Temporal Release Pattern of Copeptin and Troponin T in Patients with Suspected Acute Coronary Syndrome and Spontaneous Acute Myocardial Infarction

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BACKGROUND: The release pattern of copeptin during the initial 36 h of spontaneous acute myocardial infarction (AMI) has received relatively little investigation but may provide important information on optimal timing of diagnostic measurements.

METHODS: We investigated the release pattern of copeptin and cardiac troponin T in patients with suspected acute coronary syndrome (ACS). Blood samples were collected in the ambulance, at admission, and after 2, 4, 6, and 12–36 h. Copeptin and high-sensitivity cardiac troponin T (hs-cTnT) were measured in heparin plasma samples.

RESULTS: Of 93 patients studied, 37 (39.8%) had ST-elevation myocardial infarction (STEMI), 20 (21.5%) non-STEMI, 20 (21.5%) unstable angina pectoris (UAP), and 16 (17.2%) non-ACS diagnoses. Peak copeptin concentrations were detected during ambulance transport for NSTEMI patients [median 94.0 pmol/L, interquartile range (IQR) 53.3–302.1 pmol/L] and at admission for patients with STEMI (70.0 pmol/L, 22.0 – 144.8 pmol/L). In patients with AMI, copeptin decreased significantly over time (P < 0.0001). This was true for patients with STEMI (P = 0.005) and non-STEMI (P = 0.021). The diagnostic performance during ambulance transport was similar for hs-cTnT (area under the ROC curve 0.75, 95% CI 0.62–0.88) and copeptin (0.81, 0.69–0.92). In early presenters (n = 52), no patient with AMI was initially (in ambulance or at admission) negative for copeptin, resulting in an area under the ROC curve of 0.963 for ambulance values and a negative predictive value of 100%. In late presenters, the negative predictive value of copeptin was 50% in ambulance and at admission.

CONCLUSIONS: Our analysis is the first to show a consistent early increase in copeptin at first medical contact in the ambulance and a decrease to routine values within 12–36 h in patients presenting early with spontaneous AMI.

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The gold standard biomarkers for the diagnosis of acute myocardial infarction (AMI) are cardiac troponins I and T. Because of the delayed increase of cardiac troponins after onset of ischemia, serial measurements are obligatory to diagnose or exclude AMI and differentiate myocardial injury in patients with inconclusive electrocardiogram (ECG) at admission. For this reason, other biomarkers have been assessed with respect to their added value to diagnose or exclude AMI in combination with troponin early after admission. Measurement of copeptin, the C-terminal part of preprovasopressin, which is secreted stoichiometrically with arginine vasopressin from the neurohypophysis, has been shown to improve the diagnostic accuracy of cardiac troponins at admission. Several cohort studies investigated copeptin at four sampling time points or stratified by symptom onset time (SOT). Liebetrau et al. reported the temporal release pattern of copeptin after septal occlusion in transcatheter ablation of septal hypertrophy (TASH). Gu et al. investigated copeptin in patients with a primary diagnosis of STEMI within the first 24 h and found that copeptin values were already increased at admission, followed by normalization within 10 h.
The temporal release pattern of copeptin over a longer time period in spontaneous AMI, especially in non-STEMI (NSTEMI), has not been studied previously.

The objective of the current study was to investigate the temporal release pattern of copeptin and highsensitivity cardiac troponin T (hs-cTnT) in patients with spontaneous AMI, as well as other acute coronary syndrome (ACS) and non-ACS diagnoses, at 6 time points over 12–36 h. Secondary objectives were to illustrate the temporal release pattern of other cardiac biomarkers and to assess the diagnostic accuracy of copeptin and hs-cTnT at different time points.

Materials and Methods

STUDY DESIGN
In this prospective study, 93 patients with suspected ACS were enrolled from 1997 to 1999. The study was initially set up to evaluate the analytical and diagnostic performance of cTnT measurements (Cardiac Reader, Roche Diagnostics) in the ambulance and the emergency department. All patients were recruited in the ambulance or the emergency department of the Charité Berlin (Campus Virchow Klinikum). Patients who experienced acute chest pain and had a clinically high suspicion of AMI were enrolled. Patients with anemia (hemoglobin <10 g/dL) or known chronic renal failure, age >80 years, or life expectancy <6 months were excluded. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of our institution. All participants gave written informed consent.

