BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023994
Article Type:	Research
Date Submitted by the Author:	12-Jun-2018
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Keywords:	high sensitive cardiac troponin, non ST-elevation acute myocardial infarction, copeptin, very early presenters, chest pain onset

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Is high-sensitivity troponin (alone or in combination with copeptin) enough for ruling out NSTEMI in very early presenters at admission?

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Abstract

Objectives: Copeptin and highly-sensitive cardiac troponin (HS-cTn) assays improve the early detection of NSTEMI. Their sensitivities may however be reduced in very early presenters. *Setting:* We performed a post-hoc analysis of three prospective studies that included patients who presented to the Emergency Department for chest pain onset (CPO) of less than 6 hours. *Participants*: 449 patients were included, in whom 12% had NSTEMI. CPO occurred <2h from ED presentation in 160, between 2-4h in 143, and >4h in 146 patients. The prevalence of NSTEMI was similar in all groups (9%, 13% and 12%, respectively, p=0.281). *Measures:* Diagnostic performances of HS-cTn and copeptin at presentation were examined according to CPO <2h, 2-4h, and >4h. The discharge diagnosis was adjudicated by 2 experts, including cTnI. HS-cTn and copeptin were blindly measured. *Results:* Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT (but not HS-cTnT+copeptin) lower through CPO categories. For patients with CPO<2h, the choice of a threshold value of 14 ng/L for HS-cTnT resulted in 3 false negative (Sensitivity 80% [95%CI: 51-95]; specificity 85% [78-90]; 79% of correctly ruled-out patients) and that of 5 ng/L in 2 false negative (Sensitivity 87% [59-98]; specificity 58% [50-66]; 52% of correctly ruled-out patients). The addition of copeptin to HS-cTnT induced a decrease of misclassified patients to 1 in patients with CPO<2h (Sensitivity 93% [66-100]; specificity 41% [33-50]). *Conclusion:* A single measurement of HS-cTn, alone or in combination with copeptin at admission, does not allow safely ruling out NSTEMI in very early presenters (with CPO<2h). *Trial registrations:* French Health Ministry (no. DC-2009-1052), French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière).

Strengths

- Focus on very early chest pain presenters that was not performed before

Limitations of our study

- Small numbers of very early chest pain presenters, although the data grouping of 3 previous studies

- A single measurement of troponin at admission was considered for the analysis, but not its kinetics

- The gold standard diagnosis was based on a non-HS cTn.

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Introduction

The management of patients with acute chest pain at the Emergency Department (ED) is a major health problem and an adequate ruling out process for non-ST elevation acute myocardial infarction (NSTEMI) is crucial. Cardiac troponin (cTn) measurement with conventional assays and more recent highly-sensitive cardiac troponin assays (HS-cTn), are current diagnostic tools for the assessment of these patients (1). The European Society of Cardiology guidelines proposed a rapid rule-out strategy using low HS-cTn values (i.e. values below the Limit of Detection of the assay -LoD-) as decisional threshold for ruling out NSTEMI (1). The accuracy of a rapid 0-hour/1-hour algorithm has also been recently demonstrated in patients with CPO<6h (2) and is endorsed by the latest ESC guidelines (1). In this analysis, the authors indicated that using a single HS-cTnT cutoff value of 14 ng/L at presentation resulted in 88.7% sensitivity and 97.3% negative predictive value (NPV), but that this single assay strategy was less effective than the combination of absolute level of HS-cTnT measured at presentation and again at 1 hour, combined with the absolute difference between the two levels (2).

However, in very early presenters, such rapid and efficient triage at presentation may be uncertain (3). Indeed, very early presenters, defined as having chest pain <2h, are considered by some authors as highly vulnerable as they may not present all symptoms and signs and thus be exposed to a higher risk of misdiagnosis and therefore worse outcome (4); the application of a rapid algorithm to these patients may lead to early discharge within 3 hours from ED admission. Recently, a meta-analysis (based on eleven studies) indicated that a single HS-cTnT concentration below the limit of detection may successfully rule out AMI (5). However, concerns have been raised about the safety of a single measurement rule out protocol performed at presentation in early presenters (6,7).

Copeptin (in association with cTn) assays improve the early detection of NSTEMI (8–12).

Indeed, a randomized controlled trial demonstrated the safety of early discharge using a single

combination of copeptin + cTn at presentation for patients with CPO <6h (13).

The present study aimed to assess the diagnostic performance of HS-cTn and copeptin as a

single measurement at admission in very early ED presenters with suspected NSTEMI.

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Patients and methods

Study design and population

The study is a *post-hoc* analysis of three French prospective clinical studies (already published) of cardiac biomarker testing, each to explore the usefulness of copeptin and/or HS-cTnT testing in patients who presented to the ED with acute chest pain of less than 6h (14,15). However, none of these studies evaluated the influence of CPO value on diagnostic performances. All three trials had comparable inclusion/exclusion criteria and gathered similar clinical information (Table 1). Patients requiring renal replacement therapy were excluded. All studies received approval from local Institutional Review Board. The study complied with the principles of the Declaration of Helsinki. Recommendations of the Standards for Reporting of Diagnostic Accuracy (STARD) initiative were applied (16).

Patient Involvement

The development of the research question was not informed by patients. Patients were not involved in the design of this study. Patients were not involved in the recruitment to and conduct of the study. There was no results dissemination to study participants.

Routine assessment

All patients underwent an initial clinical evaluation (including clinical history, physical examination, 12-lead ECG, pulse oximetry, routine blood tests, and chest X-ray). Conventional cardiac Troponin I (cTnI) was measured on a venous blood collection performed at presentation and, if required, repeated after 3 to 9 h, as clinically indicated (17). The onset of chest pain (CPO), defined as the delay from symptom onset to presentation was recorded, based on patient history/declaration. When history was incomplete or inconsistent, CPO was not recorded and patient was excluded of the analysis (see flow chart, Figure 1).

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Based on all clinical, biological (including cTnI value, but not HS-cTnT and copeptin values which were blindly measured) and imaging results, a decision was made by the attending physician to admit or discharge the patient, as well as medical therapy and revascularization if indicated. Attending emergency physicians and cardiologists were blinded to the results of HS-cTnT and copeptin, and biologists were blinded to the suspected diagnosis at presentation. Patients with no cTnI results and/or no recorded CPO value, and patients with a final diagnosis of STEMI, were excluded (see flowchart, Figure 1).

Gold standard diagnosis

The gold standard diagnosis was adjudicated by two independent experts (emergency physician and cardiologist) who reviewed all available medical records (including patient history, physical findings, laboratory results including cTnI value and radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography, summary chart at discharge) pertaining to the patient from the time of ED presentation to 30-day follow-up. Experts were blind to copeptin and HS-cTnT results. In the event of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third expert.

AMI was diagnosed according to the universal definition (18). Patients with a cTnI increase (or a rise/fall pattern) above the 10%CV threshold, associated with at least one of the following: symptoms of myocardial ischemia, new ST-T changes or new Q wave on ECG, imaging of new loss of viable myocardium, or normal cTnI on admission were classified as having an MI (STEMI with a ST elevation in at least 2 continuous leads on ECG or new onset of left bundle branch block, or NSTEMI). STEMI patients were excluded from the analysis, based on ST elevation observed on the ECG (see Flow chart, Fig. 1). Patients were classified according to the CPO, <2h, from 2-4h and >4h; those with a CPO <2 hours were considered as very early presenters.

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Unstable angina (UA) diagnosis was adjudicated in patients with history or clinical symptoms consistent with ACS but without ST-T changes on the ECG and without change of cTn on serial testing. Other diagnostic categories beside NSTEMI and UA were non-ACS (e.g., stable angina, myocarditis, arrhythmias, heart failure, pulmonary embolism, and chest pain of unknown origin). UA and non-ACS chest pain were considered as non-NSTEMI in our analysis.

Troponin measurements

Plasma cTnI concentrations were routinely measured on an X-pand® HM analyzer, using the Cardiac Troponin-I immunoassay (Siemens Healthcare Diagnostics Inc., Newark, CT) in two EDs (Cochin and La Pitié Salpêtrière Hospitals). The limit of quantitation (LoQ (or 10%CV), i.e. the lowest cTn concentration that can be reproducibly measured with a between-run CV of ≤10%) was 0.14 μg/L. In Bicêtre and in Montpellier hospitals, plasma cTnI concentrations were routinely measured on an Access® analyser (Beckman Coulter, Inc., Brea, CA). According to the manufacturer's data the LoQ was 0.04 μg/L. After routine cTnI measurement, plasma samples were aliquoted and frozen (-40°C) until HS-cTnT and copeptin measurement.

