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ABSTRACT

Objective

To determine if copeptin-us can rule out diagnosis of acute myocardial infarction without prolonged monitoring and serial blood sampling in patients with suspected non–ST-segment elevation myocardial infarction (NSTEMI) and high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to the emergency department (ED).

Design

Prospective, non-randomized, individual blinded diagnostic accuracy study.

Setting

Two ED of a rural region of France.

Participants

Patient with a chest pain suspected of NSTEMI with onset within the last 12 h were considered for enrolment.

Interventions

Serial clinical, electrographical and biochemical investigations were performed at admission and after 2, 4, 6 and 12 h. Hs-cTnI was mesured using an assay with Dimension VISTA, Siemens. Copeptin was measured by the B.R.A.H.M.S copeptin-us assay on the KRYPTOR Compact Plus system. The follow-up was 90 days.

Primary and secondary outcome measures

Copeptin, troponin, myoglobin and creatin kinase values. Clinicals and paraclinicals events. The final diagnosis was adjudicated blinded to copeptin result.

Results

During 12 months, 102 patients were analysed. Final diagnosis was NSTEMI for 7.8% (n=8), unstable angina pectoris for 3.9% (n=4), cardiac but non coronary artery disease for 8.8% (n=9), non-cardiac chest pain for 52% (n=53) and unknown for 27.5% (n=28). There was no statistical difference for copeptin value between acute myocardial infarction (AMI) and non–AMI patients, respectively 5.5 pmol/L IQR[3.1-7.9] and 6.5 pmol/L IQR[3.9-12.1], p=0.4913. Only one AMI patient have a copeptin value at admission above the cut off of 95th percentile at admission.

Conclusions

In this study, copeptin show no added value for the diagnostic at admission to ED for suspected acute coronary syndrome patients without ST-segment elevation and with hs-cTnI below the 99th percentile.

Trial registration

Clinicaltrials.gov identifier: NCT01334645.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- We were not able to include the expected number of patients.
- Our prospective multicentric study is the only one that includes only patients with suspected NSTEMI and high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to ED, to limit bias spectrum.
- Despite the fact that we have not included the expected number of patients, if the required number of patients have been included to achieve 80% power (40 AMI patients), assuming that copeptin was positive for all other AMI patients, 7 of 40 AMI patients were ruled out. This risk seems too high, knowing that there is a more reliable method : troponin serial testing.

MANUSCRIPT

INTRODUCTION

Detection of a rise and/or fall of cardiac troponin with clinical symptoms of ischemia or abnormal electrocardiography (ECG) or imaging findings remains the gold standard for the identification of myocardial infarction.[1] At Emergency Department, patients with non–ST-segment elevation myocardial infarction (NSTEMI) working diagnosis requires serial measurement of troponin.[2] However, most of this patients do not have acute coronary syndrome (ACS). Identify patients suffering from non-life-threatening diseases with only one blood sample is a challenge. Many biomarkers were evaluated, alone or in combination with troponin. [3,4] Since the first publication for this indication in 2009, several studies have investigated copeptin, a surrogate marker of vasopressin. [5-30] Some of these studies suggest that the association of troponin and copeptin at the first measurement has a powerfull negative predictive value (NPV) to rule out patients with no NSTEMI.

Interpretation of the copeptin diagnostic accuracy with these studies is not evident. First, because analysis comparison are difficult due to the development of high-sensitivity cardiac troponin T and I assays and the availability of three commercial assays for copeptin (LUMItest®, Copeptin Kryptor®, Copeptin-us Kryptor®). Furthermore, many protocols included ST-segment elevation myocardial infarction (STEMI) patients and patients with a high-sensitive cardiac troponin above 99th percentile at admission. For these patients, copeptin does not add diagnostic information, urgent revascularisation or serial blood samples, respectively, remains necessary.

The aim of this study was to determine if copeptin-us can rule out diagnosis of acute myocardial infarction without prolonged monitoring and serial blood sampling in patients with suspected NSTEMI and high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to ED.

METHODS

Study design and setting

This diagnostic test evaluation is a prospective non-randomized individual blinded multicentric cohort study. The Clermont-Ferrand University Hospital designed and coordinated the study. The duration of study was one year, between march 2011 and march 2012 at the ED of two hospitals of Auvergne, a rural region of France (1.3 million people). First one, Gabriel Montpied in Clermont-Ferrand, is a teaching hospital and provincial referral center with 48000 ED admissions per year. The second hospital, Henri Mondor in Aurillac, is a general hospital with 25000 ED admissions per year. Both units are organised with a 24-hour catheterization laboratory. The study complied with the Declaration of Helsinki and was approved by the ethical committee Comité de Protection des personnes Sud-Est VI (AU 871). Before study launch, methods were registered with ClinicalTrials.gov (NCT01334645).

Population

Consecutive patients with a chest pain suspected of NSTEMI at emergency department were considered for enrolment in the study. The inclusion criteria was the following : patients older than 18 years with chest pain suggestive of ACS with onset within the last 12 hours. All patients provided written informed consent before enrolment. Patients with ST-segment elevation, legal incapacity, sepsis, shock, lung neoplasms, terminal kidney failure requiring dialysis, life expectancy of less than 6 months and refuse to consent were excluded. After the result of the first blood sample, patients with hyponatremia < 135 mmol/L or hs-cTnI > 0,045 µg/L were released of the study.

Study protocol

Upon admission, all patient underwent an initial clinical assessment, including medical history, temperature, respiratory rate, cardiac frequency, blood pressure, pulse oxymetry, 18-lead ECG, chest X-ray and screening blood test including : C-reactive protein, natremia, creatinine, hs-cTnI and creatin kinase (CK). Blood sampling were collected for hs-cTnI and CK analysis and 18-lead ECG were performed after 2, 4, 6 and 12 h. At each time point, blood sample was centrifuged and plasma was frozen at -80 °C for copeptin and myoglobin testing at the end of the study recruitment, blinded to final diagnosis. Further investigations and treatment of patients were not modified by the study. At 90 days, clinical and paraclinical events were collected.

Concentration of copeptin was measured by the B.R.A.H.M.S copeptin-us immunoluminometric assay on the KRYPTOR Compact Plus system (Thermo Fisher Scientific). The detection limit as described by the manufacturer was assessed as being 0.9 pmol/L and the lowest concentration measurable with a coefficient of variation (CV) < 10% has been reported < 4 pmol/L. Direct measuring range was 0.9 to 500 pmol/L. The 95th percentile among healthy subjects is < 12.0 pmol/L and was specified for rapid exclusion of acute myocardial infarction (AMI).

Hs-cTnI was measured using a chemiluminescence test (Dimension VISTA®, Siemens Healthcare Diagnostics). The limit of blank of hs-cTnI was 0.015 µg/L, the 99th percentile concentration was 0.045 µg/L and the lowest concentration measurable with a CV < 10% was 0.040 µg/L according to the manufacturer. The 99th percentile (0.045 µg/L) was used as diagnostic cut-off to fulfil AMI criteria.

Myoglobin was measured by Dimension VISTA® (Siemens Healthcare Diagnostics). The measuring range extended from 0.5 to 1000 ng/mL. The 95th percentile concentration was 116 ng/mL for men and 71 ng/mL for women.

Natremia, C-reactive protein, creatinin and CK, were measured using standardized methods.

Outcomes

The final diagnosis was adjudicated, blinded to copeptin results, by an expert committee of three cardiologists, four emergency physicians and two biochemists (whose one MD-PhD of each specialty), with all available medical records from the time of ED presentation to 90-day follow-up. The diagnosis was determined according to the current guidelines and universal definition of myocardial infarction. [1,2]

Each subjects was classified as one of the following categories : Non-ST Elevation Myocardial Infarction (NSTEMI), Unstable angina pectoris (UA), Cardiac but non coronary artery disease (CNCAD), non-cardiac chest pain (NCCP) and unknown cause of chest pain. NCCP were performed if a cardiac aetiology was exclude. Unknown cause of chest pain diagnosis was defined when no sufficient further diagnostic procedures were performed.

Copeptin and myoglobin measurement were performed at the end of the study recruitment, blinded to the final diagnosis.

Statistical analysis

To show a different value of copeptin between AMI subjects and non-AMI subjects, with an expected difference of 15 pmol/L, a standard deviation of 20.7 pmol/L, a significance level of 5% and a power of 95%, the number of AMI subjects needed was 40 patients.

Continuous variables were displayed either as mean ± SD or median and interquartile range (IQR). Categorical variables were described by using frequencies and percentages.

The analysis of quantitative variables was performed using the two-tailed Student's t-test after checking the assumption of equal variances (Levene test) and one way analysis of variance for variables following a normal distribution. Otherwise, the Wilcoxon rank sum tests for continuous variables and Kruskal-Wallis tests were used. Categorical variables were analysed using Chi-square analysis or the Fisher exact test (if needed). For all tests, a significance level of $p<0.05$ was used.

Statistical analysis was performed using SAS (v 9.3, SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient characteristics

During 12 months, 147 patients were assessed for eligibility in both ED. Nine presented one or more exclusion criteria, six did not give their informed consent for participation, 26 were released after the results of the first blood sample because they had hyponatremia < 135 mmol/L (n=3) or hs-cTnI > 0,045 µg/L (n=23). For three patients, blood samples at presentation were not frozen for copeptin and myoglobin measurement. Only one patient was lost of follow-up. A total of 102 patients were analysed, 62 were recruited at the Clermont-Ferrand university hospital ED and 40 at the Aurillac general hospital ED (Figure 1).

The adjudicated final diagnosis was NSTEMI for 7.8% (n=8), UA for 3.9% (n=4), CNCAD 8.8% (n=9), NCCP for 52% (n=53) and unknown for 27.5% (n=28).

CNCAD included pericarditis (3), supraventricular tachycardia (3), ventricular tachycardia (2) and left hypertrophy (1). Patients with adjudicated diagnosis NCCP included patient with anxiety (3), stomach disease (4), herpes zoster (1), neoplasms (4), breast hematoma (1), cholecystitis (1) vasovagal syncope (1) and osteoarthritis (2).

Baseline characteristics of each population are shown in Table 1.

Table 1. Baseline characteristics

Characteristics	All patients	AMI (NSTEMI)	Non-AMI	p Value
Patients	102 (100)	8 (7.8)	94 (92.2)	
Men	64 (62.7)	7 (87.5)	57 (55.9)	0.2525
Age	59.47 ± 16.05	65.75 ± 16.04	58.94 ± 16.02	0.2509
Risk factors				
Body Mass Index (kg/m^2)	26.93 ± 4.9	27.1 ± 3.7	26.9 ± 5.0	0.9416
Family history of CAD	33 (32.3)	3 (37.5)	30 (31.9)	0.7114
Hypertension	49 (48)	5 (62.5)	44 (46.8)	0.4760
Hyperlipidemia	51 (50)	4 (50)	47 (50)	1.0
Diabetes mellitus	17 (16.7)	1 (12.5)	16 (17)	1.0
Current smoking	26 (25.5)	5 (62.5)	21 (22.3)	0.0243
History of smoking	30 (29.4)	1 (12.5)	29 (31.1)	0.4302
History				
CAD	35 (34.3)	4 (50)	31 (33)	0.4410

Previous myocardial infarction	27 (26.5)	4 (50)	23 (24.5)	0.2031
Previous revascularization	26 (25.5)	3 (37.5)	23 (24.5)	0.4168
History of heart failure	5 (4.9)	0	5 (5.3)	1.0
Peripheral artery disease	6 (5.9)	2 (25)	4 (4.3)	0.0692
Previous stroke	6 (5.9)	1 (12.5)	5 (5.3)	0.3953
Clinical status				
Heart rate (beats/min)	77 ± 17	81 ± 18	77 ± 17	0.4970
Systolic blood pressure (mmHg)	141 ± 22	149 ± 28	140 ± 21	0.2888
Diastolic blood pressure (mmHg)	83 ± 15	92 ± 13	82 ± 14	0.0576
Respiratory rate	17 ± 4	16 ± 5	17 ± 4	0.5270
Temperature (^{o}C)	36.7 ± 0.5	36.9 ± 0.2	36.7 ± 0.5	0.1597
Killip class 1	97 (95)	8 (100)	89 (94.7)	1.0
Killip class 2	5 (5)	0	5 (5.3)	1.0
Time between pain onset and admission (h:min)	3:48 ± 2:50	2:27 ± 1:39	3:55 ± 2:53	0.1632
Biochemical values at admission				
Natremia (mmol/L)	140.3 ± 2.9	137.4 ± 2.3	140.5 ± 2.8	0.0022
Creatinin (µmol/L)	80.4 ± 17.5	82.3 ± 18.7	80.2 ± 17.5	0.7508
MDRD (mL/min/1.73 m^2)	85.2 ± 23.5	84.1 ± 19.1	85.3 ± 23.9	0.8950
CRP (mg/L)	4.9 ± 7.7	4.6 ± 6.1	4.9 ± 7.8	0.9323
Electrocardiographic findings at admission				
Normal	43 (42.1)	1 (12.5)	42 (44.7)	0.1339
Left bundle branch block	0	0	0	
ST segment elevation	0	0	0	
ST segment depression	9 (8.82)	2 (25)	7 (7.5)	0.1468
T wave inversion	20 (19.6)	3 (37.5)	17 (18.1)	0.1874
No significant abnormalities	30 (29.4)	2 (25)	28 (29.8)	1.0
Risk scores				
GRACE	96 ± 31	107.8 ± 25.4	95.6 ± 31.3	0.2897
TIMI 0	29 (28.4)	2 (25)	28 (29.8)	1.0
TIMI 1	26 (25.5)	0	26 (27.6)	0.1103
TIMI 2	14 (13.7)	2 (25)	12 (12.8)	0.3016
TIMI 3	21 (20.6)	1 (12.5)	20 (21.3)	1.0
TIMI 4	9 (8.8)	3 (37.5)	6 (6.4)	0.0213
TIMI 5	2 (2)	0	2 (2.1)	1.0
Explorations				
Echocardiography	61 (59.8)	7 (87.5)	54 (57.4)	0.1392
Cardiac exercice test	47 (46)	0	47 (50)	0.007
Coronary angiography	19 (18.6)	7 (87.5)	12 (12.8)	<0.0001

Values are presented as n (%) or mean +/- SD
CAD, Coronary Artery Disease; CRP, C-reactive protein; *GRACE*, Global Registery of Acute Cardiac Events; *TIMI*, Thrombosis In Myocardial Infarction.

Time between pain onset and admission was less than 3 hours for 58 patients (56.9%). Twenty-four patients were admitted between 3 and 6 h after the onset of pain (23.5%), 13 patients between 6 and 9 h (12.7%) and 7 patients between 9 and 12 h (6.9%). All patients with a diagnosis of myocardial infarction were admitted within the first 6 hours after the chest pain onset, five of them in the first 3 hours. The mean interval between chest pain onset and admission is 147,5 min ± 99 min for NSTEMI patients and 235 min ± 173 min for patients without AMI (p=0.1632).

Main results :
Serial blood testing :
At admission, all patients were recruited for blood testing. Because of therapeutic necessities after inclusion, 3 AMI patients did not have all required blood sampling. Thus, data of the 8

AMI patients are available at H0, data of 7 AMI patients are available at H2, H4 and H6, and data of 6 AMI patients at H12. Results of biomarkers are displayed in Figures 2 to 5.

Troponin

According to the inclusion criteria, all patients had hs cTnI ≤ 99th percentile at admission. The median hs cTnI value was significantly higher in patients with NSTEMI diagnosis than in patients with other diagnosis, respectively 0.021 µg/L IQR[0.015-0.04] vs 0.015 µg/L IQR[0.015-0.015], $p<0.0001$. In the five NSTEMI patients who were admitted within 3 hours after the onset of pain median troponin was 0.015 µg/L IQR[0.015-0.023] and 0.040 µg/L IQR[0.018-0.045] for the 3 NSTEMI patients who consulted between 3 and 6 hours after the onset of pain ($p=0.2090$).

Troponin is the only marker studied for which showed a significant difference between the two groups for each time performed (0, 2, 4, 6 and 12 h), including at admission.

Copeptin

The median copeptin for AMI and non-AMI patients at admission was respectively 5.5 pmol/L IQR[3.1-7.9] and 6.5 pmol/L IQR[3.9-12.1], $p=0.4913$. Only one AMI patient showed a copeptin value at admission above the cut off of 12 pmol/L (435.2 pmol/L). This patient was also the only patient who died during the follow up. For all of the samples recruited during the 12 h following admission (2, 4, 6 and 12 h) there was no significant difference in the copeptin values between patients with AMI and those with no AMI, respectively 5.9 pmol/L IQR[3.1-8.3] and 5.5 pmol/L IQR[3.5-10] at 2 h ($p=0.8617$), 4.7 pmol/L IQR[2.9-8.4] and 5.4 pmol/L IQR[3.7-9.3] at 4 h ($p=0.7430$), 5.9 pmol/L IQR[2.5-6.9] and 5.6 pmol/L IQR[3.5-8.8] at 6 h ($P=0.7695$) and 3.9 pmol/L IQR[2.8-10.2] and 6.1 pmol/L IQR[4-9.7] at 12 h ($p=0.4872$).

Myoglobin

At admission, the median myoglobin for AMI patients was 52.1 ng/mL IQR[41.1-66.1] and 47.3 ng/mL IQR[38-66.6] for patients with other diagnostics, $p=0.7060$. At 2, 4 and 6 h, median myoglobin was significantly higher in AMI patients than in patients with other diagnosis, respectively 72.9 ng/mL and 48.6 ng/mL ($p=0.012$), 102 ng/mL and 47.8 ng/mL ($p=0.0422$), 107.5 ng/mL and 49.5 ng/mL ($p=0.031$).

