjudication of the final diagnosis was performed by two independent cardiologists at the core laboratory (University Hospital Basel) applying the universal definition of AMI [29] 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 - 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 obtained from study samples, and clinical follow-up by telephone and/or mail. In situations of disagreement about the diagnosis, cases were reviewed and adjudicated in conjunction with a third cardiologist. These procedures were comparable to those in the ADAPT cohort, where the adjudication of the final diagnosis was performed by two independent cardiologists blind to results of the index-test biomarkers under investigation, but with knowledge of the clinical record, ECG, and serial cTnI results from routine care (details of adjudication are given in the Supplementary Data).

In both cohorts, AMI was defined and (hs-)cTn interpreted as recommended in the current guidelines [2,30,31]. In brief, AMI was diagnosed when there was evidence of myocardial

necrosis in association with a clinical setting consistent with myocardial ischemia. Myocardial

necrosis was diagnosed by at least one cTn value above the 99th percentile (or for the

conventional cTn assays above the 10% imprecision value if not fulfilled at the 99th percentile)

together with a significant rise and/or fall. The criteria used to define a rise and/or fall in

conventional cTn and hs-cTnT are described in detail in the method section in the data

supplement. All other patients were classified in the categories of unstable angina (UA), Non

Cardiac Chest Pain (NCCP), cardiac but non-coronary disease (e.g. tachyarrhythmias,

perimyocarditis), and symptoms of unknown origin with normal concentrations of hs-cTnT.

Measurement of hs-cTnT and hs-cTnI

After centrifugation, serum was frozen at -80°C until measurement with hs-cTn assays. Hs-cTnI

was measured by using the ARCHITECT High Sensitive STAT Troponin I assay (Abbott

Laboratories). According to the manufacturer, the 99th percentile concentration is 26.2 ng/L with

a corresponding coefficient of variation (CV) of <5% [32]. Hs-cTnT was measured with the Roche

hs-cTnT assay. The 99th percentile among healthy subjects is 14 ng/L, with a 10% analytical

variation at 13 ng/L [33]. Data presented here were not affected by the 2010–2012 hs-cTnT low-

end shift in APACE and appropriately corrected in ADAPT [34–36]. Calculation of the glomerular

filtration rate (eGFR) was performed using the abbreviated Modification of Diet in Renal

Disease (MDRD) formula [37].

Statistical analysis

We evaluated the diagnostic accuracy and performance of the combined hs-cTnI and hs-cTnT

measurement in two different ways: First, we examined sum, product and ratio. Second, we

derived and tested a combination algorithm of hs-cTnI and hs-cTnT. Data are expressed as

median ± interquartile range (IQR) for continuous variables and as numbers (n) and percentages

(%) for categorical variables. Continuous variables were compared with the Mann-Whitney U test, and categorical variables were compared by use of the Pearson χ2 test. Cohen's kappa statistic was used to examine the agreement between rule-in and rule-out at presentation based on hs-cTnT and hs-cTnI according to the two diagnostic algorithms recommended with a class I recommendation in the current European Society of Cardiology (ESC) guidelines: the 0/3h-hs-cTn-algorithm and the 0h/1h-hs-cTn-algorithm [2]. Sum, product and ratio were calculated from raw data. Undetectable low concentrations were assigned the concentration 0.1ng/L. Binary logistic regression analyses were used to calculate predicted probabilities for combined test variables.

Receiver-operating characteristics (ROC) curves were constructed to assess diagnostic performance at presentation and 1h after initial presentation including the absolute change value. Diagnostic accuracy was reported as the area under the ROC curve (AUC) and the corresponding 95% confidence intervals (95% CI). The comparison of dependent and independent AUCs was performed as recommended by Hanley and McNeil [38] and for nested models with the comparison of -2 likelihood ratios as appropriate. 0h and 0h/1h serial sampled hs-cTn blood concentrations were combined to represent the current gold standard of clinical care as suggested in the 2015 ESC guidelines [2]. Furthermore, integrated discrimination improvement (IDI) was calculated [39].

For the determination of optimal cut-off values for sum, product and ratio (minimal negative predictive value (NPV) of 99.6% and a positive predictive value (PPV) of 75.0%, respectively, to match the performance of the 0h/1h-hs-cTn-algorithm [2,40,41], the cohort was randomly divided in a derivation (80% of patients) and a validation sub-cohort (20% of patients).

For the cut-off values in the combination algorithm, the optimal rule-out combination was that which maximized the percentage ruled-out at a sensitivity of 99% and the optimal rule-in

combination was that which maximized the percentage ruled-in at a PPV of 75%. We determined the optimal combination of hs-cTn thresholds based on a smoothed average of 500 bootstraps of the original cohort, in which we varied the hs-cTn threshold for each troponin assay in steps of 0.1 ng/L. This methodology is more extensively described in the methods supplement. We used an 'AND'-approach to ensure a safe early rule-out, and an 'OR'-approach to maximize rule-in.

All hypothesis testing was two-tailed, and values of p<0.05 were considered statistically significant. We did not adjust for multiple testing. We did not adjust for multiple testing. Statistical analyses were performed with SPSS for Windows 23.0 (SPSS Inc), MedCalc 9.6.4.0 (MedCalc software) and R version 3.2.4 (with packages 'boot' v1.3-18 and 'fields' v8.10).

Results

Distribution of hs-cTn concentrations at presentation in patients with suspected AMI

Baseline characteristics of 2225 patients in the APACE cohort presenting to the ED with suspected AMI are shown in Supplementary Table 1. The adjudicated final diagnosis was AMI (NSTEMI) in 18% of patients (85% had type I and 15% type II AMI), UA in 10%, cardiac but not coronary artery disease in 14%, NCCP in 54%, and symptoms of unknown origin in 5%. AMI patients had higher concentrations of hs-cTnI and hs-cTnT at presentation compared with the no-AMI group (hs-cTnI median 115.2 ng/L (IQR: 21.7–632.9) vs. 3.5 ng/L (IQR: 2.2–7.2) P<0.001; hs-cTnT median 64.1 ng/L (IQR: 28.0–152.4) vs. 7.0 ng/L (IQR: 4.0–12.4) P<0.001; Supplementary Table 2 and Supplementary Figure 1). The correlation between hs-cTnI and hs-cTnT concentrations at presentation was high (r=0.89) (Figure 1).

Diagnostic performance of hs-cTn concentrations measured at presentation according to the ESC 0/3-hour algorithm

In the APACE cohort 721 of 2225 patients (32.4%) presented ≥6h after onset of chest pain and therefore could be assessed by the late-presenter part of the ESC 0/3h algorithm with a single blood draw. Using hs-cTnT, AMI could be ruled-out in 441 patients (19.8% of overall cohort, 61.2% of late-presenters) by a baseline hs-cTn below the 99th percentile, 4 AMI`s were missed. Adding the clinical information (GRACE score <140 and pain free) resulted in 1 missed AMI and therefore in a sensitivity of 99.3% (95%Cl 96.2-100%) and a NPV of 99.4% (95%Cl 96.8-100%).

Using hs-cTnI, in 539 patients (24.2% of overall cohort, 74.8% of late-presenters) AMI could be ruled-out by a single blood draw at presentation, 21 AMI`s were missed. Adding the clinical information reduced the number to 3 missed AMI`s; sensitivity 97.9% (95%Cl 94.0-99.6%) and NPV 98.5% (95%Cl 95.7-99.7%). The agreement on patient allocation between hs-cTnI and hs-cTnT for rule-out at presentation was good (κ=0.90) (Supplementary Table 3).

Diagnostic performance of hs-cTn concentrations measured at presentation according to the ESC 0/1-hour algorithm

AMI could be ruled-out in 149 (6.7%, sensitivity 100%, NPV 100%) and 235 (10.6%, sensitivity 100%, NPV 100%) patients after a single blood draw at presentation, using hs-cTnI and hs-cTnT, respectively. Direct rule-in could be achieved in 331 (14.9%, specificity 95.6%, PPV 75.5%) and 273 (12.3%, specificity 2.4%, PPV 84.2%) subjects, using hs-cTnI or hs-cTnT, respectively. The agreement on patient allocation at presentation between hs-cTnI and hs-cTnT was moderate for rule-out (κ=0.42) and good for rule-in (κ=0.79) (Supplementary Tables 4 and

5). Using the 0/1-hour algorithm 77-78% of patients need a second cardiac troponin measurement.

Diagnostic performance of combined hs-cTnI and hs-cTnT concentrations measured at presentation

The diagnostic accuracy in the APACE cohort, as quantified by AUC was evidently lower for the ratio than for the sum, product, or combination of hs-cTn and for the individual isoforms alone (Table 1 and Figure 2). Addition of a second isoform to 0h hs-cTn led to a numerically small increase in AUC above that for hs-cTnT alone, but not for hs-cTnI alone. Furthermore, addition of a combined measurement at presentation to the 0h and 0h/1h change concentrations led to a numerically small, but statistically significant improvement in diagnostic accuracy of hs-cTnI, but not of hs-cTnT (Supplementary Table 6). Reclassification statistics (IDI) did not uniformly show incremental value of combining cardiac troponins at presentation when applied to the APACE cohort (Supplementary Tables 7). Diagnostic performance did not increase when two different cardiac troponin I signals were combined (Siemens c-TnI Ultra, Beckman hs-cTnI and Siemens hs-cTnI Vista; Supplementary tables 8, 9 and 10). Comparable results were found when hs-cTnI and hs-cTnT were combined using logistic regression analysis (methods supplement, supplementary results).

Early allocation based on sum and product

We examined the use of sum and product on the allocation of patients at presentation. In a randomly selected derivation cohort of 1799 patients (313 AMI, 1486 no AMI), thresholds for rule-out and rule-in achieving a NPV of at least 99.6% and a PPV of 75.0%, respectively, were: rule-out cut-off for the sum of 9 ng/L and for the product of 18 ng^2/L^2 (NPV both 100% (95% CI, 99.4–100%), and a rule-in cut-off for the sum of 99 ng/L and for the product of 1608 ng^2/L^2

(PPV sum 75.1% (95% CI 69.3% − 80.3%), PPV product 75.1% (95% CI 69.5%−80.1%)). When these cut-off values were applied to the internal validation cohort of 426 patients (85 AMI, 341 no AMI), we found comparable results for sum (rule-out: sensitivity 100% (95.8%-100%), NPV 100% (97.5%-100%); rule-in: specificity 96.8% (94.3%-98.4%), PPV 83.6% (72.4%-91.6%) and product (rule-out: sensitivity 100% (95.8%-100%), NPV 100% (97.5%-100%); rule-in: specificity 96.8% (94.3%-98.4%), PPV 83.6% (72.4%-91.6%); Tables 2 and 3. Application of these cut-off values in the original cohort (APACE) would cause a 3-to-5-fold increase in the number of rule-outs at presentation as compared to the 2015 ESC algorithms. This would decrease the percentage of patients that require a second cardiac troponin measurement one hour later from 77-78% to 50-52%.

When these cut-off values were applied to the external validation cohort (for patient characteristics see Supplementary Table 11) of 2537 patients (408 AMI, 2129 no AMI), we found comparable results for sum (rule-out: sensitivity 99.0% (97.5%-99.7%); NPV 99.6%; (99.0%-99.9%) rule-in: specificity 98.2% (97.5%-98.7%); PPV 87.5% (83.3%-91.0%)) and product (rule-out: sensitivity 98.5% (96.8%-99.5%), NPV 99.4% (98.8%-99.8%); rule-in: specificity 98.0% (97.3%-98.5%), PPV 83.6% (83.2%-90.6%)); Tables 2 and 3. Applying sum and product for rule-in and rule-out would lead to 45-49% of subjects that require a second cardiac troponin measurement after an hour in the ADAPT cohort.

Details of the subjects that were falsely ruled-out using sum and product are reported in supplemental table 12 and 13.

