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High-sensitivity cardiac troponin T and copeptin assays to improve diagnostic accuracy of exercise stress test in patients with suspected coronary artery disease

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Abstract

Background: The average diagnostic sensitivity of exercise stress tests (ESTs) is lower than that of other non-invasive cardiac stress tests. The aim of the study was to examine whether high-sensitivity cardiac troponin T (hs-cTnT) or copeptin concentrations rise in response to inducible myocardial ischaemia and may improve the diagnostic accuracy of ESTs.

Methods and results: An EST was performed stepwise on a bicycle ergometer by 383 consecutive patients with suspected or progression of coronary artery disease (CAD). In addition venous blood samples for measurement of hs-cTnT and copeptin were collected prior to EST, at peak exercise, and 4 h after EST. Coronary angiography was assessed for all patients. Patients with significant CAD ($n = 224$) were more likely to be male and older compared to patients with non-significant CAD ($n = 169$). Positive EST was documented in 125 (55.8%) patients with significant CAD and in 69 (43.4%) patients with non-significant CAD. Copeptin and hs-cTnT concentrations at baseline were higher in patients with significant CAD (copeptin: 10.8 pmol/l (interquartile range (IQR) 8.1–15.6) vs 9.4 pmol/l (IQR 7.1–13.9); $p = 0.04$; hs-cTnT: 3.0 ng/l (IQR <3.0–5.4) vs <3.0 ng/l (IQR <3.0); $p = 0.006$). Hs-cTnT improved sensitivity (61.6% vs 55.8%), specificity (67.7% vs 56.6%) and the positive predictive value (PPV) (72.3% vs 64.4%) and negative (55.2% vs 47.6%) predictive value (NPV) of EST. Copeptin could not improve sensitivity (55.4% vs 55.8%) and reduced specificity, PPV and NPV.

Conclusions: The measurement of hs-cTnT during EST improves sensitivity, specificity, and positive and negative predictive values. In contrast, measurement of copeptin does not improve diagnostic sensitivity and reduces specificity.

Keywords

Coronary artery disease, cardiac troponin T, copeptin, exercise stress test, myocardial ischaemia

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Introduction

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in the industrialised world.[1] Due to demographic changes, the number of patients with CAD will increase over the coming years. The identification of patients with CAD from among the large number of elective patients with chest discomfort represents a daily clinical challenge. Accurate diagnosis and elective assessment of both symptomatic and asymptomatic patients with suspected CAD could

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lead to reduced mortality and thus, it is of substantial interest.[1]

The exercise stress test (EST) is the most common type of noninvasive diagnostic tool for the detection of myocardial ischaemia, because of its accessibility and cost efficiency.[2] There is a wide variation in the sensitivity and specificity of exercise electrocardiograms (ECGs), depending on the pretest probability (mean sensitivity, $68\% \pm 16\%$ and mean specificity, $77\% \pm 17\%$).[3,4] The average sensitivity of the EST for detecting myocardial ischaemia is considerably lower than that of other noninvasive cardiac stress tests.[5]

Various biomarkers for the assessment of exercise-induced myocardial ischaemia in stable CAD have been investigated, but to date none have been shown to improve the diagnostic accuracy of EST.[6–9] Substantial progress in assay technologies has led to high-sensitivity (hs) assays for cardiac troponin (cTnT), which can detect myocardial necrosis earlier than conventional assays.[10] Studies have shown that hs-assays can determine minimal changes in cTnT concentrations with the required accuracy in serum from healthy individuals.[11,12] In patients with CAD lately, hs-cTnT was detected (above 3 ng/l) in 78% and 23% of patients even showed values above the conventional 99th percentile (13.5 ng/l). These result implicate the role of hs-cTnT also in patients with stable CAD.[13]

Another early-onset biomarker is copeptin, which reflects individual stress levels.[21,28–33] Copeptin is cosecreted with arginine vasopressin from the posterior pituitary. Previously published data have shown the diagnostic and prognostic impact of copeptin measurements used in combination with cTnT concentrations in patients with acute myocardial infarction (AMI).[12,18–21]

The aim of the present study was to examine whether hs measured cTnT or copeptin concentrations show an increase in response to inducible myocardial ischaemia during the EST. We also sought to determine if these measurements improve the diagnostic accuracy of this noninvasive cardiac stress test.