Creatin Kinase

Median CK concentration was 156.5 U/L IQR[90-231.5] in AMI patients and 182 U/L IQR [105-277] in non-AMI patients at inclusion ($p=0.5882$). At 6 h and 12 h, CK values of AMI patients were higher than those of other patients without significant difference, respectively 183 U/L and 147 U/L ($p=0.9371$), 186 U/L and 128 U/L ($p=0.2554$).

Diagnostic accuracy

For a cut-off level of 12 pmol/L, sensitivity of copeptin for AMI diagnosis at admission was 12.5%, with a specificity of 74.5%, a predictive positive value of 4% and a NPV of 90.9%. None patients had a myoglobin value above the 95th percentile at admission.

At the sixth hour, all of 8 AMI patients had at least one troponin above the 0.045 µg/L. One patient had a troponin measured on the sample at the 6th hour already below this threshold and will continue to decrease until the twelfth hour.

LIMITATIONS OF THE STUDY

Although bicentric and carried one year period, only eight patients with NSTEMI and hs-cTnI below the 99th percentile at presentation were included. To show a significant difference between subjects with AMI and those who do not have AMI with an expected difference of 15 pmol/L, as in the princeps study of Reichlin, the number of AMI subjects needed is 40 patients.[5] We were not able to include the expected number of patients within the time allowed by the design of the study and its permissions. Thus, the area under the ROC curve and the net reclassification index can not be calculated because of too few AMI patients.
We did not assess the pre-test probability, which could increase the relevance of the biomarker in certain patient populations. However, there are no validated score to determine the clinical probability of ACS.
This study was conducted in France, with a system of prehospital medicalization. Patients supported upstream of the hospital for a very suspicious chest pain, even without ST-elevation, could be directly admitted to the cardiology department to perform immediate exploration, forming an incorporation bias. Probably, the results of this study are probably not be extrapolated to all ED.
Twelve hours after admission, there is no significant difference between the two groups (AMI vs non-AMI) for myoglobin and CK. This may be due to the fact that the population studied have low infarct size (hs-cTnI < 99th percentile at admission in the 6 hours after the pain onset) and that, two AMI patients have not been collected at the twelfth hour.

DISCUSSION

After considering the limitation, our study complements the results of previously published data. In this prospective study, we use the latest generation of troponin I and copeptin assays. We have developed the protocol in a logical form. According to previous studies, copeptin can add diagnostic value if troponin at admission is less than a threshold. Thus, we focused the study for this category of patients. Knowing that the time to result for the copeptin-us is 14 minutes, this analyse could be requested or performed automatically when the troponin is below the threshold, for a rational use of resources. To reduce the bias spectrum, we specifically explored the diagnosic value of copeptin only in patients with suspected ACS with non–ST-segment elevation and with high sensitive cardiac troponin below the 99th percentile at admission, while most of the studies have examined all patients with suspected ACS including STEMI patients and/or patients with a troponin above the 99th percentile at admission. In these populations, the prevalence of AMI is higher than patient without ST elevation and troponin below the 99th percentile. Also, for this patients, urgent care or further explorations will not be influenced by the result of copeptin. Results of these previous studies may have influenced the statistical evaluation of copeptin.
In our study population, although the copeptin NPV was 90.9%, if we would have ruled out patients on the results of copeptin on admission, seven of height AMI patients have returned at home without care. These results are consistent with COPED-MIRRO study who have a similary design but had used mostly a 4th generation troponin.[30] Even pursuing the inclusion up to 40 AMI patients as we envisage to highlight a significant difference, with these seven patients, the error seems too important to rule out patients with suspected ACS at admission in our ED. However, only a larger study could confirm or refute this assumption.
The troponin of one patient in our study had increased above the cut-off only at the sixth hour. Also, at the sixth hour, troponin of one of AMI patients had already begun its decline and was below the threshold of the 99th percentile. This observation is consistent with the precautionary statements of the Study Group on Biomarkers in Cardiology of the European Society of Cardiology Working Group on Acute Cardiac Care, advocating additional blood

sampling in patients strongly suspected of having an AMI but no significant hs-cTn increase after 3 h.[31]
A recent study suggest that it could be considered to rule out patients with undetectable Roche high sensitive cardiac troponin T at admission.[32] This algorithm is not possible with our study population and the hs-cTnI used, 3 patients had hs-cTnI undetectable at admission.
Finally, the only subject who died is the patient who had the highest value of copeptin, wich is consistent with highlight the of studies showing a prognostic role of copeptin. [15, 21, 25, 29]
In summary, our study did not reveal diagnostic value of copeptin for patients with suspected ACS without ST-elevation and with hs-cTnI below the 99th percentile at admission. Measurements of hs-cTn at presentation and after 3 h and after 6 h if necessary, remains the biochemical gold standard for AMI diagnosis. [1, 31] Using a novel marker for ACS diagnosis, alone or in a multi-marker strategy, requires at less as good sensitivity and negative predictive value than a troponin serial testing.

Table and Figure Legends

Figure 1. Flow chart

Table 1. Baseline characteristics

Figures 2 to 5. Box plots (median, interquartile range, minimal and maximal values) illustrate Troponin, Copeptin, myoglobin and CK concentration in relation to time since admission for AMI and non-AMI patients. * p<0.0001, ** p=0.012, *** p=0.0422, **** p=0.031.

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Author contributions :

JD, JS, GM, VS, NC, PM conceived the study, designed the trial.
JD undertook recruitment of participating centers and patients, managed the data, supervised the conduct of the trial, and drafted the manuscript.
SU provided statistical advice on study design and analysed the data.
SM has made monitoring and carried out biochemical assays.
LC, ND, NC JS, GM, VS, PM, LD and JD were the expert committee to adjudicate the final diagnosis.
ND, LC, SU contributed substantially to the revision of the manuscript.
JD takes responsibility for the paper as a whole.

Data Sharing

No additional data

References

1 Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. *Eur Heart J* 2012;33:2551–67.

2 Hamm CW, Bassand J-P, Agewall S, et al. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart* J 2011;32:2999–3054.

3 Lin S, Yokoyama H, Rac VE, et al. Novel biomarkers in diagnosing cardiac ischemia in the emergency department: a systematic review. *Resuscitation* 2012;83:684–91.

4 Lippi G. Biomarkers of myocardial ischemia in the emergency room: cardiospecific troponin and beyond. *Eur J Intern Med* 2013;24:97–9.

5 Reichlin T, Hochholzer W, Stelzig C, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol* 2009;54:60–8.

6 Keller T, Tzikas S, Zeller T, et al. Copeptin improves early diagnosis of acute myocardial infarction. *J Am Coll Cardiol* 2010;55:2096–106.

7 Chenevier-Gobeaux C, Freund Y, Claessens Y-E, et al. Copeptin for rapid rule out of acute myocardial infarction in emergency department. *Int J Cardiol* 2013;166:198–204.

8 Meune C, Zuily S, Wahbi K, et al. Combination of copeptin and high-sensitivity cardiac troponin T assay in unstable angina and non-ST-segment elevation myocardial infarction: a pilot study. *Arch Cardiovasc Dis* 2011;104:4–10.

9 Karakas M, Januzzi JL, Meyer J, et al. Copeptin does not add diagnostic information to

high-sensitivity troponin T in low- to intermediate-risk patients with acute chest pain: results from the rule out myocardial infarction by computed tomography (ROMICAT) study. *Clin Chem* 2011;57:1137–45.

10 Lotze U, Lemm H, Heyer A, et al. Combined determination of highly sensitive troponin T and copeptin for early exclusion of acute myocardial infarction: first experience in an emergency department of a general hospital. *Vasc Health Risk Manag* 2011;7:509–15.

11 Giavarina D, Carta M, Fortunato A, et al. Copeptin and high sensitive troponin for a rapid rule out of acute myocardial infarction? *Clin Lab* 2011;57:725–30.

12 Giannitsis E, Kehayova T, Vafaie M, et al. Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. *Clin Chem* 2011;57:1452–5.

13 Freund Y, Chenevier-Gobeaux C, Leumani F, et al. Concomitant Measurement of Copeptin and High Sensitivity Troponin for Fast and Reliable Rule Out of Acute Myocardial Infarction. *Ann Emerg Med* 2011;58:S190.

14 Dupuy A-M, Chastang E, Cristol J-P, et al. Analytical performances of the newly developed, fully automated Kryptor Copeptin assay: which impact factor for myocardial infarction rules out in the emergency department? *Clin Lab* 2012;58:635–44.

15 Potocki M, Reichlin T, Thalmann S, et al. 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. *Heart* 2012;98:558–65.

16 Ray P, Charpentier S, Chenevier-Gobeaux C, et al. Combined copeptin and troponin to rule out myocardial infarction in patients with chest pain and a history of coronary artery

disease. *Am J Emerg Med* 2012;30:440–8.

17 Charpentier S, Maupas-Schwalm F, Cournot M, et al. Combination of copeptin and troponin assays to rapidly rule out non-ST elevation myocardial infarction in the emergency department. *Acad Emerg Med* 2012;19:517–24.

18 Eggers KM, Venge P, Lindahl B. High-sensitive cardiac troponin T outperforms novel diagnostic biomarkers in patients with acute chest pain. *Clin Chim Acta* 2012;413:1135–40.

19 Folli C, Consonni D, Spessot M, et al. Diagnostic role of copeptin in patients presenting with chest pain in the emergency room. *Eur J Intern Med* 2013;24:189–93.

20 Dedic A, Kate Ten G-J, Rood PPM, et al. Copeptin in acute chest pain: identification of acute coronary syndrome and obstructive coronary artery disease on coronary CT angiography. *Emerg Med J* Published Online First: 8 November 2012. doi: 10.1136/emermed-2012-201596.

21 Bahrmann P, Bahrmann A, Breithardt O-A, et al. Additional diagnostic and prognostic value of copeptin ultra-sensitive for diagnosis of non-ST-elevation myocardial infarction in older patients presenting to the emergency department. Clin Chem Lab Med 2013;51:1307–19.

22 Ledochowski S, Fayet JM, Collin-Chavagnac D, Bonnefoy-Cudraz E. Evaluation for copeptin in NSTEMIs. *Annales Françaises de Médecine d'Urgence*. 2013;3:138-44.

23 Maisel A, Mueller C, Neath S-X, et al. Copeptin Helps in the Early Detection Of Patients with Acute Myocardial Infarction : the primary results of the CHOPIN Trial. *J Am Coll Cardiol* 2013;62:150–60.

24 Charpentier S, Lepage B, Maupas-Schwalm F, et al. Copeptin improves the diagnostic performance of sensitive troponin I-Ultra but cannot rapidly rule out non-ST-elevation

myocardial infarction at presentation to an emergency department. *Ann Emerg Med* 2013;61:549–558.

25 Afzali D, Erren M, Pavenstädt H-J, et al. Impact of copeptin on diagnosis, risk stratification, and intermediate-term prognosis of acute coronary syndromes. *Clin Res Cardiol* 2013;755-63.

26 Thelin J, Borna C, Erlinge D, et al. The combination of high sensitivity troponin T and copeptin facilitates early rule-out of ACS: a prospective observational study. BMC Cardiovasc Disord 2013;13:42.

27 Sebbane M, Lefebvre S, Kuster N, et al. Early rule out of acute myocardial infarction in ED patients: value of combined high-sensitivity cardiac troponin T and ultrasensitive copeptin assays at admission. *Am J Emerg Med* 2013;31:1302–8.

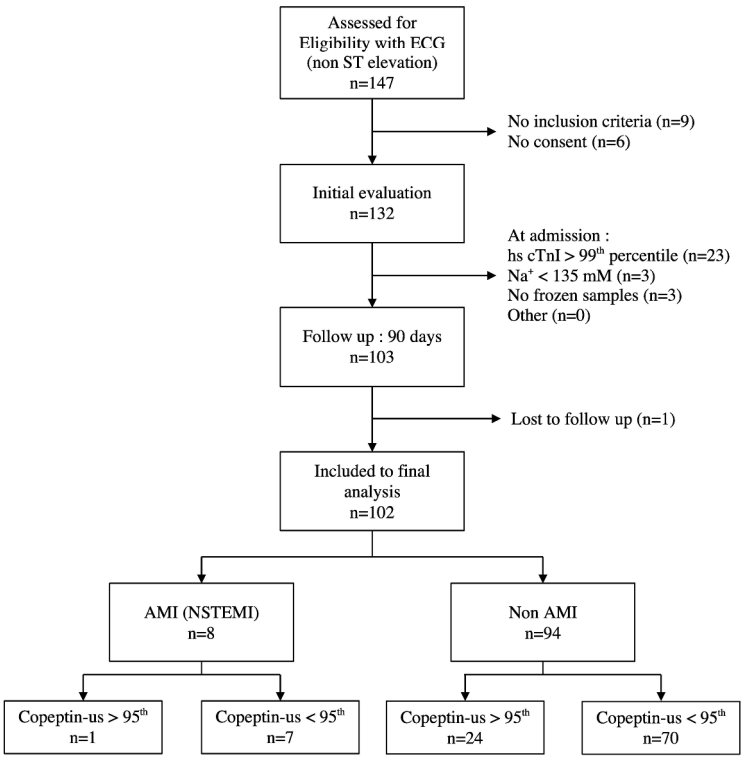
28 Balmelli C, Meune C, Twerenbold R, et al. Comparison of the performances of cardiac troponins, including sensitive assays, and copeptin in the diagnostic of acute myocardial infarction and long-term prognosis between women and men. *Am Heart J* 2013;166:30–7.

29 Maisel A, Mueller C, Neath S-X, et al. Copeptin helps in the early detection of patients with acute myocardial infarction: primary results of the CHOPIN trial (Copeptin Helps in the early detection Of Patients with acute myocardial INfarction). *J Am Coll Cardiol* 2013;62:150–60.

30 Llorens P, Sánchez M, Herrero P, et al. The utility of copeptin in the emergency department for non-ST-elevation myocardial infarction rapid rule out: COPED-MIRRO study. *Eur J Emerg Med* Published Online First: 17 July 2013. doi: 10.1097/MEJ.0b013e3283632f8b

31 Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J* 2012;33:2252–7.

32 Body R, Carley S, McDowell G, et al. Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a high-sensitivity assay. *J Am Coll Cardiol* 2011;58:1332–9.



Flow chart
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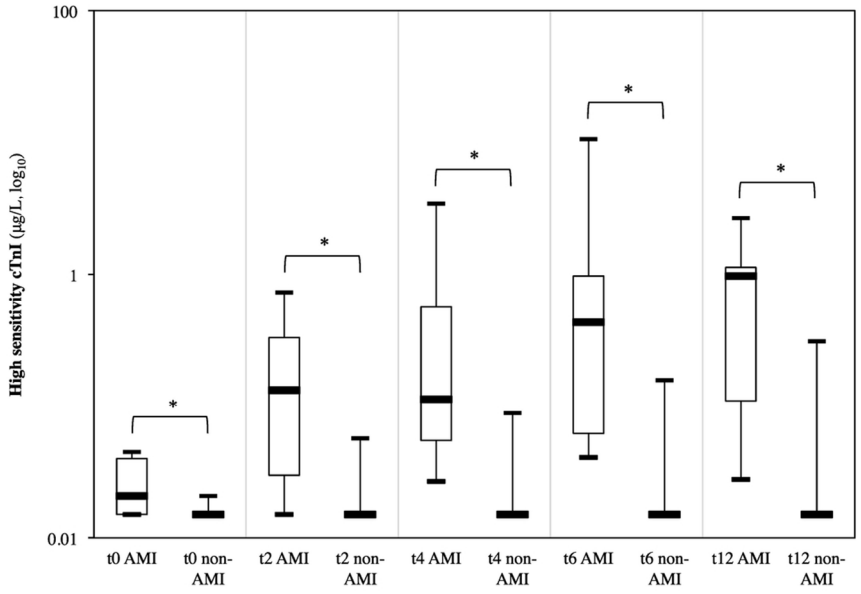

Box plots (median, interquartile range, minimal and maximal values) illustrate Troponin concentration in relation to time since admission for AMI and non-AMI patients. * p<0.0001.
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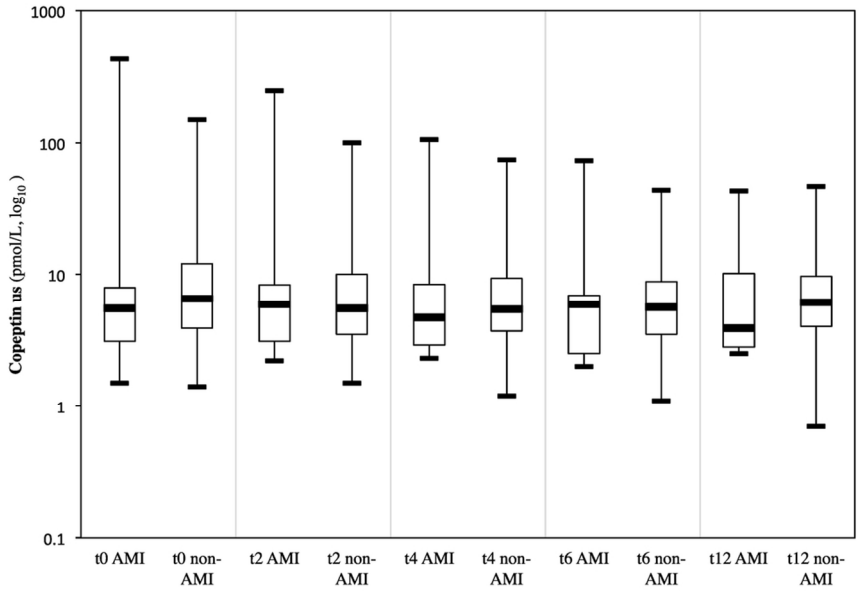