Hs-cTnT was measured in heparinized collected samples, on an Elecsys2010® analyser (Roche Diagnostics, Meylan, France). The LoD is 5 ng/L, and the 99th percentile is 14 ng/L. HS-cTnT determinations were performed blinded to the clinical assessment of the emergency physicians. Of note, the LoD is measured with a between-run CV of >10%, while the 99th percentile is a precise concentration (CV<10%) (7). HS-cTnT determinations were performed blinded to the clinical assessment of the emergency physicians

Copeptin measurement

Copeptin was measured in heparinized blood samples collected on admission. The assay was performed on a KRYPTOR® analyser using ThermoFisher Scientific sandwich immunoluminometric assay (B.R.A.H.M.S Copeptin KRYPTOR, B.R.A.H.M.S Aktiengesellschaft, Hennigsdorf, Germany). The assay principle is based on TRACE technology (Time-Resolved Amplified Cryptate Emission). The lower detection limit is 4.8 pmol/L, and the functional assay sensitivity (20%CV value) is <12 pmol/L (data from manufacturer, recommended threshold value for this method). Copeptin determinations were performed blinded to the clinical assessment of the emergency physicians.

Statistical analysis

Continuous variables are presented as means ± SD, and categorical variables are expressed as numbers (percentage). Continuous variables were compared by using the Kruskal-Wallis test, and categorical variables were assessed using Pearson's χ^2 test. Number of misclassified patients and number of correctly ruled-out patients were collected for each threshold strategy, and correspond to the false negative and the true positive patients, respectively.

Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) throughout the concentrations of cTnI, HS-cTnT and copeptin for the diagnosis of NSTEMI, and according to the CPO. cTn and copeptin values were log-transformed before combination for ROC analysis. All data are presented with their 95% confidence intervals [95%CI]. All hypothesis testing was two-tailed, and p <0.05 was considered statistically significant. Statistical analysis was performed using MedCalc (MedCalc Software, Version 12.4.0.0, Mariarkerke, Belgium).

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Results

Characteristics of the studied population

Table 2 shows the characteristics of the studied population. Briefly, in the total cohort mean age was 58 ± 17 years, and included more males. A gold standard diagnosis of NSTEMI was adjudicated in 55 patients (12%). Delay from chest pain onset to ED admission was <2 hours in 160 (36%) patients, was from 2 to 4 hours in 143 (32%) and was >4 hours (and below 6 hours) in 146 (32%) patients. Very early presenters with NSTEMI (n=15) tended to be older than those without NSTEMI (n=145) (65 *vs.* 55 years-old), were more frequently hospitalized (93% *vs.* 58%), and also more frequently underwent diagnostic coronary angiography (73% *vs.* 27%).

Diagnostic performances according to CPO

Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT lower through CPO categories, as indicated by estimated AUCs and their 95%CI intervals (Table 3). The AUCs of HS-cTnT+copeptin were not different through the CPO categories.

Diagnostic performances of both cTn, alone or in combination with copeptin, and using different decisional thresholds, are presented in Table 4. The subsequent potential misclassified patients are detailed in Online supplemental Table 5.

In very early presenters (CPO <2 hours), a single value of cTnI alone had low sensitivity (73 [73-91]%) but high specificity (97 [92-99]%), and misclassified 4 of 15 NSTEMI patients. However, this strategy could correctly rule-out 141 patients (88%). Combining copeptin with cTnI increases sensitivity, and lowers the number of misdiagnosed patients from 4 to 2, but significantly lowers the rate of ruled-out patients from 88% to 54% (Table 4). Of note, addition of copeptin also significantly lowered specificity. At a threshold of 14 ng/L, HS-cTnT had low sensitivity (80 [59-98]%), misclassified 3 NSTEMI patients but could correctly

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rule-out 123 (79%) patients, which is less than using cTnI. The sensitivity of HS-TnT alone is likely to be suboptimal in early presenters, but can be improved by using a lower threshold for positivity or adding copeptin (but this is accompagnied with a marked loss of specificity). The addition of copeptin induced a decrease in misclassified patients from 2 to 1, either with an HS-cTnT threshold at 14 or at 5 ng/L. This ultimate misdiagnosed NSTEMI patient with CPO <2h who presented with all undetectable biomarkers was a 44-years old woman with a history of smoking and no CV risk factors; the CPO was 45 min before hospital admission (Table 5).

In patients with CPO 2-4 hours, results are similar to those observed in very early presenters, although the number of misclassified patients was different (Table 4). Adding copeptin to cTnI induced a decrease of misclassified patients from 5 to 1 for an HS-cTnT threshold at 14 ng/L, and from 2 to 0 for an HS-cTnT threshold at 5 ng/L. In particular, combining copeptin to the LoD of HS-cTnT reached 100% sensitivity of the test. Indeed, all NSTEMI patients with CPO 2-4 h had a detectable HS-cTnT and/or an elevated copeptin. In this sub-group again, the use of copeptin lowered significantly the specificity of the test.

As expected, in patients with CPO >4 hours, all patients had quantitative cTnI and detectable HS-cTnT. No patient was misclassified. The addition of copeptin in this sub-group had no effect on sensitivity or misclassified patients.

Potential misdiagnosed NSTEMI

Characteristics of potential misdiagnosed NSTEMI patients are detailed in Table 5. All potentially missed NSTEMI in the very early presenters population had a CPO <1 hour. We found no distinguishing characteristics in misclassified patients when comparing to correctly diagnosed patients in terms of age, sex, and cardiovascular risks, in each CPO category. Of note, when STEMI patients were included in our analysis, results were comparable (data not shown).

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Discussion

Our results indicate that diagnostic performances of HS-cTnT values at admission, alone or combined with copeptin, are reduced in the subgroup of patients with shorter CPO. Emergency physicians should not rule out NSTEMI in very early presenters with a single low value of HS-cTnT and/or copeptin at presentation.

Our studied population included one third of early presenters, and this proportion is similar to that found by Boeddinghaus et al (26% of patients presented within 2h from CPO) (4), by Keller et al (37% and 38% of patients with CPO<3h, (8,19)), and by Reichlin et al. (222 patients with CPO<3h out of 718, i.e. 31%) (20). More recently, Stallone et al reported 519 (26%) patients that arrived within 2 hours of symptom onset to the ED (6). This may reflect that our study was performed in large urban areas equipped with pre-hospital emergency ambulances. A more prolonged delay might be expected in more rural regions. The exact definition of very early presenters is still a matter of debate. Authors nevertheless agree to define them as presenting before 2h (20). Very few studies examined the specific groups of early/very early presenters (4,6,8,20). In the ESC guidelines, a different strategy is recommended for patients with versus without CPO<6h but not for earlier presenters (1). We believe that this proportion is not negligible and the impact of this very early population might be underestimated in studies were the CPO is not evaluated.

We observed that AUCs of cTnI, cTnI+copeptin and HS-cTnT were significantly lower when CPO<4h. The NPVs were not significantly impacted by the CPO, but a suboptimal sensitivity and a non-negligible proportion of misclassified NSTEMI was observed in patients with CPO<2h for all tested thresholds and combinations, even if cTnI and HS-cTnT performances can be improved by using a lower threshold for positivity or adding copeptin. The same was true for patients with CPO 2-4h, except when using the combination of HS-cTnT<5 ng/L and copeptin <12 µmol/L. We reported the number of misclassified patients in addition to

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sensitivity and NPV, because NPV is known to be dependent of the prevalence, thus its value might be biased. The absolute number of misdiagnosed patients might be more clinically pertinent than NPV (21).

As previously suggested (1), we found that sensitivity and NPV of a single measurement of HS-cTn at admission is not enough to safely excluded a NSTEMI in very early presenters. We here show that lowering HS-cTn decisional threshold to LoD is not sufficient to detect all NSTEMI among very early chest pain presenters. Although combining copeptin to HS-cTnT increased sensitivity and lowered number of misclassified patients, none of the tested strategies allowed for identification of all NSTEMI. Results obtained in very early presenters were very similar to patients with CPO 2-4h, except that in this later sub-group combining copeptin with HS-cTnT succeeded in significantly increasing sensitivity (all NSTEMI patients had a detectable HS-cTnT and/or an elevated copeptin).

Our results can be compared to those of Boeddinghaus et al. who found that sensitivity of a single HS-cTn measurement is lower in very early presenters, in comparison to all patients (4). These authors indicated that using a single cut-off approach, 61% of the very early presenters were ruled-out, which resulted in a sensitivity of 94% and a NPV of 98%. However, the authors did not evaluate the impact of copeptin across CPO categories. In another study, the same authors indicated that the additional use of copeptin did not sufficiently improve diagnostic accuracy in early presenters (22). Here again, our results are in accordance with those of Boeddinghaus indicating that copeptin did not improve diagnostic accuracy of hs-cTnI at presentation in early presenters (22). Mueller et al showed, using the rapid 0-hour/1-hour algorithm that 63% patients with CPO<6h were classified as rule-out. However, 7 patients were missed (0.9% rate), in whom 3 had HS-cTnT<LoD at presentation and at 1 hour (2). Moreover, a single cutoff value for HS-cTnT at 14 ng/L at presentation resulted in a sensitivity of 88.7% and a NPV of 97.3%, and performed less adequately than the

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combination of HS-cTnT at presentation with 1-hour level and 1-hour absolute change (2). In a more recent study, Mokhtari et al. evaluated a rapid 0H/1H protocol for discharge chest pain patients based on a single value <LoD at admission (23). These authors found 2 missed patients in their population, and recognize that the safety of such rapid protocol is not clear in very early presenters. They further recommend additional HS-cTnT testing at 3 hours for very early presenters (23).