Methods

Study population

From January 2010–August 2011, 383 consecutive patients with suspected CAD or suspected progression of known CAD and history of percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) who were to undergo the EST were included in the study. Clinical history, physical examination, 12-lead ECG, laboratory tests, echocardiography, and coronary angiography were assessed for all patients. Exclusion criteria were AMI, unstable angina pectoris, acute heart failure, severe aortic stenosis. 47.8% ($n = 183$) of the patients showed CAD in their patient history. Altogether 137 (35.8%) patients underwent a previous PCI and 46 (12.0%) patients had a CABG previously.

Informed written consent was obtained from all patients for each investigation, as well as approval by the ethical board of the state of Hessen, Germany (FF31/2010). The investigation conforms to the principles outlined in the Declaration of Helsinki.

EST

The EST was performed stepwise on a bicycle ergometer in the erect position according to the current guidelines (World health organisation (WHO) scheme 50/25) starting with 25 or 50 W, rising 25 or 50 W every 2 min.[9] A standard 12-lead ECG was recorded regularly prior to, during every load level, and after the EST. Additionally, patients were monitored continuously by a six-lead ECG. A significant ST-segment change was defined by either a 0.1 mV horizontal or down-sloping depression or a 0.1 mV ST-segment elevation 60–80 ms after the J-point at the end of the QRS time. ECGs were recorded with a 1 mV/10 mm and 50 mm/s stylus deflection.

Criteria for a positive result from the EST were ST-segment changes as mentioned above or onset of angina pectoris during the EST. Stop criteria were relevant ST-segment changes, angina pectoris, severe dyspnoea and exhaustion, as well as significant cardiovascular disorders such as maximum heart rate, severe hypertension (>220 mm Hg systolic), and severe arrhythmias (ventricular tachycardia or arterioventricular block $>1^{\circ}$). The highest maximum heart rate was individually estimated by the formula 220–age (years).[4] Two independent cardiologists adjudicated the final EST result. If there was disagreement about the EST result, a third cardiologist was involved.

Coronary angiography

Coronary angiograms were provided for each patient after the EST according to standard clinical practice. The indication of the coronary angiography was not only based on the results of EST, but on guideline-based clinical history or prior non-invasive diagnostics.

Significant coronary artery disease was defined as a stenosis of at least one coronary artery exceeding 70%. Revascularisation, if possible, was performed by PCI or, regarding the severity of CAD, by CABG afterwards.

Laboratory assessment

Venous blood samples were collected in plain tubes for the determination of hs-cTnT and copeptin levels prior

to the EST, at peak exercise, and at 4 h after the EST. This time point could be verified by previous data showing the highest release of troponin 3–4 h after exercise tests.[22] Serum was processed immediately and frozen at −80 °C until assayed.

The cTnT was measured in serum with the hs electro-chemiluminescence immunoassay (hs-cTnT assay, Elecsys Analyzer 2010; Roche Diagnostics, Mannheim, Germany). The limit of blank (LoB) for the hs-cTnT assay is 3.0 ng/l, the limit of detection (LoD) is 5 ng/l with the 99th percentile at a concentration of 14.0 ng/l. The lowest concentration measurable with a coefficient of variation (CV) <10% for this assay is 13.5 ng/l.[12] Copeptin was measured in serum by sandwich immunoluminometric assay (CT-proAVP; ThermoFisher, Henningsdorf/Berlin, Germany). The LoB for the copeptin assay is 2.5 pmol/l, the LoD is 3.2 pmol/l, with the 99th percentile at a concentration of 13.5 pmol/l. The lowest concentration measurable with a CV < 10% for this assay is down to 5.0 pmol/l.[16]

We counted an increase of 10% of the baseline values as a significant increase.

Statistical analysis

All data for continuous variables are expressed as mean ± standard deviation (SD) or as median and interquartile range (IQR) as appropriate. Categorical variables are reported as number and percentage. After testing for normal distribution by Kolmogorov-Smirnov test, values were compared by unpaired Student's *t*-test or by Mann-Whitney test. Fisher's exact test or chi-square test was used for categorical variables with nominal scales. To evaluate test improvement due to hs-cTnT and copeptin levels, the area under the curve (AUC) of the receiver-operating characteristic (ROC) curve was plotted. To compare the AUCs a *c*-statistic was calculated. All statistical tests were two-tailed, and $p < 0.05$ was considered to indicate statistical significance. For all statistical analyses, the statistical software SPSS 19.0 (Statistical Package for the Social Sciences, Chicago, Illinois, USA) for Windows was used.