BLOOD DRAWS
Blood was drawn at 6 time points: in the doctor-led ambulance (if applicable), at hospital admission, and 2, 4, 6, and 12–36 h after admission. Ambulance blood samples were available in 53 patients. All samples were processed according to standardized operating procedures and frozen at −80 °C.

BIOMARKERS
We measured hs-cTnT and copeptin from frozen lithium heparin samples. In June 2011, we measured copeptin with a sandwich immunoluminometric assay (Copeptin US, BRAHMS ThermoFisher), which has a limit of detection of 0.9 pmol/L and a functional assay sensitivity of <2 pmol/L, assessed as interassay precision of 20% CV. The intraassay precision at 4–15 pmol/L is <8%, and the interassay precision at this concentration is <10%. The measuring range with automatic dilution is 0.9–2000 pmol/L. The copeptin cutoff recommended for rule-out of AMI at admission is 10 pmol/L (14). We performed hs-cTnT measurements in October 2014 on the Cobas Device (Cobas e602 Elecsys® hsTroponin T, Roche Diagnostics). This assay has been reported to have a limit of blank at 3 ng/L and a limit of detection of 5 ng/L (15).

END POINTS
Discharge diagnoses, documented by the treating physician, were readjudicated by a cardiologist according to the Third Universal Definition of Myocardial Infarction (1). The adjudicating physician was blinded to the hs-cTnT and copeptin results. Patients with AMI were in general immediately revascularized by lysis (STEMI) or percutaneous coronary intervention (PCI) (NSTEMI or lysis failure).

STATISTICAL ANALYSIS
We analyzed the data with SPSS, version 22. For descriptive purposes, categorical variables are shown as absolute frequencies and as relative frequencies of valid data, and numeric variables as median and interquartile range (IQR). Differences between 2 groups were tested statistically with χ² tests for categorical variables and, owing to skewed data distributions, with Mann–Whitney U tests for numeric variables. We conducted statistical tests for differences between >2 categories with χ² tests for categorical variables and Kruskal–Wallis tests for numeric variables. Statistical testing for repeated measurements was performed with a general linear model for repeated measures that was adjusted for multiple comparisons by the Bonferroni method. For the graphical illustration of biomarker kinetics over time, we used median (IQR) biomarker values. The area under the ROC curve (AUROC) and 95% CI were calculated to compare the discriminative abilities of biomarkers for the diagnosis of AMI. Biomarkers were combined for ROC analysis by logistic regression.

Results

PATIENT CHARACTERISTICS
Overall, 93 patients were enrolled in this study. Of those, 37 (39.8%) had a final diagnosis of STEMI, 20 NSTEMI (21.5%), 20 unstable angina pectoris (UAP) (21.5%), and 16 (17.2%) other, non-ACS diagnoses. The median age of all patients was 60 years (IQR 53–80 years), and the majority of them were male (81.7%, n = 76) (Table 1).

CLINICAL CHARACTERISTICS
The median SOT was 150 min (IQR 90–300 min) before admission to the ED and was shorter in patients with...
NSTEMI [120 min (75–440 min)] and UAP [120 min (90–555 min)] than in patients with STEMI [150 min (100–263 min)], but these differences were not significant (P = 0.883 and 0.452, respectively). Of all patients, 52 (55.9%) presented within 3 h after symptom onset (SOT ≤180 min), as did 35 (61.4%) patients with AMI. The majority of patients presented with typical angina pectoris symptoms (73.3%, n = 66). This finding was also consistent in diagnosis subgroups (see Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue10). Coronary angiography was performed in 76.6% (n = 69) of all patients [specifically, 34 (91.9%) STEMI patients and 16 (88.9%) NSTEMI patients]. Of all patients with UAP, 60.0% (n = 12) had coronary angiography.