Early allocation based on a combination algorithm consisting of hs-cTnI and hs-cTnT

The optimal cut-off combination with an NPV of at least 99.6% was hs-cTnT < 9.8 ng/L and hs-cTnI < 4.8 ng/L. From a pragmatic point of view, we rounded these cut-off concentrations down

to hs-cTnT <9 ng/L and hs-cTnI <4 ng/L. In the original cohort (APACE) these thresholds combine to rule-out 48.4% of patients, to a 4-to-6-fold increase in the number of rule-outs at presentation than the ESC 0/3h algorithm. In the external validation cohort the optimal rule-out combination would rule-out >50% of subjects (sensitivity 98.8% (97.2%-99.6%), NPV 99.5% (98.9%-99.9%)). The NPV in the external validation cohort was lower than the one in the original cohort (Table 4). Details of the subjects that were falsely ruled-out using this combination algorithm are reported in Supplemental Table 12 and 13.

The optimal cut-off combination for rule-in was hs-cTnT ≥ 57 ng/L OR hs-cTnI ≥ 54 ng/L which in the APACE cohort ruled-in 259 (65.1%) of AMI patients. In the external validation cohort, 293 (71.8%) patients with a final diagnosis of AMI subjects would be ruled in (specificity 97.4% (93.7%-95.6%), PPV 84.0% (79.7%-87.6%)) (Table 5). This would lead to 43% of patients that require a second cardiac troponin measurement after an hour.

Discussion

We evaluated four methods to combine cTnI and cTnT for the early diagnosis of AMI in two large prospective diagnostic multicenter studies, and report three major findings.

First, the number of direct rule-outs at presentation using the algorithms of the current ESC guidelines[2] is limited (7-13% of subjects without an AMI) and assay-dependent. Second, the difference in diagnostic accuracy between the combinations of the cTn measured by the two assays and a cTn measurement by either assay alone is numerically small (except for when combined as a ratio). In addition, the results of the reclassification statistics indicated that the application of two cTn isoforms at presentation may add incremental value, but that this is not the case for the sum and product when applied to the whole cohort. Third, combining cardiac hs-

cTnI and hs-cTnT, using the sum and product or a combination algorithm, achieved a very high NPV and lead to a 3-to-6-fold increase in the number of rule-outs after a single blood draw compared to the ESC algorithms.

The findings from this study corroborate and extend previous work aiming to further improve the safety and efficacy of the rule-out and rule-in of AMI among patients presenting with acute chest discomfort to the ED [2–4,7,8,42–45]. Including two large meta-analyses providing exact estimates for the performance of single measurement rule-out strategies using very low concentrations of hs-cTnT and hs-cTnI [46,47]. To the best of our knowledge this work is the first systematic approach testing the clinical utility of combinations of hs-cTnI and hs-cTnT, the two most accurate biochemical signals in the early diagnosis of AMI [2–4,43–45]. While there is broad agreement that hs-cTnI or hs-cTnT should be used as a key component in any AMI rule-out algorithm [2,7,10,48,49], it has remained unclear whether a second biochemical signature could provide enough incremental value to potentially justify routine clinical use.

While when used in conjunction with less sensitive cTn assays, some additional biochemical signals including copeptin and heart-type fatty acid-binding protein (hFABP) were able to provide incremental diagnostic value, this was no longer the case when using hs-cTnT or hs-cTnI as recommended in current guidelines [50–55]. The only additional analyte that recently was suggested to possibly provide incremental diagnostic value even if using hs-cTnT is cardiac myosin-binding protein C, a quantitative marker of cardiomyocyte injury that seems even more rapidly released from injured cardiomyocytes as compared to hs-cTnT and hs-cTnI [56].

The novel concept investigated in this study was based on recent studies documenting that there could be remarkable differences between the cTnI and the cTnT signal, and the moderate agreement between clinical decisions made on these concentrations [20,57,58]. We

15

hypothesized that combining the two biochemical signals might overcome independent pathophysiological, pre-analytical and analytical differences between the individual molecules such as (auto)antibodies and suggested interference with troponin released from skeletal muscle[12,15,59,60], and might therefore have higher diagnostic accuracy for AMI than either cTnI or cTnT alone.

This study shows that combining hs-cTnI and hs-cTnT may contribute to a clinically relevant increase in the number of rule-outs at presentation. The small increase in false-negative results when the derived thresholds were applied in the external validation cohort raises the question what is considered a still acceptable number of false rule-ins and rule-outs[61]. Furthermore, it illustrates the outlier-dependency of the determination of very low cut-off values, and advocates the use of extended (pooled) cohorts and the recalibration of cut-off values for the determination of more universally applicable decision rules [62]. A second point that merits attention are the, at first sight contrary, unconvincing results of the diagnostic accuracy and reclassification statistics. Because the AUC is already very high for either hs-cTn alone and because it is based on ranking with the large numbers of patients below the LoD having the same rank, the signal from an additional biomarker to increase the AUC would need to be massive and the biomarker itself may need to be a better marker even than hs-cTn. These findings are of limited additional value for the whole population, whereas combing hs-cTnI and hs-cTnT might be especially valuable in patients with low hs-cTn concentrations at presentation. Another reason for this discrepancy might be the three-group (rule-out, observational, rule-in) approach that is used for the diagnosis of AMI and its outlier dependency.

The clinical implementation of a dual-marker approach combining cTnI and cTnT would likely be associated with substantial logistic obstacles since no diagnostic company currently is

able to provide both hs-cTnT and hs-cTnI assays on the same laboratory platform. In addition, most hospitals currently do not have analyzers for both analytes running on a 24/7 basis or even have only the platform for one of the assays at all. Therefore, the cost-effective clinical implementation of the dual-marker approach would require either additional investment in infrastructure by the laboratories (installing another platform) and/or collaboration among diagnostic companies for the provision of both hs-cTnT and hs-cTnI assays on the analyzer that is used for clinical chemistry routine. The clinical implementation of a dual-marker approach combining cTnI and cTnT would likely be associated also with substantial educational efforts for clinicians working in the ED, as two similar, yet different analytes with different clinical decision values would then be in clinical use at the same institution. Nevertheless, rapid and safe clinical decision making based on a single hs-cTn measurement at presentation seems to be approaching its limits, and the exploration of new diagnostic strategies including combinations of biomarkers, risk-assessment scores, or imaging seems to be indicated [8]. From this point of view, overcoming these logistic obstacles by close collaboration between diagnostic companies, hospital laboratories, medical doctors and researchers would be able to provide substantial medical value for patients and physicians, and economic value for hospitals and the health care system in general. Future studies are necessary to identify the best strategy and to better quantify the possible clinical benefit associated with the combination of cTnI and cTnT. Considering the relevant unmet clinical need as quantified by the high percentage of rule-out mismatches, the substantial increase in early rule-outs compared to the current ESC 0h/1h-algorithm and the substantial cost savings associated with reductions in the length of stay in the ED [63], dedicated economic analyses can be expected to show substantial reductions in time to decision, time to discharge, and therefore treatment costs. Consecutive studies to objectify these claims are

indicated. Furthermore, it is important to highlight that despite the very high diagnostic accuracy, hs-cTn and their combinations will always have to be used clinically only in conjunction with full clinical assessment including detailed patient history, physical examination, and the ECG [2].

Some limitations of this study merit consideration. First, the central adjudication by two independent cardiologists based on the clinical dataset including cardiac imaging and serial measurements of the local (hs)-cTn and the study-specific dataset including 34 chest pain characteristics, serial measurements of hs-cTnT, and follow-up in the APACE study represents the highest quality possible in a diagnostic study. However, it possibly introduced a very small but unavoidable disadvantage for hs-cTnI regarding diagnostic accuracy. This is at large counterbalanced by the use of (h)s-cTnI for the adjudication ADAPT, as this possibly introduced a very small but unavoidable disadvantage for hs-cTnT regarding diagnostic accuracy. Second, patients with terminal kidney failure on chronic dialysis were excluded from APACE. Accordingly, we cannot comment on the possible clinical utility of the combination approach in these vulnerable patients. Third, the method we used to determine the cut-off values for the combination algorithm could not produce very smooth curves for rule-in. Alternative methods may therefore provide better results for rule-in. Fourth, an alternative approach to combine both cardiac troponins would be logistic regression. As shown in the supplemental, this lead to comparable results. Nevertheless, the strong correlation between cardiac troponins may lead to spurious beta coefficients, and therefore we did not use this method for our primary results [64].

In conclusion, diagnostic strategies combining cTnI and cTnT measurements, sum, product or a combination algorithm, may significantly increase the number of patients eligible for very early and safe rule-out, but does not seem helpful for the rule-in of AMI.

Authors

Noreen van der Linden, MD, PhD[1]*; Karin Wildi, MD[2,3]*; Raphael Twerenbold, MD[2];

John W. Pickering, PhD[4,5]; Martin Than, MD[4,5]; Louise Cullen, MD[6-8];

Jaimi Greenslade, PhD[7,8]; William Parsonage, MD[6-8]; Thomas Nestelberger, MD[2];

Jasper Boeddinghaus, MD[2]; Patrick Badertscher, MD[2]; Maria Rubini Giménez, MD[2,9];

Lieke J.J. Klinkenberg, PhD[1]; Otto Bekers, PhD[1]; Aline Schöni, MD[2,10];

Dagmar I. Keller, MD[10]; Zaid Sabti, MD[2]; Christian Puelacher, MD[2]; Janosch Cupa, MD[2];

Lukas Schumacher, MD[2]; Nikola Kozhuharov, MD[2]; Karin Grimm, MD[2];

Samyut Shrestha, MD[2]; Dayana Flores, MD[2]; Michael Freese, RN[2]; Claudia Stelzig, MSc[2];

Ivo Strebel, PhDc[2]; Òscar Miró, MD[11]; Katharina Rentsch, PhD[12]; Beata Morawiec, MD[13];

Damian Kawecki, MD[13]; Wanda Kloos, MD[2]; Jens Lohrmann, MD[2]; A. Mark Richards, PhD[4,5];

Richard Troughton, PhD[4,5]; Christopher Pemberton, PhD[4,5]; Stefan Osswald, MD[2];

Marja P. van Dieijen-Visser, PhD[1]; Alma M. Mingels, PhD[1]; Tobias Reichlin, MD[2];

Steven J.R. Meex, PhD[1]; Christian Mueller, MD[2]

*Both authors contributed equally and should be considered first author.

Affiliations

[1]Department of Clinical Chemistry, Central Diagnostic Laboratory, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center (MUMC), Maastricht, the Netherlands; [2]Department of Cardiology and Cardiovascular Research Institute Basel (CRIB), University Hospital Basel, Basel, Switzerland; [3]Critical Care Research Group, The Prince Charles Hospital, Brisbane, Australia; [4]Department of Medicine, University of Otago, Christchurch, New Zealand; [5]Department of Medicine, University of Otago, Christchurch, New Zealand; [6]Department of Emergency Medicine, Royal Brisbane and Women's Hospital, Brisbane, Australia; [7]School of Public Health, Queensland University of Technology, Brisbane, Australia; [8]School of Medicine, The University of Queensland, Brisbane, Australia; [9]Emergency Department, CIBERES ISC III, Hospital del Mar – IMIM, Barcelona, Spain; [10]Emergency Department, University Hospital Zürich, Zürich, Switzerland; [11]Emergency Department, Hospital Clinic, Barcelona, Spain; [12]Laboratory Medicine, University Hospital Basel, Basel, Switzerland; [13]2nd Department of Cardiology and School of Medicine with the Division of Dentistry, Zabrze, Medical University of Katowice, Katowice, Poland

References

1. Nawar EW, Niska RW, Xu J. National Hospital Ambulatory Medical Care Survey: 2005 emergency department summary. *Advance data*. 2007;29:1–32.
2. Roffi M, Patrono C, Collet J-P, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP, Gencer B, Hasenfuss G, Kjeldsen K, Lancellotti P, Landmesser U, Mehilli J, Mukherjee D, Storey RF, Windecker S. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J*. 2016;37:267–315.
3. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, Biedert S, Schaub N, Buerge C, Potocki M, Noveanu M, Breidthardt T, Twerenbold R, Winkler K, Bingisser R, Mueller C. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med*. 2009;361:858–867.
4. Reiter M, Twerenbold R, Reichlin T, Haaf P, Peter F, Meissner J, Hochholzer W, Stelzig C, Freese M, Heinisch C, Breidthardt T, Freidank H, Winkler K, Campodarve I, Gea J, Mueller C. Early diagnosis of acute myocardial infarction in the elderly using more

sensitive cardiac troponin assays. *Eur Heart J*. 2011;32:1379–89.