Results

Study population

The study enrolled 383 patients (224 with significant CAD and 159 with non-significant CAD, as revealed by angiography). Patients who required coronary revascularisation with PCI or CABG were assigned to the significant CAD group. Patients without need for revascularisation were assigned to the non-significant CAD group. Significant CAD was defined as angiographic-estimated coronary artery stenoses causing myocardial ischaemia. Non-significant CAD was defined as absence of CAD or angiographic-estimated coronary artery stenosis without causing myocardial ischaemia. The baseline characteristics of all patients are summarised in Table 1.

Baseline and patient characteristics

Patients with significant CAD were more likely to be male and older, and they had more arterial hypertension compared to patients without CAD. The Framingham Risk Score (FRS) correlated significantly with CAD ($r = 0.121$, $p = 0.036$) and with hs-cTnT increase ($r = 0.140$; $p = 0.015$). No correlation could be observed between FRS and copeptin increase ($r = 0.061$; $p = 0.291$).

EST

There were no differences in exercise capacity (CAD 119.0 ± 38.4 W vs nonsignificant CAD 121.4 ± 43.0 W; $p = 0.57$) between the two groups. The EST showed a positive result in 125 (55.8%) patients with significant CAD and in 69 (43.4%) patients with nonsignificant CAD (Table 2). This showed a low sensitivity of 55.8% and a specifity of 56.6%. A total of 39 (10.2%) patients reached their maximum heart rate, which can be explained by either symptoms or ECG changes before that point or by being on beta blocker medication. Nevertheless out of these 39 patients, 22 (56.4%) showed significant CAD. Out of these 22 patients, 17 (77.9%) patients had a positive EST. This demonstrates that in patients reaching their maximum heart rate a positive EST is more likely to prove significant CAD than patients without reaching their maximum heart rate and positive EST.

Laboratory measurement

The hs-cTnT baseline concentrations (CAD 3.0 ng/l, IQR <3.0–5.4 vs nonsignificant CAD <3.0 ng/l, IQR <3.0; $p = 0.006$) and copeptin baseline concentrations (10.8 pmol/l, IQR 8.1–15.6 vs 9.4 pmol/l, IQR 7.1–13.9; $p = 0.04$) were significantly higher in patients with CAD.

The hs-cTnT concentrations increased at at least one of the prespecified time points irrespective of the EST result in 138 (61.6%) patients with CAD and in 53 (33.3%) patients with nonsignificant CAD (Figure 1). In patients with CAD, there was no difference in the increase in hs-cTnT levels in patients with a positive EST result compared to those with a negative EST result (82 (65.6%) vs 56 (56.6%); $p = 0.21$). In patients

Table 1. Baseline characteristics

Variables	CAD $n = 224$ (58.5)	Non-significant CAD $n = 159$ (41.5)	p value
Men, *n* (%)	189 (84.4)	107 (67.3)	<0.001
Age, mean (SD)	64.7 (9.9)	60.5 (12.5)	<0.001
Cardiovascular risk factors, *n* (%)			
Hypertension	199 (88.8)	118 (74.2)	<0.001
Diabetes	70 (31.3)	39 (24.5)	0.17
Hyperlipoproteinaemia	170 (75.9)	114 (71.7)	0.18
Current smoker	32 (14.3)	38 (23.9)	0.93
Familial predisposition	68 (30.4)	49 (30.8)	0.91
Obesity	91 (40.6)	65 (40.9)	0.83
Left ventricular function, mean (SD)	54.7 (8.3)	56.1 (8.1)	0.12
Laboratory measurements, median (IQR)			
Glomerular filtration rate	91.0 (IQR 77.0–107.8)	95.5 (IQR 81.3–112.8)	0.06
Creatinine	0.9 (IQR 0.8–1.0)	0.8 (IQR 0.7–0.9)	0.005
Hs-cTnT	<3.0 (IQR <3.0–5.4)	<3.0 (IQR <3.0)	0.006
Copeptin	10.8 (IQR 8.1–15.6)	9.4 (IQR 7.1–13.9)	0.04
Medication, *n* (%)			
Beta blocker	179 (79.9)	92 (57.9)	<0.001
Digitalis	1 (0.4)	1 (0.6)	>0.99
ASA	190 (84.8)	82 (51.6)	<0.001
Statins	174 (77.7)	68 (42.8)	<0.001
ACE inhibitor	180 (80.4)	94 (59.1)	<0.001
Diuretics	83 (37.1)	40 (25.2)	0.28

