

Serial Changes in Highly Sensitive Troponin I Assay and Early Diagnosis of Myocardial Infarction

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ACUTE CHEST PAIN IS ONE OF the most common reasons patients seek care in an emergency department.¹ Early identification of individuals at high and intermediate risk for myocardial ischemia is crucial because they benefit the most from early and aggressive treatment.²

According to international consensus and task force^{3,4} definitions of myocardial infarction (MI), the diagnosis of MI is based mainly on an elevated cardiac troponin level exceeding the 99th percentile and demonstrating an increase or decrease over time. The universal definition⁴ recommends the use of a more sensitive troponin assay with a coefficient of variation of 10% or less at the diagnostic cutoff concentration representing the 99th percentile of a reference population.⁴ Sensitive tro-

Context Introduction of highly sensitive troponin assays into clinical practice has substantially improved the evaluation of patients with chest pain.

Objective To evaluate the diagnostic performance of a highly sensitive troponin I (hsTnI) assay compared with a contemporary troponin I (cTnI) assay and their serial changes in the diagnosis of acute myocardial infarction (AMI).

Design, Setting, and Patients A total of 1818 patients with suspected acute coronary syndrome were consecutively enrolled at the chest pain units of the University Heart Center Hamburg, the University Medical Center Mainz, and the Federal Armed Forces Hospital Koblenz, all in Germany, from 2007 to 2008. Twelve biomarkers including hsTnI (level of detection, 3.4 pg/mL) and cTnI (level of detection, 10 pg/mL) were measured on admission and after 3 and 6 hours.

Main Outcome Measures Diagnostic performance for AMI of baseline and serial changes in hsTnI and cTnI results at 3 hours after admission to the emergency department.

Results Of the 1818 patients, 413 (22.7%) were diagnosed as having AMI. For discrimination of AMI, the area under the receiver operating characteristic (ROC) curve was 0.96 (95% CI, 0.95-0.97) for hsTnI on admission and 0.92 (95% CI, 0.90-0.94) for cTnI on admission. Both were superior to the other evaluated diagnostic biomarkers. The use of hsTnI at admission (with the diagnostic cutoff value at the 99th percentile of 30 pg/mL) had a sensitivity of 82.3% and a negative predictive value (for ruling out AMI) of 94.7%. The use of cTnI (with the diagnostic cutoff value at the 99th percentile of 32 pg/mL) at admission had a sensitivity of 79.4% and a negative predictive value of 94.0%. Using levels obtained at 3 hours after admission, the sensitivity was 98.2% and the negative predictive value was 99.4% for both hsTnI and cTnI assays. Combining the 99th percentile cutoff at admission with the serial change in troponin concentration within 3 hours, the positive predictive value (for ruling in AMI) for hsTnI increased from 75.1% at admission to 95.8% after 3 hours, and for cTnI increased from 80.9% at admission to 96.1% after 3 hours.

Conclusions Among patients with suspected acute coronary syndrome, hsTnI or cTnI determination 3 hours after admission may facilitate early rule-out of AMI. A serial change in hsTnI or cTnI levels from admission (using the 99th percentile diagnostic cutoff value) to 3 hours after admission may facilitate an early diagnosis of AMI.

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ponin assays fulfilling these criteria have been developed, and the application of such robust commercially available sensitive troponin assays in unselected pa-

tients with chest pain facilitates early diagnosis of MI.⁵⁻⁸

Recently, highly sensitive troponin assays have been developed that reli-

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ably assess troponin levels in more than 50% of the general population.^{9,10} The reliable detection of very low troponin concentrations using these new highly sensitive assays in the acute setting might pose a challenge in everyday clinical practice. This improved sensitivity is inevitably associated with reduced specificity, leading to a substantially increased number of patients with chest pain with elevated troponin levels evaluated in emergency departments. Hence, utilization of troponin kinetics to identify troponin elevations due to chronic conditions has been proposed to overcome this dilemma.¹¹ In addition to troponin, the measurement of several other early markers representing various aspects of an evolving acute MI such as ischemia, hemodynamic stress, inflammation, oxidative stress, angiogenesis, or plaque instability has been proposed to improve rapid diagnosis. However, the diagnostic value of these markers compared with and in addition to highly sensitive troponin determination is not well defined.

The aims of this study were to evaluate the clinical applicability of a newly developed highly sensitive troponin I (hsTnI) assay in conjunction with its serial changes within 3 hours after admission for diagnosing MI in patients with suspected acute coronary syndrome (ACS). We also sought to compare the diagnostic performance of the hsTnI assay with that of a contemporary troponin I (cTnI) assay and with other biomarkers that have been shown to have diagnostic or prognostic information in this setting.

METHODS

Study Population

Between January 2007 and December 2008, patients consecutively presenting with acute chest pain and therefore suspected ACS were enrolled in a prospective biomarker assessment registry as described earlier.⁷ Patients were recruited from the chest pain units of the 3 German study centers: Johannes Gutenberg-University Medical Center Mainz, Federal Armed Forces Hospi-

tal Koblenz, and University Hospital Hamburg-Eppendorf.

All patients between 18 and 85 years of age presenting with acute angina pectoris or equivalent symptoms were eligible to participate. Exclusion criteria were major surgery or trauma within the previous 4 weeks, pregnancy, intravenous drug abuse, and anemia (hemoglobin level <10 g/dL).

Time of first onset of chest pain symptoms was assessed by independent research staff. Blood was drawn for routine workup and sample storage on admission and at 3 and 6 hours after admission to the emergency department. A 12-lead electrocardiogram was obtained at the same time points. A detailed description of the study population is provided in the eMethods (available at <http://www.jama.com>).

All patients were followed up for 30 days after initial hospitalization by standardized telephone interview and review of hospital or general practitioner charts. Additionally, the local civil registry office provided information about death. Outcomes included death, MI, or both independent of the index event. Follow-up information was available for 98.0% of the population.

The local ethics committees approved the study. Participation was voluntary; each patient gave written informed consent.

Adjudication of the Final Diagnosis

Final diagnosis was based on all available clinical, laboratory, and imaging findings and was adjudicated by 2 independent cardiologists. A third cardiologist refereed in situations of disagreement. All 3 were blinded to the investigational biomarker results including the hsTnI and cTnI measures.

Acute MI was adjudicated according to current guidelines⁴ when there was evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia and clinical symptoms of ischemia; electrocardiographic changes indicative of new ischemia (new ST-T wave changes or new left bundle-branch block); imaging evidence of new loss of viable myocar-

dium; or detection of a culprit coronary artery lesion classified according to the Ambrose criteria¹² on the coronary angiogram. Myocardial necrosis was established by at least 1 troponin measurement above the 10% imprecision cutoff of the respective in-house troponin assay together with an increasing and/or declining pattern of at least 20% within 6 hours after admission to distinguish acute troponin level elevation from background elevation due to chronic conditions. For the adjudication of the final diagnosis based on all conventional serial troponin measurements, cardiac troponin T was used at the Mainz and Hamburg sites and cardiac troponin I was used in Koblenz. A detailed description of the in-house troponin assays used is given in the eMethods.