Performance of cardiac troponins, although measured using HS assays, might be limited in very early presenters because of their kinetics of release into the blood circulation (8). The release of cTn into the circulation following cardiomyocyte damage is a time-dependent phenomenon (24), and a single measurement approach may fail at identifying AMI very early after the onset of symptoms (25). Indeed we, like other authors, found very early presenters with undetectable HS-cTnT at admission. Time-dependent release of copeptin during AMI has been described earlier, and this biomarker has been considered as an early biomarker (8). Copeptin increases immediately after induction of ischemia, and peaks 90 min after (26). However, some authors recently indicated that copeptin kinetics might be different in NSTEMI in comparison to STEMI, and that if copeptin is increased at first medical contact in the ambulance, the circulating concentrations may rapidly decrease down to normal ranges at the time of hospital admission (27). Our results are in accordance with this observation, as we found one misdiagnosed NSTEMI very early presenter with non-elevated copeptin.

Lastly, our results are reinforced by those of Stallone et al who found that the additional use of copeptin did not increase diagnostic accuracy in very early presenters (6). Furthermore, the NPV for the combination of HS-cTnT and copeptin was lower in patients arriving in the first 2 hours than in those arriving after 2 hours (6). However, these authors did not evaluate the LoD of HS-cTnT in their work. We here report that even when lowering the cutoff of HS-

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cTnT, the combination of HS-cTnT and copeptin is not enough to detect all NSTEMI among all very early presenters.

Of note, all patients with CPO >4 hours had detectable cTnI and HS-cTnT, i.e. the use of copeptin in this situation added no gain. Other studies reported that copeptin testing for the rule out of NSTEMI should be limited to CPO< 6 hours (1,13). According to our data, the added value of copeptin might be more restricted, but further studies are needed to confirm our findings.

Limitations of our study

First, it is a post-hoc analysis of three previously published studies, and some data are missing (vital signs at admission, details in ECG findings for example). Second, only a single measurement of troponin at admission was considered for this analysis, and we did not evaluate its kinetics; we therefore cannot comment on the accuracy of the recent 1h algorithm in our population (1,2). Third, different troponin assays were used across the different centers (11–13). However, all centers evaluated the same HS-cTnT and copeptin. Fourth, as the gold standard diagnosis was based on a non-HS cTn, we recognize that this could result in underdiagnosis of myocardial injury; however, our results are comparable to those of Stallone et al how recently used an HS-cTn, as suggested by our AUC (0.85[95%CI : 0.79-0.90]) in comparison to those of Stallone (0.86 [95%CI:0.82-.090]) (6). And the message on time-effect is not altered. Fifth, we examined 3 subgroups with CPO<2h, 2-4h and 4h and defined very early presenters as those having CPO <2 hours, as based on the accepted definition of early presenters (20). However, very early presenters represent more than one third of the studied population, highlighting the specific need for further studies to confirm our findings.

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Conclusion

A single measurement of HS-cTnT alone or in combination with copeptin at admission seems not sensitive enough to safely rule out NSTEMI in very early presenters (chest pain onset < 2 hours from ED admission). If other studies confirm our findings, another strategy to safely exclude NSTEMI in this specific population that represents one third of chest pain patients is warranted.

Figure legends:

Figure 1: Flow chart of the studied population.

Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

Contributorship statement:

PR, CM, MS, and NP contributed to the design and inclusions. CCG, SL, AMD and GL realized sample collections and biomarker measurements. CCG and PR realized statistical

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analysis. All authors approved the final manuscript. ThermoFisher Scientific and Roche

Diagnostic provided reagents.

Competing interests:

CCG, PR and CM received honorarias and lecture fees from Roche Diagnostics and

Thermofisher Scientific.

Funding:

None

Data sharing statement:

No data sharing

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Table 1: Main characteristics of original study designs

	Sebbane et al. (14)	**Chenevier-Gobeaux et al. (15)**
Inclusion Criteria	Prospective cohort of ED patients with CPO <12h	Consecutive patients, >18 years old, admitted to the ED or to the ICU by pre-hospital emergency ambulances
Exclusion criteria	Patients with traumatic causes of chest pain	Patients <18 years old Acute or chronic renal failure requiring dialysis
Plasma sampling and storage	Heparinized and EDTA blood collection. Storage at -80°C for later analysis	Heparinized blood collection after routine cTnI measurement. Storage at -40°C until HS-cTnT and copeptin measurement
Registration number/name	French Health Ministry (no. DC-2009-1052)	French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière)
Consent	Written informed consent	Cochin Hospital: waiver of informed consent was authorized. Pitié-Salpêtrière Hospital: informed consent was granted.

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Table 2: Main Characteristics of the studied population

	All patients	CPO <2h (very early presenters)	CPO2-4h	CPO 4-6h
n	449	160	143	146
Age (years)	58 ± 17	57 ± 16	59 ± 17	58 ± 17
Men	281 (63)	101 (63)	96 (67)	84 (58)
Past medical history:				
Familial history of CAD, present/total (%) *	104/301 (35)	63/147 (43)	26/90 (29)	15/64 (23)
Personal history of CAD	120 (27)	38 (24)	42 (29)	40 (27)
Dyslipidaemia	168 (37)	61 (38)	57 (40)	50 (34)
Diabetes	67 (15)	20 (13)	22 (15)	25 (17)
Smoking	176 (39)	72 (45)	51 (37)	53 (36)
Hypertension	158 (35)	52 (33)	58 (41)	48 (33)
Outcome:				
Coronary angiography	131 (29)	49 (31)	46 (32)	36 (25)
Admission	256 (57)	98 (61)	91 (63)	67 (46)
Final Diagnostic:				
NSTEMI	55 (12)	15 (9)	22 (13)	18 (12)
other	394 (88)	145 (91)	121 (85)	128 (88)

CAD, coronary artery disease; CPO, chest pain onset; NSTEMI, non ST-elevation myocardial infarction.

Results are expressed in mean ± SD or in number (percentage)

*, missing data exist for this variable.

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Table 3: AUC values according to CPO category

	Biomarker	AUC	95% CI
CPO <2h (very early presenters)	cTnI	0.815	0.746 to 0.872
	cTnI + copeptin	0.866	0.804 to 0.915
	HS-cTnT	0.853	0.789 to 0.904
	HS-cTnT + copeptin	0.897	0.840 to 0.940
CPO 2-4h	cTnI	0.856	0.788 to 0.909
	cTnI + copeptin	0.906	0.846 to 0.948
	HS-cTnT	0.869	0.802 to 0.919
	HS-cTnT + copeptin	0.891	0.829 to 0.937
CPO >4h	cTnI	0.989	0.955 to 0.999
	cTnI + copeptin	0.978	0.939 to 0.995
	HS-cTnT	0.980	0.942 to 0.996
	HS-cTnT + copeptin	0.953	0.905 to 0.981

AUC, area under the ROC curve; CPO, chest pain onset

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Table 4: Diagnostic performances for NSTEMI according to onset chest pain

CPO	Biomarker	Threshold	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)	Misdiagnosed (**) (n)	Correctly ruled-out, n (%)
<2h (very early presenters) **(n=160)**	cTnI	LoQ (*)	73 [45-91]	97 [92-99]	97 [92-99]	73 [45-91]	4	141 (88)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	87 [59-98]	60 [52-68] ε	98 [92-100]	18 [7-24]	2	87 (54) ε
	HScTnT	14 ng/L	80 [51-95]	85 [78-90] ε	98 [93-100]	35 [20-53]	3	123 (79) ε
		5 ng/L	87 [59-98]	58 [50-66] ε ζ	98 [92-100]	18 [10-29]	2	84 (52) ε
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	93 [66-100]	54 [46-62] ε ζ	99 [93-100]	18 [10-29]	1	79 (49) ε
		5 ng/L and 12 pmol/L	93 [66-100]	41 [33-50] ε ζ	98 [89-100]	14 [8-23]	1	59 (37) ε
2-4h **(n=143)**	cTnI	LoQ (*)	73 [50-89]	95 [89-98]	95 [89-98] ψ	73 [50-89]	6	115 (80)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	95 [75-100]	57 [48-66] ε	99 [92-100]	29 [19-41]	1	69 (48) ε
	HScTnT	14 ng/L	77 [54-91]	79 [71-86] ε	95 [88-98] ψ	40 [26-56]	5	96 (67) ε μ
		5 ng/L	91 [70-98]	55 [46-64] ε ζ	97 [89-100]	27 [18-39]	2	67 (47) ε
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	95 [75-100]	50 [41-59] ε ζ	99 [91-100]	25 [17-36]	1	58 (41) ε
		5 ng/L and 12 pmol/L	100 [82-100]	35 [27-44] ε ζ	100 [90-100]	22 [15-32]	0	42 (29) ε
>4h **(n=146)**	cTnI	LoQ (*)	100 [77-100]	96 [91-99]	100 [96-100]	77 [54-91]	0	122 (85)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	100 [77-100]	59 [50-68] ε	100 [94-100]	25 [16-37]	0	75 (54) ε
	HScTnT	14 ng/L	100 [78-100]	87 [80-92] ε	100 [96-100]	51 [34-68]	0	111 (77)
		5 ng/L	100 [78-100]	60 [51-68] ε ζ	100 [94-100]	35 [23-50]	0	77 (53) ε μ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	100 [78-100]	55 [46-64] ε ζ	100 [94-100]	24 [15-35]	0	70 (49) ε
		5 ng/L and 12 pmol/L	100 [78-100]	40 [32-49] ε ζ	100 [91-100]	19 [12-29]	0	51 (35) ε