Box plots (median, interquartile range, minimal and maximal values) illustrate Copeptin concentration in relation to time since admission for AMI and non-AMI patients.
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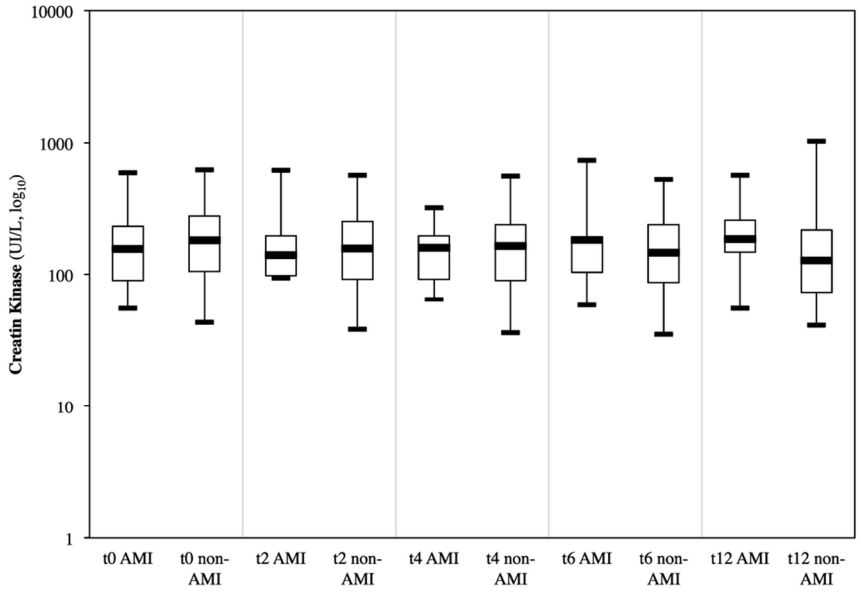

Box plots (median, interquartile range, minimal and maximal values) illustrate CK concentration in relation to time since admission for AMI and non-AMI patients.
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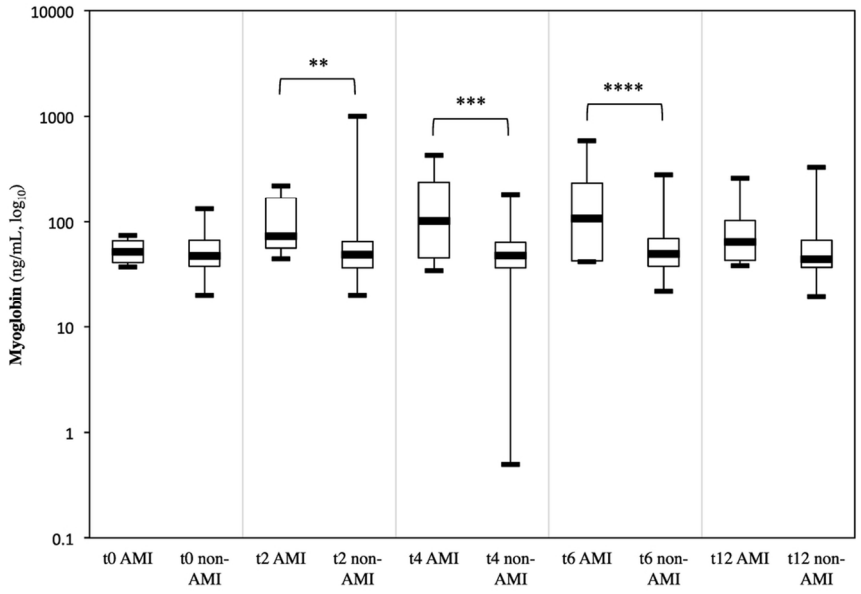

Box plots (median, interquartile range, minimal and maximal values) illustrate myoglobin concentration in relation to time since admission for AMI and non-AMI patients. ** p=0.012, *** p=0.0422, **** p=0.031.
106x65mm (300 x 300 DPI)

STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	Yes
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	1
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	1-2
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	1-2
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	1-2
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	1
Test methods	7	The reference standard and its rationale.	1
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	2
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	2
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	2
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	2
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	2-3
	13	Methods for calculating test reproducibility, if done.	3
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	1
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	3-4
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	3
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	2
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	3
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	4-5
	20	Any adverse events from performing the index tests or the reference standard.	
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	4-5
	22	How indeterminate results, missing data and outliers of the index tests were handled.	6
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	
	24	Estimates of test reproducibility, if done.	
DISCUSSION	25	Discuss the clinical applicability of the study findings.	6-7

BMJ open

Diagnostic accuracy of Copeptin sensitivity and specificity in patients with suspected non–ST-elevation myocardial infarction with troponin I below the 99th percentile at presentation.

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Title page

***Title** of the article* :

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ABSTRACT

Objective

To determine if copeptin-us can rule out diagnosis of non–ST-segment elevation myocardial infarction (NSTEMI) without prolonged monitoring and serial blood sampling in patients with high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to the emergency department (ED).

Design

Prospective, non-randomized, individual blinded diagnostic accuracy study.

Setting

Two ED of a rural region of France.

Participants

Patient with a chest pain suspected of NSTEMI with onset within the last 12 h were considered for enrolment.

Interventions

Serial clinical, electrographical and biochemical investigations were performed at admission and after 2, 4, 6 and 12 h. Hs-cTnI was mesured using an assay with Dimension VISTA, Siemens. Copeptin was measured by the B.R.A.H.M.S copeptin-us assay on the KRYPTOR Compact Plus system. The follow-up was 90 days.

Primary and secondary outcome measures

Copeptin, troponin, myoglobin and creatin kinase values. Clinicals and paraclinicals events. The final diagnosis was adjudicated blinded to copeptin result.

Results

During 12 months, 102 patients were analysed. Final diagnosis was NSTEMI for 7.8% (n=8), unstable angina for 3.9% (n=4), cardiac but non coronary artery disease for 8.8% (n=9), non-cardiac chest pain for 52% (n=53) and unknown for 27.5% (n=28). There was no statistical difference for copeptin values between NSTEMI patients and others (respectively 5.5 pmol/L IQR[3.1-7.9] and 6.5 pmol/L IQR[3.9-12.1], p=0.49). Only one NSTEMI patient had a copeptin value at admission above the cut off of 95th percentile at admission.

Conclusions

In this study, copeptin does not add a diagnostic value at admission to ED for suspected acute coronary syndrome patients without ST-segment elevation and with hs-cTnI below the 99th percentile.

Trial registration

Clinicaltrials.gov identifier: NCT01334645.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To our knowledge, our prospective multicentric study is the only one that includes only patients with suspected NSTEMI and high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to ED, to limit spectrum bias.
- The main limitation of our study is the number of patients included. Indeed we are below the prevalence. This may be explained by the fact that our study included only patients with negative ultrasensitive troponin at admission. However, this is the only group of patients for which a multimarker rule-out strategy could add diagnostic value.
- Moreover, we evaluated the sensitivity troponin and copeptin for all patients with the same assay technique which enabled to control the occurrence of methodological bias.

MANUSCRIPT

INTRODUCTION

Detection of a rise and/or fall of cardiac troponin with clinical symptoms of ischemia or abnormal electrocardiography (ECG) or imaging findings remains the gold standard for the identification of myocardial infarction.[1] At Emergency Department, patients with non–ST-segment elevation myocardial infarction (NSTEMI) working diagnosis requires serial measurement of troponin.[2] However, most of these patients do not have acute coronary syndrome (ACS). Identify patients suffering from non-life-threatening diseases with only one blood sample is a challenge. Many biomarkers were evaluated, alone or in combination with troponin.[3,4] Copeptin accuracy was explored recently in this rule out diagnostic strategy. This glycopeptide, who is the C-terminal part of the arginine vasopressin (AVP) precursor, is secreted stoichiometrically with AVP from the neurohypophysis. AVP is a marker of endogenous stress but routine measurement of AVP is limited due by its instability and difficulty of the assay.[5] Copeptin now appears to be an attractive alternative to AVP, because of its stability and development of automated technique for reliable and reproducible dosage.[6-8]
Since the first publication in this indication in 2009, several studies have investigated copeptin.[9-35] Some of these studies suggest that the association of troponin and copeptin at the first measurement has a powerfull negative predictive value (NPV) to rule out patients without NSTEMI.
Interpretation of the copeptin diagnostic accuracy through these studies is not evident. First, because analysis comparisons are disrupted by the development of high-sensitivity cardiac troponin T and I assays and the availability of three commercial assays for copeptin (LUMItest®, Copeptin Kryptor®, Copeptin-us Kryptor®). Furthermore, many protocols included ST-segment elevation myocardial infarction (STEMI) patients and patients with a high-sensitive cardiac troponin above 99th percentile at admission. For these patients, copeptin does not add diagnostic information, urgent revascularisation or serial blood samples, respectively, remains necessary.
The aim of this study was to determine if copeptin-us can rule out diagnosis of acute myocardial infarction without prolonged monitoring and serial blood sampling in patients

with suspected NSTEMI and high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to ED.

METHODS

Study design and setting

This diagnostic test evaluation is a prospective non-randomized individual blinded multicentric cohort study. The Clermont-Ferrand University Hospital designed and coordinated the study. The duration of the study was one year, between march 2011 and march 2012 at the ED of two hospitals of Auvergne, a rural region of France (1.3 million people). First one, Gabriel Montpied in Clermont-Ferrand, is a teaching hospital and provincial referral center with 48000 ED admissions per year. The second hospital, Henri Mondor in Aurillac, is a general hospital with 25000 ED admissions per year. Each units had a catheterization laboratory available 24 hours a day. The study complied with the Declaration of Helsinki and was approved by the ethical committee Comité de Protection des Personnes Sud-Est VI (AU 871). Before study launch, methods were registered with ClinicalTrials.gov (NCT01334645).

Population

Consecutive patients admitted with a chest pain suspected of NSTEMI in emergency department were considered for enrolment in the study. The inclusion criteria were the following : patients older than 18 years with chest pain suggestive of ACS of < 12 hours' duration since his onset. Atypical presentations of NSTEMI are not uncommon,[2] therefore the criteria for a pain suggestive of ACS were those of usual clinical practice of investigators. It should be non-traumatic. Written informed consent was obtained from all participating patients. Patients with ST-segment elevation, legal incapacity, sepsis, shock, lung neoplasms, terminal kidney failure requiring dialysis, life expectancy of less than 6 months were excluded. After the result of the first blood sample, patients with hyponatremia < 135 mmol/L or hs-cTnI > 0,045 μ g/L were released of the study.

ST-segment elevation, measured at the J point, was diagnosed according to the third universal definition of myocardial infarction (MI).[1] It should be found in two contiguous leads with the cut-points: ≥ 0.1 mV in all leads other than leads V2–V3 where the following cut points apply: ≥ 0.2 mV in men ≥ 40 years; ≥ 0.25 mV in men < 40 years, or ≥ 0.15 mV in women.

Sepsis, shock, lung neoplasms, terminal kidney failure requiring dialysis and hyponatremia are diseases in which the rate of vasopressin, and thus of copeptin, may be modified. These patients were not included to minimize confounding factors.

Study protocol

Upon admission, all patient underwent an initial clinical assessment, including medical history, temperature, respiratory rate, cardiac frequency, blood pressure, pulse oxymetry, 18-lead ECG, chest X-ray and screening blood test including : C-reactive protein, natremia, creatinin, hs-cTnI and creatin kinase (CK). Risk factors and previous medical history were collected as stated by the patient and its treatment. Family history of coronary artery disease (CAD) were noted if a member of the first-degree relatives had coronary artery disease before 65 years. Blood sampling were collected for hs-cTnI and CK analysis and 18-lead ECG were

performed after 2, 4, 6 and 12 h. At each time point, blood sample was centrifuged and plasma was frozen at -80 °C for copeptin and myoglobin testing at the end of the study recruitment, blinded to final diagnosis. Further investigations and treatment of patients were not modified by the study. At 90 days, clinical events were collected from the patients, theirs general practitioners and the hospitals where they were explored.

Concentration of copeptin was measured by the B.R.A.H.M.S copeptin-us immunoluminometric assay on the KRYPTOR Compact Plus system (Thermo Fisher Scientific). The detection limit as described by the manufacturer was signified as being 0.9 pmol/L and the lowest concentration measurable with a coefficient of variation (CV) < 10% has been reported < 4 pmol/L. Direct measuring range was 0.9 to 500 pmol/L. The 95th percentile among healthy subjects is < 12.0 pmol/L and was specified for rapid exclusion of acute myocardial infarction (AMI).

Hs-cTnI was measured using a chemiluminescence test (Dimension VISTA®, Siemens Healthcare Diagnostics). The limit of blank of hs-cTnI was 0.015 μg/L, the 99th percentile concentration was 0.045 μg/L and the lowest concentration measurable with a CV < 10% was 0.040 μg/L according to the manufacturer. The 99th percentile (0.045 μg/L) was used as diagnostic cut-off to fulfil AMI criteria.

Myoglobin was measured by Dimension VISTA® (Siemens Healthcare Diagnostics). The measuring range extended from 0.5 to 1000 µg/L. The 95th percentile concentration was 116 µg/L for men and 71 µg/L for women. At concentrations of 110 µg/L, the inter-assay CV was 4.9% and the intra-assay CV was 5%.

Natremia, C-reactive protein, creatinin and CK, were measured using standardized methods.

Outcomes

The final diagnosis was adjudicated, blinded to copeptin results, by an expert committee of three cardiologists, four emergency physicians and two biochemists (whose one MD-PhD of each specialty), with all available medical records from the time of ED presentation to 90-day follow-up. Each subject was classified in the following categories : Non-ST Elevation Myocardial Infarction (NSTEMI), Unstable angina (UA), Cardiac but non coronary artery disease (CNCAD), non-cardiac chest pain (NCCP) and unknown cause of chest pain. The diagnosis was determined according to the current guidelines and universal definition of myocardial infarction.[1,2] The diagnosis of NSTEMI, in these patients showing suspected symptoms of ACS, was defined by a rise and/or fall of hs-cTnI with at least one value above the 99th percentile and with the following criteria : imaging evidence of new loss of viable myocardium or new regional wall motion abnormality or identification of an intracoronary thrombus by angiography. The defining criteria unstable angina were the same as those defining the NSTEMI, without elevation of troponin. Diagnosis of CNCAD was performed if a coronary artery disease was exclude by additional testing. Diagnosis of NCCP was performed if a cardiac aetiology was exclude. Unknown cause of chest pain diagnosis was defined when no sufficient further diagnostic procedures were performed.

Copeptin and myoglobin measurements were performed at the end of the study recruitment, blinded to the final diagnosis.

Statistical analysis

In order to show a different copeptin value between NSTEMI and non NSTEMI subjects, with an expected difference of 15 pmol/L, a standard deviation of 20.7 pmol/L, a significance level of 5% and a power of 95%, 40 NSTEMI subjects were needed.

Continuous variables were displayed either as means ± SD or medians and interquartile range (IQR). Categorical variables were described by using frequencies and percentages.
The analysis of quantitative variables was performed using the two-tailed Student's t-test after checking the assumption of equal variances (Levene test) and one way analysis of variance for variables following a normal distribution. Otherwise, the Wilcoxon rank sum tests for continuous variables and Kruskal-Wallis tests were used. Categorical variables were analysed using Chi-square analysis or the Fisher exact test (if needed). For all tests, a significance level of $p<0.05$ was used.
Statistical analysis was performed using SAS (v 9.3, SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient characteristics

During 12 months, 147 patients were assessed for eligibility in both ED. Nine presented one or more exclusion criteria, six did not give their informed consent for participation, 26 were released after the results of the first blood sample because they had hyponatremia < 135 mmol/L (n=3) or hs-cTnI > 0,045 μ g/L (n=23). For three patients, blood samples at presentation were not frozen for copeptin and myoglobin measurement. Only one patient was lost of follow-up. A total of 102 patients were analysed, 62 were recruited at the Clermont-Ferrand university hospital ED and 40 at the Aurillac general hospital ED (Figure 1).
The adjudicated final diagnosis was NSTEMI for 7.8% (n=8), UA for 3.9% (n=4), CNCAD 8.8% (n=9), NCCP for 52% (n=53) and unknown for 27.5% (n=28).
CNCAD included pericarditis (3), supraventricular tachycardia (3), ventricular tachycardia (2) and left hypertrophy (1). Patients with adjudicated diagnosis NCCP included patient with anxiety (3), stomach disease (4), herpes zoster (1), neoplasms (4), breast hematoma (1), cholecystitis (1) vasovagal syncope (1) and osteoarthritis (2).
Baseline characteristics of each population are shown in Table 1.

