Diagnostic and Prognostic Impact of Copeptin and High-Sensitivity Cardiac Troponin T in Patients with Pre-existing Coronary Artery Disease and Suspected Acute Myocardial Infarction

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ABSTRACT

Objective The early diagnosis of acute myocardial infarction (AMI) can be particularly challenging in patients with known coronary artery disease (CAD) due to pre-existing ECG changes and chronic elevations of cardiac troponin (cTn).

Design We analyzed 433 patients (37%) with pre-existing CAD out of 1170 consecutive patients presenting with symptoms suggestive of AMI in a prospective, multicenter study and evaluated the diagnostic and prognostic impact of copeptin in combination with either 4th generation cTnT or high-sensitivity cTnT (hs-cTnT).

Results AMI was the final diagnosis in 78 patients with pre-existing CAD (18%). Copeptin was significantly higher in patients with AMI than without (26pmol/l [IQR 9-71] vs. 7pmol/l [IQR 4-16], p< 0.001). The diagnostic accuracy for AMI as quantified by the area under the receiver-operating characteristic curve (AUC) was significantly higher for the combination of copeptin and cTnT as compared to cTnT alone (0.94 versus 0.86 p<0.001). The combination of copeptin and hs-cTnT (0.94) was trending to superiority compared with hsTnT alone (0.92; p=0.11). The combination of Copeptin and the cTn assays was able to improve the negative predictive value up to 99.5% to rule out AMI. Copeptin was a strong and independent predictor for 1-year mortality (HR 4.18 - 4.63). Irrespective of cTn levels, patients with low levels of copeptin had an excellent prognosis compared to patients with elevation of both, copeptin and cTn (360 day mortality of 2.8%-3.6% vs. 23.1% -33.8%; p<0.001).

Conclusion **In patients with pre-existing CAD, copeptin significantly improves diagnostic accuracy if used in addition to cTnT, but only trended to superiority on top of hs-cTnT. Copeptin provides independent prognostic information, largely by overcoming the challenging interpretation of mild elevations of hs-cTnT.**

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Coronary artery disease (CAD) is estimated to affect 16.3 million people in the United States; of these, 9 million have angina pectoris, and nearly 8 million have had an acute myocardial infarction (AMI).[1]The rapid and accurate diagnosis of AMI is critical for the initiation of effective evidence based medical management and treatment.[2, 3] but still an unmet clinical need. Delayed "rule in" increases morbidity and mortality, particularly in patients with pre-existing coronary artery disease (CAD).[4, 5] Delayed "rule out" prolongs the time spent in the emergency department (ED), increasing patients' uncertainty and anxiety, and causes enormous costs for the health care system.[6]

Patients with pre-existing CAD merit particular attention: First, they are at increased risk for both: AMI as well as anxiety related to non cardiac causes of chest pain. Second, the impact of myocardial loss is particularly devastating when the myocardium already suffered previous assaults, so that delayed diagnosis of AMI yields especially severe consequences.[4, 5] Third, pre-existing ECG changes are common and render the diagnostic use of the ECG more challenging. Forth, elevated levels of sensitive cTn assays, a new diagnostic option with a lower limit of detection (LoD) below the 99th percentile of a normal reference population and

improved precision, have been found in more than 10% of patients with stable CAD.[7-11]

Recently, first pilot studies have investigated the diagnostic and prognostic value of copeptin, the c-terminal part of the vasopressin, as a novel sensitive marker of endogenous stress, in patients with acute chest pain or established AMI.[12-15] These investigations substantiated the hypothesis that the combination of two different pathophysiological processes quantified by cTn and copeptin might overcome some of the limitations of cTn.[12-16] It is unknown, whether the additional use of copeptin would help to improve the early diagnosis and risk stratification of patients with pre-existing CAD.