(*) 0.04 µg/L for Bicêtre and Montpellier hospitals, 0.14 µg/L for other sites; (**), false negative patients

ε $p<0.05$ versus cTnI alone; ζ $p<0.05$ versus HS-cTnT <14 ng/L alone; ψ $p<0.05$ versus delay >4h; μ, $p<0.05$ versus delay <2h

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Table 5: Potentially misdiagnosed patients with low cTn thresholds and copeptin

Sex	Age	Dyslipidaemia	Smoke	Diabetes	Hypertension	Personal history of CAD	Chest pain since :	CPO` category	cTnI (µg/L)	HScTnT (ng/L)	Copeptin (pmol/L)
M	89	no	no	no	yes	yes	1 hours 15 min	<2h (very early presenters)	0.04	44.9	208.7
M	86	yes	no	no	yes	yes	30 min		0.06	11.3	77.2
W	35	no	no	no	no	no	50 min		0.01	3	54.7
W	44	no	yes	no	no	no	45 min		0.01	3	10.7
W	74	yes	no	no	yes	yes	3 hrs	2-4h	0.04	8.8	10.7
M	34,2	yes	yes	no	no	no	2 hrs 35 min		0.04	3	23.5
M	55	yes	no	no	no	yes	3 hrs 50 min		0.02	6	25.9
M	34	no	yes	no	no	no	3 hrs 15 min		0.01	4	52.4
M	61	yes	yes	no	yes	yes	3 hrs		0.03	19.7	27.2
M	59	no	no	no	no	no	2 hrs 45 min		0.04	10.1	241.8

CAD, coronary artery disease; CPO, chest pain onset; M, men; W, women.

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Figure 1: Flow chart of the studied population.

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Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

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Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

254x190mm (96 x 96 DPI)

Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

254x190mm (96 x 96 DPI)

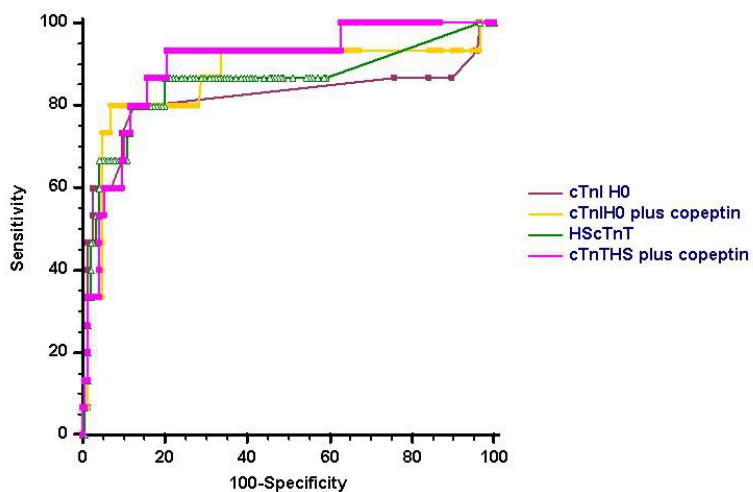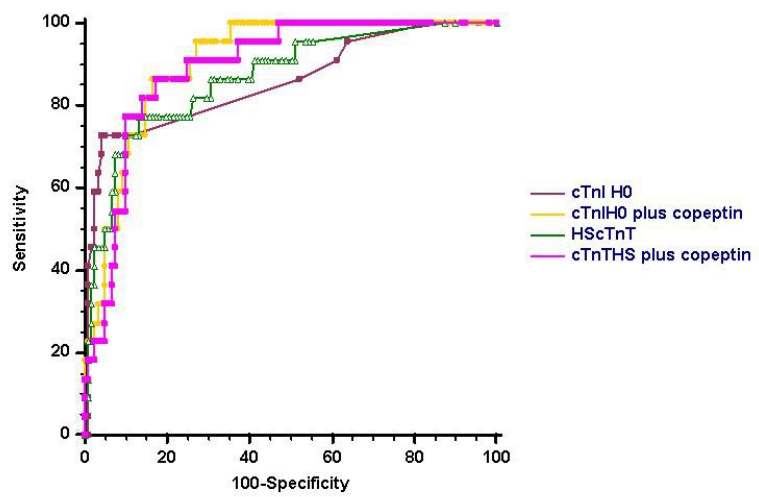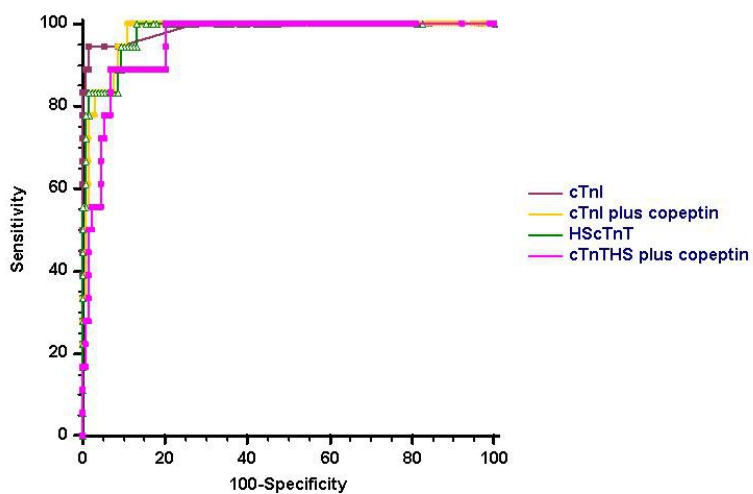| Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Page 1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Page 2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Page 4
	4	Study objectives and hypotheses	Pages 4 and 5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Page 6
Participants	**6**	Eligibility criteria	pp6&7 + Table1 p22
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Pages 6-8
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Page 6
	9	Whether participants formed a consecutive, random or convenience series	Page 6
Test methods	**10a**	Index test, in sufficient detail to allow replication	Pages 8 and 9
	10b	Reference standard, in sufficient detail to allow replication	Pages 8 and 9
	11	Rationale for choosing the reference standard (if alternatives exist)	Page 7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Page 9
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Pages 8 and 9
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Pages 6, 8 and 9
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Page 7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	Page 9
	15	How indeterminate index test or reference standard results were handled	Page 7
	16	How missing data on the index test and reference standard were handled	Flow chart (Figure1) + pages 6 and 7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Pages 6 and 7
	18	Intended sample size and how it was determined	NA
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Flow chart (Figure1) + pages 6 and 7
	20	Baseline demographic and clinical characteristics of participants	Table 2, page 23
	21a	Distribution of severity of disease in those with the target condition	Table 2, page 23
	21b	Distribution of alternative diagnoses in those without the target condition	Table 2, page 23
	22	Time interval and any clinical interventions between index test and reference standard	NA
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Tables 3&4, p.24-25
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Table 4, page25
	25	Any adverse events from performing the index test or the reference standard	Table 5 page 26
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Page 15
	27	Implications for practice, including the intended use and clinical role of the index test	Page 16
OTHER INFORMATION			
	28	Registration number and name of registry	Table 1, page22
	29	Where the full study protocol can be accessed	Table 1 page 22
	30	Sources of funding and other support; role of funders	NA

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

High-sensitivity troponin alone or in combination with copeptin is not enough for ruling out NSTEMI in very early presenters at admission in Emergency Department.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023994.R1
Article Type:	Research
Date Submitted by the Author:	17-Oct-2018
Complete List of Authors:	Chenevier-Gobeaux, Camille; Hopital Cochin, SDBA Sebbane, Mustapha; Centre Hospitalier Regional Universitaire de Montpellier, Emergency Departement Meune, Christophe; Avicenne University Hospital, Cardiology; Paris 13 University, Lefebvre, Sophie; Centre Hospitalier Regional Universitaire de Montpellier, Emergency department Dupuy, Anne-Marie; Montpellier University Hospital Centre, Department of Biochemistry Lefevre, Guillaume; Hopital Tenon, Laboratoire de Biochimie et Hormonologie Peschanski, Nicolas; CHU de Rouen, Service des Urgences Adultes; Institute for Biomedical Research and Innovation , INSERM U1096 Ray, Patrick; Hopital Tenon, Emergency Department
Primary Subject Heading:	Emergency medicine
Secondary Subject Heading:	Diagnostics
Keywords:	high sensitive cardiac troponin, non ST-elevation acute myocardial infarction, copeptin, very early presenters, chest pain onset