Table 1. Baseline characteristics

Characteristics	All patients	NSTEMI	Non-NSTEMI	p Value
Patients, n (%)	102 (100)	8 (7.8)	94 (92.2)	
Men, n (%)	64 (62.7)	7 (87.5)	57 (55.9)	0.25
Age (years), mean (SD)	59 (16)	66 (16)	59 (16)	0.25
Risk factors				
Body Mass Index, kg/m^2 (SD)	26.93 (4.9)	27.1 (3.7)	26.9 (5)	0.94
Family history of CAD, n (%)	33 (32.3)	3 (37.5)	30 (31.9)	0.71
Hypertension, n (%)	49 (48)	5 (62.5)	44 (46.8)	0.48
Hyperlipidemia, n (%)	51 (50)	4 (50)	47 (50)	1.0
Diabetes mellitus, n (%)	17 (16.7)	1 (12.5)	16 (17)	1.0
Current smoking, n (%)	26 (25.5)	5 (62.5)	21 (22.3)	0.02
History of smoking, n (%)	30 (29.4)	1 (12.5)	29 (31.1)	0.43
History, n (%)				
CAD	35 (34.3)	4 (50)	31 (33)	0.44
Previous myocardial infarction	27 (26.5)	4 (50)	23 (24.5)	0.20
Previous revascularization	26 (25.5)	3 (37.5)	23 (24.5)	0.42
History of heart failure	5 (4.9)	0	5 (5.3)	1.0
Peripheral artery disease	6 (5.9)	2 (25)	4 (4.3)	0.07
Previous stroke	6 (5.9)	1 (12.5)	5 (5.3)	0.4
Clinical status				
Heart rate, beats/min (SD)	77 (17)	81 (18)	77 (17)	0.5
Systolic blood pressure, mmHg (SD)	141 (22)	149 (28)	140 (21)	0.29
Diastolic blood pressure,mmHg (SD)	83 (15)	92 (13)	82 (14)	0.06

Respiratory rate, respiratory cycles/min (SD)	17 (4)	16 (5)	17 (4)	0.53
Temperature, °C (SD)	36.7 (0.5)	36.9 (0.2)	36.7 (0.5)	0.16
Killip class 1, n (%)	97 (95)	8 (100)	89 (94.7)	1.0
Killip class 2, n (%)	5 (5)	0	5 (5.3)	1.0
Time between pain onset and admission h:min (SD)	3:48 (2:50)	2:27 (1:39)	3:55 (2:53)	0.16
Biochemical values at admission				
Natremia, mmol/L (SD)	140.3 (2.9)	137.4 (2.3)	140.5 (2.8)	0.0022
Creatinin, μ mol/L (SD)	80.4 (17.5)	82.3 (18.7)	80.2 (17.5)	0.75
MDRD, mL/min/1.73 m^2 (SD)	85.2 (23.5)	84.1 (19.1)	85.3 (23.9)	0.9
CRP, mg/L (SD)	4.9 (7.7)	4.6 (6.1)	4.9 (7.8)	0.93
Electrocardiographic findings at admission				
Normal, n (%)	43 (42.1)	1 (12.5)	42 (44.7)	0.13
Left bundle branch block, n (%)	0	0	0	
ST segment elevation, n (%)	0	0	0	
ST segment depression, n (%)	9 (8.82)	2 (25)	7 (7.5)	0.15
T wave inversion, n (%)	20 (19.6)	3 (37.5)	17 (18.1)	0.19
No significant abnormalities, n (%)	30 (29.4)	2 (25)	28 (29.8)	1.0
Risk scores				
GRACE, score (SD)	96 (31)	107.8 (25.4)	95.6 (31.3)	0.29
TIMI 0, n (%)	29 (28.4)	2 (25)	28 (29.8)	1.0
TIMI 1, n (%)	26 (25.5)	0	26 (27.6)	0.11
TIMI 2, n (%)	14 (13.7)	2 (25)	12 (12.8)	0.30
TIMI 3, n (%)	21 (20.6)	1 (12.5)	20 (21.3)	1.0
TIMI 4, n (%)	9 (8.8)	3 (37.5)	6 (6.4)	0.02
TIMI 5, n (%)	2 (2)	0	2 (2.1)	1.0
Explorations				
Echocardiography, n (%)	61 (59.8)	7 (87.5)	54 (57.4)	0.14
Cardiac exercice test, n (%)	47 (46)	0	47 (50)	0.007
Coronary angiography, n (%)	19 (18.6)	7 (87.5)	12 (12.8)	<0.0001

Values are presented as n (%) or mean +/- SD
CAD, Coronary Artery Disease; CRP, C-reactive protein; *GRACE*, Global Registry of Acute Cardiac Events; *TIMI*, Thrombosis In Myocardial Infarction.

Time between pain onset and admission was less than 3 hours for 58 patients (56.9%). Twenty-four patients were admitted between 3 and 6 h after the onset of pain (23.5%), 13 patients between 6 and 9 h (12.7%) and 7 patients between 9 and 12 h (6.9%). All patients with a diagnosis of myocardial infarction were admitted within the first 6 hours after the chest pain onset, five of them in the first 3 hours. The mean interval between chest pain onset and admission is 147,5 min ± 99 min for NSTEMI patients and 235 min ± 173 min for patients without NSTEMI (p=0.16).

Main results :

Serial blood testing :

At admission, all patients were recruited for blood testing. Because of therapeutic necessities after inclusion, 3 NSTEMI patients did not have all required blood sampling. Thus, data of the 8 NSTEMI patients are available at H0, data of 7 NSTEMI patients are available at H2, H4 and H6, and data of 6 NSTEMI patients at H12. Results of biomarkers are displayed in Figures 2 to 5.

Troponin

According to the inclusion criteria, all patients had hs cTnI ≤ 99th percentile at admission. Troponin is the only marker studied for which showed a significant difference between the two groups for each time performed (0, 2, 4, 6 and 12 h), including at admission.

Copeptin

Median copeptin levels for NSTEMI and the others patients at admission were respectively 5.5 pmol/L IQR[3.1-7.9] and 6.5 pmol/L IQR[3.9-12.1], p=0.49. Only one NSTEMI patient showed a copeptin value at admission above the cut off of 12 pmol/L (435.2 pmol/L). This patient, who had a GRACE score of 151, was also the only patient who died during the follow up. For all of the samples recruited during the 12 h following admission (2, 4, 6 and 12 h) there was no significant difference in the copeptin values between patients with NSTEMI and those with no NSTEMI, respectively 5.9 pmol/L IQR[3.1-8.3] and 5.5 pmol/L IQR[3.5-10] at 2 h (p=0.86), 4.7 pmol/L IQR[2.9-8.4] and 5.4 pmol/L IQR[3.7-9.3] at 4 h (p=0.74), 5.9 pmol/L IQR[2.5-6.9] and 5.6 pmol/L IQR[3.5-8.8] at 6 h (P=0.77) and 3.9 pmol/L IQR[2.8-10.2] and 6.1 pmol/L IQR[4-9.7] at 12 h (p=0.49).

Myoglobin

At admission, the median myoglobin for NSTEMI patients was 52.1 µg/L IQR[41.1-66.1] and 47.3 µg/L IQR[38-66.6] for patients with other diagnostics, p=0.71. At 2, 4 and 6 h, median myoglobin was significantly higher in NSTEMI patients than in patients with other diagnosis, respectively 72.9 µg/L and 48.6 µg/L (p=0.01), 102 µg/L and 47.8 µg/L (p=0.04), 107.5 µg/L and 49.5 µg/L (p=0.03).

Creatin Kinase

At inclusion, medians CK concentrations were 156.5 U/L IQR[90-231.5] in NSTEMI patients and 182 U/L IQR [105-277] in non-NSTEMI patients (p=0.59). At 6 h and 12 h, CK values of NSTEMI patients were higher than those of other patients without significant difference, respectively 183 U/L and 147 U/L (p=0.93), 186 U/L and 128 U/L (p=0.26).

Diagnostic accuracy

For a cut-off level of 12 pmol/L, sensitivity of copeptin for NSTEMI diagnosis at admission was 12.5%, with a specificity of 74.5%, a predictive positive value of 4% and a NPV of 90.9%. None patients had a myoglobin value above the 95th percentile at admission.

At the sixth hour, all of 8 NSTEMI patients had at least one troponin above the 0.045 μ g/L. One patient had a troponin measured on the sample at the 6th hour already below this threshold and will continue to decrease until the twelfth hour.

LIMITATIONS OF THE STUDY

Despite the bicentric inclusions on a one year period, only eight patients with NSTEMI and hs-cTnI below the 99th percentile at presentation were included. To show a significant difference between subjects with NSTEMI and those who do not have NSTEMI with an expected difference of 15 pmol/L, as in the first study of Reichlin et al,[9] the number of NSTEMI subjects needed was 40 patients. We were not able to include the expected number of patients within the time allowed by the design of the study and its permissions. Thus, the area under the ROC curve (AUC) and the net reclassification index could not be calculated.

We did not assess the pre-test probability, which could increase the relevance of the biomarker in certain patient populations. However, there are no validated score to determine the clinical probability of ACS.

This study was conducted in France, with a prehospital system of medicalization. Patients supported upstream of the hospital for a very suspicious chest pain, even without ST-elevation, could be directly admitted to the cardiology department to perform immediate

exploration, forming an incorporation bias. Probably, the results of this study could probably not be extrapolated to ED collaborating with other prehospital supports.
Twelve hours after admission, there was no significant difference between the two groups (NSTEMI vs non-NSTEMI) for myoglobin and CK. This may be due to the low infarcts size observed (hs-cTnI < 99th percentile at admission in the 6 hours after the pain onset) but also to the lack of 12-hour blood samples for two NSTEMI patients.

DISCUSSION

Despite its limitations, our study complements the results of previously published data. In this prospective study, we used the latest generation of troponin I and copeptin assays. We have developed the protocol in a logical form. According to previous studies, copeptin can add a diagnostic value if there is not ST-elevation and if troponin at admission is less than a threshold. Thus, we focused the study for this category of patients to reduce spectrum bias. Knowing that only 14 minutes are needed to get a copeptin-us result, this analyse could be requested or performed automatically when the troponin is below the threshold, in a rational use of resources.
Although the copeptin NPV was 90.9% in our study, if NSTEMI diagnosis had been ruled out only regarding copeptin value at admission, seven of eight NSTEMI patients would have returned home without care. These results are consistent with COPED-MIRRO study which had a similar design but mostly used a 4th generation troponin.[33]
We identified the other studies assessing the copeptin diagnostic accuracy that used a high sensitive troponin. If we analyse the subgroups of patients similarly to our study, most results are equivalent to ours. Thus, in the latest study published, Sukul et al report that copeptin did not identify any additional patient with AMI in initial troponin-negative patients.[35] Also, the CHOPIN study, with 1967 patients analysed, had recruited 19 NSTEMI patients with a negative troponin. In this group, copeptin added to troponin testing at admission did not identify 9 NSTEMI patients (sensitivity 53%).[27] In the ROMICAT study, which did not separated the unstable angina from the NSTEMI in their analysis, as well as in the RATPAC and APACE trials, the authors report that copeptin did not provide additional significant diagnostic value to the high sensitivity troponin.[19,32,34] Charpentier and colleagues reports that the sensitivity and diagnostic accuracy were not acceptable for use in clinical practice.[28] Moreover, for the patients from the FAST II and FASTER I studies, copeptin does not detect 18 of the 27 NSTEMI patients with troponin below the 99th percentile (sensitivity = 33% in this subgroup). Bahrmann et al and Lotze et al found a negative predictive value of 100%, but each of these studies included only one NSTEMI patient with hs-cTn below the cut-off defined.[14,25] Thelin et al found a significant difference between sensitivities of single troponin versus the combination of troponin and copeptin.[30] However, regarding published data, copeptin had identified 6 of 9 NSTEMI patients (sensitivity 67%) in patients presenting a negative troponin at admission.
The first studies analysing copeptin associated with a high sensitive troponin revealed a significant diagnostic contribution of copeptin. Meune et al included 58 patients in a cardiology department where the prevalence of coronary syndromes is more important.[12] The combination of copeptin and hs-cTnT had identified all NSTEMI patients, but the status of the hs-cTnT for these patients is unknown. Keller et al showed a slightly but significant improvement of the AUC for the subgroup of patients at the ED within 3 hours after chest pain onset, but reported data do not permit to analyse the subgroup of patients with a negative troponin.
Consequently, copeptin seems to have insufficient sensitivity for NSTEMI patients with

troponin below the 99th percentile at admission. This is probably due to important similarities between this group and patients with a diagnosis of unstable angina, in which copeptin levels have not been shown as significantly different from those of non-coronary chest pain patients in most of previous studies. The hypothesis suggested in the first study on the diagnostic value of copeptin for ACS, could be that endogenous stress caused by unstable angina could be lower than in AMI patients and could be insufficient to cause a copeptin release.[9] Moreover, the authors of the ROMICAT study, regarding their results, as they corroborated Kelly et al, suggest that copeptin is a reflection of left ventricular dysfunction and not of coronary artery status. [13,36] These assumptions are consistent with the physiologic function of AVP and could explain the results of our study.

In our study, one patient had increased troponin level above the cut-off only after six hours. Still considering the sixth hour, troponin level of one NSTEMI patients had already begun its decline and was below the threshold of the 99th percentile. This observation is consistent with the precautionary statements of the Study Group on Biomarkers in Cardiology of the European Society of Cardiology Working Group on Acute Cardiac Care, advocating additional blood sampling in patients strongly suspected of having an AMI but no significant hs-cTn increase after 3 h.[37]

A recent study suggest that undetectable Roche high sensitive cardiac troponin T at admission could be considered to rule out AMI patients.[38] This algorithm could not be envisaged in our study population and the hs-cTnI used, 3 NSTEMI patients had hs-cTnI undetectable at admission.

Finally, the only subject who died is the patient who had the highest value of copeptin, wich is consistent with highlight the of studies showing a prognostic role for copeptin.[19,25,27,29]

In conclusion, our study did not show a relevant diagnostic value for copeptin in patients with suspected ACS without ST-elevation and with hs-cTnI below the 99th percentile at admission. Measurements of hs-cTn at presentation and after 3 h, and after 6 h if necessary, remains the biochemical gold standard for NSTEMI diagnosis.[1,37] Using a novel marker for NSTEMI diagnosis, alone or in a multimarker strategy, requires at least to have as good sensitivity and negative predictive value as serial troponin testing.

Table and Figure Legends

Figure 1. Flow chart

Table 1. Baseline characteristics

Figures 2 to 5. Box plots (median, interquartile range, minimal and maximal values) illustrate Troponin, Copeptin, myoglobin and CK concentration in relation to time since admission for NSTEMI and non-NSTEMI patients. * $p<0.0001$, ** $p=0.01$, *** $p=0.04$, **** $p=0.03$.

Author contributions :

JD, JS, GM, VS, NC, PM conceived the study, designed the trial.
JD undertook recruitment of participating centers and patients, managed the data, supervised the conduct of the trial, and drafted the manuscript.
SU provided statistical advice on study design and analysed the data.
SM has made monitoring and carried out biochemical assays.
LC, ND, NC JS, GM, VS, PM, LD and JD were the expert committee to adjudicate the final diagnosis.
ND, LC, SU contributed substantially to the revision of the manuscript.
JD takes responsibility for the paper as a whole.

Data Sharing Statement :

No additional data available.

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Conflicts of interest :

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References

1 Thygesen K, Alpert JS, Jaffe AS, *et al.* Third universal definition of myocardial infarction. 2012. 2551–67. doi:10.1093/eurheartj/ehs184

2 Hamm CW, Bassand J-P, Agewall S, *et al.* ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). European heart journal. 2011;**32**:2999–3054. doi:10.1093/eurheartj/ehr236

3 Lin S, Yokoyama H, Rac VE, *et al.* Novel biomarkers in diagnosing cardiac ischemia in the emergency department: a systematic review. *Resuscitation* 2012;**83**:684–91. doi:10.1016/j.resuscitation.2011.12.015

4 Lippi G. Biomarkers of myocardial ischemia in the emergency room: cardiospecific troponin and beyond. *European Journal of Internal Medicine* 2013;**24**:97–9. doi:10.1016/j.ejim.2012.10.012

5 Morgenthaler N, Struck J, Jochberger S. Copeptin: clinical use of a new biomarker. *Trends in Endocrinology & Metabolism* 2008;**19**:43–9.

6 Morgenthaler NG, Struck J, Alonso C, *et al.* Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clinical Chemistry* 2006;**52**:112–9. doi:10.1373/clinchem.2005.060038

7 Nickel CH, Bingisser R, Morgenthaler NG. The role of copeptin as a diagnostic and prognostic biomarker for risk stratification in the emergency department. *BMC Med* 2012;**10**:7. doi:10.1186/1741-7015-10-7

8 Lippi G, Plebani M, Di Somma S, *et al.* Considerations for early acute myocardial infarction rule-out for emergency department chest pain patients: the case of copeptin. *Clin Chem Lab Med* 2012;**50**:243–53. doi:10.1515/cclm-2011-0845

9 Reichlin T, Hochholzer W, Stelzig C, *et al.* Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol* 2009;**54**:60–8. doi:10.1016/j.jacc.2009.01.076

10 Keller T, Tzikas S, Zeller T, *et al.* Copeptin improves early diagnosis of acute myocardial infarction. *J Am Coll Cardiol* 2010;**55**:2096–106. doi:10.1016/j.jacc.2010.01.029

11 Chenevier-Gobeaux C, Freund Y, Claessens Y-E, *et al.* Copeptin for rapid rule out of acute myocardial infarction in emergency department. *Int J Cardiol* 2013;**166**:198–204. doi:10.1016/j.ijcard.2011.10.098

12 Meune C, Zuily S, Wahbi K, *et al.* Combination of copeptin and high-sensitivity cardiac troponin T assay in unstable angina and non-ST-segment elevation myocardial infarction: a pilot study. *Arch Cardiovasc Dis* 2011;**104**:4–10. doi:10.1016/j.acvd.2010.11.002

13 Karakas M, Januzzi JL, Meyer J, *et al.* Copeptin does not add diagnostic information to high-sensitivity troponin T in low- to intermediate-risk patients with acute chest pain: results from the rule out myocardial infarction by computed tomography (ROMICAT) study. *Clinical Chemistry* 2011;**57**:1137–45. doi:10.1373/clinchem.2010.160192

14 Lotze U, Lemm H, Heyer A, *et al.* Combined determination of highly sensitive troponin T and copeptin for early exclusion of acute myocardial infarction: first experience in an emergency department of a general hospital. *Vasc Health Risk Manag* 2011;**7**:509–15.

doi:10.2147/VHRM.S21753

15 Giavarina D, Carta M, Fortunato A, *et al.* Copeptin and high sensitive troponin for a rapid rule out of acute myocardial infarction? *Clin Lab* 2011;**57**:725–30.