Methods

Study design and population

The Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) Study is an ongoing prospective international multicenter study designed and coordinated by the University Hospital Basel. From April 2006 to June 2009, a total of 1247 consecutive patients presenting to the ED with acute chest pain with an onset or peak within the last 12 hours were recruited. Patients with end stage renal failure were excluded. Pre-existing CAD was defined as history of previous AMI, previous coronary revascularization for obstructive CAD, or known coronary artery stenosis exceeding 50%. For the current analysis 1170 patients had baseline values of the investigational biomarkers available and were included.

The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Written informed consent

was obtained from all patients. The authors designed the study, gathered and analyzed the data, vouch for the data and analysis, wrote the paper, and made the decision to submit it for publication. The assays were donated by the manufacturers, who had no role in the design of the study, the analysis of the data, the preparation of the manuscript, or the decision to submit for publication.

Routine clinical assessment

All patients underwent an initial clinical assessment that included history taking, a physical examination, 12-lead ECG, continuous ECG-monitoring, pulse oximetry, standard blood tests and chest radiography. CTnI or cTnT, CK-MB and myoglobin were measured at presentation and 6-9 hours after presentation or as long as clinically indicated. The precise timing of clinical post-baseline measurements and the treatment of patients were left to the discretion of the attending physician.

Adjudicated final diagnosis

To determine the final diagnosis for each patient, two independent cardiologists reviewed all available medical records – the clinical history, findings on physical examination and results of laboratory tests (including cTn values obtained at the participating hospitals but not those being assessed as part of this study), radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography- from the time of the patient's arrival in the ED to the end of the 60-day follow-up period. When there was disagreement about the diagnosis, cases were reviewed and adjudicated in conjunction with a third cardiologist.

An AMI was defined in accordance with current guidelines.[17] In brief, an AMI was diagnosed when there was evidence of myocardial necrosis in association with clinical signs of myocardial ischemia. Necrosis was diagnosed by a rising and/or falling pattern of the local cardiac troponin level, with at least one value above the 99th percentile, at a level of imprecision of less than 10%.[7, 18] In the absence of uniformly accepted published guidelines, a significant rise and/or fall was defined as a change of at least 30% of the 99th percentile (or the 10% CV level respectively) within 6 to 9 hours. The following cTn assays were used for the adjudication of the final diagnosis at the participating hospitals: Abbott Axsym cTnI ADV, Beckmann Coulter Accu cTnI, and Roche cTnT. All three are well-validated current cTn assays with comparable performance in the diagnosis of AMI (see supplementary appendix for details on use of local cTn assays for final diagnosis adjudication).[7, 18] Unstable angina was diagnosed when a patient had normal cTn levels and typical angina at rest, a deterioration of a previously stable angina, in cases of positive cardiac exercise testing or cardiac catheterization showing coronary arteries with stenosis of 70% or more of the vessel diameter or when the diagnosis was uncertain but follow-up information showed that the patient had an AMI or a sudden, unexpected cardiac death within 60 days after presentation. Further predefined diagnostic categories included cardiac but not coronary symptoms (e.g., perimyocarditis or tachyarrhythmias), non-cardiac causes and symptoms of unknown origin. If AMI was ruled out in the ED, but no further diagnostic procedures were performed that were sufficient to establish a conclusive diagnosis, symptoms were classified as being of unknown origin.

Follow-up and Clinical Endpoints

After hospital discharge, patients were followed after 3 and 12 months by telephone or in written form. Any clinical (cardiovascular) events since presentation to the ED were collected by establishing contact with the patient and his family physician. Information about death also was obtained from the national registry on mortality.

Outcomes

The primary diagnostic endpoint was the diagnosis of AMI by cTnT, hsTnT and copeptin and their combination. The primary prognostic endpoint was death from all causes. The secondary endpoint was a composite of all-cause death or first acute myocardial infarction in chest pain patients without acute myocardial infarction as the index event.

Investigational copeptin and cardiac troponin T analysis

Blood samples for determination of Copeptin were collected at the time of the patient's presentation to the ED in tubes containing potassium EDTA and in tubes containing serum for cTnT 4th generation assay (Roche cardiac Troponin T [cTnT],) and high-sensitive cTnT [hs-cTnT].[19, 20] After centrifugation, samples were frozen at -80°C until they were assayed in a blinded fashion in two batches in a dedicated core laboratory.