SCHOLARONE™
Manuscripts

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7 **High-sensitivity troponin alone or in combination with copeptin is not enough for ruling out**
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9 **NSTEMI in very early presenters at admission in Emergency Department.**
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11 Camille Chenevier-Gobeaux (1), Mustafa Sebbane (2), Christophe Meune (3, 4), Sophie Lefebvre
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Abstract

Objectives: Copeptin and high-sensitivity cardiac troponin (HS-cTn) assays improve the early detection of NSTEMI. Their sensitivities may however be reduced in very early presenters. *Setting:* We performed a post-hoc analysis of three prospective studies that included patients who presented to the Emergency Department for chest pain onset (CPO) of less than 6 hours. *Participants***:** 449 patients were included, in whom 12% had NSTEMI. CPO occurred <2h from ED presentation in 160, between 2-4h in 143, and >4h in 146 patients. The prevalence of NSTEMI was similar in all groups (9%, 13% and 12%, respectively, p=0.281). *Measures:* Diagnostic performances of HS-cTn and copeptin at presentation were examined according to CPO. The discharge diagnosis was adjudicated by 2 experts, including cTnI. HS-cTn and copeptin were blindly measured. *Results:* Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT (but not HS-cTnT+copeptin) lower through CPO categories. For patients with CPO<2h, the choice of a threshold value of 14 ng/L for HS-cTnT resulted in 3 false negative (Sensitivity 80% [95%CI: 51-95]; specificity 85% [78-90]; 79% of correctly ruled-out patients) and that of 5 ng/L in 2 false negative (Sensitivity 87% [59-98]; specificity 58% [50-66]; 52% of correctly ruled-out patients). The addition of copeptin to HS-cTnT induced a decrease of misclassified patients to 1 in patients with CPO<2h (Sensitivity 93% [66-100]; specificity 41% [33-50]). *Conclusion:* A single measurement of HS-cTn, alone or in combination with copeptin at admission, does not allow safely ruling out NSTEMI in very early presenters (with CPO<2h). *Trial registrations:* French Health Ministry (no. DC-2009-1052), French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière).

Strengths

- Focus on very early chest pain presenters that was not performed before

Limitations of our study

- Small numbers of very early chest pain presenters, although the data grouping of 3 previous studies

- A single measurement of troponin at admission was considered for the analysis, but not its kinetics

- The gold standard diagnosis was based on a non-HS cTn.

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Introduction

The management of patients with acute chest pain at the Emergency Department (ED) is a major health problem and an adequate ruling out process for non-ST elevation acute myocardial infarction (NSTEMI) is crucial. Cardiac troponin (cTn) measurement with conventional assays and more recent high-sensitivity cardiac troponin assays (HS-cTn), are current diagnostic tools for the assessment of these patients (1). The European Society of Cardiology guidelines proposed a rapid rule-out strategy using low HS-cTn values (i.e. values below the Limit of Detection of the assay -LoD-) as decisional threshold for ruling out NSTEMI (1). The accuracy of a rapid 0-hour/1-hour algorithm has also been recently demonstrated in patients with CPO<6h (2) and is endorsed by the latest ESC guidelines (1). In this analysis, the authors indicated that using a single HS-cTnT cutoff value of 14 ng/L at presentation resulted in 88.7% sensitivity and 97.3% negative predictive value (NPV), but that this single assay strategy was less effective than the combination of absolute level of HS-cTnT measured at presentation and again at 1 hour, combined with the absolute difference between the two levels (2).

However, in very early presenters, such rapid and efficient triage at presentation may be uncertain (3). Indeed, very early presenters, defined as having chest pain <2h, are considered by some authors as highly vulnerable as they may not present all symptoms and signs and thus be exposed to a higher risk of misdiagnosis and therefore worse outcome (4); the application of a rapid algorithm to these patients may lead to early discharge within 3 hours from ED admission. Recently, a meta-analysis (based on eleven studies) indicated that a single HS-cTnT concentration below the limit of detection may successfully rule out AMI (5). However, concerns have been raised about the safety of a single measurement rule out protocol performed at presentation in early presenters (6,7).

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Copeptin (in association with cTn) assays improve the early detection of NSTEMI (8–12). Indeed,

a randomized controlled trial demonstrated the safety of early discharge using a single

combination of copeptin + cTn at presentation for patients with CPO <6h (13).

The present study aimed to assess the diagnostic performance of HS-cTn and copeptin as a single

measurement at admission in very early ED presenters with suspected NSTEMI.

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Patients and methods

Study design and population

The study is a *post-hoc* analysis of three French prospective clinical studies (already published) of cardiac biomarker testing, each to explore the usefulness of copeptin and/or HS-cTnT testing in patients who presented to the ED with acute chest pain of less than 6h (14,15). However, none of these studies evaluated the influence of CPO value on diagnostic performances. All three trials had comparable inclusion/exclusion criteria and gathered similar clinical information (Table 1). Patients requiring renal replacement therapy were excluded. All studies received approval from local Institutional Review Board. The study complied with the principles of the Declaration of Helsinki. Recommendations of the Standards for Reporting of Diagnostic Accuracy (STARD) initiative were applied (16).

Patient Involvement

The development of the research question was not informed by patients. Patients were not involved in the design of this study. Patients were not involved in the recruitment to and conduct of the study. There was no results dissemination to study participants.

Routine assessment

All patients underwent an initial clinical evaluation (including clinical history, physical examination, 12-lead ECG, pulse oximetry, routine blood tests, and chest X-ray). Conventional cardiac Troponin I (cTnI) was measured on a venous blood collection performed at presentation and, if required, repeated after 3 to 9 h, as clinically indicated (17). The onset of chest pain (CPO), defined as the delay from symptom onset to presentation was recorded, based on patient history/declaration. When history was incomplete or inconsistent, CPO was not recorded and

patient was excluded of the analysis (see flow chart, Figure 1). Based on all clinical, biological (including cTnI value, but not HS-cTnT and copeptin values which were blindly measured) and imaging results, a decision was made by the attending physician to admit or discharge the patient, as well as medical therapy and revascularization if indicated. Attending emergency physicians and cardiologists were blinded to the results of HS-cTnT and copeptin, and biologists were blinded to the suspected diagnosis at presentation.

Patients with no cTnI results and/or no recorded CPO value, and patients with a final diagnosis of STEMI, were excluded (see flowchart, Figure 1).

Gold standard diagnosis

The gold standard diagnosis was adjudicated by two independent experts (emergency physician and cardiologist) who reviewed all available medical records (including patient history, physical findings, laboratory results including cTnI value and radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography, summary chart at discharge) pertaining to the patient from the time of ED presentation to 30-day follow-up. Experts were blind to copeptin and HS-cTnT results. In the event of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third expert.

AMI was diagnosed according to the universal definition that was in force at the time of inclusions and adapted to the use of a conventional cTn (18). Thus, patients with a cTnI increase (or a rise/fall pattern) above the 10%CV threshold, associated with at least one of the following: symptoms of myocardial ischemia, new ST-T changes or new Q wave on ECG, imaging of new loss of viable myocardium, or normal cTnI on admission, were classified as having an MI (STEMI with a ST elevation in at least 2 continuous leads on ECG or new onset of left bundle branch block, or NSTEMI). STEMI patients were excluded from the analysis, based on ST

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elevation observed on the ECG (see Flow chart, Fig. 1). Patients were classified according to the CPO, <2h, from 2-4h and >4h; those with a CPO <2 hours were considered as very early presenters.

Unstable angina (UA) diagnosis was adjudicated in patients with history or clinical symptoms consistent with ACS but without ST-T changes on the ECG and without change of cTn on serial testing. Other diagnostic categories beside NSTEMI and UA were non-ACS (e.g., stable angina, myocarditis, arrhythmias, heart failure, pulmonary embolism, and chest pain of unknown origin). UA and non-ACS chest pain were considered as non-NSTEMI in our analysis.

Troponin measurements

Plasma cTnI concentrations were routinely measured on an X-pand® HM analyzer, using the Cardiac Troponin-I immunoassay (Siemens Healthcare Diagnostics Inc., Newark, CT) in two EDs (Cochin and La Pitié Salpêtrière Hospitals). The limit of detection (LoD) was 0.04 µg/L (40 ng/L). The limit of quantitation (LoQ), i.e. the 10% imprecision point (or 10% CV), which is the lowest cTn concentration that can be reproducibly measured with a between-run CV of ≤10%, was 0.14 µg/L (140 ng/L). The 99th percentile of the assay was 0.07 µg/L (70 ng/L), with coefficients of variations (CV) between 15 to 22 %. The measuring range was 0.04 to 40 µg/L (40 to 40,000 ng/L), and the imprecision values across the measuring range were below 10%.