**TEMPORAL RELEASE PATTERN OF COPEPTIN**

The release pattern of copeptin differed between different diagnoses. In patients with NSTEMI, the peak copeptin concentration was detected in the ambulance [median 94.0 pmol/L (IQR 53.3–302.1 pmol/L)], whereas patients with STEMI had a slightly higher copeptin concentrations at admission [70.0 pmol/L (22.0–144.8 pmol/L)] compared with the ambulance values [64.5 pmol/L (22.1–196.0 pmol/L)] (Fig. 1). In patients with AMI, copeptin values differed significantly over time (P < 0.0001) and copeptin decreased rapidly within the first hours. This was true for patients with STEMI (P = 0.005) and NSTEMI (P = 0.021).

The median copeptin concentration in patients with UAP or other diagnoses was lower than in AMI patients at all time points except after 12–36 h. In the first ambulance blood draw, patients with UAP had lower values [9.2 pmol/L (4.3–29.8 pmol/L)] than the suggested cutoff value for early rule-out of AMI (10 pmol/L) (14). There was no significant change in copeptin values over time for patients with UAP (P = 0.227) or other diagnoses (P = 0.387).

**SENSITIVITY ANALYSES OF THE TEMPORAL RELEASE PATTERN OF COPEPTIN**

To address possible confounding of the temporal release pattern of copeptin caused by variability in the SOT or bias from incomplete measurements, 2 types of sensitivity analyses were performed: (a) in patients with AMI stratified by SOT (n = 35, ≤180 min; n = 20, >180 min)
min) (Fig. 2) and (b) in all patients with complete measurements \( (n = 36) \) (Fig. 3). In patients with early presentation after symptom onset, copeptin decreased significantly over time \( (P = 0.003 \) for STEMI; \( P < 0.0001 \) for NSTEMI). Patients with presentation \( > 3 \) h had comparably low copeptin values and no significant concentration changes over time \( (P = 0.671 \) for STEMI; \( P = 0.801 \) for NSTEMI). When patients with complete measurements of copeptin at all sampling time points were investigated, patients with STEMI and other diagnoses had slightly lower median copeptin values, whereas patients with UAP had slightly higher values compared with the overall cohort. Still, patients with AMI showed a decreasing release pattern over time, whereas median copeptin values in patients with non-AMI diagnoses remained constant.

**TEMPORAL RELEASE PATTERN OF hs-cTnT**

hs-cTnT values in the ambulance were increased above the 99th percentile value in ACS patients [STEMI, median 91.5 ng/L (IQR 16.5–404.1 ng/L); NSTEMI, 55.1 ng/L (17.5–146.3 ng/L); UAP, 20.0 ng/L (14.8–61.0 ng/L)] and lower in patients with other diagnoses [8.1 ng/L (5.0–41.9 ng/L)] (see online Supplemental Fig. 1). hs-cTnT increased over time in patients with STEMI [hs-cTnT after 12–36 h, 2422 ng/L (1068–4251 ng/L); \( P = 0.001 \) for admission vs 12–36 h] and NSTEMI [hs-cTnT after 12–36 h, 1242 ng/L (269–2733 ng/L); \( P = 0.035 \)]. In patients with non-AMI diagnoses, median hs-cTnT values remained low and were not found to differ significantly between time points. A majority of patients with ACS were positive for hs-cTnT in ambulance (NSTEMI, 91.7%; STEMI, 86.4%; UAP, 83.3%; other, 42.9%) and at admission (NSTEMI, 100%; STEMI, 100%; UAP, 84.2%; other, 50.0%). In the ambulance, 4 patients with AMI were negative for hs-cTnT [NSTEMI \( (n = 1) \), 12.98 ng/L; STEMI \( (n = 3) \), 7.28, 9.91, and 12.24 ng/L]. These patients became positive in the second blood draw done in hospital. Data regarding the release pattern of other cardiac markers (myoglobin, cTnT, and C-reactive protein) are included in the supplemental material (see online Supplemental Fig. 2, A–C).