Copeptin was measured using a novel commercial sandwich immunoluminometric assay (B.R.A.H.M.S. LUMItest CT-proAVP, B.R.A.H.M.S AG, Hennigsdorf/Berlin, Germany), as described in detail elsewhere.[21] Since this initial publication, the assay was modified as follows: the capture antibody was replaced by a murine monoclonal antibody directed to amino acids 137-144 (GPAGAL) of proAVP. This modification improved the sensitivity of the assay. The lower detection limit was 0.4 pmol/l, and the functional assay sensitivity (<20% interassay CV) was <1 pmol/l. The median copeptin level in 200 healthy persons was 3.7 pmol/l and the 97.5 percentile was 16.4 pmol/l. Based on a reference population of 5000 individuals of the Gutenberg Heart Study, a copeptin level of 9.8 pmol/l corresponds to the 95th percentile and 13pmol/l to the 97.5th percentile.[14] The selected diagnostic and prognostic cut-off point for copeptin of 9 pmol/l was based on our own previous study results.[13]

All Roche assays were performed with the use of the Elecsys 2010 system (Roche Diagnostics): cTnT with a limit of detection of 0.01 µg/l, a 99th-percentile cut-off point of less than 0.01 µg/l, and a coefficient of variation of less than 10% at 0.035 µg/l; hs-cTnT with a limit of blank of 0.003 µg/l, a limit of detection of 0.005 µg/l, a 99th-percentile cut-off point of 0.014 µg/l, and a coefficient of variation of less than 10% at 0.013 µg/l.[22]

Statistical analysis

Continuous variables are presented as means (±SD) or medians (with the interquartile range), and categorical variables as numbers and percentages.

Continuous variables were compared with the use of the Mann–Whitney U test and categorical variables with the use of the Pearson chi-square test. Receiver-operating-characteristic (ROC) curves were constructed to assess the sensitivity and specificity of copeptin and cTn measurements obtained at specific times and to compare their ability to diagnose AMI. Logistic regression was used to combine copeptin and cTn levels at presentation. The comparison of areas under the ROC curves (AUC) was performed as recommended by DeLong et al.[23] The Kaplan-Meier cumulative survival curves in which patients were divided into groups with biomarker levels below and above predefined cut-off values were constructed and compared by the log-rank test. We used univariate Cox proportional hazard analysis to compute hazard ratios and 95% CI of potential predictors of death. All significant variables were then tested in a multivariable model using the forward stepwise method. All hypothesis testing was two-tailed, and P values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed with the use of IBM SPSS Statistics for Windows, version 19.0 (IBM), and MedCalc software, version 10.3.0 (MedCalc).

Results

Characteristics of the patients

Of the 1247 consecutively enrolled patients, measurement of copeptin and the two investigational cTn assays were obtained at presentation from 1170 patients, of whom 433 (37%) had pre-existing CAD. Patients with pre-existing CAD differed in multiple baseline characteristics from those without pre-existing CAD including higher age, higher prevalence of cardiovascular risk factors, impaired renal function, chronic obstructive pulmonary disease, peripheral arterial disease and stroke, lower heart rate and blood pressure, as well as a higher incidence of pre-existing ECG changes including left bundle branch block and T-inversion (Table 1).

Table 1	Baseline Characteristics of the Patients			
	All patients n=1170	CAD n=433 (37%)	No CAD n=737 (63%)	p Value
Age – yr				<0.001
Median	64	72	59	
Interquartile range	51–76	59–79	49 –72	
Male gender – no. (%)	781 (67)	327 (76)	454 (62)	<0.001
Risk factors – no. (%)				
Hypertension	744 (64)	360 (83)	384 (52)	<0.001
Hypercholesterolemia	523 (45)	305 (70)	218 (30)	<0.001
Diabetes	225 (19)	130 (30)	95 (13)	<0.001
Current smoking	281 (24)	82 (19)	199 (27)	0.002
History of smoking	410 (35)	206 (48)	204 (28)	<0.001
History – no. (%)				
Chronic obstructive pulmonary disease	121 (10)	71 (16)	50 (7)	<0.001
Renal insufficiency	123 (11)	92 (21)	31 (4)	<0.001
Peripheral artery disease	80 (7)	59 (14)	21 (3)	<0.001
Previous stroke	69 (6)	34 (8)	35 (5)	0.04
Vital Status				
Heart rate – bpm				
Median (Interquartile range)	76 (66–89)	71 (62–82)	78 (68–92)	<0.001