Unstable angina pectoris was diagnosed if the electrocardiogram results were nondiagnostic, and serial in-house troponin levels were negative, but ischemia was proven by need of intervention in coronary angiography or by positive stress test findings such as ergometry and subsequent diagnosis of coronary artery disease with culprit lesion identified on coronary angiography. All patients in whom ACS was excluded were categorized as having noncoronary chest pain.

Laboratory Measures

Routine laboratory parameters including C-reactive protein, serum creatinine, and in-house troponin were measured immediately after blood draw on admission by standardized methods. Additionally, EDTA plasma, citrate plasma, and serum samples were collected at each time point, centrifuged, aliquoted, and stored at -80°C .

Biomarkers representing different aspects of an evolving ACS were measured. In addition to the 2 in-house troponins assayed routinely by the central laboratories, determination in stored frozen serial samples of the following was made: cTnI and hsTnI; biomarkers representing ischemia (heart-type fatty acid binding protein [H-FABP], glycogen phosphorylase BB [GPBB]);

hemodynamic stress (copeptin); inflammation (growth differentiation factor 15 [GDF-15]); oxidative stress (myeloperoxidase); angiogenesis (soluble vascular endothelial growth factor receptor 1 [sVEGFR-1/sFLT-1]); plaque instability (placental growth factor [PlGF]); and classic necrosis markers (creatinine kinase [CK], CK-MB, and myoglobin). Data on the individual assay characteristics and diagnostic cutoffs of the additional biomarkers appear in the eMethods.

Troponin Assays

For cTnI, the Architect STAT troponin I assay (Abbott Diagnostics) was used. For this assay, the level of detection is 10 pg/mL (range, 0-50 000 pg/mL), the 99th percentile and the concentration with coefficient of variation of 10% is 32 pg/mL.¹³ The diagnostic threshold for MI according to the World Health Organization definition is given as 300 pg/mL by the manufacturer.

For hsTnI, a prototype cardiac troponin I assay (Architect STAT High Sensitive Troponin, Abbott Diagnostics) was used. This assay is different from the assay used in our previous study.⁷ For this assay, the level of detection is 3.4 pg/mL (range, 0-50 000 pg/mL) and the coefficient of variation is 10% at a concentration of 5.2 pg/mL. The diagnostic cutoff concentration representing the 99th percentile of a reference population was determined to be 30 pg/mL in 4139 individuals of the population-based Gutenberg Health Study. If excluding individuals with elevated N-terminal B-type natriuretic peptide (above the 95th percentile) representing individuals with structural heart changes, in terms of a biomarker-guided approach as suggested recently,¹⁴ the concentration representing the 99th percentile was determined to be 24 pg/mL. Characteristics of the Gutenberg Health Study individuals and a histogram of the hsTnI distribution are given in eTable 1 and eFigure 1.

Experienced technical assistants blinded to patient characteristics measured biomarker data for all study centers at the biomarker laboratory in

Mainz. Treating physicians and research staff involved in enrollment or follow-up of study participants were unaware of the evaluated biomarker results including the cTnI and hsTnI measurements.

Statistical Analysis

Continuous skewed variables are described in quartiles and symmetric variables are presented as mean (SD). Associations between biomarkers were assessed with Spearman rank correlation coefficient. Receiver operating characteristic (ROC) curves based on continuous biomarker levels dependent on time since chest pain onset were calculated. The area under the curve (AUC) was computed for single biomarkers and combinations of hsTnI with single markers. To test for difference of AUCs and compute their confidence intervals, the method described by DeLong et al¹⁵ was used. This method provides an estimate of the covariance matrix of the AUCs. We also compared combinations of hsTnI and other markers with a simple logistic model that has hsTnI, log-transformed, as its only independent variable, via the integrated discrimination improvement. *P* values for the test that the integrated discrimination improvement=0 were computed as described by Pencina et al.¹⁶

Sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) for the individual markers and combinations were computed for marker-specific cutoff values. The relative change in hsTnI was defined as the absolute value of the difference of the hsTnI measurements at 3 hours after admission and at admission divided by the initial measurement and multiplied by 100 to calculate percentage. To represent potentially relevant hsTnI changes we included in addition to an ROC curve-based threshold, different cutoffs for the change in troponin values ranging from 20% as recommended by the National Academy of Clinical Biochemistry¹⁷ to 250% to include the diverse published cutoffs in various clinical settings.^{8,18}

The ROC-based optimized threshold was computed by finding the cutoff that maximized the sum of specificity and sensitivity. In this context all analyses were performed in patients with hsTnI values below the upper range of the highly sensitive assay of 50 000 pg/mL on admission and after 3 hours to identify a cohort in which a meaningful increase or decrease could be tested. Missing values were handled by available-case analysis. Values of biomarkers below their respective level of detection were set to this value divided by 2.

A 2-sided *P*<.05 was considered significant. All analyses were performed using R 2.13.0 (R Foundation for Statistical Computing). A detailed description of the statistical methods is given in the eMethods.

RESULTS

Patient Characteristics

Consecutive patients presenting with symptoms suggestive of ACS were screened. After exclusion of 58 individuals who met predefined exclusion criteria or refused to participate, a total of 1818 patients with suspected ACS were enrolled. Of these, 413 (22.7%) had the final discharge diagnosis of acute MI. A total of 56 patients with MI (14.1%) presented with ST-segment elevation. eFigure 3 displays the number of patients with MI according to hsTnI determination on admission, after 3 hours, and according to the relative change of hsTnI between these 2 points.

Baseline characteristics for the overall study population are provided in TABLE 1 and TABLE 2. Traditional risk factors were more common in patients with a discharge diagnosis of ACS. The time between chest pain onset and hospital admission was similar in all diagnosis groups. Patients categorized as having MI experienced more events than those with unstable angina pectoris or those with noncardiac chest pain (1.1% vs 0.1% and 0.3%). Classification by hsTnI exceeding the 99th percentile cutoff on admission resulted in a comparable event rate (1.1%) (eTable 5).

Data on correlations among the evaluated biomarkers as well as data on the time course of the biomarkers in patients with a discharge diagnosis of MI are provided in the eMethods and eFigure 2.

Diagnostic Accuracy of Troponin and Other Biomarkers

The ROC curves and AUC values for all measured biomarkers according to time after chest pain onset are shown in FIGURE 1 and eFigure 4.

The hsTnI provided the highest diagnostic information of these biomarkers, with an AUC of 0.962, followed by cTnI with an AUC of 0.921 (vs hsTnI, $P < .001$) and H-FABP with an AUC of 0.892 (vs hsTnI, $P < .001$) (eFigure 4). The ROC curves of the 2 troponin I assays were similar except for those parts of the curves representing lower concentrations of troponin I, which were detectable only with the highly sensitive assay, accounting for the observed AUC difference (Figure 1). The difference in the diagnostic performance between hsTnI and cTnI was apparent in the first hours after chest pain onset (FIGURE 2). In the subgroup of patients ($n=407$) with chest pain onset time of less than 2 hours, hsTnI had an AUC of 0.970 whereas cTnI had an AUC of 0.888 ($P < .001$).