In Bicêtre and in Montpellier hospitals, plasma cTnI concentrations were routinely measured on an Access® analyser (Beckman Coulter, Inc., Brea, CA). According to the manufacturer's data, the limit of detection (LoD) was 0.01 µg/L (10 ng/L), the 20% point on the imprecision curve was 0.02 µg/L (20 ng/L). The LoQ/10%CV was 0.04 µg/L (40 ng/L). The 99th percentile of the

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assay was 0.04 µg/L (40 ng/L). The measuring range was 0.01 to 100 µg/L (10 to 100,000 ng/L), and the imprecision values across the measuring range were below 10%.

After routine cTnI measurement, plasma samples were aliquoted and frozen (-40°C) until HS-cTnT and copeptin measurement.

Hs-cTnT was measured in heparinized collected samples, on an Elecsys2010® analyser (Roche Diagnostics, Meylan, France). The limit of blanck (LoB) *was 3 ng/L, the LoD was 5 ng/L, and the 99th percentile was 14 ng/L. The measuring range was 3 to 10,000 ng/L. In our laboratory, CVs obtained in Roche quality controls containing 27.5 and 2,360 ng/L of cTnT were 3.6 and 2.8 % (between-run precision) and 1.4 and 0.4 % (within-run precision). Of note, the LoD is measured with a between-run CV of >10%, while the 99th percentile is a precise concentration (CV<10%) (7). HS-cTnT determinations were performed blinded to the clinical assessment of the emergency physicians.

Copeptin measurement

Copeptin was measured in heparinized blood samples collected on admission. The assay was performed on a KRYPTOR® analyser using ThermoFisher Scientific sandwich immunoluminometric assay (B.R.A.H.M.S Copeptin KRYPTOR, B.R.A.H.M.S Aktiengesellschaft, Hennigsdorf, Germany). The assay principle is based on TRACE technology (Time-Resolved Amplified Cryptate Emission). The lower detection limit is 4.8 pmol/L, and the functional assay sensitivity (20%CV value) is <12 pmol/L (data from manufacturer, recommended threshold value for this method). Copeptin determinations were performed blinded to the clinical assessment of the emergency physicians.

Statistical analysis

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Continuous variables are presented as means ± SD, and categorical variables are expressed as numbers (percentage). Continuous variables were compared by using the Kruskal-Wallis test, and categorical variables were assessed using Pearson's χ^2 test. Number of misclassified patients and number of correctly ruled-out patients were collected for each threshold strategy, and correspond to the false negative and the true positive patients, respectively.

Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) throughout the concentrations of cTnI, HS-cTnT and copeptin for the diagnosis of NSTEMI, and according to the CPO. cTn and copeptin values were log-transformed before combination for ROC analysis. For cTnI values, as they were obtained from two non-standardized methods, values were normalized by factorizing to the 99th percentile of the method prior to ROC analysis in order to remove any bias due to methodological differences.

Diagnostic thresholds that were used for classification of the data are:

- For cTnI, the LoQ values: 0.04 μg/L (40 ng/L) for Bicêtre and Montpellier hospitals, 0.14 μg/L (140 ng/L) for other sites,

- For HS-cTnT, The LoB (3 ng/L), the LoD (5 ng/L) and the 99th percentile (14 ng/L),

- For copeptin, the manufacturer's recommended threshold at 12 pmol/L.

All data are presented with their 95% confidence intervals [95%CI]. All hypothesis testing was two-tailed, and $p < 0.05$ was considered statistically significant. Statistical analysis was performed using MedCalc (MedCalc Software, Version 12.4.0.0, Mariarkerke, Belgium).

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Results

Characteristics of the studied population

Table 2 shows the characteristics of the studied population. Briefly, in the total cohort mean age was 58 ± 17 years, and included more males. A gold standard diagnosis of NSTEMI was adjudicated in 55 patients (12%). Delay from chest pain onset to ED admission was <2 hours in 160 (36%) patients, was from 2 to 4 hours in 143 (32%) and was >4 hours (and below 6 hours) in 146 (32%) patients. Very early presenters with NSTEMI (n=15) tended to be older than those without NSTEMI (n=145) (65 *vs.* 55 years-old), were more frequently hospitalized (93% *vs.* 58%), and also more frequently underwent diagnostic coronary angiography (73% *vs.* 27%).

Diagnostic performances according to CPO

ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin are presented in Figure 2. Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT are reduced when time to onset of chest pain gets less, as indicated by estimated AUCs and their 95%CI intervals (Table 3). The AUCs of HS-cTnT+copeptin were not different through the CPO categories.

Diagnostic performances of cTn, alone or in combination with copeptin, and using different decisional thresholds, are presented in Table 4.

In very early presenters (CPO <2 hours), a single value of cTnI alone had low sensitivity (73 [73-91] %) but high specificity (97 [92-99]%), and misclassified 4 of 15 NSTEMI patients. However, this strategy could correctly rule-out 141 patients (88%). Combining copeptin with cTnI increases sensitivity, and lowers the number of misdiagnosed patients from 4 to 2, but significantly lowers the rate of ruled-out patients from 88% to 54% (Table 4). Of note, addition

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of copeptin also significantly lowered specificity. At a threshold of 14 ng/L, HS-cTnT had low sensitivity (80 [59-98] %), misclassified 3 NSTEMI patients but could correctly rule-out 123 (79%) patients, which is less than using cTnI. The sensitivity of HS-TnT alone is likely to be suboptimal in early presenters, but can be improved by using a lower threshold for positivity or adding copeptin (but this is accompanied with a marked loss of specificity). The addition of copeptin induced a decrease in misclassified patients from 2 to 1, either with an HS-cTnT threshold at 14 or at 5 ng/L. This ultimate misdiagnosed NSTEMI patient with CPO <2h who presented with all undetectable biomarkers was a 44-years old woman with a history of smoking and no CV risk factors; the CPO was 45 min before hospital admission (Table 5).

In patients with CPO 2-4 hours, results are similar to those observed in very early presenters, although the number of misclassified patients was different (Table 4). Adding copeptin to cTnI induced a decrease of misclassified patients from 5 to 1 for an HS-cTnT threshold at 14 ng/L, and from 2 to 0 for an HS-cTnT threshold at 5 ng/L. In particular, combining copeptin to the LoD (5 ng/L) of HS-cTnT reached 100% sensitivity of the test. Similar performance was observed using the LoB (3ng/L) of HS-cTnT. Indeed, all NSTEMI patients with CPO 2-4 h had a detectable HS-cTnT and/or an elevated copeptin. In this sub-group again, the use of copeptin lowered significantly the specificity of the test.

As expected, in patients with CPO >4 hours, all patients had quantitative cTnI and detectable HS-cTnT. No patient was misclassified. The addition of copeptin in this sub-group had no effect on sensitivity or misclassified patients.

Potential misdiagnosed NSTEMI

Characteristics of potential misdiagnosed NSTEMI patients are detailed in Table 5. All potentially missed NSTEMI in the very early presenters population had a CPO <1 hour. We

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found no distinguishing characteristics in misclassified patients when comparing to correctly

diagnosed patients in terms of age, sex, and cardiovascular risks, in each CPO category. Of note,

when STEMI patients were included in our analysis, results were comparable (data not shown).

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Discussion

Our results indicate that diagnostic performances of HS-cTnT values at admission, alone or combined with copeptin, are reduced in the subgroup of patients with shorter CPO. Emergency physicians should not rule out NSTEMI in very early presenters with a single low value of HS-cTnT and/or copeptin at presentation.

Our studied population included one third of early presenters, and this proportion is similar to that found by Boeddinghaus et al (26% of patients presented within 2h from CPO) (4), by Keller et al (37% and 38% of patients with CPO<3h, (8,19)), and by Reichlin et al. (222 patients with CPO<3h out of 718, i.e. 31%) (20). More recently, Stallone et al reported 519 (26%) patients that arrived within 2 hours of symptom onset to the ED (6). This may reflect that our study was performed in large urban areas equipped with pre-hospital emergency ambulances. A more prolonged delay might be expected in more rural regions. The exact definition of very early presenters is still a matter of debate. Authors nevertheless agree to define them as presenting before 2h (20). Very few studies examined the specific groups of early/very early presenters (4,6,8,20). In the ESC guidelines, a different strategy is recommended for patients with versus without CPO<6h but not for earlier presenters (1). We believe that this proportion is not negligible and the impact of this very early population might be underestimated in studies were the CPO is not evaluated.