ASA: acetylsalicylic acid; ACE: angiotensin-converting enzyme; CAD: coronary artery disease; IQR: interquartile range; SD: standard deviation

with nonsignificant CAD, no difference in hs-cTnT concentrations was observed irrespective of the EST results (26 (37.7%) vs 27 (30.0%); $p = 0.39$). A significant increase in hs-cTnT concentrations was seen more often in patients with multivessel CAD compared to patients with one-vessel disease or without significant CAD (143 (64.4%) vs 48 (29.8%); $p < 0.001$). The hs-cTnT measurements during EST showed improved sensitivity (61.6% vs 55.8%), specificity (67.7% vs 56.6%), and, therefore, the positive predictive value (PPV) (72.3% vs 64.4%) and negative (55.2% vs 47.6%) predictive values (NPV) of EST (Table 3).

Copeptin concentrations increased at at least one of the prespecified time points in 124 (55.4%) patients with CAD and 92 (57.9%) patients with non-significant CAD (Figure 2). Regarding the EST result, there was no difference in the increase in copeptin levels in patients with CAD (positive EST: 61 (48.8%) vs negative EST: 63 (63.6%); $p = 0.03$) compared to those with nonsignificant CAD (positive EST: 37 (53.6%) vs negative EST: 55 (61.1%); $p = 0.43$). The hs-cTnT and copeptin concentrations are displayed in Figures 1 and 2. Copeptin measurement did not improve sensitivity (55.4% vs 55.8%), and it reduced the specificity (42.1% vs 56.6%) and positive (57.4% vs 64.4%) and negative (40.1% vs 47.6%) predictive values of the EST (Table 3).

Regarding the ROC curves, hs-cTnT determination during the EST provided the best result, with an AUC of 0.646 (95% confidence interval (CI) 0.59–0.70; $p < 0.001$) vs EST with an AUC of 0.56 (95% CI 0.50–0.62; $p = 0.038$) and copeptin with an AUC of 0.55 (95% CI 0.49–0.61; $p = 0.09$) (Figure 3). The AUC of all three parameters were shown to be of significant difference due to the *c*-statistics ($p < 0.001$). AUC of hs-cTnT ruled out EST ($p = 0.02$) and copeptin ($p < 0.001$).

Discussion

The number of patients with cardiovascular disease is growing steadily.[23] Consequently, suspected CAD is one of the most frequent reasons for medical consultation. The EST is widely used, but it has considerably

Table 2. Procedural and laboratory characteristics

Variables	CAD $n = 224$ (58.5%)	Non-significant CAD $n = 159$ (41.5%)	p value
Watt, mean (SD)	119.0 ± 38.4	121.4 ± 43.0	0.57
Reason for halting EST, *n* (%)			
Angina pectoris	99 (44.2)	53 (33.3)	0.03
Dyspnoea	52 (23.2)	40 (25.2)	0.72
Exhaustion	135 (60.3)	102 (64.2)	0.46
Hypertensive disorder	17 (7.6)	17 (10.7)	0.37
ST-segment changes, *n* (%)	108 (48.2)	56 (35.2)	0.01
Positive EST result, *n* (%)	125 (55.8)	69 (43.4)	0.01
Haemodynamic parameters, mean (SD)			
Heart rate baseline (bpm)	67.1 ± 13.2	70.4 ± 13.8	0.04
Heart rate maximum (bpm)	116.6 ± 22.0	122.6 ± 22.7	0.02
Systolic blood pressure baseline (mm Hg)	128.1 ± 21.8	127.7 ± 17.5	0.99
Systolic blood pressure maximum (mm Hg)	177.9 ± 30.2	184.8 ± 32.1	0.02
Diastolic blood pressure baseline (mm Hg)	78.7 ± 12.1	81.1 ± 11.7	0.07
Diastolic blood pressure maximum (mm Hg)	86.6 ± 16.1	87.9 ± 15.0	0.22
Hs-cTnT increase, *n* (%)	138 (61.6)	53 (33.3)	<0.001
Hs-cTnT at maximum stress	<3.0 (IQR 3.0–6.5)	<3.0 (IQR <3.0–3.5)	<0.001
Hs-cTnT 4 h after EST	3.4 (IQR <3.0–7.7)	<3.0 (IQR <3.0–3.7)	<0.001
Copeptin increase, *n* (%)	124 (55.4)	92 (57.9)	0.68
Copeptin at maximum stress	14.9 (IQR 9.7–26.8)	10.8 (IQR 8.5–18.9)	0.003
Copeptin 4 h after EST	11.7 (IQR 8.2–14.9)	9.6 (IQR 6.5–14.8)	0.006