Among the early biomarkers, H-FABP and copeptin provided the best diagnostic performance, both with highest AUCs of 0.904 and 0.806 as early as 2 hours after chest pain onset.

The potential added value, reflected by improvement in AUC, of an individual early biomarker or the relative change in hsTnI concentration within 3 hours after admission in combination with hsTnI assayed on admission is shown in TABLE 3. The AUC of hsTnI of 0.962 was increased slightly by the addition of the biomarker copeptin, reaching an AUC of 0.968 ($P = .01$), H-FABP (AUC, 0.967; $P = .02$), or sVEGFR-1/sFLT-1 (AUC, 0.966; $P = .03$). Addition of the information from the 3-hour relative change in hsTnI concentration improved the AUC to 0.985 ($P < .001$). The 3 biomarkers

improved the relative integrated discrimination improvement model based on hsTnI by 5.6%, 7.4%, and 4.0%, respectively (all $P < .001$). The relative change in hsTnI improved the integrated discrimination improvement model by 31.3% ($P < .001$).

For the cTnI assay and the combination with its relative change in concentration within 3 hours after admission, the AUC improved from 0.92 (95% CI, 0.90-0.94) to 0.98 (95% CI, 0.98-0.99; $P < .001$). This was not statistically different from the AUC of

the combination of hsTnI with its relative change at 3 hours (AUC difference of 0.0022; 95% CI, 0.0026-0.007; $P = .37$).

Among subgroups of patients presenting within 3 hours after onset of symptoms ($n=696$), sex-specific and age-specific analyses comparing the relative change in hsTnI concentration showed robust additional diagnostic information to hsTnI determined on admission. However, the additive effect of the other biomarkers diminished (eTable 3).

Table 1. Baseline Demographic and Electrocardiographic Characteristics of 1818 Consecutive Patients Presenting With Chest Pain

	No.	No. (%)			
		Noncoronary Chest Pain (n = 1165)	Unstable Angina Pectoris (n = 240)	Acute MI (n = 413)	All (N = 1818)
Age, mean (SD), y	1818	59.7 (14.3)	65.2 (10.5)	64.0 (11.8)	61.4 (13.5)
Male sex	1818	729 (62.6)	165 (68.8)	314 (76.0)	1208 (66.4)
Risk factors					
Body mass index, mean (SD) ^a	1694	27.7 (4.9)	27.9 (4.4)	27.9 (4.6)	27.8 (4.8)
Hypertension	1818	822 (70.6)	204 (85.0)	313 (75.8)	1339 (73.7)
Diabetes mellitus	1746	140 (12.5)	53 (23.0)	80 (20.0)	273 (15.6)
Smoking status					
Current	1802	254 (21.9)	40 (17.1)	143 (35.1)	437 (24.3)
Former	1774	326 (28.5)	76 (33.2)	124 (31.0)	526 (29.7)
Never	1771	564 (49.3)	111 (48.9)	133 (33.2)	808 (45.6)
Hyperlipidemia	1818	824 (70.7)	193 (80.4)	311 (75.3)	1328 (73.0)
Lipids, mean (SD), mg/dL					
Total cholesterol	1605	197.7 (49.0)	196.7 (47.5)	205.0 (50.1)	199.2 (49.1)
HDL cholesterol	1601	51.8 (15.9)	49.3 (14.0)	47.6 (13.8)	50.5 (15.3)
LDL cholesterol	1600	117.2 (40.8)	116.9 (41.9)	129.6 (43.8)	119.9 (41.9)
Parental CAD	1750	379 (33.4)	71 (32.3)	118 (29.9)	568 (32.5)
Known CAD	1771	361 (31.8)	137 (58.5)	136 (33.7)	634 (35.8)
Electrocardiographic results on admission	1789				
ST-segment elevation		27 (2.3)	6 (2.5)	56 (14.1)	89 (5.0)
ST-segment depression		69 (6.0)	27 (11.3)	109 (27.5)	205 (11.5)
T-wave inversion		295 (25.6)	77 (32.2)	174 (43.8)	546 (30.5)
Left or right bundle-branch block		149 (12.9)	35 (14.6)	61 (15.4)	245 (13.7)
Time between chest pain onset and admission, median (IQR), h	1818	4.2 (2.0-11.7)	4.6 (2.1-15.0)	4.3 (1.9-15.4)	4.3 (2.0-13.0)
<3		446 (38.28)	84 (35.00)	166 (40.19)	696 (38.28)
<6		693 (59.48)	139 (57.92)	237 (57.38)	1069 (58.80)
<12		877 (75.28)	171 (71.25)	289 (69.98)	1337 (73.54)
≥12		288 (24.72)	69 (28.75)	124 (30.02)	481 (26.46)

Abbreviations: CAD, coronary artery disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; MI, myocardial infarction.

SI conversion factors: To convert cholesterol to mmol/L, multiply values by 0.0258.

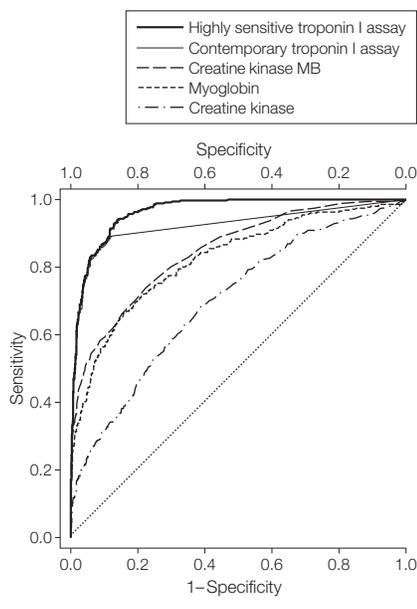
^aBody mass index is calculated as weight in kilograms divided by height in meters squared.

Table 2. Baseline Laboratory Parameters of 1818 Consecutive Patients Presenting With Chest Pain

