Optimizing Early Rule-Out Strategies for Acute Myocardial Infarction: Utility of 1-Hour Copeptin

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BACKGROUND: Combined testing of high-sensitivity cardiac troponin T (hs-cTnT) and copeptin at presentation provides a very high—although still imperfect—negative predictive value (NPV) for the early rule-out of acute myocardial infarction (AMI). We hypothesized that a second copeptin measurement at 1 h might further increase the NPV.

METHODS: In a prospective diagnostic multicenter study, we measured hs-cTnT and copeptin concentrations at presentation and at 1 h in 1439 unselected patients presenting to the emergency department with suspected AMI. The final diagnosis was adjudicated by 2 independent cardiologists blinded to copeptin concentrations. We investigated the incremental value of 1-h copeptin in the rule-out setting (0-h hs-cTnT negative and 0-h copeptin negative) and the intermediate-risk setting (0-h hs-cTnT negative and 0-h copeptin positive).

RESULTS: The adjudicated diagnosis was AMI in 267 patients (18.6%). For measurements obtained at presentation, the NPV in the rule-out setting was 98.6% (95% CI 97.4%–99.3%). Whereas 1-h copeptin did not increase the NPV significantly, 1-h hs-cTnT did, to 99.6% (95% CI 98.7%–99.9%, P = 0.008). Similarly, in the intermediate-risk setting (NPV 92.8%, 95% CI 88.7%–95.8%), 1-h copeptin did not significantly increase the NPV (P = 0.751), but 1-h hs-cTnT did, to 98.6 (95% CI 96%–99.7%, P < 0.001).

CONCLUSIONS: One-hour copeptin increased neither the safety of the rule-out process nor the NPV in the intermediate-risk setting. In contrast, the incremental value of 1-h hs-cTnT was substantial in both settings.

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Symptoms suggestive of acute myocardial infarction (AMI)7 are among the leading causes for presentation at the emergency department (ED) (1, 2). Rapid identification of AMI as a life-threatening disorder is important for the early initiation of appropriate, evidence-based therapy. The diagnosis of AMI is based on clinical assessment, 12-lead electrocardiogram (ECG), and biomarkers of myocardial necrosis (cardiac troponin) (3–5). Since the introduction of high-sensitivity cardiac troponin assays, AMI can be diagnosed earlier and more frequently (6, 7). Nonetheless, safe rule-out of AMI is still time-consuming, especially in patients who present early after symptom onset (5).

Because the vast majority of suspected AMI patients eventually do not suffer from AMI, fast and safe rule-out is of paramount importance to prevent delayed consideration of alternative diagnoses and expensive overcrowding of the ED (8–10).

A promising strategy to accelerate rule-out of AMI is the combination of high-sensitivity cardiac troponin and copeptin measurements at presentation. Several studies have shown a very high (but still imperfect) negative predictive value (NPV) of the combination of high-sensitivity cardiac troponin within reference intervals and low copeptin concentrations at presentation for rapid and safe rule-out of AMI (11–15). Circulating copeptin concentrations peak early after chest pain onset, making them a useful tool for AMI rule-out, especially in early
Methods

STUDY DESIGN AND POPULATION

Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) is an ongoing prospective international multicenter study designed to advance the early diagnosis of AMI. From April 2006 to November 2011, 2305 consecutive women and men >18 years old, who presented to the ED with symptoms suggestive of AMI with an onset or peak within the last 12 h, were recruited in 9 sites in 3 countries (Switzerland, Spain, and Italy) after written informed consent was obtained. Enrollment was independent of renal function; however, patients with terminal kidney failure requiring regular dialysis were excluded. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees.

For this analysis, patients were excluded if (a) the final diagnosis remained unclear after adjudication and ≥1 high-sensitivity cardiac troponin T (hs-cTnT) concentration was increased, thus possibly indicating the presence of an AMI (n = 61), or (b) ST-segment elevation myocardial infarction was the adjudicated final diagnosis (n = 85), as biomarkers are considered of limited clinical value in these patients. Detailed characteristics of patients in whom the final diagnosis remained unclear after adjudication and who had ≥1 increased hs-cTnT concentration are reported in Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue11.

Among the remaining 2159 patients, measurements of hs-cTnT and copeptin at both presentation and 1 h were available in 1439 patients.