Systolic blood pressure – mm Hg				
Median (Interquartile range)	142 (127–160)	139 (124–157)	145 (129–161)	<0.001
Diastolic blood pressure – mm Hg				
Median (Interquartile range)	84 (74–93)	80 (70–89)	86 (77–96)	<0.001
Body-mass index				
Median (Interquartile range)	26 (24–30)	27 (24–30)	26 (24–29)	0.08
Electrocardiographic findings – no. (%)				
Left bundle branch block	42 (4)	25 (6)	17 (2)	0.003
ST-segment elevation	62 (5)	19 (4)	43 (6)	0.35
ST-segment depression	118 (10)	51 (12)	67 (9)	0.16
T-wave inversion	89 (8)	46 (11)	43 (6)	0.004
No significant ECG abnormalities	859 (73)	292 (68)	567 (77)	<0.001
Glomerular filtration rate – ml/min/1.73m^2				
Median (Interquartile range)	89 (71–107)	80 (61–99)	94 (75–109)	<0.001
Adjudicated final diagnosis - no. (%)				<0.001
Acute myocardial infarction	184 (16)	78 (18)	106 (14)	0.59
Unstable angina	164 (14)	118 (27)	46 (6)	0.006
Cardiac symptoms from causes other than CAD	154 (13)	42 (10)	112 (15)	0.59
Non-cardiac causes	565 (48)	149 (34)	416 (56)	<0.001
Symptoms of unknown origin	103 (9)	46 (11)	57 (8)	0.85

Patients with pre-existing CAD had more often unstable angina (27% versus 6%, p=0.006) but showed no difference in AMI (18% versus 14%, p=0.59) than patient without pre-existing CAD.

Levels of Copeptin, cTnT and hs-cTnT at presentation

Median levels of copeptin, cTnT and hs-cTnT were all significantly higher in patients with than without pre-existing CAD (Table 2).

Table 2	Copeptin and troponin levels according to pre-existing CAD			
	All patients n=1170	CAD n=433 (37%)	No CAD n=737 (63%)	p Value
Copeptin				
Median (Interquartile range) – pmol/l	6.8 (3.5–15.6)	8.8 (4.1–23.1)	6.1 (3.2–12.6)	<0.001
Roche troponin T 4th Generation (cTnT)				
Median (Interquartile range) - µg/l	≤0.01 (≤0.01–≤0.01)	≤0.01 (≤0.01–≤0.01)	≤0.01 (≤0.01–≤0.01)	0.03
Roche high-sensitive troponin T (hs-cTnT)				
Median (Interquartile range) - µg/l	0.009 (0.004–0.024)	0.014 (0.008–0.031)	0.007 (0.003–0.018)	<0.001

The levels of copeptin and the cTn assays according to the final diagnosis and the presence of CAD are shown in Figure 1.

Diagnostic utility of individual markers

Among CAD-patients with a final diagnosis of AMI, baseline values were elevated in 78.2% for cTnT (>0.01µg/l), in 93.6% for hsTnT (>0.014µg/l) and in 75.6% for copeptin (>9pmol/l). The corresponding sensitivities, specificities, negative and positive predictive values are given in Table 3A. Among patients without a history of CAD, the baselines values were elevated in 83.0% for cTnT, 94.3% for hsTnT and 67.9% for copeptin (Table 3B).