We observed that AUCs of cTnI, cTnI+copeptin and HS-cTnT were significantly lower when CPO<4h. The NPVs were not significantly impacted by the CPO, but a suboptimal sensitivity and a non-negligible proportion of misclassified NSTEMI was observed in patients with CPO<2h for all tested thresholds and combinations, even if cTnI and HS-cTnT performances can be improved by using a lower threshold for positivity or adding copeptin. The same was true for patients with CPO 2-4h, except when using the combination of HS-cTnT<5 ng/L and copeptin

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<12 µmol/L. We reported the number of misclassified patients in addition to sensitivity and NPV, because NPV is known to be dependent of the prevalence, thus its value might be biased. The absolute number of misdiagnosed patients might be more clinically pertinent than NPV (21).

As previously suggested (1), we found that sensitivity and NPV of a single measurement of HS-cTn at admission is not enough to safely excluded a NSTEMI in very early presenters. We here show that lowering HS-cTn decisional threshold to LoD or to LoB is not sufficient to detect all NSTEMI among very early chest pain presenters. Although combining copeptin to HS-cTnT increased sensitivity and lowered number of misclassified patients, none of the tested strategies allowed for identification of all NSTEMI. Results obtained in very early presenters were very similar to patients with CPO 2-4h, except that in this later sub-group combining copeptin with HS-cTnT succeeded in significantly increasing sensitivity (all NSTEMI patients had a detectable HS-cTnT and/or an elevated copeptin).

Our results can be compared to those of Boeddinghaus et al. who found that sensitivity of a single HS-cTn measurement is lower in very early presenters, in comparison to all patients (4). These authors indicated that using a single cut-off approach, 61% of the very early presenters were ruled-out, which resulted in a sensitivity of 94% and a NPV of 98%. They conclude that the single cutoff strategy should not be applied in early presenters. However, the authors did not evaluate the impact of copeptin across CPO categories. In another study, the same authors indicated that the additional use of copeptin did not sufficiently improve diagnostic accuracy in early presenters (22). Here again, our results are in accordance with those of Boeddinghaus indicating that copeptin did not improve diagnostic accuracy of hs-cTnI at presentation in early presenters (22). Mueller et al showed, using the rapid 0-hour/1-hour algorithm that 63% patients with CPO<6h were classified as rule-out. However, 7 patients were missed (0.9% rate), in whom 3 had HS-cTnT<LoD at presentation and at 1 hour (2). Moreover, a single cutoff value for HS-

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cTnT at 14 ng/L at presentation resulted in a sensitivity of 88.7% and a NPV of 97.3%, and performed less adequately than the combination of HS-cTnT at presentation with 1-hour level and 1-hour absolute change (2). In a more recent study, Mokhtari et al. evaluated a rapid 0H/1H protocol for discharge chest pain patients based on a single value <LoD at admission (23). These authors found 2 missed patients in their population, and recognize that the safety of such rapid protocol is not clear in very early presenters. They further recommend additional HS-cTnT testing at 3 hours for very early presenters (23).

Performance of cardiac troponins, although measured using HS assays, might be limited in very early presenters because of their kinetics of release into the blood circulation (8). The release of cTn into the circulation following cardiomyocyte damage is a time-dependent phenomenon (24), and a single measurement approach may fail at identifying AMI very early after the onset of symptoms (25). Indeed we, like other authors, found very early presenters with undetectable HS-cTnT at admission. Time-dependent release of copeptin during AMI has been described earlier, and this biomarker has been considered as an early biomarker (8). Copeptin increases immediately after induction of ischemia, and peaks 90 min after (26). However, some authors recently indicated that copeptin kinetics might be different in NSTEMI in comparison to STEMI, and that if copeptin is increased at first medical contact in the ambulance, the circulating concentrations may rapidly decrease down to normal ranges at the time of hospital admission (27). Our results are in accordance with this observation, as we found one misdiagnosed NSTEMI very early presenter with non-elevated copeptin.

Lastly, our results are reinforced by those of Stallone et al who found that the additional use of copeptin did not increase diagnostic accuracy in very early presenters (6). Furthermore, the NPV for the combination of HS-cTnT and copeptin was lower in patients arriving in the first 2 hours than in those arriving after 2 hours (6). However, these authors did not evaluate the LoD nor

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LoB ? of HS-cTnT in their work. We here report that even when lowering the cutoff of HS-cTnT, the combination of HS-cTnT and copeptin is not enough to detect all NSTEMI among all very early presenters. Our study and the one of Stallone et al. (6) are in accordance with previous studies that have shown that there is no or marginal benefit when adding copeptin to HS-cTn assays; indeed, Wildi et al. indicated that copeptin provides no significant increase in AUC when combined to HS-cTn (28), either in their all population or in patients with CPO<4h. These authors found an incremental value in sensitivities, NPV and calculating the integrated discrimination improvement index (IDI), but they did not evaluated low HS-cTn thresholds such as LoB and LoD values (28).

Of note, all patients with CPO >4 hours had detectable cTnI and HS-cTnT, i.e. the use of copeptin in this situation added no gain. Other studies reported that copeptin testing for the rule out of NSTEMI should be limited to CPO< 6 hours (1,13). According to our data, the added value of copeptin might be more restricted, but further studies are needed to confirm our findings. The current ESC guidelines incorporate an additional criterion for direct rule-out of patients that are not very early presenters; indeed, the rapid rule-out is possible only if CPO is >3h (1). Furthermore, this rapid algorithm can be used only for 3 HS-cTn assays, including HS-cTnT. Considering our data about patients with CPO >4h, we note that our conclusions are in line with the recommendations. Therefore, the use of a 0/1h-algorithm might be used for a safe rule-out in patients that are not very early presenters.

Limitations of our study

First, it is a post-hoc analysis of three previously published studies, and some data are missing (vital signs at admission, details in ECG findings for example). Second, only a single measurement of troponin at admission was considered for this analysis, and we did not evaluate

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its kinetics; we therefore cannot comment on the accuracy of the recent 1h algorithm in our population (1,2). Third, different cTn assays were used across the different centers (11–13) and we had to normalize cTnI values before analysis in order to minimize bias. However, all centers evaluated the same HS-cTnT and copeptin. Fourth, as the gold standard diagnosis was based on a non-HS cTn, we recognize that this could result in underdiagnosis of myocardial injury. Previous studies have shown that an early rule-out of NSTEMI using hs-cTn alone, also in the vulnerable subgroup of early presenters, is safe (with high AUC, sensitivities and NPV in patients presenting with CPO<3h) (20).In our study, the combined biomarkers are not safe-enough for early rule-out of NSTEMI in patients presenting very early (CPO<2h). The fact that gold standard diagnoses were adjudicated by the use of a conventional but not a hs-cTn assay, and that different assays where used, may have led to underdiagnose patients. This point limits the generalizability of our findings and explains why sensitivities and NPVs were much lower as compared to previous studies. However, our results are comparable to those of Stallone et al how recently used an HS-cTn, as suggested by our AUC (0.85[95%CI : 0.79-0.90]) in comparison to those of Stallone (0.86 [95%CI:0.82-.090]) (6). However, the aim of our work is not to evaluate another time global HS-cTn accuracy, but to highlight CPO effects on diagnostic accuracy of HS-cTn combined or not to copeptin. Fifth, we examined 3 subgroups with CPO<2h, 2-4h and 4h and defined very early presenters as those having CPO <2 hours, as based on the accepted definition of early presenters (20). Even if very early presenters represent more than one third of the studied population, the number of very early presenters (CPO<2h) is relatively small, although data from three cohorts was used. This explains why false rule-out of 3 patients results in a significant drop in sensitivity and NPV. Many previous studies investigated the rule-out performance in early presenters using e.g. the LoD of hs-cTnT and hs-cTnI and found much higher sensitivities and NPVs. This can be explained due to a larger number of patients (4, 20).

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Conclusion

A single measurement of HS-cTnT alone or in combination with copeptin at admission seems not sensitive enough to safely rule out NSTEMI in very early presenters (chest pain onset < 2 hours from ED admission). If other studies confirm our findings, another strategy to safely exclude NSTEMI in this specific population that represents one third of chest pain patients is warranted.

Acknowledgments

The authors would like to thank again the nursing, technical and medical staff at the emergency departments and biochemistry laboratories for their assistance throughout the original studies. Reagents for the Kryptor ® Compact Plus and HS-cTnT assays used for the 3 studies were kindly provided by ThermoFisher Scientific and Roche Diagnostic, France, respectively.

Figure legends:

Figure 1: Flow chart of the studied population.

Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

Contributorship statement:

PR, CM, MS, and NP contributed to the design and inclusions. CCG, SL, AMD and GL realized sample collections and biomarker measurements. CCG and PR realized statistical analysis. All

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authors approved the final manuscript. ThermoFisher Scientific and Roche Diagnostic provided reagents.

Competing interests:

CCG, PR and CM received honorarias and lecture fees from Roche Diagnostics and Thermofisher Scientific.