In 329 patients, the blood sample at presentation or 1 h was taken, but hs-cTnT or copeptin measurement was not performed (Fig. 1). Baseline characteristics of the 391 patients in whom the 1-h blood sample was missing due to logistical issues in the ED were compared with those of the study population. Reasons for missing 1-h blood samples are listed in the online Supplement. We found no significant differences in baseline characteristics between patients with and without a sample drawn at 1 h except for a higher incidence of diabetes mellitus and a higher percentage of early presenters among patients who had a 1-h sample drawn (see online Supplemental Table 4). Samples for study laboratory measurements were drawn independently from those for other on-site laboratory measurements, and ED physicians were blinded to patients’ copeptin concentrations.

ROUTINE CLINICAL ASSESSMENT

All patients underwent a clinical assessment that included medical history, physical examination, 12-lead ECG, continuous ECG monitoring, pulse oximetry, standard blood tests, and chest radiography. Concentrations of cardiac troponin were measured at presentation and serially thereafter as long as clinically indicated. Troponin was tested per routine practice at the individual sites. The following sensitive cardiac troponin assays were used at the different study sites: Roche cTnT fourth-generation assay, Abbott AxSymcTnI ADV, and Beckmann Coulter AccuTnI. At 1 study site, the standard assay was changed to the Roche hs-cTnT assay in January 2010. Local cardiac troponin assays with their clinical decision values are shown in the online Supplement.

Tables for the latest hs-cTnT/cTnT value available after symptom onset and after presentation are displayed in online Supplemental Tables 2 and 3. Timing of the assessments and treatment of patients were left to the discretion of the attending physician.

ADJUDICATED FINAL DIAGNOSIS

Final diagnosis was adjudicated centrally in a core laboratory (University Hospital Basel) and also included serial concentrations of hs-cTnT (Roche) to take advantage of the higher sensitivity and higher overall diagnostic accuracy offered by high-sensitivity cardiac troponin assays (23); this allowed the additional detection of small
AMI that were missed by the adjudication on the basis of conventional cardiac troponin assays. Two independent cardiologists reviewed all available medical records—patient history, physical examination, results of laboratory testing (including hs-cTnT concentrations), radiologic testing, ECG, echocardiography, cardiac exercise stress test, and lesion severity and morphology in coronary angiography—pertaining to the patient from the time of ED presentation to 90-day follow-up. In situations of disagreement about the diagnosis, cases were reviewed and adjudicated in conjunction with a third cardiologist.

We defined AMI and interpreted cardiac troponin concentrations as recommended in current guidelines (5, 24). In brief, AMI was diagnosed when there was evidence of myocardial necrosis in association with a clinical setting consistent with myocardial ischemia. Myocardial necrosis was diagnosed by a cardiac troponin value >99th percentile together with a significant increase and/or decrease (3–5, 24, 25). We used absolute changes in hs-cTnT to determine significant changes on the basis of the diagnostic superiority of absolute over relative changes (26, 27).

On the basis of studies of the biological variation of cardiac troponin (28, 29) as well as data from previous chest pain cohort studies (7, 30, 31), we defined a significant absolute change as a rise or fall of ≥10 ng/L within 6 h or 6 ng/L within 3 h.

Type 1 and type 2 AMI were adjudicated according to the universal definition of myocardial infarction (24). All other patients were classified as “no AMI” for this analysis, including the categories of unstable angina, cardiac but noncoronary disease (e.g., tachyarrhythmia, perimyocarditis), and noncardiac chest pain.

Unstable angina was diagnosed in patients with healthy cardiac troponin concentrations and typical angina at rest as recommended in current guidelines (4), if additional clinical information made the presence of unstable angina more likely and no other cause of acute chest discomfort was considered to have a higher likelihood. Additional arguments in favor of unstable angina were ECG changes indicative of myocardial ischemia, positive cardiac exercise testing, cardiac catheterization with coronary arteries found to have stenosis of ≥70%, follow-up information revealing AMI or cardiovascular

Fig. 1. Patient flow diagram.
death within 60 days, and complete resolution of symp-
toms after revascularization.

MEASUREMENT OF COPEPTIN AND hs-cTnT
At the time of patients’ presentation to the ED, blood
samples for determination of copeptin and hs-cTnT were
collected into tubes containing potassium EDTA and
serum, respectively. Additional samples were collected at
1, 2, 3, and 6 h after presentation. Serial sampling was
discontinued when a patient was released or transferred
to the catheter laboratory for acute treatment. After cen-
trifugation, samples were frozen at −80 °C until they
were assayed in a blinded fashion in 2 batches in a dedi-
cated core laboratory.