16 Giannitsis E, Kehayova T, Vafaie M, *et al.* Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. *Clinical Chemistry* 2011;**57**:1452–5. doi:10.1373/clinchem.2010.161265

17 Freund Y, Chenevier-Gobeaux C, Leumani F, *et al.* Concomitant Measurement of Copeptin and High Sensitivity Troponin for Fast and Reliable Rule Out of Acute Myocardial Infarction. *Ann Emerg Med* 2011;**58**:S190. doi:10.1016/j.annemergmed.2011.06.061

18 Dupuy A-M, Chastang E, Cristol J-P, *et al.* Analytical performances of the newly developed, fully automated Kryptor Copeptin assay: which impact factor for myocardial infarction rules out in the emergency department? *Clin Lab* 2012;**58**:635–44.

19 Potocki M, Reichlin T, Thalmann S, *et al.* 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. *Heart* 2012;**98**:558–65. doi:10.1136/heartjnl-2011-301269

20 Ray P, Charpentier S, Chenevier-Gobeaux C, *et al.* Combined copeptin and troponin to rule out myocardial infarction in patients with chest pain and a history of coronary artery disease. *Am J Emerg Med* 2012;**30**:440–8. doi:10.1016/j.ajem.2011.12.008

21 Charpentier S, Maupas-Schwalm F, Cournot M, *et al.* Combination of copeptin and

troponin assays to rapidly rule out non-ST elevation myocardial infarction in the emergency department. *Acad Emerg Med* 2012;**19**:517–24. doi:10.1111/j.1553-2712.2012.01350.x

22 Eggers KM, Venge P, Lindahl B. High-sensitive cardiac troponin T outperforms novel diagnostic biomarkers in patients with acute chest pain. *Clin Chim Acta* 2012;**413**:1135–40. doi:10.1016/j.cca.2012.03.011

23 Folli C, Consonni D, Spessot M, *et al.* Diagnostic role of copeptin in patients presenting with chest pain in the emergency room. *European Journal of Internal Medicine* 2013;**24**:189–93. doi:10.1016/j.ejim.2012.09.006

24 Dedic A, Kate Ten G-J, Rood PPM, *et al.* Copeptin in acute chest pain: identification of acute coronary syndrome and obstructive coronary artery disease on coronary CT angiography. *Emergency Medicine Journal* Published Online First: 8 November 2012. doi:10.1136/emermed-2012-201596

25 Bahrmann P, Bahrmann A, Breithardt O-A, *et al.* Additional diagnostic and prognostic value of copeptin ultra-sensitive for diagnosis of non-ST-elevation myocardial infarction in older patients presenting to the emergency department. *Clin Chem Lab Med* 2013;**51**:1307–19. doi:10.1515/cclm-2012-0401

26 Ledochowski S, Fayet JM, Collin-Chavagnac D, *et al.* Évaluation de la copeptine dans les syndromes coronaires aigus non ST. *Ann Fr Med Urgence* 2013;:138–44. doi:10.1007/s13341-013-0303-4

27 Maisel A, Mueller C, Neath S-X, *et al.* Copeptin helps in the early detection of patients with acute myocardial infarction: primary results of the CHOPIN trial (Copeptin Helps in the early detection Of Patients with acute myocardial INfarction). *J Am Coll Cardiol*

2013;**62**:150–60. doi:10.1016/j.jacc.2013.04.011

28 Charpentier S, Lepage B, Maupas-Schwalm F, *et al.* Copeptin improves the diagnostic performance of sensitive troponin I-Ultra but cannot rapidly rule out non-ST-elevation myocardial infarction at presentation to an emergency department. *Ann Emerg Med* 2013;**61**:549–558.e1. doi:10.1016/j.annemergmed.2012.12.018

29 Afzali D, Erren M, Pavenstädt H-J, *et al.* Impact of copeptin on diagnosis, risk stratification, and intermediate-term prognosis of acute coronary syndromes. *Clin Res Cardiol* 2013;**102**:755–63. doi:10.1007/s00392-013-0583-0

30 Thelin J, Borna C, Erlinge D, *et al.* The combination of high sensitivity troponin T and copeptin facilitates early rule-out of ACS: a prospective observational study. *BMC Cardiovasc Disord* 2013;**13**:42. doi:10.1186/1471-2261-13-42

31 Sebbane M, Lefebvre S, Kuster N, *et al.* Early rule out of acute myocardial infarction in ED patients: value of combined high-sensitivity cardiac troponin T and ultrasensitive copeptin assays at admission. *Am J Emerg Med* 2013;**31**:1302–8. doi:10.1016/j.ajem.2013.04.033

32 Balmelli C, Meune C, Twerenbold R, *et al.* Comparison of the performances of cardiac troponins, including sensitive assays, and copeptin in the diagnostic of acute myocardial infarction and long-term prognosis between women and men. *Am Heart J* 2013;**166**:30–7. doi:10.1016/j.ahj.2013.03.014

33 Llorens P, Sánchez M, Herrero P, *et al.* The utility of copeptin in the emergency department for non-ST-elevation myocardial infarction rapid rule out: COPED-MIRRO study. *Eur J Emerg Med* Published Online First: 17 July 2013. doi:10.1097/MEJ.0b013e3283632f8b

34 Collinson P, Gaze D, Goodacre S. Comparison of contemporary troponin assays with the novel biomarkers, heart fatty acid binding protein and copeptin, for the early confirmation or exclusion of myocardial infarction in patients presenting to the emergency department with chest pain. *Heart* 2014;**100**:140–5. doi:10.1136/heartjnl-2013-304716

35 Sukul D, Bonaca MP, Ruff CT, *et al.* Diagnostic Performance of Copeptin in Patients With Acute Nontraumatic Chest Pain: BWH-TIMI ED Chest Pain Study. *Clin Cardiol* Published Online First: 22 January 2014. doi:10.1002/clc.22244

36 Kelly D, Squire IB, Khan SQ, *et al.* C-terminal provasopressin (copeptin) is associated with left ventricular dysfunction, remodeling, and clinical heart failure in survivors of myocardial infarction. *Journal of cardiac failure* 2008;**14**:739–45. doi:10.1016/j.cardfail.2008.07.231

37 Thygesen K, Mair J, Giannitsis E, *et al.* How to use high-sensitivity cardiac troponins in acute cardiac care. *European heart journal* 2012;**33**:2252–7. doi:10.1093/eurheartj/ehs154

38 Body R, Carley S, McDowell G, *et al.* Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a high-sensitivity assay. *J Am Coll Cardiol* 2011;**58**:1332–9. doi:10.1016/j.jacc.2011.06.026

Diagnostic accuracy of Copeptin sensitivity and specificity in patients with suspected non–ST-elevation myocardial infarction with troponin I below the 99th percentile at presentation.

ABSTRACT

Objective

To determine if copeptin-us can rule out diagnosis of non–ST-segment elevation myocardial infarction (NSTEMI) ~~acute myocardial infarction~~ without prolonged monitoring and serial blood sampling in patients with ~~suspected non-ST-segment elevation myocardial infarction (NSTEMI) and~~ high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to the emergency department (ED).

Design

Prospective, non-randomized, individual blinded diagnostic accuracy study.

Setting

Two ED of a rural region of France.

Participants

Patient with a chest pain suspected of NSTEMI with onset within the last 12 h were considered for enrolment.

Interventions

Serial clinical, electrographical and biochemical investigations were performed at admission and after 2, 4, 6 and 12 h. Hs-cTnI was mesured using an assay with Dimension VISTA, Siemens. Copeptin was measured by the B.R.A.H.M.S copeptin-us assay on the KRYPTOR Compact Plus system. The follow-up was 90 days.

Primary and secondary outcome measures

Copeptin, troponin, myoglobin and creatin kinase values. Clinicals and paraclinicals events. The final diagnosis was adjudicated blinded to copeptin result.

Results

During 12 months, 102 patients were analysed. Final diagnosis was NSTEMI for 7.8% (n=8), unstable angina ~~pectoris~~ for 3.9% (n=4), cardiac but non coronary artery disease for 8.8% (n=9), non-cardiac chest pain for 52% (n=53) and unknown for 27.5% (n=28). There was no statistical difference for copeptin values between ~~acute myocardial infarction (AMI)~~NSTEMI patients and ~~non AMI patients,~~ others (respectively 5.5 pmol/L IQR[3.1-7.9] and 6.5 pmol/L IQR[3.9-12.1], p=0.49)~~13~~. Only one ~~AMI~~ NSTEMI patient ~~have~~ had a copeptin value at admission above the cut off of 95th percentile at admission.

Conclusions

In this study, copeptin ~~show no added value for the diagnostic~~ does not add a diagnostic value at admission to ED for suspected acute coronary syndrome patients without ST-segment elevation and with hs-cTnI below the 99th percentile.

Trial registration

Clinicaltrials.gov identifier: NCT01334645.

STRENGTHS AND LIMITATIONS OF THIS STUDY

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~~- We were not able to include the expected number of patients.~~

- To our knowledge, o~~O~~ur prospective multicentric study is the only one that includes only patients with suspected NSTEMI and high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to ED, to limit ~~bias~~ spectrum bias.

- The main limitation of our study is the number of patients included. Indeed we are below the prevalence. This may be explained by the fact that our study included only patients with negative ultrasensitive troponin at admission. However, this is the only group of patients for which a multimarker rule-out strategy could add diagnostic value.

- Moreover, we evaluated the sensitivity troponin and copeptin for all patients with the same assay technique which enabled to control the occurrence of methodological bias.

~~- Despite the fact that we have not included the expected number of patients, if the required number of patients have been included to achieve 80% power (40 AMI patients), assuming that copeptin was positive for all other AMI patients, 7 of 40 AMI patients were ruled out. This risk seems too high, knowing that there is a more reliable method : troponin serial testing.~~

MANUSCRIPT

INTRODUCTION

Detection of a rise and/or fall of cardiac troponin with clinical symptoms of ischemia or abnormal electrocardiography (ECG) or imaging findings remains the gold standard for the identification of myocardial infarction.[1]~~[1]~~ At Emergency Department, patients with non–ST-segment elevation myocardial infarction (NSTEMI) working diagnosis requires serial measurement of troponin.[2]~~[2]~~ However, most of ~~this~~ these patients do not have acute coronary syndrome (ACS). Identify patients suffering from non-life-threatening diseases with only one blood sample is a challenge. Many biomarkers were evaluated, alone or in combination with troponin.~~[3,4]~~[3,4] Copeptin accuracy was explored recently in this rule out diagnostic strategy. This glycopeptide, who is the C-terminal part of the arginine vasopressin (AVP) precursor, is secreted stoichiometrically with AVP from the neurohypophysis. AVP is a marker of endogenous stress but routine measurement of AVP is limited due by its instability and difficulty of the assay.[5] Copeptin now appears to be an attractive alternative to AVP, because of its stability and development of automated technique for reliable and reproducible dosage.[6-8]

Since the first publication in~~for~~ this indication in 2009, several studies have investigated copeptin~~, a surrogate marker of vasopressin~~.~~[9-35]~~[5-30]~~ Some of these studies suggest that the association of troponin and copeptin at the first measurement has a powerfull negative predictive value (NPV) to rule out patients with~~without~~ ~~no~~ NSTEMI.

Interpretation of the copeptin diagnostic accuracy ~~with~~ through these studies is not evident. First, because analysis comparisons are ~~difficult due to~~disrupted by the development of high-sensitivity cardiac troponin T and I assays and the availability of three commercial assays for copeptin (LUMItest®, Copeptin Kryptor®, Copeptin-us Kryptor®). Furthermore, many protocols included ST-segment elevation myocardial infarction (STEMI) patients and patients with a high-sensitive cardiac troponin above 99th percentile at admission. For these patients,

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copeptin does not add diagnostic information, urgent revascularisation or serial blood samples, respectively, remains necessary.
The aim of this study was to determine if copeptin-us can rule out diagnosis of acute myocardial infarction without prolonged monitoring and serial blood sampling in patients with suspected NSTEMI and high sensitive cardiac troponin I (hs-cTnI) below the 99th percentile at presentation to ED.

METHODS

Study design and setting

This diagnostic test evaluation is a prospective non-randomized individual blinded multicentric cohort study. The Clermont-Ferrand University Hospital designed and coordinated the study. The duration of the study was one year, between march 2011 and march 2012 at the ED of two hospitals of Auvergne, a rural region of France (1.3 million people). First one, Gabriel Montpied in Clermont-Ferrand, is a teaching hospital and provincial referral center with 48000 ED admissions per year. The second hospital, Henri Mondor in Aurillac, is a general hospital with 25000 ED admissions per year. EachBoth units ~~are organised with a 24-hour~~had a catheterization laboratory available 24 hours a day. The study complied with the Declaration of Helsinki and was approved by the ethical committee Comité de Protection des Ppersonnes Sud-Est VI (AU 871). Before study launch, methods were registered with ClinicalTrials.gov (NCT01334645).

Population

Consecutive patients admitted with a chest pain suspected of NSTEMI inat emergency department were considered for enrolment in the study. The inclusion criteria werewas the following : patients older than 18 years with chest pain suggestive of ACS of < 12 hours' duration since his onset~~with onset within the last 12 hours~~. Atypical presentations of NSTEMI are not uncommon,[2] therefore the criteria for a pain suggestive of ACS were those of usual clinical practice of investigators. It should be non-traumatic. Written informed consent was obtained from all participating patients. ~~All patients provided written informed consent before enrolment.~~ Patients with ST-segment elevation, legal incapacity, sepsis, shock, lung neoplasms, terminal kidney failure requiring dialysis, life expectancy of less than 6 months ~~and refuse to consent~~ were excluded. After the result of the first blood sample, patients with hyponatremia < 135 mmol/L or hs-cTnI > 0,045 μ g/L were released of the study.

ST-segment elevation, measured at the J point, was diagnosed according to the third universal definition of myocardial infarction (MI),[1] It should be found in two contiguous leads with the cut-points: ≥ 0.1 mV in all leads other than leads V2–V3 where the following cut points apply: ≥ 0.2 mV in men ≥ 40 years; ≥ 0.25 mV in men < 40 years, or ≥ 0.15 mV in women.

Sepsis, shock, lung neoplasms, terminal kidney failure requiring dialysis and hyponatremia are diseases in which the rate of vasopressin, and thus of copeptin, may be modified. These patients were not included to minimize confounding factors.

Study protocol

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Upon admission, all patient underwent an initial clinical assessment, including medical history, temperature, respiratory rate, cardiac frequency, blood pressure, pulse oxymetry, 18-lead ECG, chest X-ray and screening blood test including : C-reactive protein, natremia, creatinine, hs-cTnI and creatin kinase (CK). Risk factors and previous medical history were collected as stated by the patient and its treatment. Family history of coronary artery disease (CAD) were noted if a member of the first-degree relatives had coronary artery disease before 65 years. Blood sampling were collected for hs-cTnI and CK analysis and 18-lead ECG were performed after 2, 4, 6 and 12 h. At each time point, blood sample was centrifuged and plasma was frozen at -80 °C for copeptin and myoglobin testing at the end of the study recruitment, blinded to final diagnosis. Further investigations and treatment of patients were not modified by the study. At 90 days, clinical and paraclinical events were collected from the patients, theirs general practitioners and the hospitals where they were explored.

Concentration of copeptin was measured by the B.R.A.H.M.S copeptin-us immunoluminometric assay on the KRYPTOR Compact Plus system (Thermo Fisher Scientific). The detection limit as described by the manufacturer was assessed signified as being 0.9 pmol/L and the lowest concentration measurable with a coefficient of variation (CV) < 10% has been reported < 4 pmol/L. Direct measuring range was 0.9 to 500 pmol/L. The 95th percentile among healthy subjects is < 12.0 pmol/L and was specified for rapid exclusion of acute myocardial infarction (AMI).

Hs-cTnI was measured using a chemiluminescence test (Dimension VISTA®, Siemens Healthcare Diagnostics). The limit of blank of hs-cTnI was 0.015 μ g/L, the 99th percentile concentration was 0.045 μ g/L and the lowest concentration measurable with a CV < 10% was 0.040 μ g/L according to the manufacturer. The 99th percentile (0.045 μ g/L) was used as diagnostic cut-off to fulfil AMI criteria.

Myoglobin was measured by Dimension VISTA® (Siemens Healthcare Diagnostics). The measuring range extended from 0.5 to 1000 µng/mL. The 95th percentile concentration was 116 µng/mL for men and 71 µng/mL for women. At concentrations of 110 µg/L, the inter-assay CV was 4.9% and the intra-assay CV was 5%.

Natremia, C-reactive protein, creatinin and CK, were measured using standardized methods.