Among CAD-patients with final diagnoses other than AMI, baseline values were elevated above the decision cut-point in 9.9% for cTnT, in 39.7% for hs-cTnT and in 43.7% for copeptin giving specificities of 90.1, 60.3 and 56.3, respectively (Table 3A). In contrast, among patients without a history of CAD and with final diagnoses other than AMI the percentage was 5.4% for cTnT, 18.2% for hs-cTnT ($p<0.001$ for comparison of hs-cTnT) and 30.6% for copeptin (Table 3B). Due to the higher incidence of elevated hs-cTnT levels in non-AMI patients, the cut off for hs-cTnT > 0.014µg/l showed a lower specificity for AMI in patients with pre-existing CAD (60%) as compared to those without (82%). For the rule out of AMI in patients with pre-existing CAD, the negative predictive values are shown in Table 3A. The diagnostic accuracy as quantified by the AUC

to diagnose AMI was 0.86 (95% CI, 0.80–0.92) for cTnT, 0.92 (95% CI, 0.89–0.96) for hs-cTnT and 0.76 (95% CI, 0.70–0.81) for copeptin.

Diagnostic utility of combined markers

Among CAD-patients with a final diagnosis of AMI, baseline values of either copeptin or cTnT or both together, were elevated in 98.7% of patients. The same proportion had elevated values for copeptin and hsTnT (Table 3A). Among patients without a history of CAD, the baselines values were elevated in 98.1% for copeptin with cTnT and in 99.1% for copetin with hsTnT.

Among CAD-patients with final diagnoses other than AMI, baseline values of either copeptin or cTnT or both together, were elevated in 46.5% of patients and in 59.6% for copeptin and hsTnT giving specificities of 53.5 and 41.4 (Table 3A). In contrast, among patients without a history of CAD and with final diagnoses other than AMI the percentage was 33.1% for copeptin with cTnT and 39.9% for copeptin with hsTnT (Table 3B). All negative predictive values are also shown in Table 3A and 3B with the highest NPV of 99.5 for the combination of copeptin with cTnT.

The combination of copeptin and cTnT significantly increased the diagnostic accuracy provided by cTnT alone to 0.94 (95% CI, 0.91–0.97; $p<0.001$, Figure 2). The combination of copeptin and the hs-cTnT assay trended to superiority with an AUC of 0.94 (95% CI, 0.91–0.97) compared to hsTnT alone ($p=0.11$). The diagnostic performance of copeptin and its

combination with cTnT and hs-TnT was similar in patients with and without pre-existing CAD (p=ns for all comparisons; Figure 2).

Figure 3 shows the different AUCs among patients with pre-existing CAD and who presented within different time points since the onset of chest pain. The difference among the AUC for the different markers was most pronounced in patients who presented within 3 hours after onset of chest pain. The AUC of hs-cTnT and the combination of Copeptin with either cTnT or hs-cTnT steadily increased since onset of chest pain.

Table 3A	Diagnostic performance of copeptin and different troponin assays and its combination at presentation in patients with pre-existing coronary artery disease			
	Sensitivity (95% CI)	Specificity (95% CI)	Negative Predictive Value (95% CI)	Positive Predictive Value (95% CI)
Copeptin 9pmol/l (cut off)	75.6 (64.6-84.7)	56.3 (51.0-61.6)	91.3 (86.8-94.7)	27.6 (21.7-34.1)
Roche troponin T 4th generation (cTnT)				
cTnT 0.01µg/l (cut off)	78.2 (67.4-86.8)	90.1 (86.6-93.0)	95.0 (92.1-97.0)	63.5 (53.1-73.1)
+ Copeptin 9pmol/l (cut off)	98.7 (93.0-99.8)	41.4 (36.2-46.7)	99.3 (96.3-99.9)	27.0 (22.0-32.6)
Roche high-sensitive troponin T (hs-cTnT)				
hs-TnT 0.014µg/l (cut off)	93.6 (85.7-97.9)	60.2 (55.0-65.4)	97.7 (94.8-99.3)	34.1 (27.8-40.9)
+ Copeptin 9pmol/l (cut off)	98.7 (93.0-99.8)	53.5 (48.2-58.8)	99.5 (97.1-99.9)	31.8 (26.0-38.1)