5. Lipinski MJ, Escárcega RO, D'Ascenzo F, Magalhães M a., Baker NC, Torguson R, Chen F, Epstein SE, Miró Ò, Llorens P, Giannitsis E, Lotze U, Lefebvre S, Sebbane M, Cristol JP, Chenevier-Gobeaux C, Meune C, Eggers KM, Charpentier S, Twerenbold R, Mueller C, Biondi-Zoccai G, Waksman R. A systematic review and collaborative meta-analysis to determine the incremental value of copeptin for rapid rule-out of acute myocardial infarction. *Am J Cardiol*. 2014;113:1581–1591.

6. Mockel M, Searle J, Hamm C, Slagman A, Blankenberg S, Huber K, Katus H, Liebetrau C, Muller C, Muller R, Peitsmeyer P, von Recum J, Tajsic M, Vollert JO, Giannitsis E. Early discharge using single cardiac troponin and copeptin testing in patients with suspected acute coronary syndrome (ACS): a randomized, controlled clinical process study. *Eur Heart* J. 2015;36:369–376.

7. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, Huber K, Plebani M, Biasucci LM, Tubaro M, Collinson P, Venge P, Hasin Y, Galvani M, Koenig W, Hamm C, Alpert JS, Katus H, Jaffe AS. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J*. 2012;33:2252–2257. A

8. Nestelberger T, Wildi K, Boeddinghaus J, Twerenbold R, Reichlin T, Giménez MR, Puelacher C, Jaeger C, Grimm K, Sabti Z, Hillinger P, Kozhuharov N, du Fay de Lavallaz J, Pinck F, Lopez B, Salgado E, Miró Ò, Bingisser R, Lohrmann J, Osswald S, Mueller C. Characterization of the observe zone of the ESC 2015 high-sensitivity cardiac troponin 0h/1h-algorithm for the early diagnosis of acute myocardial infarction. *Int J Cardiol*. 2016;207:238–245.

9. Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Ganiats TG, Holmes DR, Jaffe AS, Jneid H, Kelly RF, Kontos MC, Levine GN, Liebson PR, Mukherjee D, Peterson ED, Sabatine MS, Smalling RW, Zieman SJ. 2014 AHA/ACC Guideline for the Management of Patients With Non–ST-Elevation Acute Coronary Syndromes. *Circulation*. 2014;130:e344-426.

10. Thygesen K, Mair J, Katus H, Plebani M, Venge P, Collinson P, Lindahl B, Giannitsis E, Hasin Y, Galvani M, Tubaro M, Alpert JS, Biasucci LM, Koenig W, Mueller C, Huber K, Hamm C, Jaffe AS, Study Group on Biomarkers in Cardiology of the ESCWG on ACC. Recommendations for the use of cardiac troponin measurement in acute cardiac care. *Eur Heart J*. 2010;31:2197–2204.

11. Klinkenberg LJJ, Wildi K, van der Linden N, Kouw IWK, Niens M, Twerenbold R, Rubini Gimenez M, Puelacher C, Daniel Neuhaus J, Hillinger P, Nestelberger T, Boeddinghaus J, Grimm K, Sabti Z, Bons JAP, van Suijlen JDE, Tan FES, ten Kate J, Bekers O, van Loon LJC, van Dieijen-Visser MP, Mueller C, Meex SJR. Diurnal rhythm of cardiac troponin: consequences for the diagnosis of acute myocardial infarction. *Clin Chem*. 2016;62:1602–1611.

12. Rubini Gimenez M, Twerenbold R, Reichlin T, Wildi K, Haaf P, Schaefer M, Zellweger C, Moehring B, Stallone F, Sou S, Mueller M, Denhaerynck K, Mosimann T, Reiter M, Freese M, Stelzig C, I K, Voegele J, Hartmann B, Rentsch K, Osswald S, Mueller C. Direct Comparison of High-sensitivity Cardiac Troponin I versus T for the Early Diagnosis of Acute Myocardial Infarction. *Eur Heart J*. 2014;35:2303–2311.

13. Artunc F, Mueller C, Breidthardt T, Twerenbold R, Peter A, Thamer C, Weyrich P, Haering H-U, Friedrich B. Sensitive Troponins – Which Suits Better for Hemodialysis Patients? Associated Factors and Prediction of Mortality. *PLoS ONE* . 2012;7:e47610.

14. Bais R. The effect of sample hemolysis on cardiac troponin I and T assays. *Clin Chem*. 2010;56:1357–9.

15. Lippi G, Aloe R, Meschi T, Borghi L, Cervellin G. Interference from heterophilic antibodies in troponin testing. Case report and systematic review of the literature. *Clin Chim Acta*. 2013;426:79–84.

16. van der Linden N, Cornelis T, Klinkenberg LJJ, Kimenai DM, Hilderink JM, Litjens EJR, Kooman JP, Bekers O, van Dieijen-Visser MP, Meex SJR. Strong diurnal rhythm of troponin T, but not troponin I, in a patient with renal dysfunction. *Intl J Cardiol*. 2016;221:287–288.

17. van der Linden N, Klinkenberg LJJ, Bekers O, Loon LJC van, Dieijen-Visser MP van, Zeegers MP, Meex SJR. Prognostic value of basal high-sensitive cardiac troponin levels on mortality in the general population. *Med*. 2016;95:e5703.

18. Katrukha IA. Human cardiac troponin complex. Structure and functions. *Biochem*. 2013;78:1447–1465.

19. Wu AH, Feng YJ. Biochemical differences between cTnT and cTnI and their significance for diagnosis of acute coronary syndromes. *Eur Heart J*. 1998;19 Suppl N:N25-9.

20. Kimenai DM, Henry RM, van der Kallen CJ, Dagnelie PC, Schram MT, Stehouwer C DA, van Suijlen J DE, Niens M, Bekers O, Sep SJ, Schaper NC, van Dieijen-Visser MP, Meex SJ. Direct comparison of clinical decision limits for cardiac troponin T and I. *Heart*. 2016;102:610–616.

21. Wildi K, Rubini Gimenez M, Twerenbold R, Reichlin T, Jaeger C, Heinzelmann A, Arnold C, Nelles B, Druey S, Haaf P, Hillinger P, Schaerli N, Kreutzinger P, Tanglay Y, Herrmann T, Moreno Weidmann Z, Krivoshei L, Freese M, Stelzig C, Puelacher C, Rentsch K, Osswald S, Mueller C. Misdiagnosis of Myocardial Infarction Related to Limitations of the Current Regulatory Approach to Define Clinical Decision Values for Cardiac Troponin. *Circulation*. 2015;131:2032–2040.

22. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS, CKD-EPI Investigators. Estimating Glomerular Filtration Rate from Serum Creatinine and Cystatin C. *N Engl J Med*. 2012;367:20–29.

23. Haaf P, Reichlin T, Twerenbold R, Hoeller R, Rubini Gimenez M, Zellweger C, Moehring B, Fischer C, Meller B, Wildi K, Freese M, Stelzig C, Mosimann T, Reiter M, Mueller M, Hochgruber T, Sou SM, Murray K, Minners J, Freidank H, Osswald S, Mueller C. Risk stratification in patients with acute chest pain using three high-sensitivity cardiac troponin assays. *Eur Heart J*. 2014;35:365–75.

24. Reiter M, Twerenbold R, Reichlin T, Benz B, Haaf P, Meissner J, Hochholzer W, Stelzig C, Freese M, Heinisch C, Balmelli C, Drexler B, Freidank H, Winkler K, Campodarve I, Gea J, Mueller C. Early diagnosis of acute myocardial infarction in patients with pre-existing coronary artery disease using more sensitive cardiac troponin assays. *Eur Heart* J. 2012;33:988–97.

25. Than M, Cullen L, Aldous S, Parsonage WA, Reid CM, Greenslade J, Flaws D, Hammett CJ, Beam DM, Ardagh MW, Troughton R, Brown AF, George P, Florkowski CM, Kline JA, Peacock WF, Maisel AS, Lim SH, Lamanna A, Richards AM. 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;59:2091–2098.

26. Than M, Aldous S, Lord SJ, Goodacre S, Frampton CMA, Troughton R, George P, Florkowski CM, Ardagh M, Smyth D, Jardine DL, Peacock WF, Young J, Hamilton G, Deely JM, Cullen L, Richards AM. A 2-hour diagnostic protocol for possible cardiac chest pain in the emergency department: a randomized clinical trial. *J Am Med Assoc Int Med*. 2014;174:51–8.

27. Than MP, Pickering JW, Aldous SJ, Cullen L, A Frampton CM, Frank Peacock W, Jaffe AS, Goodacre SW, Mark Richards A, Ardagh MW, Deely JM, Florkowski CM, George P, Hamilton GJ, Jardine DL, Troughton RW, van Wyk P, Young JM, Bannister L, Lord SJ. Effectiveness of EDACS Versus ADAPT Accelerated Diagnostic Pathways for Chest Pain: A Pragmatic Randomized Controlled Trial Embedded Within Practice. *Ann Emerg Med*. 2016;68:93–102.

28. Luepker R V., Apple FS, Christenson RH, Crow RS, Fortmann SP, Goff D, Goldberg RJ, Hand MM, Jaffe AS, Julian DG, Levy D, Manolio T, Mendis S, Mensah G, Pajak A, Prineas RJ, Reddy KS, Roger VL, Rosamond WD, Shahar E, Sharrett AR, Sorlie P, Tunstall-Pedoe H, AHA Council on Epidemiology and Prevention, AHA Statistics Committee, World Heart Federation Council on Epidemiology and Prevention, European Society of Cardiology Working Group on Epidemiology and Prevention, Centers for Disease Control and Prevention, National Heart, Lung, and Blood Institute. Case Definitions for Acute Coronary Heart Disease in Epidemiology and Clinical Research Studies: A Statement From the AHA Council on Epidemiology and Prevention; AHA Statistics Committee; World Heart Federation Council on Epidemiology and Prevention; the Centers for Disease Control and Prevention; and the National Heart, Lung and Blood Institute. *Circulation*. 2003;108:2543–2549.

29. Thygesen K, Alpert JS, White HD, Jaffe AS, Apple FS, Galvani M, Katus H a, Newby LK, Ravkilde J, Chaitman B, Clemmensen PM, Dellborg M, Hod H, Porela P, Underwood R, Bax JJ, Beller G a, Bonow R, Van der Wall EE, Bassand J-P, Wijns W, Ferguson TB, Steg PG, Uretsky BF, Williams DO, Armstrong PW, Antman EM, Fox K a, Hamm CW, Ohman EM, Simoons ML, Poole-Wilson P a, Gurfinkel EP, Lopez-Sendon J-L, Pais P, Mendis S, Zhu J-R, Wallentin LC, Fernández-Avilés F, Fox KM, Parkhomenko AN, Priori SG, Tendera M, Voipio-Pulkki L-M, Vahanian A, Camm a J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Widimsky P, Zamorano JL, Morais J, Brener S, Harrington R, Morrow D, Lim M, Martinez-Rios M a, Steinhubl S, Levine GN, Gibler WB, Goff D, Tubaro M, Dudek D, Al-Attar N. Universal definition of myocardial infarction. *Circulation*. 2007;116:2634–53.

30. Apple FS, Wu AHB, Jaffe AS. European Society of Cardiology and American College of Cardiology guidelines for redefinition of myocardial infarction: how to use existing assays clinically and for clinical trials. *Am Heart J*. 2002;144:981–6.