**DIAGNOSTIC PERFORMANCE OF hs-cTnT AND COPEPTIN AT DIFFERENT TIME POINTS**

For the diagnosis of AMI, the AUROC for hs-cTnT was 0.748 (95% CI 0.615–0.880) in the ambulance, 0.891 (0.811–0.970) at admission, 0.946 (0.895–0.997) after 2 h, 0.981 (0.951–1.000) after 4 h, 0.984 (0.957–1.000) after 6 h, and 0.975 (0.947–1.000) after 12–36 h (Fig. 4A). The diagnostic performance of copeptin was similar to that of hs-cTnT only at time of ambulance transport [AUROC 0.805 (95% CI 0.686–0.925)] and less at later time points as shown by lower AUROC values [admission, 0.687 (0.566–0.808); 2 h, 0.653 (0.527–0.779);
4 h, 0.666 (0.543–0.789); 6 h, 0.683 (0.561–0.805); 12–36 h, 0.583 (0.451–0.714)]. The combination of both markers was superior to the single markers in the ambulance only [0.883 (0.788–0.978)]. When the diagnostic performance was analyzed in early presenters (n = 52), copeptin alone [0.963 (0.899–1.000)] was superior to hs-cTnT alone [0.579 (0.352–0.806)] and higher than the combination of copeptin and hs-cTnT [0.890 (0.748–1.000)] in the ambulance (Fig. 4B).

**Fig. 2.** Copeptin in patients with AMI stratified by SOT (n = 55).

Thirty-five patients with AMI and SOT ≤180 min and 20 patients with AMI and SOT >180 min were included in this study. No patient with AMI and SOT ≤180 min had an initial copeptin value below the suggested rule-out cutoff for AMI (10 pmol/L).

**EARLY RULE-OUT BY COPEPTIN AND hs-cTnT**

The majority of copeptin values in patients with AMI were above the suggested rule-out cutoff (Fig. 5, A and B). Three patients with STEMI and 4 patients with NSTEMI were negative for copeptin in ambulance and at admission. All of these patients had hs-cTnT values >99th percentile of a healthy reference population in the corresponding blood draws and presented late after SOT (>180 min after symptom onset) (see online Supplemental Table 2). Thus, the negative predictive value (NPV) of the marker combination was 100% in ambulance and at admission in all patients. In early presenters (SOT ≤180 min), no patient with AMI was negative for copeptin in ambulance and at admission, resulting in an NPV of 100% for copeptin alone. In late presenters, the NPV of copeptin decreased to 50% in ambulance and at admission, as 35.0% of late presenters with AMI (7/20) were copeptin negative.

**Discussion**

Our study complements previous studies in several aspects. Copeptin values in AMI patients were increased at time of transport in the ambulance and provided excellent diagnostic accuracy, especially in patients presenting within the first 3 h after symptom onset. Median copeptin values were increased early after symptom onset in patients with AMI and decreased rapidly within 12–36 h, including patients with NSTEMI. In contrast with the excellent diagnostic accuracy of measurements before hospital arrival, the diagnostic accuracy of copeptin at admission was lower than that of hs-cTnT alone when all patients (early and late presenters) were considered.

The temporal release pattern of copeptin in our study was characterized by increased values very early after symptom onset, a peak in ambulance or at admission, and a rapid decrease within the first hours after admission. Copeptin concentrations returned to baseline in all diagnoses after 12–36 h. Several studies have shown that copeptin values are increased in patients with AMI at admission, especially in early presenters (4, 6, 10, 11, 17–23). We were able to illustrate for the first time that copeptin values were al-
ready increased at time of first medical contact in the ambulance. The plausibility of these results is supported by a study in patients with TASH, in whom copeptin was increased as early as 30 min after induction of ischemia (13), with the limitation that TASH patients have no coronary artery disease. Thus, our study provides proof of concept for ACS patients. Reichlin et al. investigated the release pattern of copeptin in a small set of serial blood samples in patients with AMI (n = 25) (4) but could not show a decrease over time in median copeptin values. When copeptin values were stratified by SOT, copeptin concentrations in AMI patients were higher in patients presenting early after symptom onset, decreased over time, and had a negative association with SOT. In a study by Gu et al., the relation of copeptin and SOT as well as the rapid decrease of copeptin within 10 h after admission were shown in 145 patients with STEMI (12). Keller et al. (6) compared copeptin concentrations between patients with AMI (n = 75) and an SOT < 2 h at admission and after 3 and 6 h. The results of their study are in line with our results. In our study, patients with NSTEMI had higher copeptin values in ambulance and at admission compared with patients with STEMI. It is likely that this observation is influenced by the fact that SOT was shorter in NSTEMI patients than in patients with STEMI. In a sensitivity analysis, when patients with AMI were stratified by SOT, higher copeptin values became evident in early presenters with STEMI vs NSTEMI. Copeptin values in our AMI patients were higher in ambulance and at admission compared with Keller et al. (6) and Reichlin et al. (4). This could have resulted from our samples being drawn earlier after symptom onset. Another possible explanation might be that our study enrolled high-risk patients with typical symptoms who were mainly transported by ambulance. These patients might have more severe symptoms due to larger infarct sizes that are hemodynamically more relevant and thus trigger a stronger copeptin release from the pituitary gland (24, 25). The exact pathophysiologic stimulus of copeptin release in AMI is currently unknown. Hemodynamic changes or endogenous stress occurring during AMI might be causative (3), but secretion of arginine vasopressin directly from myocytes of pressure-overloaded hearts has also been reported (26), further supporting the hypothesis of a correlation between copeptin and infarct size (25).