Table 3B	Diagnostic performance of copeptin and different troponin assays and its combination at presentation in patients without pre-existing coronary artery disease			
	Sensitivity (95% CI)	Specificity (95% CI)	Negative Predictive Value (95% CI)	Positive Predictive Value (95% CI)
Copeptin 9pmol/l (cut off)	67.9 (58.2-76.7)	69.4 (65.7-73.0)	92.8 (90.1-95.0)	27.2 (21.9-33.0)
Roche troponin T 4th generation (cTnT)				
cTnT 0.01µg/l (cut off)	83.0 (74.5-89.6)	94.6 (92.6-96.2)	97.1 (95.4-98.3)	72.1 (63.3-79.9)
+ Copeptin 9pmol/l (cut off)	98.1 (93.3-99.7)	66.9 (63.1-70.5)	99.5 (98.3-99.9)	33.2 (28.0-38.8)
Roche high-sensitive troponin T (hs-cTnT)				
hs-TnT 0.014µg/l (cut off)	94.3 (88.1-97.9)	81.8 (78.5-84.7)	98.9 (97.5-99.6)	46.5 (39.7-52.4)
+ Copeptin 9pmol/l (cut off)	99,1 (94.8-99.8)	60.1 (56.1-63.9)	99.7 (98.5-100.0)	29.4 (24.7-34.4)

Prognostic impact of Copeptin in patients with pre-existing CAD

During a median follow up time of 497 days (376-791days) in survivors, 41 patients died (10%). Baseline copeptin levels were higher among patients who died in the ensuing 360-day period after presentation than in survivors (60pmol/l [14-103] vs. 8pmol/l [4-21]; p<0.001 for 30-day mortality and 41pmol/l [18-93] vs. 8pmol/l [4-18]; p<0.001 for 360-day mortality). Kaplan-Meier analysis demonstrated a low 360-day mortality rate of 3.2% in patients with copeptin ≤9 pmol/l compared to a mortality rate of 26% in patients with a copeptin levels above the cut off (p<0.001). Kaplan-Meier analyses using strata defined by cut off of cTnT (0.01 µg/l) and the 99th percentile of hs-cTnT (0.014 µg/l) showed that copeptin with a cut off of 9 pmol/l clearly separate low and high risk patients (Figure 4).. Irrespective of elevated troponin levels, patients with low copeptin levels had an excellent prognosis with a 360-day mortality rate of 2.8% (hs-cTnT>0.014 µg/l) respectively 3.6% (cTnT>0.01 µg/l). In contrast, patients with both, elevated copeptin and troponin levels had a mortality rate of 23.1% (hs-cTnT>0.014 µg/l) respectively 33.8% (cTnT>0.01 µg/l; p<0.001 for difference).

Univariable Cox regression analysis confirmed copeptin > 9 pmol/l (HR 5.4, 95% CI 2.4-12.2; p<0.001) as a strong predictor of mortality. In multivariable analysis together with all significant baseline variables including either cTnT or hs-cTnT, copeptin was the strongest independent predictor. Of note, in the model with hs-cTnT, elevated levels of hs-cTnT was not longer a predictor of mortality (Table 4). Similar findings were obtained in the Cox regression analyses, assessing the combined endpoint of death and non-fatal AMI in patients with index diagnoses other than AMI (data not shown).