31. Apple FS, Wu AH, Jaffe AS, Panteghini M, Christenson RH, Cannon CP, Francis G, Jesse RL, Morrow DA, Newby LK, Storrow AB, Tang WH, Pagani F, Tate J, Ordonez-Llanos J, Mair J. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine practice guidelines: Analytical issues for biomarkers of heart failure. *Circulation*. 2007;116:e95-8.

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

33. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. *Clin Chem*. 2010;56:254–261.

34. Wildi K, Twerenbold R, Jaeger C, Rubini Giménez M, Reichlin T, Stoll M, Hillinger P, Puelacher C, Boeddinghaus J, Nestelberger T, Grimm K, Grob M, Rentsch K, Arnold C, Mueller C, Müller C. Clinical impact of the 2010–2012 low-end shift of high-sensitivity cardiac troponin T. *Eur Heart J Acute Cardiovas Care*. 2016;5:399–408.

35. Hallermayer K, Jarausch J, Menassanch-Volker S, Zaugg C, Ziegler R. Implications of adjustment of high-sensitivity cardiac troponin T assay. *Clin Chem*. 2013;59:572–4.

36. Kavsak P, Hill S, McQueen MJ, Devereaux PJ. Implications of adjustment of high-sensitivity cardiac troponin T assay. *Clin Chem*. 2013;59:574–6.

37. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F, Chronic Kidney Disease Epidemiology C. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 2006;145:247–254.

38. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiol*. 1983;148:839–843.

39. Pencina MJ, D'Agostino RB, Demler O V. Novel metrics for evaluating improvement in discrimination: net reclassification and integrated discrimination improvement for normal variables and nested models. *Stat Med*. 2012;31:101–13.

40. Reichlin T, Schindler C, Drexler B, Twerenbold R, Reiter M, Zellweger C, Moehring B, Ziller R, Hoeller R, Rubini Gimenez M, Haaf P, Potocki M, Wildi K, Balmelli C, Freese M, Stelzig C, Freidank H, Osswald S, Mueller C. One-Hour Rule-out and Rule-in of Acute Myocardial Infarction Using High-Sensitivity Cardiac Troponin T. *Arch Int Med*. 2012;172:1211.

41. Jaeger C, Wildi K, Twerenbold R, Reichlin T, Rubini Gimenez M, Neuhaus JD, Grimm K, Boeddinghaus J, Hillinger P, Nestelberger T, Singeisen H, Gugala M, Pretre G, Puelacher C, Wagener M, Honegger U, Schumacher C, Moreno Weidmann Z, Kreutzinger P, Krivoshei L, Freese M, Stelzig C, Dietsche S, Ernst S, Rentsch K, Osswald S, Mueller C. One-hour rule-in and rule-out of acute myocardial infarction using high-sensitivity cardiac troponin I. *Am Heart J*. 2016;171:92–102.

42. Raskovalova T, Twerenbold R, Collinson PO, Keller T, Bouvaist H, Folli C, Giavarina D, Lotze U, Eggers KM, Dupuy A-M, Chenevier-Gobeaux C, Meune C, Maisel A, Mueller C, Labarère J. Diagnostic accuracy of combined cardiac troponin and copeptin assessment for early rule-out of myocardial infarction: a systematic review and meta-analysis. *Eur Heart J Acute Cardiovasc Care*. 2014;3:18–27.

43. Lipinski MJ, Baker NC, Escárcega RO, Torguson R, Chen F, Aldous SJ, Christ M, Collinson PO, Goodacre SW, Mair J, Inoue K, Lotze U, Sebbane M, Cristol J-P, Freund Y, Chenevier-Gobeaux C, Meune C, Eggers KM, Pracoń R, Schreiber DH, Wu AHB, Ordoñez-Llanos J, Jaffe AS, Twerenbold R, Mueller C, Waksman R. Comparison of conventional and high-sensitivity troponin in patients with chest pain: A collaborative meta-analysis. *Am Heart J*. 2015;169:6–16.

44. Möckel M, Searle J, Hamm C, Slagman A, Blankenberg S, Huber K, Katus H, Liebetrau C, Müller C, Muller R, Peitsmeyer P, von Recum J, Tajsic M, Vollert JO, Giannitsis E. Early discharge using single cardiac troponin and copeptin testing in patients with suspected acute coronary syndrome (ACS): a randomized, controlled clinical process study. *Eur Heart J*. 2014;36:369–376.

45. Collinson P, Gaze D, Goodacre S. Comparison of contemporary troponin assays with the novel biomarkers, heart fatty acid binding protein and copeptin, for the early confirmation or exclusion of myocardial infarction in patients presenting to the emergency department with chest pain. *Heart*. 2014;100:140–145.

46. Chapman AR, Lee KK, McAllister DA, Cullen L, Greenslade JH, Parsonage W, Worster A, Kavsak PA, Blankenberg S, Neumann J, Söerensen NA, Westermann D, Buijs MM, Verdel GJE, Pickering JW, Than MP, Twerenbold R, Badertscher P, Sabti Z, Mueller C, Anand A, Adamson P, Strachan FE, Ferry A, Sandeman D, Gray A, Body R, Keevil B, Carlton E, Greaves K, Korley FK, Metkus TS, Sandoval Y, Apple FS, Newby DE, Shah AS V., Mills NL. Association of High-Sensitivity Cardiac Troponin I Concentration With Cardiac Outcomes in Patients With Suspected Acute Coronary Syndrome. *J Am Med Assoc*. 2017;318:1913.

47. Pickering JW, Than MP, Cullen L, Aldous S, Ter Avest E, Body R, Carlton EW, Collinson P, Dupuy AM, Ekelund U, Eggers KM, Florkowski CM, Freund Y, George P, Goodacre S, Greenslade JH, Jaffe AS, Lord SJ, Mokhtari A, Mueller C, Munro A, Mustapha S, Parsonage W, Peacock WF, Pemberton C, Richards AM, Sanchis J, Staub LP, Troughton R, Twerenbold R, Wildi K, Young J. Rapid Rule-out of Acute Myocardial Infarction With a Single High-Sensitivity Cardiac Troponin T Measurement Below the Limit of Detection: A Collaborative Meta-analysis. *Ann Int Med*. 2017;166:715–724.

48. Hollander JE, Than M, Mueller C. State-of-the-Art Evaluation of Emergency Department Patients Presenting With Potential Acute Coronary Syndromes. *Circulation*. 2016;134:547–564.

49. Mueller C, Giannitsis E, Möckel M, Huber K, Mair J, Plebani M, Thygesen K, Jaffe AS, Lindahl B, Biomarker Study Group of the ESC Acute Cardiovascular Care Association. Rapid rule out of acute myocardial infarction: novel biomarker-based strategies. *Eur Heart J Acute Cardiovasc Care*. 2017;6:218–222.

50. Devaux Y, Mueller M, Haaf P, Goretti E, Twerenbold R, Zangrando J, Vausort M, Reichlin T, Wildi K, Moehring B, Wagner DR, Mueller C. Diagnostic and prognostic value of circulating microRNAs in patients with acute chest pain. *J Int Med*. 2015;277:260–271.

51. Haaf P, Zellweger C, Reichlin T, Zbinden A, Wildi K, Mosimann T, Twerenbold R, Reiter M, Balmelli C, Freidank H, Gimenez MR, Peter F, Freese M, Stelzig C, Hartmann B, Dinter C, Osswald S, Mueller C. Utility of C-terminal Proendothelin in the Early Diagnosis and Risk Stratification of Patients With Suspected Acute Myocardial Infarction. *Can J Cardiol*. 2014;30:195–203.

52. Wildi K, Haaf P, Reichlin T, Acemoglu R, Schneider J, Balmelli C, Drexler B, Twerenbold R, Mosimann T, Reiter M, Mueller M, Ernst S, Ballarino P, Zellweger C, Moehring B, Vilaplana C, Freidank H, Mueller C. Uric acid for diagnosis and risk stratification in suspected myocardial infarction. *Eur J Clin Invest*. 2013 ;43:174–82.

53. Haaf P, Balmelli C, Reichlin T, Twerenbold R, Reiter M, Meissner J, Schaub N, Stelzig C, Freese M, Paniz P, Meune C, Drexler B, Freidank H, Winkler K, Hochholzer W, Mueller C. N-terminal pro B-type natriuretic peptide in the early evaluation of suspected acute myocardial infarction. *Am J Med*. 2011;124:731–739.

54. Reiter M, Twerenbold R, Reichlin T, Mueller M, Hoeller R, Moehring B, Haaf P, Wildi K, Merk S, Bernhard D, Mueller CZ, Freese M, Freidank H, Campodarve Botet I, Mueller C. Heart-type fatty acid-binding protein in the early diagnosis of acute myocardial

infarction. *Heart*. 2013;99:708–714.

55. Reichlin T, Hochholzer W, Stelzig C, Laule K, Freidank H, Morgenthaler NG, Bergmann A, Potocki M, Noveanu M, Breidthardt T, Christ A, Boldanova T, Merki R, Schaub N, Bingisser R, Christ M, Mueller C. Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol*. 2009;54:60–8.

56. Kaier TE, Twerenbold R, Puelacher C, Marjot J, Imambaccus N, Boeddinghaus J, Nestelberger T, Badertscher P, Sabti Z, Giménez MR, Wildi K, Hillinger P, Grimm K, Loeffel S, Shrestha S, Widmer DF, Cupa J, Kozhuharov N, Miró Ò, Martín-Sánchez FJ, Morawiec B, Rentsch K, Lohrmann J, Kloos W, Osswald S, Reichlin T, Weber E, Marber M, Mueller C. Direct Comparison of Cardiac Myosin-Binding Protein C With Cardiac Troponins for the Early Diagnosis of Acute Myocardial Infarction. *Circulation*. 2017;136:1495–1508.

57. Reichlin T, Cullen L, Parsonage W a., Greenslade J, Twerenbold R, Moehring B, Wildi K, Mueller S, Zellweger C, Mosimann T, Rubini Gimenez M, Rentsch K, Osswald S, Müller C. 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;128:369–379.

58. Mueller C, Giannitsis E, Christ M, Ordóñez-Llanos J, deFilippi C, McCord J, Body R, Panteghini M, Jernberg T, Plebani M, Verschuren F, French J, Christenson R, Weiser S, Bendig G, Dilba P, Lindahl B. 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.

59. Guclu T, Bolat S, Şenes M, Yucel D. Relationship between high sensitivity troponins and estimated glomerular filtration rate. *Clin Biochem*. 2016;49:467–471.

60. Adamczyk M, Brashear RJ, Mattingly PG. Circulating cardiac troponin-I autoantibodies in human plasma and serum. *Ann NY Acad Sci*. 2009;1173:67–74.

61. Than M, Herbert M, Flaws D, Cullen L, Hess E, Hollander JE, Diercks D, Ardagh MW, Kline JA, Munro PT, Jaffe A. What is an acceptable risk of major adverse cardiac event in chest pain patients soon after discharge from the Emergency Department? A clinical survey. *Int J Cardiol*. 2013;166:752–754.

62. Pickering JW, Than MP. The small number problem in diagnostic algorithms and why we need to bootstrap. *Clin Biochem*. 2017;50:540–541.

63. Shortt C, Xie F, Whitlock R, Ma J, Clayton N, Sherbino J, Hill SA, Pare G, McQueen M, Mehta SR, Devereaux PJ, Worster A, Kavsak P. Economic Considerations of Early Rule-In/Rule-Out Algorithms for The Diagnosis of Myocardial Infarction in The Emergency Department Using Cardiac Troponin and Glycemic Biomarkers. *Clin Chem*. 2017;63:593–602.