CAD: coronary artery disease; EST: exercise stress test; hs-cTnT: high-sensitivity cardiac troponin T; SD: standard deviation.

low diagnostic accuracy. Improvements in the diagnostic accuracy of the EST are of major clinical and health system importance. We therefore evaluated whether additional measurements of hs-cTnT and copeptin levels would improve the diagnostic accuracy of the EST.

The new generation of troponin assays has a higher analytical sensitivity and the ability to detect even minor releases of cTnT; using one of these hs-assays, our study showed an improvement in the diagnostic accuracy of the EST by additional measurements of hs-cTnT during the EST. Previously published data demonstrated that cTnT increases proportionately after exercise stress with the degree of myocardial ischaemia found in nuclear imaging.[24] These data support our results and show that hs-cTnT concentrations increased below the 99th percentile significantly in patients with multi-vessel CAD while they underwent the EST compared to patients with one-vessel CAD that resulted from a greater myocardial ischaemia.[24,25]

In contrast, approximately one-third of the patients without significant CAD showed increased hs-cTnT concentrations below the 99th percentile during the EST. It is also known that cTnT is released independently of CAD during extreme physical stress or high ventricular rates.[26] The elevation of hs-cTnT levels in the absence of obstructive CAD might therefore be explained by the fact that physical stress is accompanied by higher blood pressures and higher heart rates, which may eventually lead to myocardial necrosis or apoptosis.[27]

In our cohort, baseline copeptin concentrations were higher in patients with CAD. During the EST, patients with significant CAD did not show an increase in copeptin concentrations more often than patients without significant CAD. Measurement of copeptin concentrations during the EST was not of additive value for making a diagnosis of CAD. As a biomarker, copeptin appears to be related more to individual stress levels than to myocardial ischaemia. This is in line with previous studies, which showed elevations in copeptin levels due not only to myocardial ischaemia, but also due to stroke, septic shock, heart failure, or other stress-related conditions such as Tako-Tsubo cardiomyopathy.[28–31] Previous studies also indicated that copeptin might function as an outcome parameter after resuscitation or myocardial infarction.[18,32]