We measured copeptin with a novel commercial
sandwich immunoluminometric assay (LUMITest CT-
proAVP, Brahms) (19). Since initial publication, the as-
say was modified: the capture antibody was replaced by a
murine monoclonal antibody directed to amino acids
137–144 (GPAGAL) of proAVP. This modification im-
proved the sensitivity of the assay (13). The lower limit of
detection (LoD) was 0.4 pmol/L, and the functional assay
sensitivity threshold (<20% interassay CV) was 1 pmol/L.
In healthy individuals, a copeptin concentration of 9.8
pmol/L corresponds to the 95th percentile, and 13 pmol/L
to the 97.5th percentile (11). The selected prognostic cutoff
point for copeptin of 10 pmol/L, corresponding to the 95th
percentile, was based on previous results (13, 14).

The Roche hs-cTnT assay was measured on the
Elecsys 2010 (Roche Diagnostics). The limit of blank
(LoB) and LoD were determined to be 3 and 5 ng/L, re-
spectively. The 99th percentile of a healthy reference pop-
ulation was reported at 14 ng/L, with imprecision corre-
spanding to 10% CV at 13 ng/L (32). As recommended in
current guidelines (27), we chose the 99th percentile (14
ng/L) as the cutoff concentration for hs-cTnT.

DEFINITION OF RULE-OUT AND INTERMEDIATE-RISK SETTINGS
We defined the rule-out setting as copeptin and hs-cTnT
measurements below the cutoff concentrations at presenta-
tion (<10 pmol/L and <14 ng/L, respectively). We
defined the intermediate-risk setting as hs-cTnT <14
ng/L but copeptin concentrations ≥10 pmol/L at pre-
sentation. Patients with hs-cTnT concentrations ≥14
ng/L at presentation were defined as high-risk patients in
whom a second copeptin measurement would not add
any further information, as a second troponin measure-
ment is mandatory in these patients and they would be
treated as having acute coronary syndrome in the ade-
quate clinical context.

FOLLOW-UP
After hospital discharge, patients were followed for 3, 12,
and 24 months by telephone or mail. Any clinical (car-
diovascular) events since presentation to the ED were
collected by establishing contact with the patient and
family physician. Information regarding death was also
obtained from the national mortality registry. The pri-
mary prognostic endpoint was all-cause mortality.

STATISTICAL ANALYSIS
Our primary hypothesis was that a second copeptin mea-
surement 1 h after presentation would improve risk strat-
ification in acute chest pain patients with initially nega-
tive hs-cTnT and negative copeptin concentrations as
well as in patients with initially negative hs-cTnT and
positive copeptin concentrations, thus leading to a safer
and faster rule-out of AMI. The primary endpoint of this
analysis was the rate of AMI diagnosis.

Continuous variables (all non–normally distrib-
uted) are presented as medians with interquartile ranges
(IQRs), and categorical variables are expressed as counts
and percentages. We used Mann–Whitney U-test and
Kruskal–Wallis 1-way analysis of variance to compare
continuous data between study groups, and Pearson χ²
test to compare categorical variables.

Sensitivity and NPV were calculated for both the
second copeptin and the second hs-cTnT measurement
for patients in the rule-out setting (hs-cTnT and copep-
tin below the cutoff concentration at presentation) as
well as for patients in the intermediate-risk setting (hs-
cTnT negative and copeptin positive at presentation).
Subgroup analyses were performed in patients who pre-
sented very early after symptom onset (<2 h) and in
patients who presented with undetectable concentrations
of hs-cTnT (<5 ng/L).

Glomerular filtration rate was estimated (eGFR)
with the abbreviated Modification of Diet in Renal Dis-
ease (MDRD) formula (33).

All hypothesis testing was 2-tailed, and a P value of
<0.05 was considered statistically significant. All statis-
tical analyses were performed with IBM SPSS for Win-
dows 21.0 (SPSS), MedCalc 9.6.4.0 (MedCalc), and R,
version 3.1.1.

Results

BASELINE CHARACTERISTICS
Baseline characteristics of patients are shown in Table 1.
Among 1439 unselected patients, 267 (18.6%) had an
adjudicated final diagnosis of AMI; 17% of these patients
had a type 2 AMI. Patients with AMI were significantly
older, had more cardiovascular risk factors, more often
had a history of coronary artery disease or other cardio-
vascular disease, and more often had ECG abnormalities
than patients with chest discomfort of other origin. hs-
cTnT and copeptin concentrations at presentation and
1 h were significantly higher, and eGFR was lower, than
in patients with other final diagnoses.
Overall, 941 patients (65.4%) had initial hs-cTnT concentrations $\leq$ 14 ng/L. Twenty-seven of these (2.9%) had an adjudicated final diagnosis of AMI (NPV 97.1%, 95% CI 95.9%–98.1%) (Figs. 1 and 2). Nine hundred twenty patients presented with copeptin concentrations $\leq$ 10 pmol/L. Of these, 705 had initially healthy hs-cTnT concentrations. Ten (1.4%) of 705 patients had an adjudicated final diagnosis of AMI despite being negative for hs-cTnT and copeptin.
at presentation (NPV 98.6%, 95% CI 97.4%–99.3%) (Figs. 1 and 2).