64. Bewick V, Cheek L, Ball J. Statistics review 14: Logistic regression. *Crit Care*. 2005;9:112–118.

Table 1. Diagnostic accuracy of hs-cTnI, hs-cTnT, the combination, sum, product and ratio for the diagnosis of AMI at presentation

Parameters	AUC (95% CI)	Compared with hs-cTnI alone (p-value)	Compared with hs-cTnT alone (p-value)
hs-cTnI alone	0.93 (0.92 – 0.94)		0.714
hs-cTnT alone	0.93 (0.92 – 0.94)	0.714	
hs-cTnI <4ng/L & hs-cTnT <9ng/L	0.93 (0.92 – 0.94)	0.789	0.002
Sum (hs-cTnI + hs-cTnT)	0.94 (0.93 – 0.95)	0.053	0.114
Product (hs-cTnI x hs-cTnT)	0.94 (0.93 – 0.95)	0.007	0.078
Ratio (hs-cTnI/hs-cTnT)	0.79 (0.78 – 0.81)	<0.001	<0.001

Table 2. Performance of sum and product for rule-out.

	Original cohort (N=2225; 398 AMI, 1827 NO AMI)	External validation cohort (N=2537; 408 AMI, 2129 NO AMI)
Sum < 9 ng/L		
All subjects	746 (33.5%)	988 (38.9%)
AMI	0 (0.0 %)	4 (1.0%)
No AMI	746 (40.8 %)	984 (46.2%)
NPV	*100%* *(99.5% - 100%)*	*99.6%* *(99.0% – 99.9%)*
Product < 18 ng^2/L^2		
All subjects	782 (35.1%)	1047 (41.3%)
AMI	0 (0.0 %)	6 (1.5%)
No AMI	782 (42.8 %)	1041 (48.9%)
NPV	*100%* *(99.5% - 100%)*	*99.4%* *(98.8% – 99.8%)*

Table 3. Performance of sum and product for rule-in.

	Original cohort (N=2225; 398 AMI, 1827 NO AMI)	External validation cohort (N=2537; 408 AMI, 2129 NO AMI)
Sum > 99 ng/L		
All subjects	324 (14.6%)	312 (12.3%)
AMI	249 (62.2%)	273 (66.9%)
No AMI	75 (4.1%)	39 (1.8%)
PPV	*76.9%* *(71.8% – 81.3%)*	*87.5%* *(83.3% - 91.0%)*
Product > 1608 ng^2/L^2		
All subjects	340 (15.3%)	337 (13.3%)
AMI	261 (65.6 %)	294 (72.1%)
No AMI	79 (4.3 %)	43 (2.0%)
PPV	*76.8%* *(71.9% – 81.2%)*	*87.2%* *(83.2% - 90.6%)*

Table 4. Performance of the combination approach for rule-out.

	Original cohort (N=2225; 398 AMI, 1827 NO AMI)	External validation cohort (N=2537; 408 AMI, 2129 NO AMI)
hs-cTnI < 4 ng/L AND hs-cTnT <9 ng/L		
All subjects	886 (39.8%)	1088 (42.9%)
AMI	1 (0.3%)	5 (1.2%)
No AMI	885 (48.4%)	1083 (50.9%)
NPV	*99.9% (99.2% – 100%)*	*99.5% (98.9% – 99.8%)*
hs-cTnI < 4 ng/L		
All subjects	1021 (45.9%)	1210 (47.7%)
AMI	5 (1.3%)	6 (1.5%)
No AMI	1016 (55.6%)	1204 (56.6%)
NPV	*99.5% (98.9% – 99.8%)*	*99.5% (98.9% – 99.8%)*
hs-cTnT <9 ng/L		
All subjects	1117 (50.2%)	1440 (56.8%)
AMI	12 (3.0%)	16 (3.9%)
No AMI	1105 (60.5%)	1424 (66.9%)
NPV	*98.9% (98.1% - 99.4%)*	*98.9% (93.7% – 97.7%)*

Table 5. Performance of the combination approach for rule-in.

	Original cohort (N=2225; 398 AMI, 1827 NO AMI)	External validation cohort (N=2537; 408 AMI, 2129 NO AMI)
hs-cTnI ≥ 54 ng/L OR hs-cTnT ≥ 57 ng/L		
All subjects	348 (15.6%)	349 (13.8%)
AMI	259 (65.1%)	293 (71.8%)
No AMI	89 (4.9%)	56 (2.6%)
PPV	74.4% (69.6% – 78.8%)	84.0% (79.7% - 87.6%)
hs-cTnI ≥ 54 ng/L		
All subjects	327 (14.7%)	322 (12.7%)
AMI	247 (62.1%)	283 (69.4%)
No AMI	80 (4.4%)	39 (1.8%)
PPV	75.5% (70.4% - 80.0%)	87.9% (83.8% - 91.2%)
hs-cTnT ≥ 57 ng/L		
All subjects	256 (11.5%)	240 (9.5%)
AMI	218 (54.8%)	206 (50.5%)
No AMI	38 (2.1%)	34 (1.6%)
PPV	85.2% (80.1% - 89.2%)	85.8% (80.8% - 90.0%)

32

Figure Legends

Figure 1. Log (base 10)-scale scatter plot of hs-cTnT and hs-cTnI at presentation in the APACE cohort

Log-scale scatter plot displaying hs-cTnI and hs-cTnT concentrations at presentation in the APACE cohort (n=2225). The correlation coefficient is high (Pearson's r=0.89).

Figure 2. ROC curves of the diagnostic performance of high-sensitivity cTn and their ratio, sum and product for NSTEMI in the APACE cohort

Diagnostic performance of high-sensitive cTn for non-ST segment myocardial infarction at presentation to the emergency department with acute chest pain. Receiver-operating-characteristic curves show the diagnostic accuracy of high-sensitive cardiac troponins I and T, their ratio, sum and product.
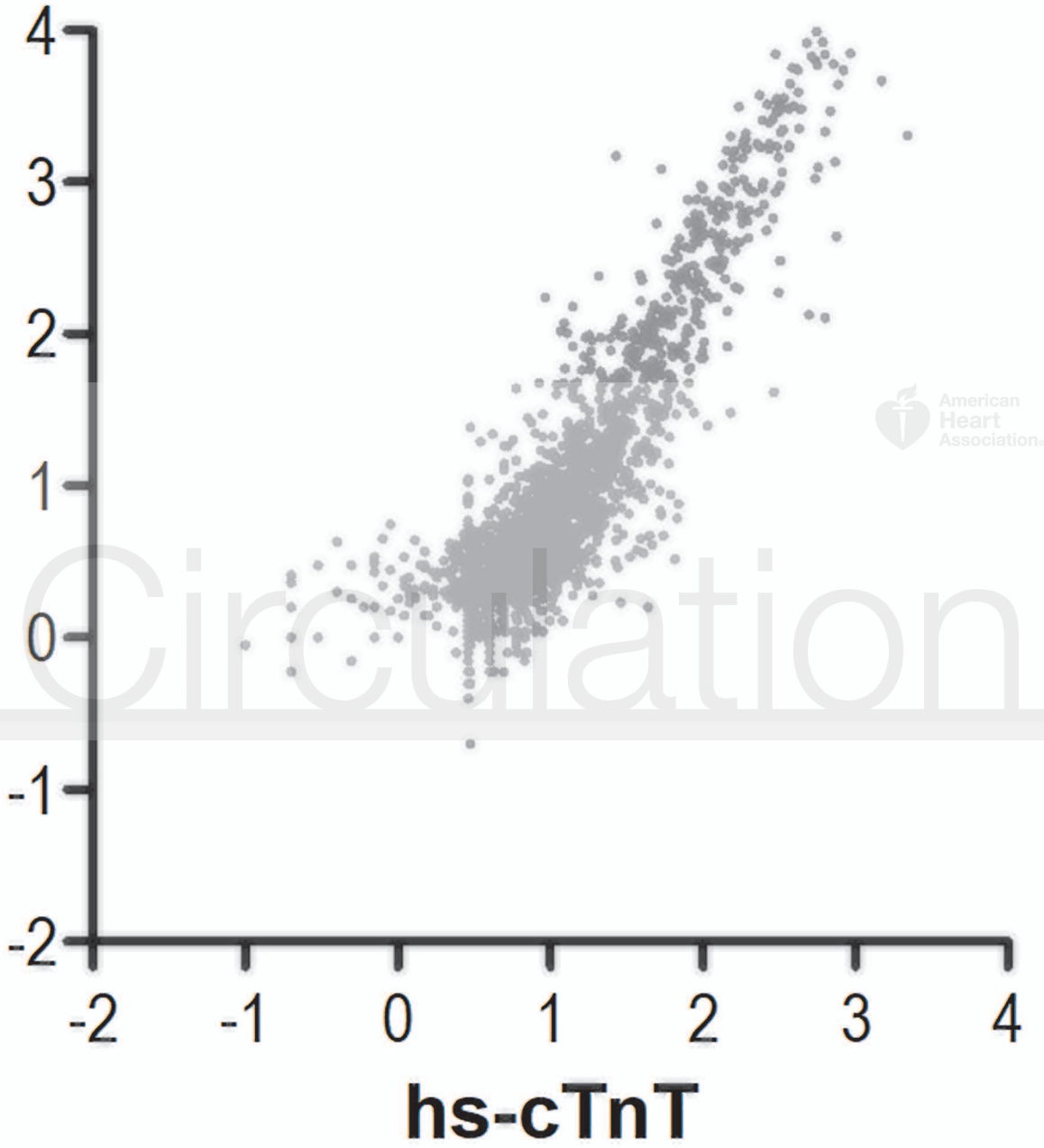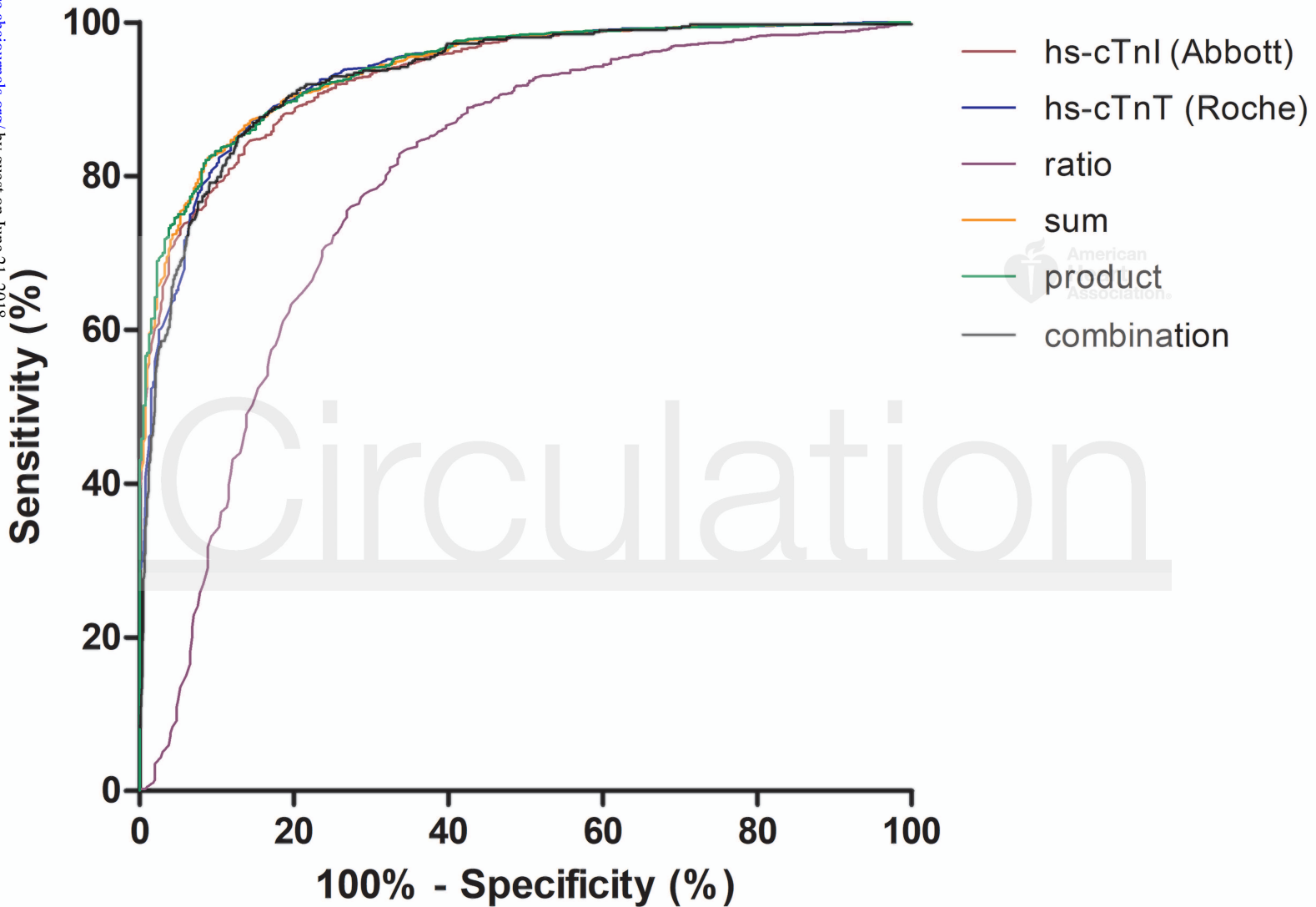
ROC analysis 0h