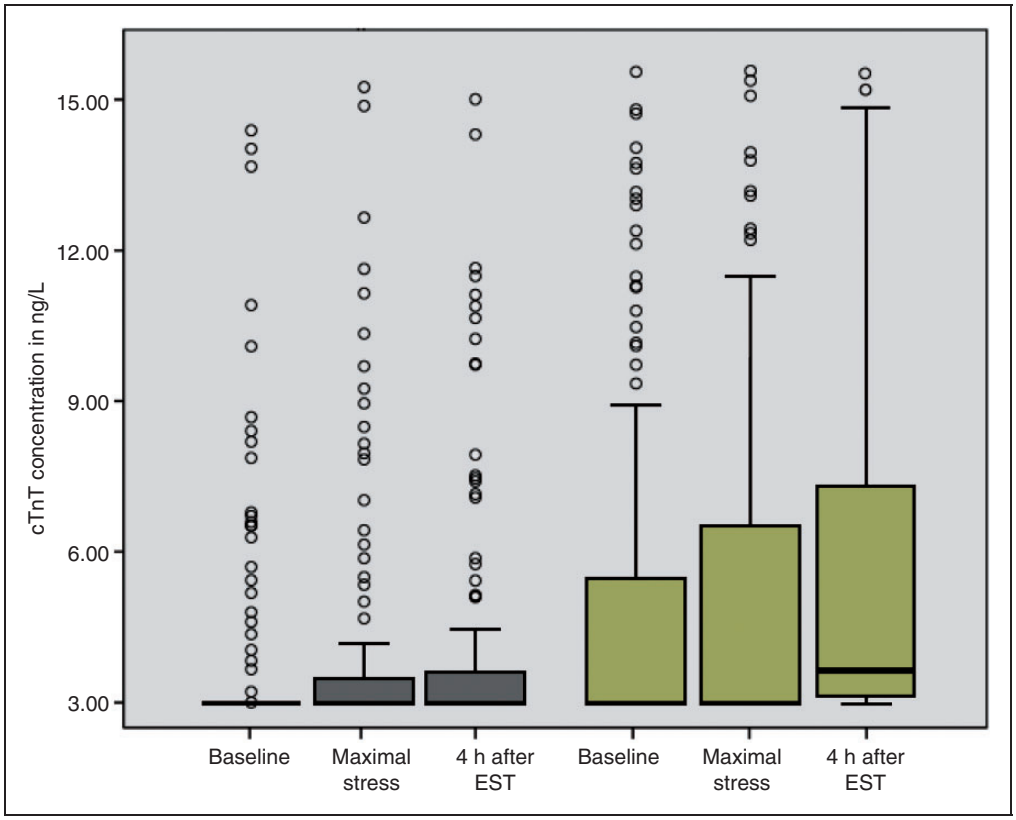

Figure 1. Cardiac troponin T (cTnT) serum concentrations (median fold-change (interquartile range (IQR)) of all patients at baseline and throughout the study. Grey bars indicate patients without coronary artery disease (CAD). Green bars indicate patients with CAD. Circles indicate outlier data points. EST: exercise stress test.

Table 3. Test accuracy for diagnosis of coronary artery disease (CAD)

Tests	Sensitivity	Specificity	NPV	PPV
Exercise stress test	55.8%	56.6%	64.4%	47.6%
Troponin T increase				
At maximum stress	45.1%	72.3%	69.7%	48.3%
At 4 h after EST	51.8%	72.3%	72.5%	51.6%
At at least one of the time points	61.6%	66.7%	72.3%	55.2%
Copeptin increase				
At maximum stress	50.0%	45.3%	56.3%	39.1%
At 4 h after EST	30.8%	76.1%	64.4%	43.8%
At at least one of the time points	55.4%	42.1%	57.4%	40.1%

EST: exercise stress test; NPV: negative predictive value; PPV: positive predictive value.

Increased copeptin levels correlated more strongly with the peak exercise level than with obstructive CAD. This confirms the findings by Maeder and colleagues, who reported that higher absolute exercise capacity and higher exercise intensity are associated with greater changes in copeptin concentrations.[33]

If the additional determination of hs-cTnT during EST reduces false-positive and false-negative EST results, then the number of unnecessary coronary angiographies could be reduced, and patients with CAD could be treated earlier. This would have major implications for health care resources. However, a validation needs to be performed in a large-scale, real-world scenario to establish new work-up protocols for the EST.

Limitations

The results of our study should be interpreted in the context of several limitations. The EST results in previous studies as well as in our study show a very low sensitivity and specificity, but provide insight in daily clinical practice. The troponin concentrations in both groups are mainly close to the LoB. Values below the LoD have a higher imprecision (CV = 20%). The new nano particle troponin assay provides an imprecision of <10% at very low cTnT concentrations which would be of interest.[34] The availability of these new assays is