	No.	Median (IQR)			
		Noncoronary Chest Pain (n = 1165)	Unstable Angina Pectoris (n = 240)	Acute MI (n = 413)	All (N = 1818)
cTnI, pg/mL	1786	5.0 (5.0-5.0)	5.0 (5.0-9.2)	281.0 (59.0-1918.0)	5.0 (5.0-27.0)
hsTnI, pg/mL	1606	4.8 (1.7-9.0)	9.0 (4.5-26.0)	271.5 (60.1-1864.5)	7.4 (3.5-37.8)
Creatine kinase, U/L	1721	74 (51-114)	74 (53-108)	121 (79-224)	82 (56-131)
Creatine kinase MB, ng/mL	1709	1.0 (0.6-1.5)	1.1 (0.7-1.7)	3.3 (1.5-10.5)	1.2 (0.7-2.0)
Myoglobin, µg/L	1705	46.9 (32.4-69.6)	52.4 (35.2-75.7)	130.2 (69.6-283.3)	55.0 (35.5-92.1)
C-reactive protein, mg/L	1778	2.3 (1.1-5.4)	2.3 (1.3-4.5)	3.4 (1.7-8.8)	2.5 (1.3-5.8)
Creatinine, mg/dL	1809	0.94 (0.82-1.08)	0.93 (0.82-1.06)	0.99 (0.88-1.16)	0.95 (0.83-1.09)
eGFR, mL/min/1.73 m ² , mean (SD)	1809	80.1 (21.0)	79.8 (21.1)	75.5 (22.3)	79.0 (21.4)
sVEGFR-1/sFLT-1, pg/mL	1778	302.40 (228.5-7851.6)	294.3 (227.5-8286.6)	5710.7 (301.5-10 649.5)	362.4 (237.0-9055.2)
GDF15, pg/mL	1703	692.2 (495.8-1068.6)	803.7 (606.6-1182.1)	967.1 (662.3-1340.5)	772.9 (534.8-1164.2)
PlGF, pg/mL	1786	16.6 (13.5-20.2)	17.7 (14.6-21.5)	18.1 (14.99-23.0)	17.2 (13.9-20.9)
H-FABP, ng/mL	1696	2.1 (1.5-3.0)	2.2 (1.7-3.1)	8.3 (4.1-21.0)	2.4 (1.6-4.3)
Myeloperoxidase, pmol/L	1782	561.7 (386.5-1231.7)	564.4 (373.4-1114.5)	1070.7 (498.7-1521.3)	645.5 (400.4-1279.8)
GPBB, ng/mL	1679	4.7 (3.7-6.2)	4.9 (3.8-6.5)	5.7 (4.2-8.5)	4.8 (3.8-6.8)
Copeptin, pmol/L	1382	5.4 (2.9-11.9)	5.1 (3.0-11.9)	18.5 (7.0-50.1)	6.7 (3.3-16.5)

Abbreviations: cTnI, contemporary sensitive troponin I; eGFR, estimated glomerular filtration rate; GDF15, growth differentiation factor 15; GPBB, glycogen phosphorylase BB; H-FABP, heart-type fatty acid binding protein; hsTnI, highly sensitive troponin I; IQR, interquartile range; MI, myocardial infarction; PlGF, placental growth factor; sVEGFR-1/sFLT-1, soluble vascular endothelial growth factor receptor 1.

Figure 1. Receiver Operating Characteristic Curves for Identification of Acute MI by Troponin Assays and Necrosis Markers in the Overall Cohort



Different Relative Changes in Troponin Concentration in Diagnosis of MI

Data on application of serial cTnI and hsTnI measurements using the diagnostic 99th percentile cutoff on admis-

sion and after 3 hours are shown in TABLE 4. The level of detection (LoD) as cutoff and different tested relative changes are also provided in Table 4, TABLE 5, and TABLE 6.

Using the 99th percentile cutoff, hsTnI on admission had a sensitivity of 82.3% and NPV of 94.7%; hsTnI determined after 3 hours had a sensitivity of 98.2% with NPV of 99.4%. Using the LoD as diagnostic threshold, hsTnI at admission had a sensitivity and NPV of 100%, with specificity of 35.3% because 74% of patients had hsTnI values above the LoD on admission. Different relative changes did not further improve the NPV and therefore a safe rule-out of MI.

Compared with hsTnI, the cTnI assay (using the 99th percentile as cutoff) had comparable sensitivity and NPV: 79.4% sensitivity and 94.0% NPV on admission, and 98.2% sensitivity and 99.4% NPV after 3 hours. Using the LoD as diagnostic threshold was associated with sensitivity of 87.4% and NPV of 96.0% on admission, less than the NPVs of the hsTnI assay when using the LoD as cutoff.

To clinically establish the diagnosis of MI, troponin I determination using the recommended 99th percentile was associated with a PPV of 75.1% on ad-

mission and 74.7% after 3 hours for the hsTnI, and with a PPV of 80.9% on admission and 73.9% after 3 hours for the cTnI assay. The relative change within the first 3 hours after admission in concentration alone yielded a comparable PPV up to 65.1% for hsTnI and up to 64.8% for cTnI, whereas the combination of the relative change and the 99th percentile cutoff substantially improved the PPV up to 95.8% for the hsTnI assay (Table 5). Combination of the respective relative change in troponin I concentration and exceeding of the 99th percentile cutoff on admission if using the cTnI assay improved the PPV up to 96.1% (Table 6).

In patients presenting with hsTnI levels that were below the 99th percentile on admission but increased above this cutoff within 3 hours after admission (n=951), the relative change in combination with the positive troponin level after 3 hours had a PPV up to 83.0% when using the hsTnI assay, whereas the cTnI assay combined with the relative change after 3 hours had a PPV of 57.4%.

Additionally, an optimized relative change, based on ROC analyses, calculated for the hsTnI assay was at a cutoff of 266%. Using such a change of 266% in combination with the 99th per-

centile cutoff on admission, the specificity and the PPV of hsTnI increased to 99.6% and 95.8%, respectively, within a rule-in approach. The combination of the 99th percentile with such a high relative change cutoff of 250% or higher was associated with a sensitivity of only 32.9%; therefore, lower potential relative change thresholds could be of interest. For example, a cutoff of 50% shows an inflection in specificity compared with lower relative changes (Table 5) and could be an alternative threshold.

If using a lower 99th percentile cutoff of 24 pg/mL for the hsTnI assay based on a reference population without structural heart changes defined by normal N-terminal B-type natriuretic peptide values, the PPV and NPV were 71.8% and 95.2%, respectively, for hsTnI on admission and 69.8% and 99.5%, respectively, for hsTnI at 3 hours after admission.

Data on the discriminatory information of hsTnI on admission and after 3 hours as well as the relative changes and their combination for identification of patients with non-ST-elevation acute coronary syndrome are given in eTable 4. In this context, the relative change provided added diagnostic value, although lower than for identification of MI.

Validation and Clinical Application

An additional analysis excluded patients with ST-elevation or clearly elevated troponin on admission because these patients have to be treated as having acute MI with all consequences (n=1176, n=128 with final diagnosis of MI). Elevated troponin was defined as a cTnI above the World Health Organization cutoff (300 pg/mL) of this assay on admission. Characteristics of this cohort are given in eTable 6. In this post hoc-defined cohort, hsTnI determined on admission yielded an AUC of 0.95 (95% CI, 0.94-0.97) that was improved to 0.98 (95% CI, 0.97-1.00; P<.001) when combined with the hsTnI relative change between admission and 3 hours.

The previously derived ROC optimized cutoff of 266%, a cutoff of 50%

showing an improvement in specificity in the overall population (Table 3), and 82% as middle-ground cutoff representing the biological and assay variability as reference change value¹⁹ were applied. Data on sensitivity, specificity, NPV, and PPV of hsTnI determination alone and in combination with the hsTnI relative change and all other evaluated biomarkers are shown in eFigure 5.

Serial hsTnI determination provided the highest diagnostic information regarding rule-out of MI. Considering all combinations of an individual biomarker with hsTnI and troponin I kinetic, the highest NPV (99.6%) is provided by hsTnI 3 hours after admission using the 99th percentile cutoff. The combination of copeptin or sVEGFR-1/sFLT-1 and hsTnI on admission provided a comparable NPV of 98.4%.