Legend:
- hs-cTnI (Abbott)
- hs-cTnT (Roche)
- ratio
- sum
- product
- combination

Y-axis: Sensitivity (%)
X-axis: 100% - Specificity (%)

Circulation

Combining High Sensitivity Cardiac Troponin I and Cardiac Troponin T in the Early Diagnosis of Acute Myocardial Infarction

Noreen van der Linden, Karin Wildi, Raphael Twerenbold, John W. Pickering, Martin Than, Louise Cullen, Jaimi Greenslade, William Parsonage, Thomas Nestelberger, Jasper Boeddinghaus, Patrick Badertscher, Maria Rubini Giménez, Lieke J. J. Klinkenberg, Otto Bekers, Aline Schöni, Dagmar I. Keller, Zaid Sabti, Christian Puelacher, Janosch Cupa, Lukas Schumacher, Nikola Kozhuharov, Karin Grimm, Samyut Shrestha, Dayana Flores, Michael Freese, Claudia Stelzig, Ivo Strebel, Òscar Miró, Katharina Rentsch, Beata Morawiec, Damian Kawecki, Wanda Kloos, Jens Lohrmann, A. Mark Richards, Richard Troughton, Christopher Pemberton, Stefan Osswald, Marja P. van Dieijen-Visser, Alma M. Mingels, Tobias Reichlin, Steven J. R. Meex and Christian Mueller

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/early/2018/04/20/CIRCULATIONAHA.117.032003

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2018/04/20/CIRCULATIONAHA.117.032003.DC1

SUPPLEMENTAL MATERIAL

Combining Cardiac Troponin I and Cardiac Troponin T in the Early Diagnosis of Acute Myocardial Infarction

Methods supplement

Supplementary results

Supplementary Table 1. Baseline characteristics of patients in the APACE cohort

Supplementary Table 2. Median hs-cTn concentrations at presentation in the APACE cohort

Supplementary Table 3. Baseline characteristics of patients in the ADAPT cohort

Supplementary Table 4. Agreement on rule-out at presentation using the 0h/3h algorithm in the APACE cohort

Supplementary Table 5. Agreement on rule-out at presentation using the 0h/1h algorithm in the APACE cohort

Supplementary Table 6. Agreement on rule-in at presentation using the 0h/1h algorithm in the APACE cohort

Supplementary Table 7. Diagnostic accuracy of hs-cTnT and hs-cTnI after the addition of the alternative signal or sum, product or ratio at presentation in the APACE cohort

Supplementary Table 8. IDI for events and non-events in the APACE cohort

Supplementary Table 9. Comparison of the combination of signals from Abbott hs-cTnTI and different (hs) cTnI assays at presentation

Supplementary Table 10. Comparison of the sum of the signals from Abbott hs-cTnTI and different (hs) cTnI assays at presentation

Supplementary Table 11. Comparison of the product of the signals from Abbott hs-cTnI and different (hs) cTnI assays at presentation

Supplementary Table 12. Descriptions of falsely ruled-out patients in the APACE cohort

Supplementary Table 13. Descriptions of falsely ruled-out patients in the ADAPT cohort

Supplementary Figure 1. hs-cTn concentrations at presentation in the APACE cohort

Methods supplement

Use of local conventional cTn values and hs-cTnT values for adjudication of final diagnoses in APACE cohort

The cTn assays used clinically in most of the participating institutions changed during the study from a conventional cTn assay to the hs-cTnT assay. In this analysis 40% of patients in APACE were managed clinically with a high-sensitivity troponin. In order to take advantage of the higher sensitivity and higher overall diagnostic accuracy offered by the hs-cTnT assay, patients were adjudicated using the hs-cTnT values in all patients. In patients in whom clinically a conventional cTn assay was used, the conventional cTn values and the hs-cTnT values were available for the adjudication. In patients in whom clinically the hs-cTnT assay was used, only the hs-cTnT values were available for the adjudication.

The following conventional cTn assays were used: For the Roche cTnT 4th generation assay, the 10% CV level is 0.035ug/l. The laboratories of the participating sites reported only two decimals; therefore 0.04ug/l was used as a cut-off for myocardial necrosis. In order to fulfil the criteria of a significant change (30% of 99th percentile or 10% CV level), a patient would e.g. need to have a level of <0.01ug/l at presentation and 0.04ug/l at 6h. A patient would also qualify if the first level is 0.02ug/l and the second 0.04ug/l. A patient would not fulfil the criteria if the first level is 0.03ug/l and the second is 0.04ug/l. If the first level is 0.04ug/l, the second level needs to be at least 0.06ug/l.

For the Abbott Axsym cTnI ADV, the 10% CV level is 0.16ug/l. A patient having 0.16ug/l at presentation would meet the criteria for significant change if the second was ≥0.21ug/l. A patient having <0.12ug/l at presentation (limit of detection) would qualify if the second is >0.16ug/l.

For the Beckmann Coulter Accu cTnI, the 10% CV level is 0.06ug/l. A patient having 0.06ug/l at presentation would qualify if the second is ≥0.08ug/l. A patient having 0.05 at presentation would qualify if the second is 0.07ug/l, but not 0.06ug/l. A patient having undetectable cTnI (cTnI<0.01ug/l) at presentation would qualify if the second is ≥0.06ug/l.

For hs-cTnT the 99th percentile (14ng/l) was used as cut-off for myocardial necrosis [1,2]. Absolute changes in hs-cTnT were used to determine significant changes based on the diagnostic superiority of

absolute over relative changes [3,4]. Based on studies of the biological variation of cTn [5,6] as well as on data from previous chest pain cohort studies [7,8], a significant absolute change was defined as a rise or fall of at least 10ng/l within six hours. In patients, in whom a 6 hour hs-cTnT level was not available, changes were assessed at earlier time points. In an assumption of linearity, an absolute change of 6ng/l within three hours was considered.

Adjudication of final diagnosis in ADAPT cohort

Outcomes were adjudicated independently by local cardiologists using predefined standardized reporting guidelines. Cardiologists had knowledge of the clinical record, ECG, troponin results and objective testing from standard care. A second cardiologist conducted a blind review of all ACS cases and 10% of non-ACS cases. In cases of disagreement, endpoints were agreed by consensus.

Diagnosis of AMI was according to international guidelines and based on evidence of myocardial necrosis and ischemia. Evidence of ischaemia included at least one of ECG changes or positive imaging results from exercise tolerance testing, myocardial perfusion scan, stress echocardiography, computed tomographic coronary angiography or coronary angiography during catheterization. Necrosis was diagnosed based on a rise or fall of cardiac troponin concentration over at least six hours with at least one value above the 99th percentile of the normal reference range at a level of assay imprecision near to 10%. If the troponin was greater than the reference range but no rise or fall was recorded, other causes of raised troponin were considered. If no alternative cause for the troponin rise was apparent and if the clinical presentation was suggestive of ACS, an adjudicated diagnosis of AMI was made.

Emergency revascularisation was defined as PCI or CABG in a symptomatic patient where the clinical status includes either 1) ischaemic dysfunction (ongoing ischaemia despite maximal medical therapy, acute evolving myocardial within 24 hours before intervention or pulmonary oedema requiring intubation) or 2) mechanical dysfunction (shock with or without circulatory support). Urgent revascularisation included PCI or CABG that did not meet the emergency criteria above but was

required during the same hospitalization to minimize chance of further clinical deterioration. Elective revascularization, or those procedures that could be deferred without increased risk of compromised cardiovascular outcome, were not included in the endpoint.

Assumption of linearity of absolute changes of hs-cTnT within the first hours

The assumption of linearity of absolute changes within the first hours is based on unpublished internal data as well as recent data from Ola Hammarsten et al. showing a near-linear increase in levels of hs-cTnT with increasing time from symptom onset in their NSTEMI cohort [9].

Determination of cut-offs for the combination algorithm

We aimed to determine the safe, and optimal combination of hs-cTnI concentration and hs-cTnT concentration. A test was considered positive if either hs-cTn concentration was greater than or equal to an hs-cTn threshold for that troponin assay. We varied the hs-cTn threshold for each troponin assay in steps of 0.1 ng/L across the range from the LoD to 26 ng/L. At each combination of hs-cTn thresholds we calculated the sensitivity and specificity for the diagnosis of AMI. We created 500 bootstraps of the data, and to average the results we then fitted a smooth surface (thin plate spline) for sensitivity on the hs-cTnI threshold – hs-cTnT threshold grid from which we could determine the 99% sensitivity contour and contours for specificity. The optimal threshold was defined as the combination with 99% sensitivity and maximized specificity.

Combining cardiac troponin T and cardiac troponin I using logistic regression

In addition, we performed a logistic regression model for the index admission of AMI using a single blood draw at presentation. The log-10-transformed hs-cTnI and hs-cTnT concentrations were combined in a logistic regression model in order to calculate the probability of AMI for each patient.

SUPPLEMENTAL REFERENCES

1. Apple FS, Wu AH, Jaffe AS. European Society of Cardiology and American College of Cardiology guidelines for redefinition of myocardial infarction: how to use existing assays clinically and for clinical trials. *Am Heart J*. 2002;144:981–986.

2. Apple FS, Jesse RL, Newby LK, Wu AHB, Christenson RH. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: Analytical issues for biochemical markers of acute coronary syndromes. *Circ*. 2007;115:e352-5.

3. Reichlin T, Irfan A, Twerenbold R, Reiter M, Hochholzer W, Burkhalter H, Bassetti S, Steuer S, Winkler K, Peter F, Meissner J, Haaf P, Potocki M, Drexler B, Osswald S, Mueller C. Utility of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. *Circ*. 2011;124:136–45.

4. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, Huber K, Plebani M, Biasucci LM, Tubaro M, Collinson P, Venge P, Hasin Y, Galvani M, Koenig W, Hamm C, Alpert JS, Katus H, Jaffe AS, the Study Group on Biomarkers in Cardiology of the ESCWG on ACC. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J*. 2012;33:2252-2257.

5. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. *Clin Chem*. 2010;56:1086–1090.

6. Wu A, Lu QA, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. *Clin Chem*. 2009;55:52–58.

7. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, Bickel C, Baldus S, Warnholtz A, Frohlich M, Sinning CR, Eleftheriadis MS, Wild PS, Schnabel RB, Lubos E, Jachmann N, Genth-Zotz S, Post F, Nicaud V, Tiret L, Lackner KJ, Munzel TF, Blankenberg S. Sensitive troponin I assay in early diagnosis of acute myocardial infarction. *N Engl J Med*. 2009;361:868–877.

8. Apple FS, Pearce LA, Smith SW, Kaczmarek JM, Murakami MM. Role of monitoring changes in sensitive cardiac troponin I assay results for early diagnosis of myocardial infarction and prediction of risk of adverse events. *Clin Chem*. 2009;55:930–937.