A second copeptin measurement drawn after 1 h did not give additional information for the rule-out of AMI compared with baseline. Six hundred eighty-eight patients had negative copeptin concentrations at 1 h, with an AMI prevalence of 1.4% (NPV 98.6%, 95% CI 97.3%–99.3%, not significant) (Figs. 1 and 3); none of the 10 patients was identified as having an AMI. In contrast, a second hs-cTnT measurement 1 h after presentation identified 7 of 10 AMI patients and improved the NPV to 99.6% (95% CI 98.7%–99.9%, \( P = 0.008 \) vs baseline) (Figs. 1 and 3). Three of 1439 unselected patients (0.2%), all presenting within 3 h after chest pain onset, could not be identified with this 1-h algorithm.

PREDICTIVE VALUES FOR THE INTERMEDIATE-RISK SETTING

Increased concentrations of copeptin (\( \geq 10 \) pmol/L) at presentation were measured in 519 patients, and 236 (45.5%) of them presented with initially negative hs-cTnT concentrations (<14 ng/L). Seventeen (7.2%) of these 236 intermediate-risk patients had a final diagnosis of AMI (NPV 92.8%, 95% CI 88.7%–95.8%) (Figs. 1 and 4). A second copeptin measurement did not significantly improve the NPV (93.7%, 95% CI 84.5%–98.2%, \( P = 0.751 \)). Compared with baseline, a second hs-cTnT measurement improved the NPV to 98.6%.
(95% CI 96%–99.7%; P < 0.001) (Figs. 1 and 4). Two hundred fourteen patients had repeatedly negative hs-cTnT concentrations 1 h after presentation, 74% of whom had persistently increased copeptin concentrations. Three of these 158 patients (1.9%) had a final adjudicated diagnosis of AMI (Fig. 4). By combining hs-cTnT and copeptin measurement 1 h after presentation, all AMIs were detected (NPV 100%, 95% CI 93.6%–100%) (Figs. 1 and 4).

VERY EARLY PRESENTERS
Overall, 105 patients presented within 2 h after symptom onset, 17 of whom (16.2%) had an adjudicated final diagnosis of AMI. Eighty-nine patients (84.8%) had initial hs-cTnT concentrations <14 ng/L, and 10 of these patients had a final diagnosis of AMI (NPV 88.8%, 95% CI 80.3%–94.5%).

In the rule-out setting (hs-cTnT <14 ng/L and copeptin <10 pmol/L), NPV was 95.9% (95% CI 86%–99.5%). A negative copeptin concentration at 1 h did not improve the NPV to rule out AMI (no additional patient with AMI was detected). A second hs-cTnT improved the NPV to 97.9% (95% CI 88.7%–100%), but no significant difference could be observed compared with combined measurement at baseline (P = 0.324).

In the intermediate-risk setting (hs-cTnT <14 ng/L and copeptin ≥10 pmol/L), the NPV was 80% (95% CI 64.4%–91%). In patients with copeptin concentrations <10 pmol/L at 1 h, the NPV to rule out AMI did not significantly improve (84.6%, 95% CI 54.6%–98.1%, P = 0.595). In contrast, persistently negative hs-cTnT concentrations at 1 h improved the NPV to 96.9% (95% CI 83.8%–99.9%, P = 0.004).

PREDICTIVE VALUES FOR PATIENTS WITH UNDETECTABLE VALUES OF hs-cTnT AT PRESENTATION
Three hundred sixty-five patients (25.4%) presented with undetectable concentrations of hs-cTnT (<5 ng/L). For these patients, the NPV to rule out AMI was 99.7% (95% CI 98.5%–100%). Additional measurement of copeptin at presentation (<10 pmol/L) did not significantly improve the diagnostic accuracy to rule out AMI (NPV 100%, 95% CI 98.6%–100%, P = 0.317).

Discussion
In this prospective diagnostic multicenter study, we aimed to investigate the incremental value of a second copeptin measurement at 1 h to accelerate early rule-out of AMI while ensuring patient safety.