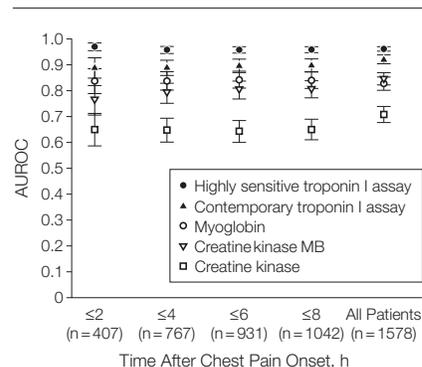
Regarding rule-in of MI, the highest PPV (up to 96.5%) is provided by the relative change in hsTnI within 3 hours after admission in addition to the 99th percentile cutoff. None of the combi-

nations of an early biomarker with hsTnI on admission was able to reach comparable results.

COMMENT

The advancement in diagnostic discrimination of MI³⁻⁷ based on higher

Figure 2. Area Under the Receiver Operating Characteristic (AUROC) Curve on Admission According to Chest Pain Onset Time for Both Troponin I Assays and Necrosis Markers



Error bars indicate 95% CIs.

Table 3. Diagnostic Performance of Individual Biomarkers and in Combination With hsTnI Assay for Identification of Acute Myocardial Infarction^a

	AUROC (95% CI)		P Value	Relative IDI Addition to Model With hsTnI, % (95% CI)	P Value
	Individual Biomarker	Combined With hsTnI			
cTnI					
On admission	0.92 (0.90 to 0.94)				
Change at 3 h ^b	0.89 (0.88 to 0.91)	0.98 (0.98 to 0.99)	<.001	33.4 (24.4 to 44.5)	<.001
hsTnI					
On admission	0.96 (0.95 to 0.97)				
Change at 3 h	0.66 (0.62 to 0.70)	0.99 (0.98 to 0.99)	<.001	31.3 (20.8 to 41.2)	<.001
Creatine kinase	0.71 (0.68 to 0.74)	0.96 (0.95 to 0.97)	.90	0.1 (-0.1 to 1.0)	.07
Creatine kinase MB	0.85 (0.82 to 0.87)	0.96 (0.95 to 0.97)	.90	0.4 (0.0 to 1.5)	.04
Myoglobin	0.83 (0.80 to 0.85)	0.96 (0.95 to 0.97)	.82	2.4 (0.9 to 5.1)	.002
sVEGFR-1/sFLT-1	0.65 (0.62 to 0.68)	0.97 (0.96 to 0.97)	.03	4.0 (2.0 to 7.0)	<.001
GDF15	0.64 (0.61 to 0.68)	0.96 (0.95 to 0.97)	.12	0.2 (-0.2 to 1.3)	.03
PIGF	0.59 (0.56 to 0.63)	0.96 (0.95 to 0.97)	.11	0.1 (0.0 to 1.1)	.39
H-FABP	0.89 (0.87 to 0.91)	0.97 (0.96 to 0.98)	.02	7.4 (4.2 to 13.2)	<.001
Myeloperoxidase	0.63 (0.60 to 0.66)	0.96 (0.95 to 0.97)	.39	1.5 (0.2 to 3.4)	.004
GPBB	0.63 (0.59 to 0.66)	0.96 (0.95 to 0.97)	.99	0.5 (0.0 to 1.6)	.10
Copeptin	0.74 (0.70 to 0.77)	0.97 (0.96 to 0.98)	.01	5.6 (2.5 to 10.6)	<.001

Abbreviations: cTnI, contemporary sensitive troponin I; GDF15, growth differentiation factor 15; GPBB, glycogen phosphorylase BB; H-FABP, heart-type fatty acid binding protein; hsTnI, highly sensitive troponin I; PIGF, placental growth factor; sVEGFR-1/sFLT-1, soluble vascular endothelial growth factor receptor 1.

^aArea under the receiver operating characteristics curve (AUROC) for individual diagnostic biomarkers and their combination with hsTnI on admission as well as the diagnostic ability of the change between admission and 3 hours in hsTnI and its combination with the concentration on admission. The relative integrated discrimination improvement (IDI) is shown for an individual biomarker or the change added to a model based on hsTnI on admission.

^bCombination of cTnI on admission with the relative change in cTnI concentration within 3 hours after admission.

sensitivity of troponin assays is accompanied by the loss of specificity. Consideration of troponin kinetics to identify patients with chronic or subacute troponin elevations originating from causes other than coronary disease has been recommended.⁴

In the present study, application of a next-generation hsTnI assay pro-

vided excellent diagnostic discrimination for both the rule-out and rule-in of MI even in patients presenting within the first hours after chest pain onset. Rule-in of MI with ensuing adequate treatment initiation is facilitated by the utilization of serial hsTnI changes. The relative change in concentration of hsTnI within 3 hours after admission

in addition to the 99th percentile diagnostic cutoff provides a substantially improved PPV for the diagnosis of MI when compared with hsTnI determination on admission alone.

In comparison, the application of the 99th percentile cutoff using a cTnI assay, already established in clinical practice, provided comparable results for

Table 4. Diagnostic Performance for Identification of Acute Myocardial Infarction by Use of Serial cTnI and hsTnI Determination

	cTnI, % (95% CI)				hsTnI, % (95% CI)			
	On Admission		At 3 Hours		On Admission		At 3 Hours	
	>LoD	>99th Percentile	>LoD	>99th Percentile	>LoD	>99th Percentile	>LoD	>99th Percentile
Sensitivity	87.4 (83.3-90.8)	79.4 (74.6-83.7)	98.8 (96.9-99.7)	98.2 (96.0-99.3)	100.0 (98.0-100.0)	82.3 (77.3-86.5)	100.0 (98.0-100.0)	98.2 (95.9-99.4)
Specificity	88.6 (86.6-90.4)	94.5 (93.0-95.7)	78.9 (76.4-81.3)	89.8 (87.8-91.5)	35.3 (32.3-38.4)	92.1 (90.3-93.7)	1.9 (1.2-3.0)	90.4 (88.4-92.2)
Positive predictive value	69.3 (64.6-73.8)	80.9 (76.2-85.1)	58.0 (53.8-62.2)	73.9 (69.5-78.0)	30.8 (27.8-33.9)	75.1 (69.9-79.8)	22.7 (20.4-25.2)	74.7 (69.9-79.0)
Negative predictive value	96.0 (94.6-97.1)	94.0 (92.4-95.3)	99.5 (98.8-99.9)	99.4 (98.7-99.8)	100.0 (98.4-100.0)	94.7 (93.1-96.1)	100.0 (75.1-100.0)	99.4 (98.7-99.8)
No. positive/total ^a	411/1430	320/1430	555/1430	433/1430	915/1260	309/1260	1241/1260	371/1260

Abbreviations: cTnI, contemporary sensitive troponin I; hsTnI, highly sensitive troponin I; LoD, level of detection.

^aNumber of patients with positive test criteria/number of patients with available data on criteria.