9. Hammarsten O, Fu ML, Sigurjonsdottir R, Petzold M, Said L, Landin-Wilhelmsen K, Widgren B, Larsson M, Johanson P. Troponin T percentiles from a random population sample, emergency room patients and patients with myocardial infarction. *Clin Chem*. 2012;58:628–637.

Supplementary results

Combining cardiac troponin T and cardiac troponin I using logistic regression

Applying a logistic regression model (described in the methods supplement) to the original cohort (APACE) resulted in: $p = 1/(1+\exp(-X_{Beta}))$ where $X_{Beta} = -5.75 + 1.1 * \log(\text{hs-TnI}) + 2.34* \log (\text{hs-TnT})$. The corresponding AUC was 0.940 (95%CI: 0.929 to 0.952).

In the original cohort, the determined probability threshold (p) for a NPV of 99.5% was p=0.05. This resulted in a NPV of 99.5% (95% CI 98.9% to 99.8%) and allocation of 82.1% of patients to the rule-out category. The probability threshold of p=0.38 corresponded to a PPV of 75%, and led to a PPV of 75.5% (70.7% to 79.9%) and the allocation of 17.9% of patients to the rule-in category.

When the beta coefficients that were derived from the logistic regression model in the original cohort were applied to the validation cohort (ADAPT), the AUC was 0.959 (95%CI: 0.949 to 0.959). Using the probability thresholds from the original cohort (p=0.05 and p=0.38 for rule-out and rule-in, respectively), the NPV was 99.3% (98.6% to 99.6%) and the PPV was 82.5% (78.2% to 86.2%). This would lead to the allocation of 83.9% and 16.1% of patients to the rule-out and the rule-in category, respectively.

Supplementary Table 1. Baseline characteristics of patients in the APACE cohort

Characteristic	All	AMI	No AMI
	n= 2225	n= 398	n= 1827
Age, y	62 (49 – 75)	72 (61 – 80)	60 (47 – 73)
Male sex, n (%)	1511 (68)	290 (73)	1221 (67)
Risk factors, n (%)			
Hypertension	1382 (62)	318 (80)	1064 (58)
Hypercholesterolemia	1110 (50)	272 (68)	838 (46)
Diabetes Mellitus	384 (17)	108 (27)	276 (15)
Current or previous smoking	1366 (61)	253 (64)	1113 (61)
Family history	549 (25)	117 (29)	432 (24)
History, n (%)			
Coronary Artery Disease	776 (35)	202 (51)	574 (31)
Previous AMI	513 (23)	136 (34)	377 (21)
Previous revascularisation	612 (28)	147 (37)	465 (26)
Peripheral artery disease	141 (6)	56 (14)	85 (5)
Previous stroke	122 (6)	39 (10)	83 (5)
Body-mass index, kg/m^2	26 (24 – 30)	26 (24 – 29)	26 (24 – 30)
eGFR, ml·min^{-1}·1.73m^{-2}	85 (69 – 101)	73 (55 – 93)	87 (71 – 103)
ECG changes, n (%)			
ST segment depression	232 (10)	121 (30)	111 (6)
T wave inversion	296 (13)	100 (25)	196 (11)
LBBB	25 (1)	11 (3)	14 (1)
Medication at presentation			
ASA	808 (36)	199 (50)	609 (33)
Vitamin K antagonist	191 (9)	41 (10)	150 (8)
β-blockers	770 (35)	174 (44)	596 (33)
Statins	777 (35)	178 (45)	599 (33)
ACEIs/ARBs	842 (38)	206 (52)	636 (35)
Calcium antagonists	321 (14)	82 (21)	239 (13)
Nitrates	257 (12)	85 (21)	172 (9)

APACE denotes Advantageous Predictors of Acute Coronary Syndrome Evaluation; AMI, acute myocardial infarction; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; LBBB, left bundle branch block; ASA, acetyl salicylic acid; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Values are expressed in percentage or medians ± IQR.

Supplementary Table 2. Hs-cTn concentrations at presentation in the APACE cohort

	hs-cTnI (ng/L)	hs-cTnT (ng/L)	Sum (ng/L)	Product (ng²/L²)	Ratio
AMI (n=398)	115.2 (21.7 – 632.9)	64.1 (28.0 – 152.4)	184.2 (52.4 – 812.8)	7258 (596 – 91526)	1.84 (0.82 – 4.72)
No AMI (n=1827)	3.5 (2.2 – 7.2)	7.0 (4.0 – 12.4)	10.6 (6.5 – 20.0)	23 (9 – 86)	0.57 (0.38 – 0.90)
UA (n=216)	6.4 (3.6 – 12.1)	10.7 (6.9 – 16.0)	17.5 (11.1 – 29.6)	63 (25 – 199)	0.65 (0.42 – 1.12)
Cardiac, non-coronary (n=307)	8.4 (3.4 – 28.6)	13.4 (7.0 – 29.0)	10.6 (23.3 – 59.5)	112 (24 – 727)	0.70 (0.45 – 1.31)
NCPP (n=1202)	3.0 (1.9 – 5.1)	6.0 (3.9 – 9.7)	8.9 (5.9 – 15.1)	17 (8 – 47)	0.52 (0.36 – 0.81)
Unknown (n=102)	2.9 (2.3 – 4.8)	5.8 (3.0 – 9.4)	8.8 (5.8 – 14.9)	17 (8 – 48)	0.58 (0.39 – 0.76)
p-value *(AMI compared with no AMI)*	*p<0.001*	*p<0.001*	*p<0.001*	*p<0.001*	*p<0.001*

Median (IQR)

Supplementary Table 3. Baseline characteristics of patients in the ADAPT cohort

Characteristic	All	AMI	No AMI
	n=2537	n=408	n=2129
Age, y	60 (51 – 71)	59 (49 – 69)	69 (59 – 78)
Male sex, n (%)	1535 (61)	292 (72)	1243 (58)
Risk factors, n (%)			
Hypertension	1316 (52)	254 (62)	1062 (50)
Hypercholesterolemia	1309 (52)	228 (56)	1081 (51)
Diabetes Mellitus	377 (15)	81 (20)	296 (14)
Current smoking	462 (18)	72 (18)	390 (18)
Family history of CAD	1393 (55)	245 (60)	1148 (54)
History, n (%)			
Previous AMI	627 (25)	128 (31)	499 (23)
Previous PCI	525 (21)	85 (21)	440 (21)
Previous CABG	219 (9)	55 (13)	164 (8)
Peripheral artery disease	94 (4)	25 (6)	69 (3)
Previous stroke	205 (8)	39 (10)	166 (8)
Body-mass index, kg/m^2	27 (24 – 31)	28 (24 – 31)	27 (24 – 31)

ADAPT denotes Accelerated Diagnostic Protocol to Assess Patients Witch Chest Pain Symptoms Using Contemporary Troponins as the Only Biomarker; AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention.

Values are expressed in percentage or medians ± IQR.

Supplementary Table 4. Agreement on rule-out at presentation using the 0h/3h-algorithm in the APACE cohort

		hs-cTnT	
		Rule-out*	No rule-out
hs-cTnI	**Rule-out***	132 (5.9%)	25 (1.1%)
	No rule-out	1 (0.0%)	2067 (92.9%)

* if hs-cTnT < 14 ng/L or hs-cTnI < 26.2 ng/L, >6h after onset of symptoms and painfree, and GRACE score < 140

Supplementary Table 5. Agreement on rule-out at presentation using the 0h/1h-algorithm in the APACE cohort

		hs-cTnT	
		Rule-out*	No rule-out
hs-cTnI	**Rule-out***	98 (4.4%)	51 (2.3%)
	No rule-out	137 (6.2%)	1939 (87.1%)

* if hs-cTnT < 5 ng/L or hs-cTnI < 2 ng/L and >3h after onset of symptoms

Supplementary Table 6. Agreement on rule-in at presentation using the 0/1h-algorithm in the APACE cohort

		hs-cTnT	
		Rule-in*	No rule-in
hs-cTnI	**Rule-in***	246 (11.1%)	85 (3.8%)
	No rule-in	27 (1.2%)	1867 (83.9%)

* if hs-cTnT > 52 ng/L or hs-cTnI > 52 ng/L

Supplementary Table 7. Diagnostic accuracy of hs-cTnT and hs-cTnI after the addition of the alternative signal or sum, product or ratio at presentation in the APACE cohort

	Time point	AUC (95% CI; SE)	AUC (95% CI; SE) after addition of hs-cTnI or hs-cTnT	p-value	AUC (95% CI;SE) after addition of sum	p-value	AUC (95% CI;SE) after addition of product	p-value	AUC (95% CI;SE) after addition of ratio	p-value
hs-cTnT	0h*	0.93 (0.92 – 0.94; 0.009)	0.94 (0.93 – 0.95; 0.008)	*0.002*	0.94 (0.93 – 0.95; 0.009)	*0.006*	0.94 (0.93 – 0.95; 0.008)	*0.002*	0.94 (0.93 – 0.95; 0.008)	*0.002*
	0h + 1h†	0.96 (0.95 – 0.97; 0.008)	0.96 (0.95– 0.97; 0.008)	*0.795*	0.96 (0.95 – 0.97; 0.008)	*0.838*	0.96 (0.95– 0.97; 0.008)	*0.795*	0.96 (0.95– 0.97; 0.008)	*0.795*†
	0h + 0h/1h-delta†	0.95 (0.93 – 0.96; 0.009)	0.95 (0.94 – 0.96; 0.009)	*0.142*	0.95 (0.94 – 0.96; 0.009)	*0.217*	0.95 (0.94 – 0.96; 0.009)	*0.142*	0.95 (0.94 – 0.96; 0.009)	*0.142*
hs-cTnI	0h*	0.93 (0.92 – 0.94; 0.009)	0.94 (0.93 – 0.95; 0.008)	*0.118*	0.94 (0.93 – 0.95; 0.008)	*0.157*	0.94 (0.93 – 0.95; 0.008)	*0.118*	0.94 (0.93 – 0.95; 0.008)	*0.118*
	0h + 1h‡	0.95 (0.94 – 0.96; 0.009)	0.96 (0.95 – 0.97; 0.007)	*0.006*	0.96 (0.95 – 0.97; 0.008)	*0.007*	0.96 (0.95 – 0.97; 0.007)	*0.006*	0.96 (0.95 – 0.97; 0.007)	*0.006*
	0h + 0h/1h-delta‡	0.94 (0.93 – 0.95; 0.009)	0.95 (0.94 – 0.96; 0.009)	*0.014*	0.95 (0.94 – 0.96; 0.009)	*0.021*	0.95 (0.94 – 0.96; 0.009)	*0.014*	0.95 (0.94 – 0.96; 0.009)	*0.014*

*2225 patients, †1786 patients, ‡1752 patients

Supplementary Table 8. IDI for events and non-events in the APACE cohort

		combination	p-value	sum	p-value	Product	p-value	ratio	p-value
hs-cTnT	IDI event	0.013	< 0.001	-0.131	1.0	-0.244	1.0	-0.230	1.00
	IDI non event	-0.003		0.028		0.053		0.050	
hs-cTnI	IDI event	0.174	<0.001	0.031	<0.001	-0.082	1.00	-0.068	1.00
	IDI non event	-0.038		-0.007		0.018		0.015	

dedicates hs-cTnT resp. hs-cTnI alone (old model) versus combinations of hs-cTnT and hs-cTnI (new model)

Supplementary Table 9. Comparison of the combination of signals from Abbott hs-cTnI and different (hs) cTnI assays at presentation

		Abbott hs-cTnI (n=2225)	Siemens c-TnI Ultra (n=2127)	Beckman hs-cTnI (n=1028)	Siemens hs-cTnI Vista (n=1348)	Combination T and I
Abbott hs-cTnI and …		AUC: 0.93 (0.92 – 0.94)	AUC: 0.91 (0.90 – 0.92)	AUC: 0.92 (0.90 – 0.94)	AUC: 0.92 (0.90 – 0.93)	AUC: 0.94 (0.93 – 0.95)
Siemens c-TnI Ultra (n=2127)	AUC: 0.89 (0.88 – 0.90)	P < 0.001	P = 0.006			P < 0.001
Beckman hs-cTnI (n=1028)	AUC: 0.93 (0.91 – 0.94)	P = 0.635		P = 0.119		P = 0.570
Siemens hs-cTnI Vista (n=1348)	AUC: 0.91 (0.90 – 0.93)	P = 0.073			P = 0.066	P = 0.057