Outcomes

The final diagnosis was adjudicated, blinded to copeptin results, by an expert committee of three cardiologists, four emergency physicians and two biochemists (whose one MD-PhD of each specialty), with all available medical records from the time of ED presentation to 90-day follow-up. The diagnosis was determined according to the current guidelines and universal definition of myocardial infarction.[1,2]

Each subject was classified in the following categories Each subjects was classified as one of the following categories : Non-ST Elevation Myocardial Infarction (NSTEMI), Unstable angina pectoris (UA), Cardiac but non coronary artery disease (CNCAD), non-cardiac chest pain (NCCP) and unknown cause of chest pain. The diagnosis was determined according to the current guidelines and universal definition of myocardial infarction.[1,2] NCCP were performed if a cardiac aetiology was exclude. The diagnosis of NSTEMI, in these patients showing suspected symptoms of ACS, was defined by a rise and/or fall of hs-cTnI with at least one value above the 99th percentile and with the following criteria : imaging evidence of new loss of viable myocardium or new regional wall motion abnormality or identification of an intracoronary thrombus by angiography. The defining criteria unstable angina were the same as those defining the NSTEMI, without elevation of troponin. Diagnosis of CNCAD was performed if a coronary artery disease was exclude by additional testing. Diagnosis of NCCP was performed if a cardiac aetiology was exclude. NCCP were performed if a cardiac

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~~aetiology was exclude.~~Unknown cause of chest pain diagnosis was defined when no sufficient further diagnostic procedures were performed.
Copeptin and myoglobin measurements were performed at the end of the study recruitment, blinded to the final diagnosis.

Statistical analysis

In order ~~T~~to show a different copeptin value ~~of copeptin~~ between ~~AMI~~ NSTEMI ~~subjects~~ and non NSTEMI~~non AMI~~ subjects, with an expected difference of 15 pmol/L, a standard deviation of 20.7 pmol/L, a significance level of 5% and a power of 95%, ~~the number of~~ 40 ~~AMI~~ NSTEMI subjects were needed ~~was 40 patients~~.
Continuous variables were displayed either as means ± SD or medians and interquartile range (IQR). Categorical variables were described by using frequencies and percentages.
The analysis of quantitative variables was performed using the two-tailed Student's t-test after checking the assumption of equal variances (Levene test) and one way analysis of variance for variables following a normal distribution. Otherwise, the Wilcoxon rank sum tests for continuous variables and Kruskal-Wallis tests were used. Categorical variables were analysed using Chi-square analysis or the Fisher exact test (if needed). For all tests, a significance level of $p<0.05$ was used.
Statistical analysis was performed using SAS (v 9.3, SAS Institute Inc., Cary, NC, USA).

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RESULTS

Patient characteristics

During 12 months, 147 patients were assessed for eligibility in both ED. Nine presented one or more exclusion criteria, six did not give their informed consent for participation, 26 were released after the results of the first blood sample because they had hyponatremia < 135 mmol/L (n=3) or hs-cTnI > 0,045 μ g/L (n=23). For three patients, blood samples at presentation were not frozen for copeptin and myoglobin measurement. Only one patient was lost of follow-up. A total of 102 patients were analysed, 62 were recruited at the Clermont-Ferrand university hospital ED and 40 at the Aurillac general hospital ED (Figure 1).
The adjudicated final diagnosis was NSTEMI for 7.8% (n=8), UA for 3.9% (n=4), CNCAD 8.8% (n=9), NCCP for 52% (n=53) and unknown for 27.5% (n=28).
CNCAD included pericarditis (3), supraventricular tachycardia (3), ventricular tachycardia (2) and left hypertrophy (1). Patients with adjudicated diagnosis NCCP included patient with anxiety (3), stomach disease (4), herpes zoster (1), neoplasms (4), breast hematoma (1), cholecystitis (1) vasovagal syncope (1) and osteoarthritis (2).
Baseline characteristics of each population are shown in Table 1.

Table 1. Baseline characteristics

Characteristics	All patients	~~AMI~~ (NSTEMI)	Non-~~AMI~~NSTEMI	p Value
Patients, n (%)	102 (100)	8 (7.8)	94 (92.2)	
Men, n (%)	64 (62.7)	7 (87.5)	57 (55.9)	0.25~~25~~
Age (years), mean (SD)	59.~~47 ±~~ (16)~~.05~~	6~~65.75 ±~~ (16)~~.04~~	5~~98.94 (±~~ 16)~~.02~~	0.25~~09~~
Risk factors				
Body Mass Index, ~~(~~kg/m^2 (SD)~~)~~	26.93~~ ±~~ (4.9)	27.1~~ ±~~ (3.7)	26.9~~ ±~~ (5.~~0~~)	0.94~~16~~
Family history of CAD, n (%)	33 (32.3)	3 (37.5)	30 (31.9)	0.71~~14~~

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Hypertension, n (%)	49 (48)	5 (62.5)	44 (46.8)	0.48760
Hyperlipidemia, n (%)	51 (50)	4 (50)	47 (50)	1.0
Diabetes mellitus, n (%)	17 (16.7)	1 (12.5)	16 (17)	1.0
Current smoking, n (%)	26 (25.5)	5 (62.5)	21 (22.3)	0.0243
History of smoking, n (%)	30 (29.4)	1 (12.5)	29 (31.1)	0.4302
History, n (%)				
CAD	35 (34.3)	4 (50)	31 (33)	0.4410
Previous myocardial infarction	27 (26.5)	4 (50)	23 (24.5)	0.2031
Previous revascularization	26 (25.5)	3 (37.5)	23 (24.5)	0.42168
History of heart failure	5 (4.9)	0	5 (5.3)	1.0
Peripheral artery disease	6 (5.9)	2 (25)	4 (4.3)	0.07692
Previous stroke	6 (5.9)	1 (12.5)	5 (5.3)	0.43953
Clinical status				
Heart rate, (beats/min (SD))	77 (±17)	81 (±18)	77 (±17)	0.54970
Systolic blood pressure, (mmHg (SD))	141 (±22)	149 (±28)	140 (±21)	0.29888
Diastolic blood pressure, (mmHg (SD))	83 (±15)	92 (±13)	82 (±14)	0.06576
Respiratory rate, respiratory cycles/min (SD)	17 (±4)	16 (±5)	17 (±4)	0.53270
Temperature, (°C (SD))	36.7 (±0.5)	36.9 (±0.2)	36.7 (±0.5)	0.16597
Killip class 1, n (%)	97 (95)	8 (100)	89 (94.7)	1.0
Killip class 2, n (%)	5 (5)	0	5 (5.3)	1.0
Time between pain onset and admission, (h:min (SD))	3:48 (± 2:50)	2:27 (± 1:39)	3:55 (± 2:53)	0.1632
Biochemical values at admission				
Natremia, (mmol/L (SD))	140.3 (± 2.9)	137.4 (± 2.3)	140.5 (± 2.8)	0.0022
Creatinin, (μ mol/L (SD))	80.4 (± 17.5)	82.3 (± 18.7)	80.2 (± 17.5)	0.7508
MDRD, (mL/min/1.73 m² (SD))	85.2 (± 23.5)	84.1 (± 19.1)	85.3 (± 23.9)	0.98950
CRP, (mg/L (SD))	4.9 (±7.7)	4.6 (±6.1)	4.9 (±7.8)	0.9323
Electrocardiographic findings at admission				
Normal, n (%)	43 (42.1)	1 (12.5)	42 (44.7)	0.1339
Left bundle branch block, n (%)	0	0	0	
ST segment elevation, n (%)	0	0	0	
ST segment depression, n (%)	9 (8.82)	2 (25)	7 (7.5)	0.15468
T wave inversion, n (%)	20 (19.6)	3 (37.5)	17 (18.1)	0.19874
No significant abnormalities, n (%)	30 (29.4)	2 (25)	28 (29.8)	1.0
Risk scores				
GRACE, score (SD)	96 (±31)	107.8 (± 25.4)	95.6 (± 31.3)	0.29897
TIMI 0, n (%)	29 (28.4)	2 (25)	28 (29.8)	1.0
TIMI 1, n (%)	26 (25.5)	0	26 (27.6)	0.1103
TIMI 2, n (%)	14 (13.7)	2 (25)	12 (12.8)	0.3016
TIMI 3, n (%)	21 (20.6)	1 (12.5)	20 (21.3)	1.0
TIMI 4, n (%)	9 (8.8)	3 (37.5)	6 (6.4)	0.0213
TIMI 5, n (%)	2 (2)	0	2 (2.1)	1.0
Explorations				
Echocardiography, n (%)	61 (59.8)	7 (87.5)	54 (57.4)	0.14392
Cardiac exercice test, n (%)	47 (46)	0	47 (50)	0.007
Coronary angiography, n (%)	19 (18.6)	7 (87.5)	12 (12.8)	<0.0001

Values are presented as n (%) or mean +/- SD

CAD, Coronary Artery Disease; CRP, C-reactive protein; *GRACE*, Global Registery of Acute Cardiac Events; *TIMI*, Thrombosis In Myocardial Infarction.

Time between pain onset and admission was less than 3 hours for 58 patients (56.9%). Twenty-four patients were admitted between 3 and 6 h after the onset of pain (23.5%), 13 patients between 6 and 9 h (12.7%) and 7 patients between 9 and 12 h (6.9%). All patients

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with a diagnosis of myocardial infarction were admitted within the first 6 hours after the chest pain onset, five of them in the first 3 hours. The mean interval between chest pain onset and admission is 147,5 min ± 99 min for NSTEMI patients and 235 min ± 173 min for patients without ~~AMI~~ NSTEMI (*p*=0.16~~32~~).

Main results :

Serial blood testing :

At admission, all patients were recruited for blood testing. Because of therapeutic necessities after inclusion, 3 ~~AMI~~ NSTEMI patients did not have all required blood sampling. Thus, data of the 8 ~~AMI~~ NSTEMI patients are available at H0, data of 7 ~~AMI~~ NSTEMI patients are available at H2, H4 and H6, and data of 6 ~~AMI~~ NSTEMI patients at H12. Results of biomarkers are displayed in Figures 2 to 5.

Troponin

According to the inclusion criteria, all patients had hs cTnI ≤ 99th percentile at admission. ~~The median hs cTnI value was significantly higher in patients with NSTEMI diagnosis than in patients with other diagnosis, respectively 0.021 µ g/L IQR[0.015-0.04] vs 0.015 µ g/L IQR[0.015-0.015], *p*<0.0001. In the five NSTEMI patients who were admitted within 3 hours after the onset of pain median troponin was 0.015 µ g/L IQR[0.015-0.023] and 0.040 µ g/L IQR[0.018-0.045] for the 3 NSTEMI patients who consulted between 3 and 6 hours after the onset of pain (*p*=0.2090).~~

Troponin is the only marker studied for which showed a significant difference between the two groups for each time performed (0, 2, 4, 6 and 12 h), including at admission.

Copeptin

~~The m~~Median copeptin levels for ~~AMI~~ NSTEMI and ~~non AMI patients~~the others patients at admission were ~~was~~ respectively 5.5 pmol/L IQR[3.1-7.9] and 6.5 pmol/L IQR[3.9-12.1], *p*=0.49~~13~~. Only one ~~AMI~~ NSTEMI patient showed a copeptin value at admission above the cut off of 12 pmol/L (435.2 pmol/L). This patient, who had a GRACE score of 151, was also the only patient who died during the follow up. For all of the samples recruited during the 12 h following admission (2, 4, 6 and 12 h) there was no significant difference in the copeptin values between patients with ~~AMI~~ NSTEMI and those with no ~~AMI~~NSTEMI, respectively 5.9 pmol/L IQR[3.1-8.3] and 5.5 pmol/L IQR[3.5-10] at 2 h (*p*=0.86~~17~~), 4.7 pmol/L IQR[2.9-8.4] and 5.4 pmol/L IQR[3.7-9.3] at 4 h (*p*=0.74~~30~~), 5.9 pmol/L IQR[2.5-6.9] and 5.6 pmol/L IQR[3.5-8.8] at 6 h (*P*=0.77~~695~~) and 3.9 pmol/L IQR[2.8-10.2] and 6.1 pmol/L IQR[4-9.7] at 12 h (*p*=0.49~~872~~).

Myoglobin

At admission, the median myoglobin for ~~AMI~~ NSTEMI patients was 52.1 µ~~n~~g/~~m~~L IQR[41.1-66.1] and 47.3 µ~~n~~g/~~m~~L IQR[38-66.6] for patients with other diagnostics, *p*=0.71~~060~~. At 2, 4 and 6 h, median myoglobin was significantly higher in ~~AMI~~ NSTEMI patients than in patients with other diagnosis, respectively 72.9 µ~~n~~g/~~m~~L and 48.6 µ~~n~~g/~~m~~L (*p*=0.01~~2~~), 102 µ~~n~~g/~~m~~L and 47.8 µ~~n~~g/~~m~~L (*p*=0.04~~22~~), 107.5 µ~~n~~g/~~m~~L and 49.5 µ~~n~~g/~~m~~L (*p*=0.03~~1~~).

Creatin Kinase

At inclusion, m~~M~~edians CK concentrations were~~as~~ 156.5 U/L IQR[90-231.5] in ~~AMI~~ NSTEMI patients and 182 U/L IQR [105-277] in non-~~AMI~~ NSTEMI patients ~~at inclusion~~ (*p*=0.59~~882~~). At 6 h and 12 h, CK values of ~~AMI~~ NSTEMI patients were higher than those of other patients without significant difference, respectively 183 U/L and 147 U/L (*p*=0.93~~71~~), 186 U/L and 128 U/L (*p*=0.26~~554~~).

Diagnostic accuracy

For a cut-off level of 12 pmol/L, sensitivity of copeptin for ~~AMI~~ NSTEMI diagnosis at admission was 12.5%, with a specificity of 74.5%, a predictive positive value of 4% and a NPV of 90.9%. None patients had a myoglobin value above the 95th percentile at admission.

At the sixth hour, all of 8 ~~AMI~~ NSTEMI patients had at least one troponin above the 0.045 μ g/L. One patient had a troponin measured on the sample at the 6th hour already below this threshold and will continue to decrease until the twelfth hour.

LIMITATIONS OF THE STUDY

~~Although~~ Despite the bicentric inclusions ~~and carried on~~one a one year period, only eight patients with NSTEMI and hs-cTnI below the 99th percentile at presentation were included. To show a significant difference between subjects with ~~AMI~~ NSTEMI and those who do not have ~~AMI~~ NSTEMI with an expected difference of 15 pmol/L, as in the ~~princeps~~ first study of Reichlin et al,[9] the number of ~~AMI~~ NSTEMI subjects needed was~~is~~ 40 patients.~~[5]~~ We were not able to include the expected number of patients within the time allowed by the design of the study and its permissions. Thus, the area under the ROC curve (AUC) and the net reclassification index could not~~can not~~ be calculated ~~because of too few AMI patients~~.

We did not assess the pre-test probability, which could increase the relevance of the biomarker in certain patient populations. However, there are no validated score to determine the clinical probability of ACS.

This study was conducted in France, with a ~~system of~~ prehospital system of medicalization. Patients supported upstream of the hospital for a very suspicious chest pain, even without ST-elevation, could be directly admitted to the cardiology department to perform immediate exploration, forming an incorporation bias. Probably, the results of this study could~~are~~ probably not be extrapolated to ~~all~~ ED collaborating with other prehospital supports.

Twelve hours after admission, there ~~is~~ was no significant difference between the two groups (~~AMI~~ NSTEMI vs non-~~AMI~~NSTEMI) for myoglobin and CK. This may be due to the ~~fact that the population studied have~~ low infarcts size observed (hs-cTnI < 99th percentile at admission in the 6 hours after the pain onset) ~~and that~~but also~~,~~ to the lack of 12-hour blood samples for two ~~AMI~~ NSTEMI patients ~~have not been collected at the twelfth hour~~.

DISCUSSION

~~After considering the~~Despite its limitations, our study complements the results of previously published data. In this prospective study, we used the latest generation of troponin I and copeptin assays. We have developed the protocol in a logical form. According to previous studies, copeptin can add a diagnostic value if there is not ST-elevation and if troponin at admission is less than a threshold. Thus, we focused the study for this category of patients to reduce spectrum bias. Knowing that only ~~that the time to result for the copeptin-us is~~ 14 minutes are needed to get a copeptin-us result, this analyse could be requested or performed automatically when the troponin is below the threshold, in~~for~~ a rational use of resources. ~~To reduce the bias spectrum, we specifically explored the diagnosic value of copeptin only in patients with suspected ACS with non-ST segment elevation and with high sensitive cardiac troponin below the 99th percentile at admission, while most of the studies have examined all patients with suspected ACS including STEMI patients and/or patients with a troponin above the 99th percentile at admission. In these populations, the prevalence of~~

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~~AMI is higher than patient without ST elevation and troponin below the 99th percentile. Also, for this patients, urgent care or further explorations will not be influenced by the result of copeptin. Results of these previous studies may have influenced the statistical evaluation of copeptin.~~

~~In our study population, a~~Although the copeptin NPV was 90.9% in our study, if NSTEMI diagnosis had been ruled out only regarding ~~we would have ruled out patients on the results of~~ copeptin value at ~~on~~ admission, seven of ~~h~~eight ~~AMI~~ NSTEMI patients would have returned ~~at~~ home without care. These results are consistent with COPED-MIRRO study wh~~wh~~ich~~o~~ had~~have~~ a similar~~y~~ design but ~~had used~~ mostly used a 4th generation troponin.[33]~~[30]~~

We identified the other studies assessing the copeptin diagnostic accuracy that used a high sensitive troponin. If we analyse the subgroups of patients similarly to our study, most results are equivalent to ours. Thus, in the latest study published, Sukul et al report that copeptin did not identify any additional patient with AMI in initial troponin-negative patients.[35] Also, the CHOPIN study, with 1967 patients analysed, had recruited 19 NSTEMI patients with a negative troponin. In this group, copeptin added to troponin testing at admission did not identify 9 NSTEMI patients (sensitivity 53%).[27] In the ROMICAT study, which did not separated the unstable angina from the NSTEMI in their analysis, as well as in the RATPAC and APACE trials, the authors report that copeptin did not provide additional significant diagnostic value to the high sensitivity troponin.[19,32,34] Charpentier and colleagues reports that the sensitivity and diagnostic accuracy were not acceptable for use in clinical practice.[28] Moreover, for the patients from the FAST II and FASTER I studies, copeptin does not detect 18 of the 27 NSTEMI patients with troponin below the 99th percentile (sensitivity = 33% in this subgroup). Bahrmann et al and Lotze et al found a negative predictive value of 100%, but each of these studies included only one NSTEMI patient with hs-cTn below the cut-off defined.[14,25] Thelin et al found a significant difference between sensitivities of single troponin versus the combination of troponin and copeptin.[30] However, regarding published data, copeptin had identified 6 of 9 NSTEMI patients (sensitivity 67%) in patients presenting a negative troponin at admission.

