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

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Running title: combination of troponin T and I for early diagnosis of AMI

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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: www.clinicaltrial.gov, NCT00470587; ADAPT: www.anzctr.org.au, ACTRN12611001069943

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

Clinical implications

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 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 $\chi 2$ 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 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.

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Conflict of interest

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Tables

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%)

Figures

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.