AUC (and the corresponding 95% confidence intervals), the comparison between AUCs was performed as recommended by Hanley and McNeil

Supplementary Table 10. Comparison of the sum of the signals from Abbott hs-cTnTI and different (hs) cTnI assays at presentation

		Abbott hs-cTnI (n=2225)	Siemens c-TnI Ultra (n=2127)	Beckman hs-cTnI (n=1028)	Siemens hs-cTnI Vista (n=1348)	Sum T and I
Abbott hs-cTnI and …		AUC: 0.93 (0.92 – 0.94)	AUC:0.91 (0.90 – 0.92)	AUC: 0.92 (0.90 – 0.94)	AUC: 0.92 (0.90 – 0.93)	AUC: 0.94 (0.93 – 0.95)
Siemens c-TnI Ultra (n=2127)	AUC: 0.93 (0.91 – 0.94)	P = 0.286	P < 0.001			P = 0.021
Beckman hs-cTnI (n=1028)	AUC: 0.93 (0.91 – 0.94)	P = 0.715		P = 0.135		P = 0.143
Siemens hs-cTnI Vista (n=1348)	AUC: 0.93 (0.91 – 0.94)	P = 0.539			P = 0.013	P = 0.053

AUC (and the corresponding 95% confidence intervals), the comparison between AUCs was performed as recommended by Hanley and McNeil

Supplementary Table 11. Comparison of the product of the signals from Abbott hs-cTnI and different (hs) cTnI assays at presentation

		Abbott hs-cTnI (n=2225)	Siemens c-TnI Ultra (n=2127)	Beckman hs-cTnI (n=1028)	Siemens hs-cTnI Vista (n=1348)	Product T and I
*Abbott hs-cTnI * …*		AUC: 0.93 (0.92 – 0.94)	AUC:0.91 (0.90 – 0.92)	AUC: 0.92 (0.90 – 0.94)	AUC: 0.92 (0.90 – 0.93)	AUC: 0.94 (0.93 – 0.95)
Siemens c-TnI Ultra (n=2127)	AUC: 0.93 (0.92 – 0.94)	P = 0.449	P < 0.001			P = 0.017
Beckman hs-cTnI (n=1028)	AUC: 0.93 (0.91 – 0.95)	P = 0.962		P = 0.030		P = 0.146
Siemens hs-cTnI Vista (n=1348)	AUC: 0.93 (0.91 – 0.94)	P = 0.560			P = 0.004	P = 0.031

AUC (and the corresponding 95% confidence intervals), the comparison between AUCs was performed as recommended by Hanley and McNeil

Supplementary Table 12. Descriptions of falsely ruled-out patients using the combination algorithm in the APACE cohort

missed by	age	sex	time since CPO, in h	history of CAD	0h hs-cTnT (ng/l)	0h hs-cTnI (ng/l)	ECG changes	discharge diagnosis	PCI performed	PCI results
combination algorithm	75	1	1	yes	6.3	2.9	no	stable angina	no	
hs-cTnI <4ng/l	75	m	1	yes	6.3	2.9	no	stable angina	no	
	73	m	4	yes	33.3	3.4	no	unclear	no	
	74	f	1	no	10.0	3.1	no	other cardiopathy	yes	LAD, LCX
	93	f	9	yes	41.0	3.6	no	unclear	no	
	79	f	1	yes	18.0	3.9	ST-depression	rhythm disorder	no	
hs-cTnT <9ng/l	75	m	1	yes	6.3	2.9	no	stable angina	no	
	72	m	4	yes	8.0	7.5	RBBB	NSTEMI	yes	LAD, LCX, RCA
	51	m	1	yes	7.1	27.9	no	NSTEMI	yes	LAD, LCX, RCA
	66	m	1	yes	4.8	6.4	no	unclear	yes	LAD, LCX
	57	f	0	yes	8.9	8.9	no	unstable angina	yes	LAD, RCA
	47	f	2	no	4.5	5.3	ST-depression	NSTEMI	yes	LAD
	61	m	2	no	7.3	12.0	no	unstable angina	yes	RCA
	71	m	12	yes	8.8	4.5	no	unstable angina	yes	LAD, LCX, Bypass
	46	m	1	no	6.0	6.0	ST-depression	NSTEMI	yes	LCX

| missed by	age	sex	time since CPO, in h	history of CAD	0h hs-cTnT (ng/l)	0h hs-cTnI (ng/l)	ECG changes	discharge diagnosis	PCI performed	PCI results
	44	m	2	no	5.0	4.1	not sig. ST-elev.	STEMI	yes	LCX, RCA
	64	m	1	no	8.0	10.7	ST-depression	STEMI	yes	LCA, LCX
	54	f	12	no	6.0	9.9	ST-depression	NSTEMI	yes	LCX

Supplementary Table 13. Descriptions of falsely ruled-out patients in the ADAPT cohort

missed by	age	sex	time since CPO, in h	history of CAD	0h hs-cTnT (ng/l)	0h hs-cTnI (ng/l)	ECG changes	discharge diagnosis	PCI performed	PCI results
Sum	81	m	8	yes	6.5	2.1	no	NSTEMI	yes	LAD, RCA
	68	f	2	yes	0.1	2.4	no	NSTEMI	no	
	65	f	9	no	5.0	3.8	no	NSTEMI	no	
	60	m	1	no	0.1	0.1	yes	STEMI	yes	LAD, LCX
Product	81	m	8	yes	6.5	2.1	no	NSTEMI	yes	LAD, RCA
	60	m	1	no	0.1	0.1	yes	STEMI	yes	LAD, LCX
	68	f	2	yes	0.1	2.4	no	NSTEMI	no	
	59	m	2	yes	0.1	108.5	no	NSTEMI	no	
	54	m	9	yes	0.1	43.3	no	NSTEMI	yes	No
combination algorithm	81	m	8	yes	6.5	2.1	no	NSTEMI	yes	LAD, RCA
	60	m	1	no	0.1	0.1	yes	STEMI	yes	LAD, LCX

	Age	Sex								
	71	m	4	yes	7.8	4.6	no	NSTEMI	no	
	68	f	2	yes	0.1	2.4	no	NSTEMI	no	
	68	f	2	yes	0.1	2.4	no	NSTEMI	no	
hs-cTnI <4ng/l	81	m	8	yes	6.5	2.1	no	NSTEMI	yes	LAD, RCA
	60	m	1	no	0.1	0.1	yes	STEMI	yes	LAD, LCX
	71	m	4	yes	7.8	4.6	no	NSTEMI	no	
	68	f	2	yes	0.1	2.4	no	NSTEMI	no	
	65	f	9	no	5.0	3.8	no	NSTEMI	no	
hs-cTnT <9ng/l	81	M	7,73	yes	6.45	2.1	no	NSTEMI	yes	LAD, RCA
	40	M	9,68	yes	6.85	4.35	no	NSTEMI	yes	LCA
	79	F	2,65	no	6.67	5.5	yes	STEMI	no	
	60	M	0,97	no	0.1	0.1	yes	NSTEMI	yes	LAD, LCX
	59	M	2,33	yes	0.1	108.5	no	NSTEMI	no	
	42	M	21,43	no	8.41	22.7	no	NSTEMI	yes	LCX, RCA
	60	F	16,58	no	5.92	49.6	yes	NSTEMI	no	
	44	M	2,58	yes	6.93	41.85	no	NSTEMI	yes	LCX, RCA
	59	F	6,25	yes	7.6	28.5	no	NSTEMI	yes	LCX, RCA
	48	M	2,33	no	8.03	10	no	NSTEMI	yes	LCX, RCA
	71	M	3,53	yes	7.75	3.55	no	NSTEMI	no	
	58	F	0,60	no	7.39	10.2	yes	NSTEMI	yes	n.a.

68	F	1,62	yes	0.1	2.4	no	NSTEMI	no	
56	M	5,63	no	8.15	114.2	no	NSTEMI	yes	n.a.
65	F	9,2	no	5.06	3.8	no	NSTEMI	no	
54	M	9,0	yes	0.1	43.3	no	NSTEMI	yes	n.a.

Supplementary Figure 1. hs-cTn concentrations at presentation in the APACE cohort
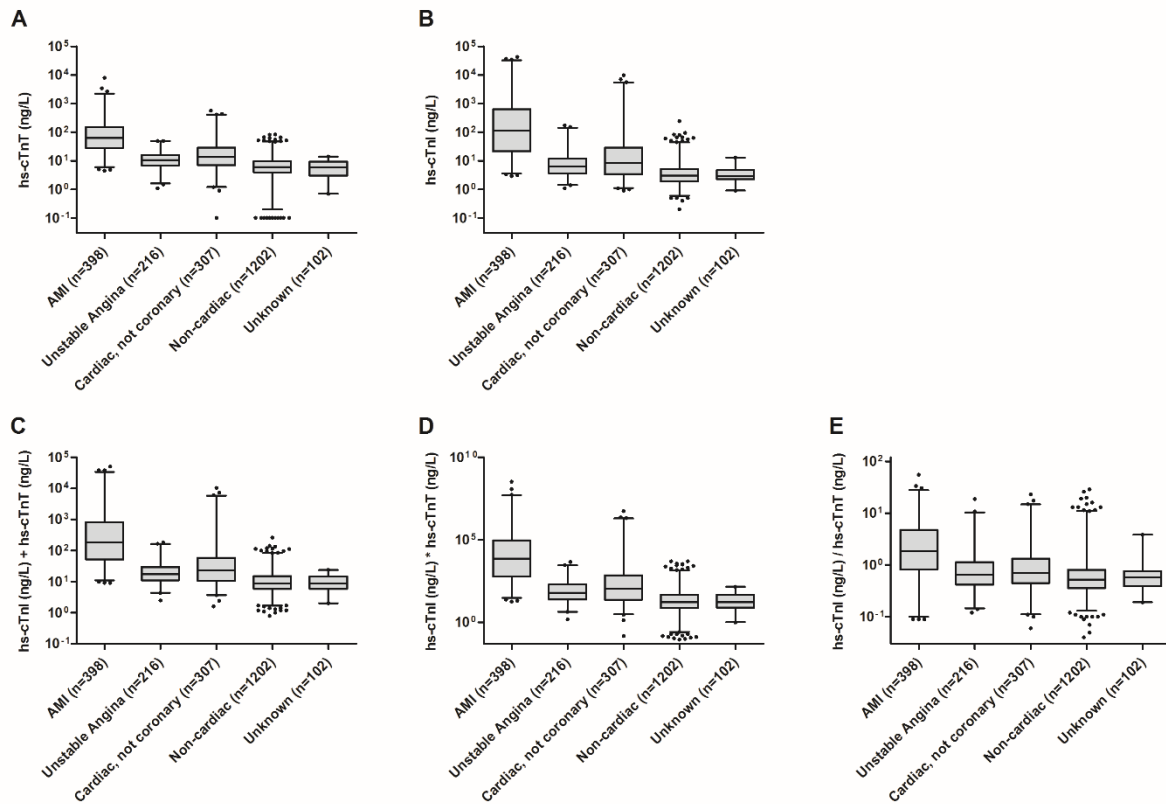
A) in hs-cTnT B) in hs-cTnI C) in the sum of hs-cTnT and hs-cTnI D) in the product of hs-cTnT and hs-cTnI E) in the ratio of hs-cTnT and hs-cTnI. The boxes represent median and inter-quartile ranges, the whiskers 1st-99th percentile. hs-cTn, high-sensitivity cardiac troponin; AMI, acute myocardial infarction; UA, unstable angina; CNC, cardiac non-coronary disease; NCCP, non-cardiac chest pain.