The first studies analysing copeptin associated with a high sensitive troponin revealed a significant diagnostic contribution of copeptin. Meune et al included 58 patients in a cardiology department where the prevalence of coronary syndromes is more important.[12] The combination of copeptin and hs-cTnT had identified all NSTEMI patients, but the status of the hs-cTnT for these patients is unknown. Keller et al showed a slightly but significant improvement of the AUC for the subgroup of patients at the ED within 3 hours after chest pain onset, but reported data do not permit to analyse the subgroup of patients with a negative troponin.

Consequently, copeptin seems to have insufficient sensitivity for NSTEMI patients with troponin below the 99th percentile at admission. This is probably due to important similarities between this group and patients with a diagnosis of unstable angina, in which copeptin levels have not been shown as significantly different from those of non-coronary chest pain patients in most of previous studies. The hypothesis suggested in the first study on the diagnostic value of copeptin for ACS, could be that endogenous stress caused by unstable angina could be lower than in AMI patients and could be insufficient to cause a copeptin release.[9] Moreover, the authors of the ROMICAT study, regarding their results, as they corroborated Kelly et al, suggest that copeptin is a reflection of left ventricular dysfunction and not of coronary artery status. [13,36] These assumptions are consistent with the physiologic function of AVP and could explain the results of our study.

In our study, one patient had increased ~~Even pursuing the inclusion up to 40 AMI patients as we envisage to highlight a significant difference, with these seven patients, the error seems~~

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~~too important to rule out patients with suspected ACS at admission in our ED. However, only a larger study could confirm or refute this assumption.~~

~~The~~ troponin~~-~~ level above the cut-off only after six hours. ~~of one patient in our study had increased above the cut-off only at the sixth hour. Also,~~Still considering ~~at~~ the sixth hour, troponin level of one ~~of AMI~~ NSTEMI patients had already begun its decline and was below the threshold of the 99th percentile. This observation is consistent with the precautionary statements of the Study Group on Biomarkers in Cardiology of the European Society of Cardiology Working Group on Acute Cardiac Care, advocating additional blood sampling in patients strongly suspected of having an AMI but no significant hs-cTn increase after 3 h.[37]~~[31]~~

A recent study suggest that undetectable Roche high sensitive cardiac troponin T at admission ~~it~~ could be considered to rule out AMI patients ~~with undetectable Roche high sensitive cardiac troponin T at admission~~.[38]~~[32]~~ This algorithm could~~is~~ not be ~~possible~~ envisaged in~~with~~ our study population and the hs-cTnI used~~,~~ 3 NSTEMI patients had hs-cTnI undetectable at admission.

Finally, the only subject who died is the patient who had the highest value of copeptin, wich is consistent with highlight the of studies showing a prognostic role for~~of~~ copeptin. [19,25,27,29]~~[15, 21, 25, 29]~~

In ~~summary~~conclusion, our study did not ~~reveal~~ show a relevant diagnostic value for~~of~~ copeptin in~~for~~ patients with suspected ACS without ST-elevation and with hs-cTnI below the 99th percentile at admission. Measurements of hs-cTn at presentation and after 3 h, and after 6 h if necessary, remains the biochemical gold standard for ~~AMI~~ NSTEMI diagnosis.~~-~~[1,37]~~[1, 31]~~ Using a novel marker for ~~ACS~~ NSTEMI diagnosis, alone or in a multi~~-~~marker strategy, requires at least to have~~ss~~ as good sensitivity and negative predictive value ~~than as a troponin~~ serial troponin testing.

Table and Figure Legends

Figure 1. Flow chart

Table 1. Baseline characteristics

Figures 2 to 5. Box plots (median, interquartile range, minimal and maximal values) illustrate Troponin, Copeptin, myoglobin and CK concentration in relation to time since admission for ~~AMI~~ NSTEMI and non-~~AMI~~ NSTEMI patients. * p<0.0001, ** p=0.01~~2~~, *** p=0.04~~22~~, **** p=0.03~~1~~.

References

1 Thygesen K, Alpert JS, Jaffe AS, *et al.* Third universal definition of myocardial infarction. 2012. 2551–67. doi:10.1093/eurheartj/ehs184

2 Hamm CW, Bassand J-P, Agewall S, *et al.* ESC Guidelines for the management of acute

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coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). European heart journal. 2011;**32**:2999–3054. doi:10.1093/eurheartj/ehr236

3 Lin S, Yokoyama H, Rac VE, *et al.* Novel biomarkers in diagnosing cardiac ischemia in the emergency department: a systematic review. *Resuscitation* 2012;**83**:684–91. doi:10.1016/j.resuscitation.2011.12.015

4 Lippi G. Biomarkers of myocardial ischemia in the emergency room: cardiospecific troponin and beyond. *European Journal of Internal Medicine* 2013;**24**:97–9. doi:10.1016/j.ejim.2012.10.012

5 Morgenthaler N, Struck J, Jochberger S. Copeptin: clinical use of a new biomarker. *Trends in Endocrinology & Metabolism* 2008;**19**:43–9.

6 Morgenthaler NG, Struck J, Alonso C, *et al.* Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clinical Chemistry* 2006;**52**:112–9. doi:10.1373/clinchem.2005.060038

7 Nickel CH, Bingisser R, Morgenthaler NG. The role of copeptin as a diagnostic and prognostic biomarker for risk stratification in the emergency department. *BMC Med* 2012;**10**:7. doi:10.1186/1741-7015-10-7

8 Lippi G, Plebani M, Di Somma S, *et al.* Considerations for early acute myocardial infarction rule-out for emergency department chest pain patients: the case of copeptin. *Clin Chem Lab Med* 2012;**50**:243–53. doi:10.1515/cclm-2011-0845

9 Reichlin T, Hochholzer W, Stelzig C, *et al.* Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol* 2009;**54**:60–8. doi:10.1016/j.jacc.2009.01.076

10 Keller T, Tzikas S, Zeller T, *et al.* Copeptin improves early diagnosis of acute myocardial infarction. *J Am Coll Cardiol* 2010;**55**:2096–106. doi:10.1016/j.jacc.2010.01.029

11 Chenevier-Gobeaux C, Freund Y, Claessens Y-E, *et al.* Copeptin for rapid rule out of acute myocardial infarction in emergency department. *Int J Cardiol* 2013;**166**:198–204. doi:10.1016/j.ijcard.2011.10.098

12 Meune C, Zuily S, Wahbi K, *et al.* Combination of copeptin and high-sensitivity cardiac troponin T assay in unstable angina and non-ST-segment elevation myocardial infarction: a pilot study. *Arch Cardiovasc Dis* 2011;**104**:4–10. doi:10.1016/j.acvd.2010.11.002

13 Karakas M, Januzzi JL, Meyer J, *et al.* Copeptin does not add diagnostic information to high-sensitivity troponin T in low- to intermediate-risk patients with acute chest pain: results from the rule out myocardial infarction by computed tomography (ROMICAT) study. *Clinical Chemistry* 2011;**57**:1137–45. doi:10.1373/clinchem.2010.160192

14 Lotze U, Lemm H, Heyer A, *et al.* Combined determination of highly sensitive troponin T and copeptin for early exclusion of acute myocardial infarction: first experience in an emergency department of a general hospital. *Vasc Health Risk Manag* 2011;**7**:509–15. doi:10.2147/VHRM.S21753

15 Giavarina D, Carta M, Fortunato A, *et al.* Copeptin and high sensitive troponin for a rapid rule out of acute myocardial infarction? *Clin Lab* 2011;**57**:725–30.

16 Giannitsis E, Kehayova T, Vafaie M, *et al.* Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. *Clinical Chemistry* 2011;**57**:1452–5. doi:10.1373/clinchem.2010.161265

17 Freund Y, Chenevier-Gobeaux C, Leumani F, *et al.* Concomitant Measurement of Copeptin and High Sensitivity Troponin for Fast and Reliable Rule Out of Acute Myocardial Infarction. *Ann Emerg Med* 2011;**58**:S190. doi:10.1016/j.annemergmed.2011.06.061

18 Dupuy A-M, Chastang E, Cristol J-P, *et al.* Analytical performances of the newly developed, fully automated Kryptor Copeptin assay: which impact factor for myocardial infarction rules out in the emergency department? *Clin Lab* 2012;**58**:635–44.

19 Potocki M, Reichlin T, Thalmann S, *et al.* 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. *Heart* 2012;**98**:558–65. doi:10.1136/heartjnl-2011-301269

20 Ray P, Charpentier S, Chenevier-Gobeaux C, *et al.* Combined copeptin and troponin to rule out myocardial infarction in patients with chest pain and a history of coronary artery disease. *Am J Emerg Med* 2012;**30**:440–8. doi:10.1016/j.ajem.2011.12.008

21 Charpentier S, Maupas-Schwalm F, Cournot M, *et al.* Combination of copeptin and troponin assays to rapidly rule out non-ST elevation myocardial infarction in the emergency department. *Acad Emerg Med* 2012;**19**:517–24. doi:10.1111/j.1553-2712.2012.01350.x

22 Eggers KM, Venge P, Lindahl B. High-sensitive cardiac troponin T outperforms novel

diagnostic biomarkers in patients with acute chest pain. *Clin Chim Acta* 2012;**413**:1135–40. doi:10.1016/j.cca.2012.03.011

23 Folli C, Consonni D, Spessot M, *et al.* Diagnostic role of copeptin in patients presenting with chest pain in the emergency room. *European Journal of Internal Medicine* 2013;**24**:189–93. doi:10.1016/j.ejim.2012.09.006

24 Dedic A, Kate Ten G-J, Rood PPM, *et al.* Copeptin in acute chest pain: identification of acute coronary syndrome and obstructive coronary artery disease on coronary CT angiography. *Emergency Medicine Journal* Published Online First: 8 November 2012. doi:10.1136/emermed-2012-201596

25 Bahrmann P, Bahrmann A, Breithardt O-A, *et al.* Additional diagnostic and prognostic value of copeptin ultra-sensitive for diagnosis of non-ST-elevation myocardial infarction in older patients presenting to the emergency department. *Clin Chem Lab Med* 2013;**51**:1307–19. doi:10.1515/cclm-2012-0401

26 Ledochowski S, Fayet JM, Collin-Chavagnac D, *et al.* Évaluation de la copeptine dans les syndromes coronaires aigus non ST. *Ann Fr Med Urgence* 2013;:138–44. doi:10.1007/s13341-013-0303-4

27 Maisel A, Mueller C, Neath S-X, *et al.* Copeptin helps in the early detection of patients with acute myocardial infarction: primary results of the CHOPIN trial (Copeptin Helps in the early detection Of Patients with acute myocardial INfarction). *J Am Coll Cardiol* 2013;**62**:150–60. doi:10.1016/j.jacc.2013.04.011

28 Charpentier S, Lepage B, Maupas-Schwalm F, *et al.* Copeptin improves the diagnostic performance of sensitive troponin I-Ultra but cannot rapidly rule out non-ST-elevation myocardial infarction at presentation to an emergency department. *Ann Emerg Med*

2013;**61**:549–558.e1. doi:10.1016/j.annemergmed.2012.12.018

29 Afzali D, Erren M, Pavenstädt H-J, *et al.* Impact of copeptin on diagnosis, risk stratification, and intermediate-term prognosis of acute coronary syndromes. *Clin Res Cardiol* 2013;**102**:755–63. doi:10.1007/s00392-013-0583-0

30 Thelin J, Borna C, Erlinge D, *et al.* The combination of high sensitivity troponin T and copeptin facilitates early rule-out of ACS: a prospective observational study. *BMC Cardiovasc Disord* 2013;**13**:42. doi:10.1186/1471-2261-13-42

31 Sebbane M, Lefebvre S, Kuster N, *et al.* Early rule out of acute myocardial infarction in ED patients: value of combined high-sensitivity cardiac troponin T and ultrasensitive copeptin assays at admission. *Am J Emerg Med* 2013;**31**:1302–8. doi:10.1016/j.ajem.2013.04.033

32 Balmelli C, Meune C, Twerenbold R, *et al.* Comparison of the performances of cardiac troponins, including sensitive assays, and copeptin in the diagnostic of acute myocardial infarction and long-term prognosis between women and men. *Am Heart J* 2013;**166**:30–7. doi:10.1016/j.ahj.2013.03.014

33 Llorens P, Sánchez M, Herrero P, *et al.* The utility of copeptin in the emergency department for non-ST-elevation myocardial infarction rapid rule out: COPED-MIRRO study. *Eur J Emerg Med* Published Online First: 17 July 2013. doi:10.1097/MEJ.0b013e3283632f8b

34 Collinson P, Gaze D, Goodacre S. Comparison of contemporary troponin assays with the novel biomarkers, heart fatty acid binding protein and copeptin, for the early confirmation or exclusion of myocardial infarction in patients presenting to the emergency department with chest pain. *Heart* 2014;**100**:140–5. doi:10.1136/heartjnl-

2013-304716

35 Sukul D, Bonaca MP, Ruff CT, *et al.* Diagnostic Performance of Copeptin in Patients With Acute Nontraumatic Chest Pain: BWH-TIMI ED Chest Pain Study. *Clin Cardiol* Published Online First: 22 January 2014. doi:10.1002/clc.22244

36 Kelly D, Squire IB, Khan SQ, *et al.* C-terminal provasopressin (copeptin) is associated with left ventricular dysfunction, remodeling, and clinical heart failure in survivors of myocardial infarction. *Journal of cardiac failure* 2008;**14**:739–45. doi:10.1016/j.cardfail.2008.07.231

37 Thygesen K, Mair J, Giannitsis E, *et al.* How to use high-sensitivity cardiac troponins in acute cardiac care. *European heart journal* 2012;**33**:2252–7. doi:10.1093/eurheartj/ehs154

38 Body R, Carley S, McDowell G, *et al.* Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a high-sensitivity assay. *J Am Coll Cardiol* 2011;**58**:1332–9. doi:10.1016/j.jacc.2011.06.026

~~1 Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. *Eur Heart J* 2012;33:2551–67.~~

~~2 Hamm CW, Bassand J-P, Agewall S, et al. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2011;32:2999–3054.~~

~~3 Lin S, Yokoyama H, Rac VE, et al. Novel biomarkers in diagnosing cardiac ischemia in the~~

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emergency department: a systematic review. *Resuscitation* 2012;83:684–91.

4 Lippi G. Biomarkers of myocardial ischemia in the emergency room: cardiospecific troponin and beyond. *Eur J Intern Med* 2013;24:97–9.

5 Reichlin T, Hochholzer W, Stelzig C, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol* 2009;54:60–8.

6 Keller T, Tzikas S, Zeller T, et al. Copeptin improves early diagnosis of acute myocardial infarction. *J Am Coll Cardiol* 2010;55:2096–106.

7 Chenevier-Gobeaux C, Freund Y, Claessens Y-E, et al. Copeptin for rapid rule out of acute myocardial infarction in emergency department. *Int J Cardiol* 2013;166:198–204.

8 Meune C, Zuily S, Wahbi K, et al. Combination of copeptin and high-sensitivity cardiac troponin T assay in unstable angina and non-ST-segment elevation myocardial infarction: a pilot study. *Arch Cardiovasc Dis* 2011;104:4–10.

9 Karakas M, Januzzi JL, Meyer J, et al. Copeptin does not add diagnostic information to high-sensitivity troponin T in low- to intermediate-risk patients with acute chest pain: results from the rule-out myocardial infarction by computed tomography (ROMICAT) study. *Clin Chem* 2011;57:1137–45.

10 Lotze U, Lemm H, Heyer A, et al. Combined determination of highly sensitive troponin T and copeptin for early exclusion of acute myocardial infarction: first experience in an emergency department of a general hospital. *Vasc Health Risk Manag* 2011;7:509–15.

11 Giavarina D, Carta M, Fortunato A, et al. Copeptin and high sensitive troponin for a rapid rule out of acute myocardial infarction? *Clin Lab* 2011;57:725–30.

12 Giannitsis E, Kehayova T, Vafaie M, et al. Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. *Clin Chem* 2011;57:1452–5.