We report 3 major findings. First, in patients with negative hs-cTnT and copeptin concentrations at presentation, a second copeptin measurement did not significantly increase the NPV for AMI; in contrast, a second hs-cTnT did. This finding extends and corroborates previous studies that focused on the combination of hs-cTnT and copeptin at presentation (14, 15). It is a matter of ongoing discussion whether the NPV for AMI achieved with the dual-marker strategy at presentation (pooled NPV 98%) (14) is sufficiently high to justify clinical use, and this strategy has not been implemented widely (11–15). Whereas data from a recent randomized controlled trial (34) provided additional support for the safety of this concept, its high but still suboptimal NPV requires detailed comparison with other emerging rule-out strategies (27, 30, 35, 36), including the implications in terms of cost-effectiveness. The dual-marker approach with measurements of copeptin at baseline and 1 h would imply significant additional costs (e.g., approximately US$75 000 for a first and second copeptin measurement in the analyzed patient cohort). The need for this comparison is further highlighted by the observation that in contrast to copeptin, a second measurement of hs-cTnT improves the NPV for AMI in our study. These findings extend and corroborate previous observations regarding the reciprocal kinetics of copeptin and cardiac troponin (11–13). When most AMI patients present to the emergency department, concentrations of copeptin seem already to be declining, whereas concentrations of cardiac troponin are rising. Accordingly, if copeptin fails to detect AMI at presentation, later measurements would also fail. Future studies need to investigate in more detail the AMI patient phenotype that does not result in a release of copeptin, to identify other complementary diagnostic tools for these individuals.

Second, similar findings were observed in the intermediate-risk group. In patients with negative hs-cTnT but increased copeptin concentrations at presentation, a second copeptin measurement did not significantly increase the NPV to rule out AMI, whereas a second hs-cTnT measurement was of incremental value for the early rule-out of AMI. The combined measurement of copeptin and hs-cTnT at 1 h improved the NPV to rule-out AMI to 100% in the intermediate-risk group in our cohort. The ability to detect all AMIs was at the expense of a very low specificity and mainly due to the second hs-cTnT measurement, as a second copeptin measurement alone did not help to optimize the rule-out of AMI. Likewise, in patients who presented very early after symptom onset, a second copeptin measurement did not improve the diagnostic accuracy to rule out AMI.

Again, these findings extend and corroborate previous observations regarding the reciprocal kinetics of copeptin and cardiac troponin (11–13) and do not support the routine use of copeptin for diagnostic purposes in the intermediate-risk group.

Third, in patients with hs-cTnT concentrations <5 ng/L at presentation, the diagnostic accuracy to rule out AMI was already very high, as previously observed in very early presenters.
a large observational study by Bandstein et al. (35). No additional benefit of a copeptin measurement could be detected.

Some limitations merit consideration when interpreting the findings of this study. First, this was a secondary analysis from a large ongoing multicenter study designed to improve the early diagnosis of AMI. As such, no specific power analysis was performed to justify the sample size for this hypothesis. Accordingly, despite its high number of patients, our sample size might have been too small to detect a small diagnostic benefit of a 1-h copeptin measurement. Second, not all patients with acute chest pain had a second set of laboratory measurements at 1 h. Because baseline characteristics did not differ significantly between patients with and without measurements at 1 h, and because the most common reasons for missing blood samples were logistic issues in the ED that precluded blood draw around the 1-h window, it is unlikely that the absence of these patients significantly influenced our results. Third, the 2 independent cardiologists were blinded to the patients’ copeptin concentrations. However, to apply the universal definition of MI, all serial hs-cTnT measurements complemented all other clinical information pertaining to the individual patient and were available during adjudication of the final diagnosis (5, 24). Accordingly, by design, our study introduces a small but inherent bias in favor of 1-h hs-cTnT concentrations. Fourth, even by experienced cardiologists using current guideline recommendations (5, 24, 27), AMI could not be surely excluded in a small number of patients (3%-5%), although further clinical course did not reveal additional information indicating the diagnosis of AMI. Therefore, this subgroup had to be excluded from analysis. As can be seen in online Supplemental Table 1, these patients often present with complex clinical findings. Fifth, we cannot generalize these findings to patients with terminal kidney failure requiring dialysis, since they were excluded from this study.

In conclusion, a second copeptin measurement at 1 h increased neither the safety of the rule-out process in low-risk patients nor the NPV to rule out AMI in intermediate-risk patients. In contrast, the 1-h hs-cTnT concentration provided incremental value in both settings.

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