Table 5. Diagnostic Performance for Identification of Acute Myocardial Infarction by Use of hsTnI Determination and Its Relative Change in Troponin Concentration^a

	Change 0 to 3 Hours After Admission, % (95% CI)							
	≥20	≥30	≥50	≥75	≥100	≥150	≥200	≥250
hsTnI Change 0 to 3 Hours								
Sensitivity	77.3 (72.0-82.1)	72.7 (67.1-77.8)	67.0 (61.2-72.5)	61.0 (55.0-66.7)	57.4 (51.4-63.3)	52.8 (46.8-58.8)	50.4 (44.4-56.3)	48.2 (42.3-54.2)
Specificity	26.1 (23.3-28.9)	33.6 (30.7-36.7)	46.8 (43.7-50.0)	55.5 (52.3-58.7)	59.7 (56.6-62.8)	69.3 (66.3-72.2)	83.9 (81.5-86.2)	92.5 (90.7-94.1)
PPV	23.2 (20.5-26.0)	24.0 (21.2-27.0)	26.7 (23.4-30.1)	28.3 (24.8-32.1)	29.1 (25.4-33.1)	33.2 (28.8-37.8)	47.5 (41.7-53.3)	65.1 (58.2-71.5)
NPV	79.9 (75.1-84.2)	81.0 (76.9-84.7)	83.1 (79.7-86.2)	83.2 (80.1-85.9)	83.0 (80.0-85.7)	83.6 (80.9-86.1)	85.4 (83.0-87.6)	86.1 (83.9-88.1)
No. positive/total ^b	941/1260	854/1260	709/1260	607/1260	556/1260	449/1260	299/1260	209/1260
hsTnI >LoD on Admission and hsTnI Change 0 to 3 Hours								
Sensitivity	77.3 (72.0-82.1)	72.7 (67.1-77.8)	67.0 (61.2-72.5)	61.0 (55.0-66.7)	57.4 (51.4-63.3)	52.8 (46.8-58.8)	50.4 (44.4-56.3)	48.2 (42.3-54.2)
Specificity	60.2 (57.1-63.3)	67.8 (64.8-70.7)	80.9 (78.3-83.3)	89.6 (87.5-91.4)	93.4 (91.6-94.8)	96.8 (95.5-97.8)	98.1 (97.0-98.8)	98.6 (97.6-99.2)
PPV	35.9 (32.1-39.9)	39.4 (35.2-43.8)	50.3 (45.1-55.4)	62.8 (56.8-68.5)	71.4 (65.0-77.2)	82.8 (76.5-88.0)	88.2 (82.2-92.7)	90.7 (84.8-94.8)
NPV	90.2 (87.7-92.4)	89.6 (87.2-91.7)	89.5 (87.3-91.4)	88.8 (86.7-90.7)	88.4 (86.3-90.3)	87.7 (85.6-89.6)	87.3 (85.1-89.2)	86.8 (84.7-88.8)
No. positive/total ^b	708/1260	606/1260	434/1260	310/1260	254/1260	197/1260	171/1260	157/1260
hsTnI >99th Percentile on Admission and hsTnI Change 0 to 3 Hours								
Sensitivity	60.3 (54.3-66.0)	55.7 (49.7-61.6)	50.0 (44.0-56.0)	44.3 (38.4-50.3)	41.5 (35.7-47.5)	36.9 (31.2-42.8)	34.4 (28.9-40.3)	32.6 (27.2-38.4)
Specificity	96.8 (95.5-97.8)	98.0 (96.9-98.7)	99.1 (98.3-99.6)	99.2 (98.4-99.6)	99.4 (98.7-99.8)	99.4 (98.7-99.8)	99.5 (98.8-99.8)	99.6 (99.0-99.9)
PPV	84.6 (78.8-89.3)	88.7 (83.1-93.0)	94.0 (88.9-97.2)	94.0 (88.5-97.4)	95.1 (89.7-98.2)	94.5 (88.5-98.0)	95.1 (88.9-98.4)	95.8 (89.7-98.9)
NPV	89.4 (87.4-91.2)	88.5 (86.4-90.3)	87.3 (85.2-89.2)	86.1 (83.9-88.0)	85.5 (83.3-87.5)	84.5 (82.3-86.6)	84.0 (81.8-86.1)	83.7 (81.4-85.8)
No. positive/total ^b	221/1260	193/1260	161/1260	141/1260	131/1260	116/1260	107/1260	101/1260
hsTnI Change 0 to 3 Hours and hsTnI >99th Percentile After 3 Hours in Patients With hsTnI <99th Percentile on Admission								
Sensitivity	92.0 (80.8-97.8)	92.0 (80.8-97.8)	92.0 (80.8-97.8)	92.0 (80.8-97.8)	90.0 (78.2-96.7)	90.0 (78.2-96.7)	90.0 (78.2-96.7)	88.0 (75.7-95.5)
Specificity	97.4 (96.2-98.4)	97.6 (96.3-98.5)	97.9 (96.7-98.7)	97.9 (96.7-98.7)	98.1 (97.0-98.9)	98.1 (97.0-98.9)	98.7 (97.7-99.3)	99.0 (98.1-99.5)
PPV	66.7 (54.3-77.6)	67.6 (55.2-78.5)	70.8 (58.2-81.4)	70.8 (58.2-81.4)	72.6 (59.8-83.1)	72.6 (59.8-83.1)	78.9 (66.1-88.6)	83.0 (70.2-91.9)
NPV	99.5 (98.8-99.9)	99.5 (98.8-99.9)	99.5 (98.8-99.9)	99.5 (98.8-99.9)	99.4 (98.7-99.8)	99.4 (98.7-99.8)	99.4 (98.7-99.8)	99.3 (98.6-99.8)
No. positive/total ^b	78/951	76/951	72/951	71/951	68/951	67/951	59/951	54/951

Abbreviations: hsTnI, highly sensitive troponin I; LoD, level of detection; NPV, negative predictive value; PPV, positive predictive value.

^aCutoff of 32 pg/mL for the cTnI and 30 pg/mL for hTnI assays and LoD of 10 pg/mL for cTnI and 3.4 pg/mL for hTnI. Various relative troponin changes between admission and 3 hours and their combination with cTnI and hsTnI are presented. For each assay, analyses were performed on patients with their corresponding troponin I values below the upper range of the used assay on admission and 3 hours after admission.

^bNumber of patients with positive test criteria/number of patients with available data on criteria.

rule-out MI after 3 hours (NPV, 99.4%). Similarly, this approach using cTnI facilitated early rule-in of MI using the 99th percentile with the relative change in concentration within 3 hours. However, the hsTnI assay provided higher PPV compared with cTnI among the subgroup of patients in this study (n=951) who presented with initially very low troponin I values that exceeded the 99th percentile at 3 hours.

Ruling out MI appears feasible as early as 3 hours after admission in a large proportion of patients with chest pain by applying the 99th percentile cutoff to a second hsTnI or cTnI measurement after 3 hours, which yields an NPV of more than 99%. If considering the baseline blood draw only, the combination of hsTnI with copeptin or sVEGFR-1/sFLT-1 assessed once on admission provided an NPV of 97% to 98% that is nearly comparable to the 3-hour hsTnI determination.