13 Freund Y, Chenevier-Gobeaux C, Leumani F, et al. Concomitant Measurement of Copeptin and High-Sensitivity Troponin for Fast and Reliable Rule-Out of Acute Myocardial Infarction. *Ann Emerg Med* 2011;58:S190.

14 Dupuy A-M, Chastang E, Cristol J-P, et al. Analytical performances of the newly developed, fully automated Kryptor Copeptin assay: which impact factor for myocardial infarction rules out in the emergency department? *Clin Lab* 2012;58:635–44.

15 Potocki M, Reichlin T, Thalmann S, et al. 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. *Heart* 2012;98:558–65.

16 Ray P, Charpentier S, Chenevier-Gobeaux C, et al. Combined copeptin and troponin to rule-out myocardial infarction in patients with chest pain and a history of coronary artery disease. *Am J Emerg Med* 2012;30:440–8.

17 Charpentier S, Maupas-Schwalm F, Cournot M, et al. Combination of copeptin and troponin assays to rapidly rule out non-ST-elevation myocardial infarction in the emergency department. *Acad Emerg Med* 2012;19:517–24.

18 Eggers KM, Venge P, Lindahl B. High-sensitive cardiac troponin T outperforms novel diagnostic biomarkers in patients with acute chest pain. *Clin Chim Acta* 2012;413:1135–40.

19 Folli C, Consonni D, Spessot M, et al. Diagnostic role of copeptin in patients presenting with chest pain in the emergency room. *Eur J Intern Med* 2013;24:189–93.

20 Dedic A, Kate Ten G J, Rood PPM, et al. Copeptin in acute chest pain: identification of acute coronary syndrome and obstructive coronary artery disease on coronary CT angiography. *Emerg Med J* Published Online First: 8 November 2012. doi: 10.1136/emermed-2012-201596.

21 Bahrmann P, Bahrmann A, Breithardt O-A, et al. Additional diagnostic and prognostic value of copeptin ultra-sensitive for diagnosis of non-ST-elevation myocardial infarction in older patients presenting to the emergency department. Clin Chem Lab Med 2013;51:1307–19.

22 Ledochowski S, Fayet JM, Collin-Chavagnac D, Bonnefoy-Cudraz E. Evaluation for copeptin in NSTEMIs. *Annales Françaises de Médecine d'Urgence*. 2013;3:138–44.

23 Maisel A, Mueller C, Neath S-X, et al. Copeptin Helps in the Early Detection Of Patients with Acute Myocardial Infarction : the primary results of the CHOPIN Trial. *J Am Coll Cardiol* 2013;62:150–60.

24 Charpentier S, Lepage B, Maupas-Schwalm F, et al. Copeptin improves the diagnostic performance of sensitive troponin I Ultra but cannot rapidly rule out non-ST-elevation myocardial infarction at presentation to an emergency department. *Ann Emerg Med* 2013;61:549–558.

25 Afzali D, Erren M, Pavenstädt H J, et al. Impact of copeptin on diagnosis, risk stratification, and intermediate-term prognosis of acute coronary syndromes. *Clin Res Cardiol* 2013;755–63.

26 Thelin J, Borna C, Erlinge D, et al. The combination of high sensitivity troponin T and copeptin facilitates early rule-out of ACS: a prospective observational study. BMC Cardiovasc Disord 2013;13:42.

27 Sebbane M, Lefebvre S, Kuster N, et al. Early rule out of acute myocardial infarction in ED patients: value of combined high-sensitivity cardiac troponin T and ultrasensitive copeptin assays at admission. *Am J Emerg Med* 2013;31:1302–8.

28 Balmelli C, Meune C, Twerenbold R, et al. Comparison of the performances of cardiac troponins, including sensitive assays, and copeptin in the diagnostic of acute myocardial infarction and long-term prognosis between women and men. *Am Heart J* 2013;166:30–7.

29 Maisel A, Mueller C, Neath S X, et al. Copeptin helps in the early detection of patients with acute myocardial infarction: primary results of the CHOPIN trial (Copeptin Helps in the early detection Of Patients with acute myocardial INfarction). *J Am Coll Cardiol* 2013;62:150–60.

30 Llorens P, Sánchez M, Herrero P, et al. The utility of copeptin in the emergency department for non-ST-elevation myocardial infarction rapid rule out: COPED-MIRRO study. *Eur J Emerg Med* Published Online First: 17 July 2013. doi: 10.1097/MEJ.0b013e3283632f8b

31 Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J* 2012;33:2252–7.

32 Body R, Carley S, McDowell G, et al. Rapid exclusion of acute myocardial infarction in patients with undetectable troponin using a high-sensitivity assay. *J Am Coll Cardiol* 2011;58:1332–9.

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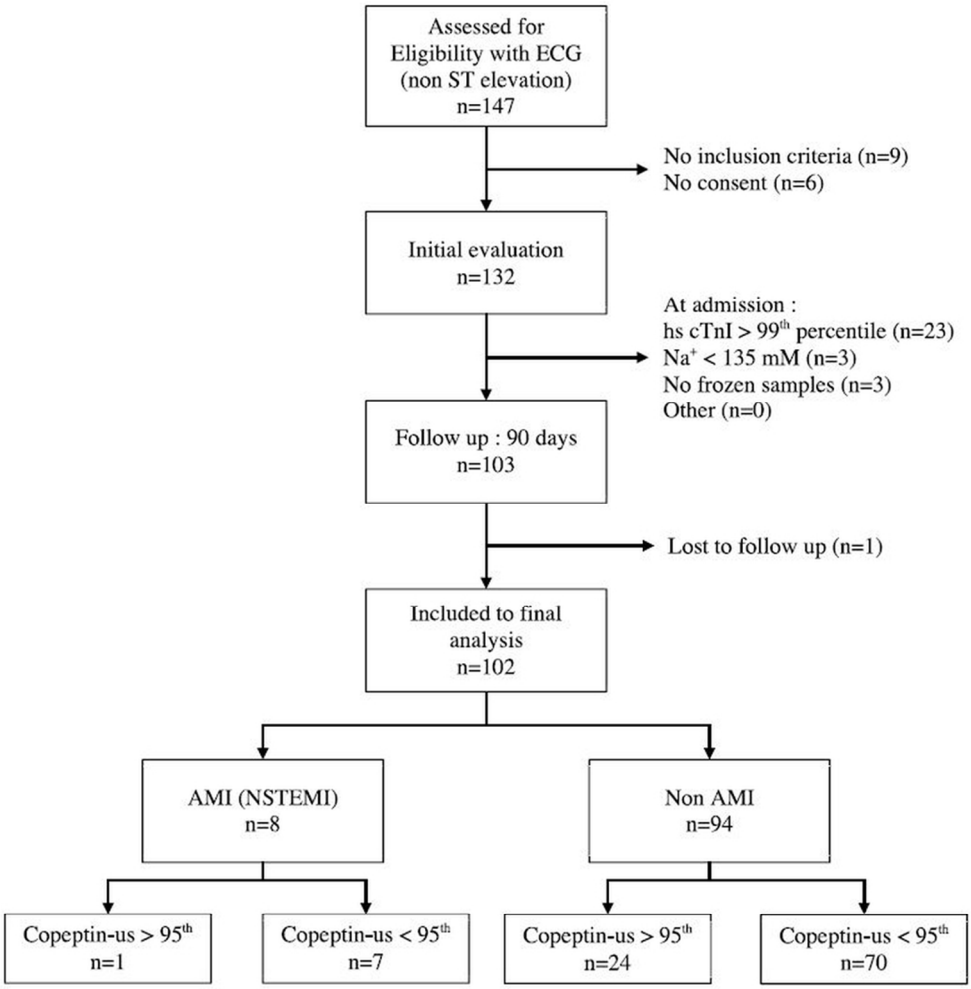

Flow chart
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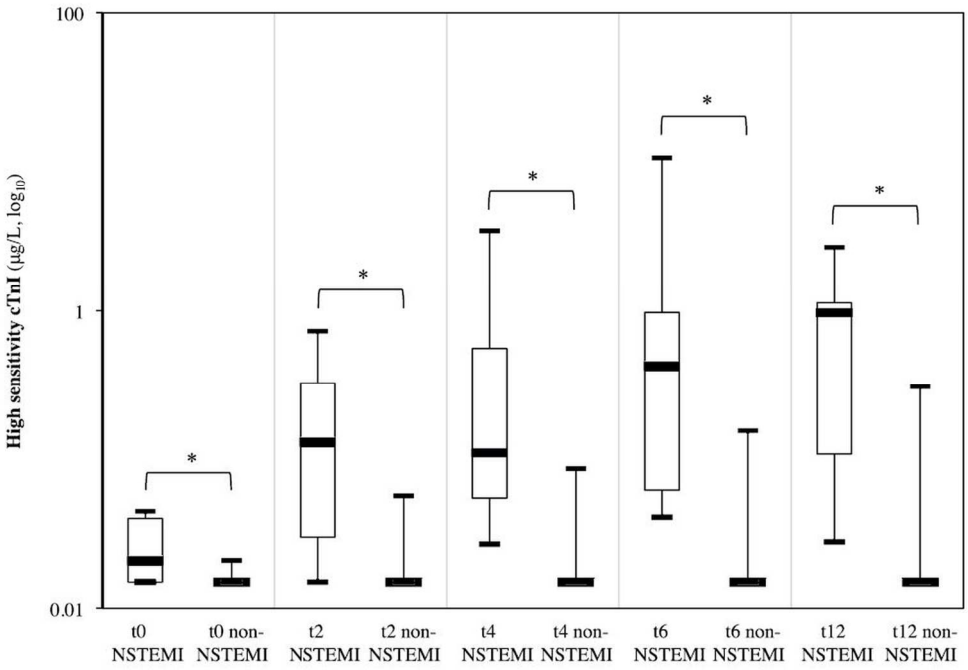
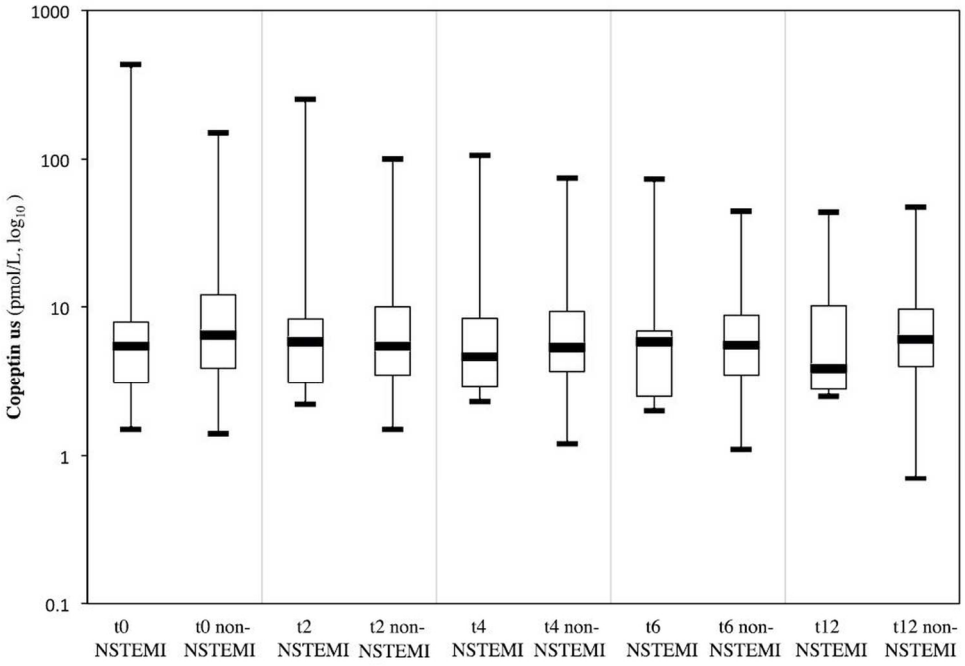


Box plots (median, interquartile range, minimal and maximal values) illustrate Troponin concentration in relation to time since admission for NSTEMI and non-NSTEMI patients. * p<0.0001.
130x90mm (300 x 300 DPI)



Box plots (median, interquartile range, minimal and maximal values) illustrate Copeptin concentration in relation to time since admission for NSTEMI and non-NSTEMI patients.
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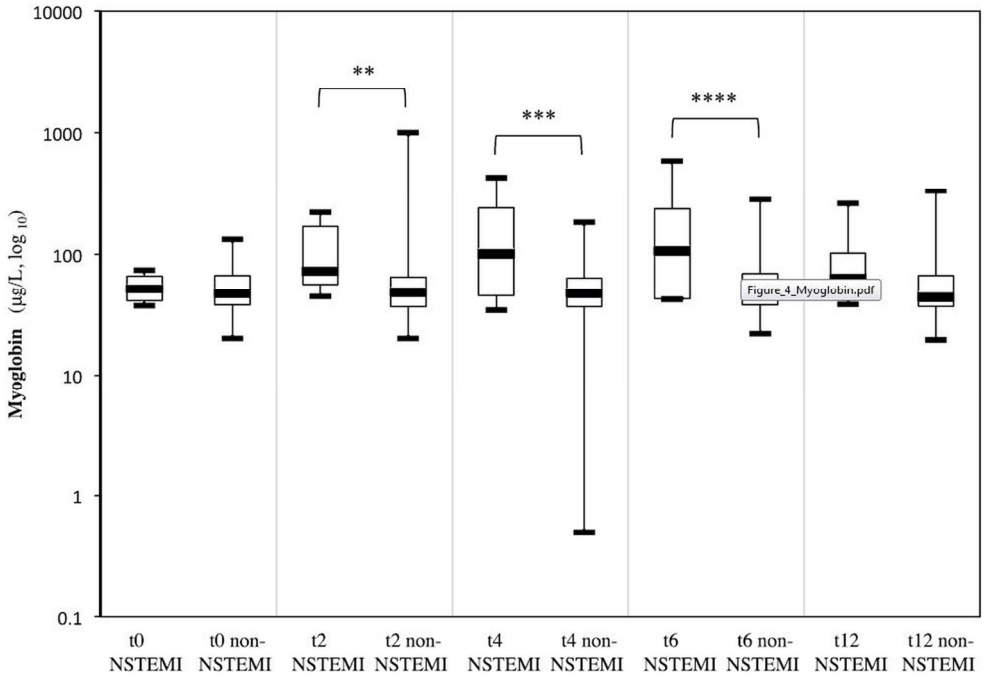

Box plots (median, interquartile range, minimal and maximal values) illustrate myoglobin concentration in relation to time since admission for NSTEMI and non-NSTEMI patients. ** p=0.01, *** p=0.04, **** p=0.03.
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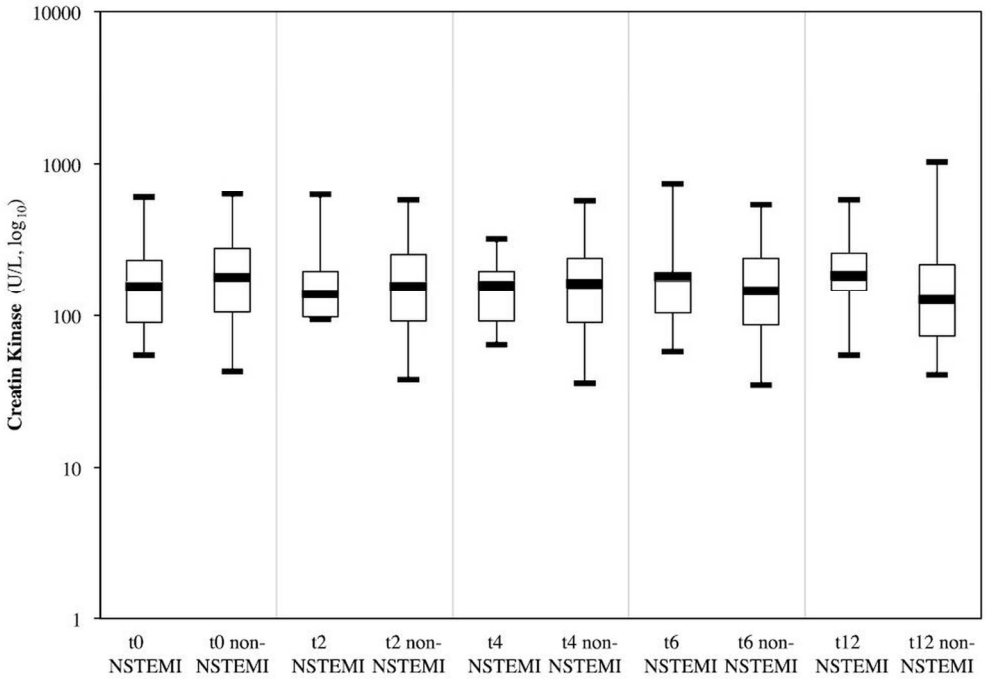

Box plots (median, interquartile range, minimal and maximal values) illustrate CK concentration in relation to time since admission for NSTEMI and non-NSTEMI patients.
129x90mm (300 x 300 DPI)

STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	Yes
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	1
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	1-2
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	1-2
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	1-2
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	1
Test methods	7	The reference standard and its rationale.	1
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	2
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	2
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	2
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	2
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	2-3
	13	Methods for calculating test reproducibility, if done.	3
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	1
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	3-4
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	3
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	2
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	3
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	4-5
	20	Any adverse events from performing the index tests or the reference standard.	
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	4-5
	22	How indeterminate results, missing data and outliers of the index tests were handled.	6
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	
	24	Estimates of test reproducibility, if done.	
DISCUSSION	25	Discuss the clinical applicability of the study findings.	6-7