Table 4 Multivariate Cox regression analysis for mortality during follow-up

	Univariate			Multivariate					
				Model with hs-cTnT			Model with cTnT		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Age (years)	1.09	1.06-1.13	<0.001	1.08	1.04-1.12	<0.001	1.06	1.03-1.10	0.001
Gender	1.02	0.50-2.07	0.97						
Diabetes mellitus	1.72	0.92-3.20	0.09						
Previous stroke	3.63	1.73-7.60	0.001						
Heart rate	1.03	1.02-1.04	0.001	1.03	1.02-1.04	<0.001	1.02	1.01-1.04	0.001
Systolic blood pressure	0.98	0.96-0.99	<0.001						
Diastolic blood pressure	0.95	0.93-0.97	<0.001	0.95	0.93-0.97	<0.001	0.95	0.93-0.97	<0.001
Body-mass index	0.90	0.83-0.98	0.02						
Glomerular filtration rate	0.98	0.97-0.99	0.001	1.02	1.00-1.03	0.05	1.02	1.00-1.03	0.03
hs-cTnT >0.014 µg/l	6.33	2.66-15.04	<0.001						
cTnT >0.01 µg/l	5.45	2.93-10.15	<0.001				2.40	1.15-4.99	0.02
Copeptin > 9pmol/l	5.42	2.40-12.22	<0.001	4.63	1.83-11.71	0.001	4.18	1.64-10.61	0.003

Discussion

This prospective, multicenter study involving unselected patients presenting to the ED with acute chest pain examined the value of a dual marker strategy using cTn, a marker of cardiac necrosis, and copeptin, a marker of endogenous stress, for diagnostic and prognostic purposes with a special focus on the important subgroup of patients with pre-existing CAD. We report four major findings:

First, the specificity for the diagnosis of AMI of an elevated hs-cTnT level was significantly lower in CAD-patients compared to non CAD-patients (60% vs 82%) because of a marked proportion of non-AMI patients with elevated hs-cTnT levels (40%). Second, as a stand-alone test, copeptin is inferior to cTnT and hs-cTnT in the diagnosis of AMI but in combination with copeptin, the diagnostic accuracy significantly increased in patients with pre-existing CAD. Third, the NPV to rule-out AMI already at ED presentation was improved up to 99.3% with cTnT, respectively, 99.5% with hs-cTnT in combination with Copeptin. Fourth, Copeptin was a powerful independent predictor for mortality. Patients with low copeptin values had an excellent prognosis even with elevated levels of cTnT or hs-cTnT, while patients with elevations in both copeptin and cTn levels comprised a high risk population with impaired prognosis.

Our findings extend and corroborate previous studies describing the potential use of copeptin to rule-out AMI in patients with acute chest pain and have the potential to improve diagnosis of AMI and triage of patients with pre-existing CAD at the time of presentation to the ED, [13, 24, 25] Copeptin, the c-terminal part of the vasopressin, is secreted stoichiometrically with arginin-vasopressin from the neurohypophysis and plays a crucial role in the regulation of the individual

endogenous stress response. Routine measurement of arginin-vasopressin in clinical practice has been limited so far because of instability (half-life: 5 to 15 min) and substantial attachment to platelets.[26, 27] Copeptin, is much more stable, thus overcoming the limitations and difficulties of assessing the arginin-vasopressin system.[21] Our findings highlight that the benefits of the combination of copeptin with the standard cTnT assay can be extended to the high-risk group of patients with pre-existing CAD. The diagnostic gain when added to hs-cTnT was smaller, as expected due to the substantially higher diagnostic accuracy of hs-cTnT. Regarding rule-out of AMI, the combination of copeptin with the hs-cTnT, increased the sensitivity from 93.6% to 98.7%, providing an excellent NPV of 99.3%. In contrast, regarding rule-in of AMI, the combination of a positive hs-cTnT and a positive copeptin provides only a positive predictive value of 38% with a specificity of 75%, limiting the value for this purpose. The guidelines of the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation recommends serial troponin measurement at presentation and after 3 hours. [28] Taking together our results, measuring troponin and copeptin together at presentation, might obviate a prolonged stay in the emergency department. To our knowledge, there exists only one small study by Karakas et al., who states that copeptin does not add diagnostic information to patients with acute chest pain. A major limitation of the study is the very low number of patients with acute coronary syndrome. Of 366 patients enrolled, only 35 patients had an acute coronary syndrome, of whom 27 were classified as having unstable angina and 8 patients had acute myocardial infarction. Furthermore, they draw the blood samples after a median of 4.5 hours, which is

too late for chest pain patients, because an early rule out of myocardial infarction at admission is what clinically matters. [25]