None of the individual early biomarkers representing different pathophysiological aspects of an evolving ACS exceeded the diagnostic performance of hsTnI. Furthermore, combining a single biomarker with hsTnI on admission did not achieve the high PPV of the relative change within a rule-in approach.

In terms of an early definitive diagnosis with possible subsequent diagnostic or therapeutic interventions, we used a time interval of only 3 hours between the troponin determinations because it has been shown that this approach delivers comparable results^{7,20} to the recommended approach with a second troponin determination after 6 to 9 hours.⁴

Applicability of truly highly sensitive troponin assays in clinical routine and in comparison with cTnI assays should be discussed with respect to 2 main aspects. The first issue is the safe rule-out of MI accounting for the large number

of patients with chest pain seeking medical attention and second, the early and accurate rule-in of MI as patients at risk benefit the most from a modern and invasive therapeutic strategy.²

For ruling out MI, the use of refined troponin assays with their improved precision in very low troponin concentrations and therefore improved NPV based on better sensitivity has the potential to influence patient care. Applying the LoD as cutoff for hsTnI determined on admission yields a sensitivity and NPV of 100%. Because 74% of the enrolled patients had detectable troponin values on admission, a rule-out with such a low diagnostic cutoff is possible in only one-fourth of patients. To improve specificity and enable a broader usage a stepwise rule-out approach seems feasible.

One possible approach would be first to apply the LoD as the diagnostic cutoff to hsTnI determined on admission, thereby allowing a rule-out of a

Table 6. Diagnostic Performance for Identification of Acute Myocardial Infarction by Use of cTnI Determination and Its Relative Change in Troponin Concentration^a

	Change 0 to 3 Hours After Admission, % (95% CI)							
	≥20	≥30	≥50	≥75	≥100	≥150	≥200	≥250
cTnI Change 0 to 3 Hours								
Sensitivity	81.9 (77.3-85.9)	77.3 (72.4-81.7)	69.0 (63.7-74.0)	63.5 (58.0-68.7)	60.7 (55.2-66.1)	55.8 (50.3-61.3)	51.5 (46.0-57.1)	49.7 (44.1-55.3)
Specificity	79.7 (77.2-82.0)	81.0 (78.5-83.3)	82.0 (79.6-84.2)	83.8 (81.5-85.9)	84.9 (82.6-86.9)	89.0 (87.0-90.8)	90.7 (88.8-92.3)	92.0 (90.3-93.6)
PPV	54.4 (49.9-58.8)	54.5 (49.9-59.2)	53.1 (48.2-57.9)	53.6 (48.5-58.7)	54.2 (49.0-59.4)	60.1 (54.3-65.6)	62.0 (55.9-67.8)	64.8 (58.5-70.7)
NPV	93.7 (92.0-95.2)	92.4 (90.5-93.9)	90.0 (87.9-91.7)	88.6 (86.5-90.5)	88.0 (85.9-89.9)	87.2 (85.1-89.1)	86.4 (84.3-88.3)	86.1 (84.0-88.0)
No. positive/total ^b	491/1430	462/1430	424/1430	386/1430	365/1430	303/1430	271/1430	250/1430
cTnI >LoD on Admission and cTnI Change 0 to 3 Hours								
Sensitivity	70.2 (65.0-75.2)	65.6 (60.2-70.8)	57.4 (51.8-62.8)	51.8 (46.3-57.4)	49.1 (43.5-54.6)	44.2 (38.7-49.7)	39.9 (34.5-45.4)	38.0 (32.7-43.5)
Specificity	92.5 (90.8-94.0)	93.8 (92.2-95.1)	94.7 (93.3-96.0)	96.6 (95.3-97.6)	97.6 (96.6-98.5)	98.6 (97.8-99.2)	98.7 (97.9-99.3)	98.9 (98.1-99.4)
PPV	73.4 (68.1-78.2)	75.6 (70.2-80.5)	76.3 (70.5-81.5)	81.6 (75.7-86.7)	86.0 (80.2-90.7)	90.6 (84.9-94.6)	90.3 (84.2-94.6)	91.2 (85.1-95.4)
NPV	91.3 (89.5-92.9)	90.2 (88.4-91.9)	88.3 (86.3-90.0)	87.2 (85.2-89.0)	86.7 (84.6-88.5)	85.7 (83.6-87.6)	84.8 (82.7-86.7)	84.4 (82.3-86.3)
No. positive/total ^a	349/1430	317/1430	270/1430	226/1430	202/1430	171/1430	153/1430	144/1430
cTnI >99th Percentile on Admission and cTnI Change 0 to 3 Hours								
Sensitivity	62.3 (56.8-67.6)	58.0 (52.4-63.4)	49.7 (44.1-55.3)	44.2 (38.7-49.7)	41.4 (36.0-47.0)	36.5 (31.3-42.0)	32.2 (27.2-37.6)	30.4 (25.4-35.7)
Specificity	97.5 (96.4-98.3)	98.4 (97.4-99.0)	98.6 (97.8-99.2)	98.8 (98.0-99.4)	99.3 (98.6-99.7)	99.5 (98.9-99.9)	99.5 (98.9-99.9)	99.6 (99.1-99.9)
PPV	87.9 (83.0-91.8)	91.3 (86.6-94.8)	91.5 (86.4-95.2)	91.7 (86.3-95.5)	94.4 (89.3-97.6)	96.0 (90.8-98.7)	95.5 (89.7-98.5)	96.1 (90.4-98.9)
NPV	89.7 (87.9-91.4)	88.8 (86.9-90.5)	86.9 (84.9-88.7)	85.7 (83.7-87.6)	85.2 (83.1-87.1)	84.2 (82.1-86.1)	83.3 (81.1-85.2)	82.9 (80.8-84.9)
No. positive/total ^b	252/1430	226/1430	188/1430	164/1430	149/1430	129/1430	113/1430	105/1430
cTnI Change 0 to 3 Hours and cTnI >99th Percentile After 3 Hours in Patients With cTnI <99th Percentile on Admission								
Sensitivity	92.5 (83.4-97.5)	92.5 (83.4-97.5)	92.5 (83.4-97.5)	92.5 (83.4-97.5)	92.5 (83.4-97.5)	92.5 (83.4-97.5)	92.5 (83.4-97.5)	92.5 (83.4-97.5)
Specificity	94.5 (93.0-95.8)	94.5 (93.0-95.8)	94.6 (93.1-95.9)	94.9 (93.4-96.2)	95.2 (93.7-96.4)	95.5 (94.1-96.7)	95.5 (94.1-96.7)	95.6 (94.2-96.8)
PPV	52.1 (42.8-61.3)	52.1 (42.8-61.3)	52.5 (43.1-61.8)	53.9 (44.4-63.2)	55.4 (45.7-64.8)	56.9 (47.0-66.3)	56.9 (47.0-66.3)	57.4 (47.5-66.9)
NPV	99.5 (98.8-99.8)	99.5 (98.8-99.8)	99.5 (98.8-99.8)	99.5 (98.8-99.8)	99.5 (98.8-99.8)	99.5 (98.8-99.8)	99.5 (98.8-99.8)	99.5 (98.8-99.8)
No. positive/total ^b	134/1110	134/1110	133/1110	130/1110	126/1110	122/1110	121/1110	120/1110

Abbreviations: cTnI, contemporary sensitive troponin I; LoD, level of detection; NPV, negative predictive value; PPV, positive predictive value.