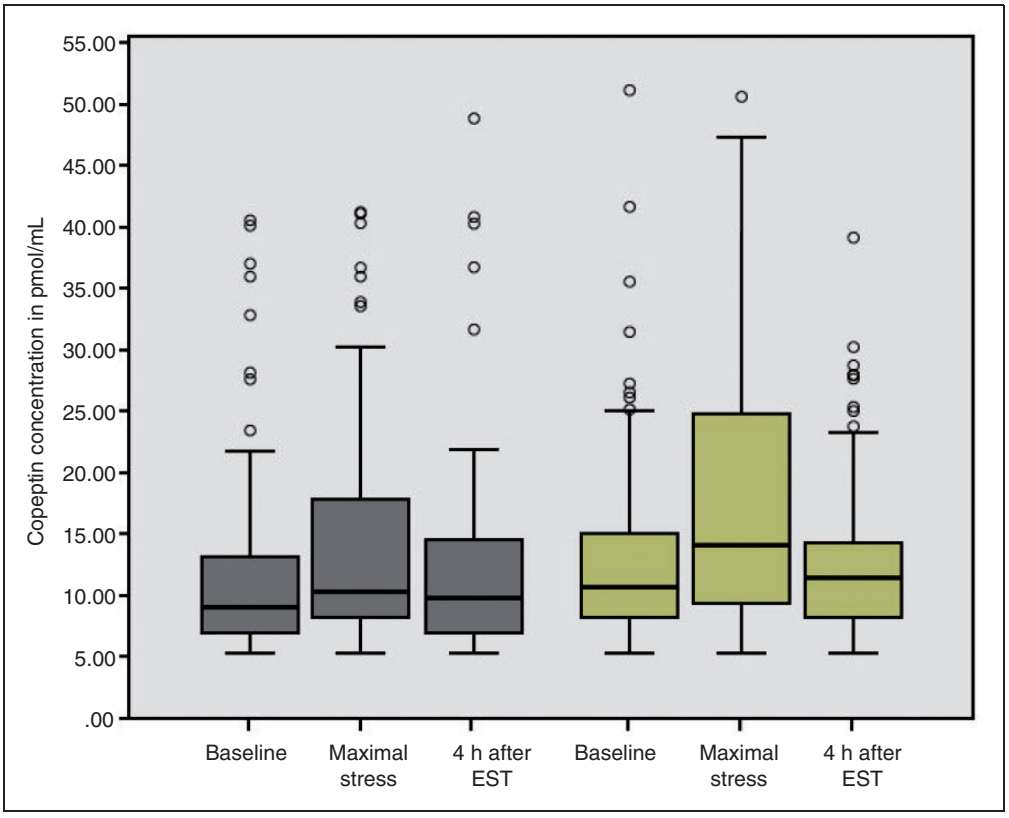

Figure 2. Copeptin serum concentrations (median fold-change (interquartile range (IQR)) of all patients at baseline and throughout the study. Grey bars indicate patients without coronary artery disease (CAD). Green bars indicate patients with CAD. Circles indicate outlier data points. EST: exercise stress test.

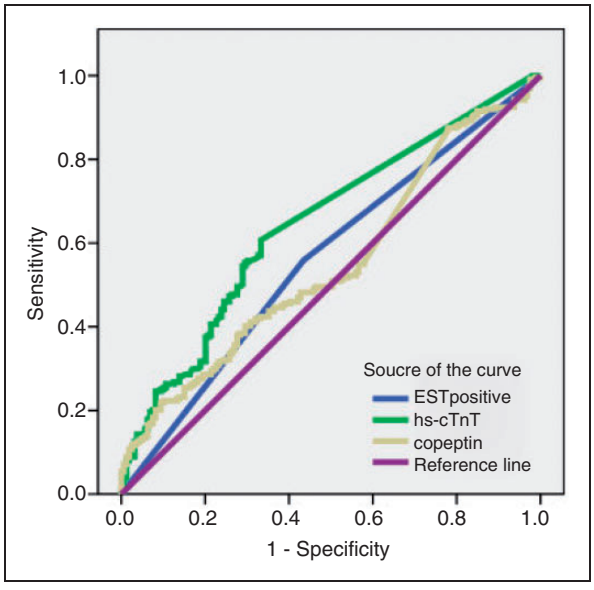

Figure 3. Area under the curve of the receiver-operating characteristic curve for the prediction of coronary artery disease (CAD). cTnT: cardiac troponin T; EST: exercise stress test.

limited and due to costs and time-consuming analysis without clinical implications at the moment.

Conclusions

The EST alone has a low diagnostic accuracy for CAD. Analysing hs-cTnT during the EST improves the sensitivity and specificity, as well as the PPV and NPV of the EST. In contrast, measurement of copeptin levels improves the diagnostic sensitivity of the EST, but worsens the specificity and therefore the PPV and NPV. Only hs-cTnT measurements improve the diagnostic accuracy of the EST in patients with suspected CAD.

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Conflict of interest

C Hamm has received research grants as well as speaker honoraria from Roche and Brahms. All other authors declare that they have no conflict of interest with this study.

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