In our study, copeptin was a strong and independent prognostic marker in patients with pre-existing CAD. Copeptin levels measured at presentation provided powerful information towards the risk of death during the subsequent twelve months. Only patients characterized by both, elevated copeptin and cTn levels were at markedly increased risk, whereas patients with elevated hs-cTnT levels and normal Copeptin values had a similar prognosis as patients with normal hs-cTnT levels. This point merits special attention: The clinical use of high-sensitive cTn assays leads to a marked increase of patients with cTn elevations in settings other than AMI.[10, 11, 29] This has caused some confusion among clinicians and makes the interpretation of elevated troponin values more challenging.[30-32] Having a second marker that identifies patients with a good prognosis despite an elevated high-sensitive cTn value is therefore of great clinical value. Copeptin might help emergency physicians to tailor the therapy in view of the relative risk and allocate resources accordingly. Tailored therapy in high-risk patients may include consultation of specialists, early invasive strategy or admission to the intensive care unit. Whether this risk stratification guided strategy might affect outcome needs to be evaluated prospectively.

Our findings corroborate and extend previous studies investigating the prognostic role of copeptin in different various settings such as AMI, unstable angina and heart failure.[12, 15, 33-35] Khan et al. showed that copeptin was elevated in nearly all patients after AMI and that copeptin levels were closely associated with the risk of death. [12] Interestingly, an animal study documented

the release of vasopressin from cardiomyocytes indicating a vasopressin system in rat hearts.[36] This experiment showed that vasopressin is synthesized and released on stimulation with cardiac pressure overload or stimulation with nitric oxide resulting in systemic effects such as osmoregulation, regulation of cardiac function, perfusion, and cardiac neurohormone secretion. These findings could explain the higher copeptin concentrations in patients with pre-existing CAD even in the absence of AMI because of increased nitric oxide synthesis.[37]

In conclusion, in patients with pre-existing CAD the additional use of copeptin seems to be an attractive option to overcome at least some of the limitations of the respective cTn assay used. When copeptin is used in combination with cTnT, it significantly improved diagnostic and prognostic accuracy, largely by overcoming the sensitivity deficit. The additional use of copeptin results in a very high NPV and seems to allow the rapid rule-out of AMI at presentation. When copeptin is used in combination with hs-cTnT, it significantly increased prognostic accuracy, largely by overcoming the challenging interpretation of mild elevations of hs-cTnT that are common in patients with chronic cardiac disorders.

The following limitations of the current study merit consideration. First, we evaluated two cTn assays with widely different sensitivity to examine the added value of copeptin at both ends of the sensitivity spectrum of cTn assays. Whether these findings can be generalized to other cTn assays requires validation in future studies. Second, in this ongoing prospective study, the subgroup analysis of patients with pre-existing CAD was not pre-defined at the time of the initial protocol written in 2005. It was added while we were still blinded to the results in 2009, regarding to previous investigations, challenging the diagnostic accuracy of

sensitive cTn assays in patients with pre-existing CAD.[29, 38] Third, this observational study cannot quantify exactly the clinical benefit associated with the combination of copeptin and the cTn. To add this important information intervention studies seem warranted.

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Competing interests

We disclose that Dr. Mueller has received speaker honoraria from Abbott, ALERE, BRAHMS, Roche, and Siemens. Dr. Potocki has received speaker honoraria from Abbott, BRAHMS and Roche. Dr. Twerenbold has received speaker honoraria from BRAHMS. All other authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Copeptin and cardiac troponin levels according to the final diagnosis

Figure 2. ROC curves at presentation for the diagnosis of AMI

Figure 3. Diagnostic accuracy of Copeptin and troponin assays and its combination at presentation according to time since onset of chest pain in patients with pre-existing CAD

Figure 4. Mortality within 360 days according to Copeptin and cardiac troponin cut-off levels

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