^aCutoff of 32 pg/mL for the cTnI and 30 pg/mL for hTnI assays and LoD of 10 pg/mL for cTnI and 3.4 pg/mL for hTnI. Various relative troponin changes between admission and 3 hours and their combination with cTnI and hsTnI are presented. For each assay, analyses were performed on patients with their corresponding troponin I values below the upper range of the used assay on admission and 3 hours after admission.

^bNumber of patients with positive test criteria/number of patients with available data on criteria.

minority of patients. Then, using the 99th percentile as the diagnostic cutoff for hsTnI determined 3 hours after admission in patients with detectable troponin could allow a safe rule-out of MI in a larger proportion of patients with chest pain. However, due to the inherent lower sensitivity of cTnI assays, such a stepwise approach with rule-out in a relevant proportion of patients directly on presentation might not be recommended because the higher LoD of the cTnI assay applied as cutoff on admission yields an NPV of 96% compared with 100% for the hsTnI assay. Importantly, such a stepwise approach, using either hsTnI or cTnI, will require validation in a rigorous investigation in a separate cohort.

To address the rule-in of MI in patients presenting to the emergency department potentially with detectable or elevated troponin levels, 2 concerns exist. The first issue is identification of individuals with troponin exceeding the 99th percentile threshold who are in need of further diagnostic workup and potential treatment as having MI. The second is definition of high-risk patients with elevated troponin levels who potentially benefit the most from urgent treatment.

To define a relative change in biomarker concentration, which reflects a relevant difference of 2 consecutive measurements, the calculation of reference change values²¹ considers the variation explainable by assay imprecision and by the biological variability of the analyte. Reference change values for an hsTnI assay have been determined to be +46%/-32%,²² for an hsTnT assay to be +90%/-47%,²³ and for the hsTnI assay we used to be at least 82%.¹⁹ Application of this 82% reference change value relative change cutoff in low-risk patients in our study improved the PPV of hsTnI alone from 66.4% to 93%. A relative change cutoff of 235% has been independently established for an hsTnI assay in the setting of short-term prognosis of patients with chest pain.²⁴ Therefore, such a high relative change cutoff might help in the selection of patients with high-risk non-

ST-elevation MI for urgent and potentially invasive treatment. Our data show that a relative change cutoff of 266% provided the highest PPV in low-risk patients of 96.5%.

Integrating the result of the present analyses with data in the literature, a rule-in approach to MI including 2 aspects seems potentially useful. Consideration of the relative change in troponin levels within 3 hours after admission is essential in patients with a nondiagnostic electrocardiogram and slightly elevated troponin above the 99th percentile cutoff. To interpret these relative changes, the magnitude of the change can provide helpful diagnostic information. First, if this relative change exceeds the reference change value of the respective assay, the observed increase or decline is not explainable by biological variability or assay imprecision. Therefore, the patient should receive further cardiac diagnostic workup. Second, if the relative change exceeds a higher cutoff (in our study the evaluated hsTnI of 266%), the patient should be treated as having an acute MI.

The cTnI assay we evaluated herein provided comparable results to those of the hsTnI assay, with a PPV of up to 96.1% with the relative change within 3 hours and the 99th percentile cutoff on admission. Thus, the expected robust specificity of the cTnI assay could allow for its use in a similar diagnostic approach.

However, in the subgroup of patients presenting with troponin I values below the 99th percentile cutoff on admission increasing above this threshold within 3 hours, the enhanced sensitivity of the hsTnI assay could lead to an improved identification of MI if using the relative change as criterion (PPV of 83.0% vs PPV of 57.4% with the cTnI assay). Hence, in the setting of very low troponin values between the level of detection and the 99th percentile at baseline, application of an hsTnI assay may have advantages compared with the cTnI assay. However, such an approach, especially the specific relative change cutoff of 266% calculated from our data for the

used hsTnI assay, has to be independently validated.

The shortcoming of conventional troponin assays with low sensitivity within the first hours after chest pain onset led to the evaluation of various so-called early biomarkers in the diagnosis of MI. In our study, the diagnostic information of hsTnI was superior to all other evaluated biomarkers alone. Confirming previously published data, the combined determination of hsTnI and sVEGFR-1/sFLT-1²⁵ or copeptin^{26,27} on admission might facilitate early rule-out of MI. Whether the use of serial changes in cTnI or hsTnI improves patient care and outcome has to be investigated in further prospective studies.

Our study had several limitations. First, the final diagnosis of acute MI was based substantially on in-house troponin measurements, which might bias the biomarker evaluation toward troponin assays. Because both the index test and reference standard included a change in troponin levels over time, there is the potential for a type of incorporation bias, which may overestimate the measure of diagnostic accuracy of serial hsTnI levels. However, hsTnI appeared to facilitate identification of patients with non-ST-elevation ACS, a diagnosis independent of troponin values. Second, the number of patients with availability of biomarker values differed, which potentially could affect the results. Third, the proportion of patients with MI was rather high compared with that of other studies involving consecutive patients with chest pain, but the number is in line with different European cohorts.^{6,28} Still, this and the fact that only white European patients were enrolled might limit the generalizability of the findings to other populations.

CONCLUSIONS

Use of hsTnI and cTnI assays in patients with suspected MI provides useful diagnostic information. Various early biomarkers representing different aspects of an evolving ACS are less sensitive than cardiac troponin I for timely diagnosis of MI. Determination

of hsTnI and cTnI values 3 hours after admission to the emergency department with use of the 99th percentile cutoff provides an NPV greater than 99%, potentially allowing a safe rule-out of MI. Application of the relative change in hsTnI or cTnI concentration within 3 hours after admission in combination with the 99th percentile diagnostic cutoff value on admission improves specificity and may facilitate an accurate early rule-in of MI.

Author Contributions: Drs Keller and Blankenberg had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Keller, Lackner, Münzel, Blankenberg.

Acquisition of data: Keller, Zeller, Tzikas, Lillpopp, Sinning, Genth-Zotz, Bickel, Peetz, Lackner, Baldus.

Analysis and interpretation of data: Keller, Ojeda, Wild, Genth-Zotz, Warnholtz, Giannitsis, Möckel, Bickel, Peetz, Lackner, Baldus, Münzel, Blankenberg.

Drafting of the manuscript: Keller, Blankenberg.

Critical revision of the manuscript for important intellectual content: Zeller, Ojeda, Tzikas, Lillpopp, Sinning, Wild, Genth-Zotz, Warnholtz, Giannitsis, Möckel, Bickel, Peetz, Lackner, Baldus, Münzel.

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