An early rule-out strategy by combined measurement of copeptin and cTnT at admission is supported by the findings of several cohort studies (4, 6, 10), a randomized control trial (14), and 2 metaanalyses (27, 28). Our study extends these findings on an earlier blood draw during ambulance transport with an excellent NPV of 100% for the combination of hs-cTnT and copeptin, as well as for copeptin alone in early presenters among a
population at high clinical suspicion for ACS. Regarding the overall diagnostic accuracy as assessed by AUROC, there was no additional value of copeptin at admission and later time points. Because of the small sample size of our study, further studies in larger cohorts should be undertaken to address the diagnostic value of combined measurement of copeptin and cTnT in a prehospital setting.

Regarding limitations, this was a single-center study initially set up to evaluate the analytical and diagnostic performance of cTnT measurements (Cardiac Reader, Roche Diagnostics) in the ambulance and the emergency room.
Blood samples were drawn approximately 15 years ago, and patients were treated according to standard management at that time, although in our center primary PCI had already been established, as well as cardiac catheterization after fibrinolysis within 24 h. Final diagnoses were readjudicated to match the current definition of AMI.

The current analysis focuses on copeptin and hscTnT kinetics in patients with a high suspicion of ACS and early presentation after onset of symptoms. The number of patients is small with respect to the diagnostic evaluation but is the largest cohort and the longest time frame reported currently for patients with NSTEMI with respect to kinetic analyses. Our results have high external validity.

Fig. 5. Release pattern of copeptin in STEMI (A) and NSTEMI (B). Median copeptin values. Error bars represent IQRs at the respective time points. The suggested rule-out cutoff for AMI is shown as a black line at 10 pmol/L.
and internal validity, as they are in line with previously reported studies from other centers, and sensitivity analyses did not reveal major discrepancies.

The exact time point of reperfusion in patients with AMI is unknown in this study, but according to standard practice at the time the study was performed, was within 2 h after first medical contact in patients with STEMI and usually within 24 h in patients with NSTEMI. Thus, the release pattern of all biomarkers applies for AMI patients who underwent ly sis or PCI during sampling, which is the current standard of therapy. It is possible that median copeptin values could be higher and remain at higher concentrations in patients without reperfusion, but this would have no clinical impact.

The concentration of copeptin was measured in samples that were obtained several years ago. The samples were stored at −80 °C for the entire period from collection until measurement of copeptin; thus no freeze-thaw cycles affected sample quality. The stability of copeptin in different materials was investigated by Morgenthaler et al., who found that copeptin was stable for ≥7 days at room temperature and ≥14 days at 4 °C. (29). The copeptin concentration decreased in serum and heparin plasma after 14 days at room temperature. If there is any bias in the absolute concentration of copeptin due to sample stability issues, the values would be expected to be lower compared with the original concentration at the time of sample acquisition.

In conclusion, our analysis is the first to show a consistent early increase in copeptin at first medical contact in the ambulance and a decrease to routine values within 12–36 h in patients presenting early with spontaneous AMI (STEMI as well as NSTEMI).

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