Funding:

None

Data sharing statement:

No data sharing

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Table 1: Main characteristics of original study designs

	Sebbane et al. (14)	**Chenevier-Gobeaux et al. (15)**
Inclusion Criteria	Prospective cohort of ED patients with CPO <12h	Consecutive patients, >18 years old, admitted to the ED or to the ICU by pre-hospital emergency ambulances
Exclusion criteria	Patients with traumatic causes of chest pain	Patients <18 years old Acute or chronic renal failure requiring dialysis
Plasma sampling and storage	Heparinized and EDTA blood collection. Storage at -80°C for later analysis	Heparinized blood collection after routine cTnI measurement. Storage at -40°C until HS-cTnT and copeptin measurement
Registration number/name	French Health Ministry (no. DC-2009-1052)	French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière)
Consent	Written informed consent	Cochin Hospital: waiver of informed consent was authorized. Pitié-Salpêtrière Hospital: informed consent was granted.

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Table 2: Main Characteristics of the studied population

	All patients	**CPO <2h (very early presenters)**	**CPO 2-4h**	**CPO 4-6h**
n	449	160	143	146
Age (years)	58 ± 17	57 ± 16	59 ± 17	58 ± 17
Men	281 (63)	101 (63)	96 (67)	84 (58)
Past medical history:				
Familial history of CAD, present/total (%) *	104/301 (35)	63/147 (43)	26/90 (29)	15/64 (23)
Personal history of CAD	120 (27)	38 (24)	42 (29)	40 (27)
Dyslipidaemia	168 (37)	61 (38)	57 (40)	50 (34)
Diabetes	67 (15)	20 (13)	22 (15)	25 (17)
Smoking	176 (39)	72 (45)	51 (37)	53 (36)
Hypertension	158 (35)	52 (33)	58 (41)	48 (33)
Outcome:				
Coronary angiography	131 (29)	49 (31)	46 (32)	36 (25)
Admission	256 (57)	98 (61)	91 (63)	67 (46)
Final Diagnostic:				
NSTEMI	55 (12)	15 (9)	22 (13)	18 (12)
other	394 (88)	145 (91)	121 (85)	128 (88)

CAD, coronary artery disease; CPO, chest pain onset; NSTEMI, non ST-elevation myocardial infarction. Results are expressed in mean $\pm$ SD or in number (percentage)

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*, missing data exist for this variable.

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Table 3: AUC values according to CPO category

	Biomarker	AUC	95% CI
CPO <2h (very early presenters)	cTnI	0.841	0.775 to 0.894
	cTnI + copeptin	0.880	0.819 to 0.926
	HS-cTnT	0.853	0.789 to 0.904
	HS-cTnT + copeptin	0.897	0.840 to 0.940
CPO 2-4h	cTnI	0.886	0.823 to 0.933
	cTnI + copeptin	0.915	0.857 to 0.955
	HS-cTnT	0.869	0.802 to 0.919
	HS-cTnT + copeptin	0.891	0.829 to 0.937
CPO >4h	cTnI	0.995	0.965 to 1.000
	cTnI + copeptin	0.979	0.940 to 0.995
	HS-cTnT	0.980	0.942 to 0.996
	HS-cTnT + copeptin	0.953	0.905 to 0.981

AUC, area under the ROC curve; CPO, chest pain onset

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Table 4: Diagnostic performances for NSTEMI according to onset chest pain

CPO	Biomarker	Threshold	Sensitivity (%, [95%CI])	Specificity (%, [95%CI])	Negative predictive value (%, [95%CI])	Positive predictive value (%, [95%CI])	Misdiagnosed (**) (n)	Correctly ruled-out, n (%)
<2h (very early presenters) (n=160)	cTnI	LoQ (*)	73 [45-91]	97 [92-99]	97 [92-99]	73 [45-91]	4	141 (88)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	87 [59-98]	60 [52-68] ε	98 [92-100]	18 [7-24] ε	2	87 (54) ε
	HScTnT	14 ng/L	80 [51-95]	85 [78-90] ε	98 [93-100]	35 [20-53]	3	123 (79) ε
		5 ng/L	87 [59-98]	58 [50-66] ε ζ	98 [92-100]	18 [10-29] ε	2	84 (52) ε ζ
		3 ng/L	87[58-98]	40 [32-49] ε ζ	97 [88-99]	13 [7-22] ε	2	58 (36) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	93 [66-100]	54 [46-62] ε ζ	99 [93-100]	18 [10-29] ε	1	79 (49) ε ζ
		5 ng/L and 12 pmol/L	93 [66-100]	41 [33-50] ε ζ	98 [89-100]	14 [8-23] ε	1	59 (37) ε ζ
		3 ng/L and 12 pmol/L	93 [66-100]	25 [19-34] ε ζ	97 [85-100]	12 [7-19] ε	1	37 (23) ε ζ
2-4h (n=143)	cTnI	LoQ (*)	73 [50-89]	95 [89-98]	95 [89-98] ψ	73 [50-89]	6	115 (80)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	95 [75-100]	57 [48-66] ε	99 [92-100]	29 [19-41] ε	1	69 (48) ε
	HScTnT	14 ng/L	77 [54-91]	79 [71-86] ε	95 [88-98] ψ	40 [26-56]	5	96 (67) ε μ
		5 ng/L	91 [70-98]	55 [46-64] ε ζ	97 [89-100]	27 [18-39] ε	2	67 (47) ε ζ
		3 ng/L	96 [75-100]	45 [36-54] ε ζ	98 [89-100]	24 [16-34] ε	1	54 (38) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	95 [75-100]	50 [41-59] ε ζ	99 [91-100]	25 [17-36] ε	1	58 (41) ε ζ
		5 ng/L and 12 pmol/L	100 [82-100]	35 [27-44] ε ζ	100 [90-100]	22 [15-32] ε	0	42 (29) ε ζ
		3 ng/L and 12 pmol/L	100 [82-100]	29 [21-38] ε ζ	100 [88-100]	20 [14-30] ε	0	35 (25) ε ζ
>4h (n=146)	cTnI	LoQ (*)	100 [77-100]	96 [91-99]	100 [96-100]	77 [54-91]	0	122 (85)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	100 [77-100]	59 [50-68] ε	100 [94-100]	25 [16-37] ε	0	75 (54) ε
	HScTnT	14 ng/L	100 [78-100]	87 [80-92] ε	100 [96-100]	51 [34-68]	0	111 (77)
		5 ng/L	100 [78-100]	60 [51-68] ε ζ	100 [94-100]	35 [23-50] ε	0	77 (53) ε μ ζ
		3 ng/L	100 [78-100]	52 [43-61] ε ζ	100 [96-100]	23 [14-34] ε	0	67 (47) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	100 [78-100]	55 [46-64] ε ζ	100 [94-100]	24 [15-35] ε	0	70 (49) ε ζ
		5 ng/L and 12 pmol/L	100 [78-100]	40 [32-49] ε ζ	100 [91-100]	19 [12-29] ε ζ	0	51 (35) ε ζ
		3 ng/L and 12 pmol/L	100 [78-100]	34 [26-43] ε ζ	100 [96-100]	18 [11-27] ε ζ	0	43 (30) ε ζ

(*) 0.04 µg/L (40 ng/L) for Bicêtre and Montpellier hospitals, 0.14 µg/L (140 ng/L) for other sites; (**), false negative patients

ε p<0.05 versus cTnI alone; ζ p<0.05 versus HS-cTnT <14 ng/L alone; ψ p<0.05 versus delay >4h; μ, p<0.05 versus delay <2h

Table 5: Potentially misdiagnosed patients with low cTn thresholds and copeptin

Sex	Age	Dyslipidaemia	Smoke	Diabetes	Hypertension	Personal history of CAD	Chest pain since :	CPO` category	cTnI	HScTnT	Copeptin
M	89	no	no	no	yes	yes	1 hr 15 min	<2h (very early presenters)	0.04 µg/L (40 ng/L)	44.9 ng/L	208.7 pmol/L
M	86	yes	no	no	yes	yes	30 min		0.06 µg/L (60 ng/L)	11.3 ng/L	77.2 pmol/L
W	35	no	no	no	no	no	50 min		0.01 µg/L (10 ng/L)	<3 ng/L	54.7 pmol/L
W	44	no	yes	no	no	no	45 min		0.01 µg/L (10 ng/L)	<3 ng/L	10.7 pmol/L
W	74	yes	no	no	yes	yes	3 hrs	2-4h	0.04 µg/L (40 ng/L)	8.8 ng/L	10.7 pmol/L
M	34,2	yes	yes	no	no	no	2 hrs 35 min		0.04 µg/L (40 ng/L)	<3 ng/L	23.5 pmol/L
M	55	yes	no	no	no	yes	3 hrs 50 min		0.02 µg/L (20 ng/L)	6 ng/L	25.9 pmol/L
M	34	no	yes	no	no	no	3 hrs 15 min		0.01 µg/L (10 ng/L)	4 ng/L	52.4 pmol/L
M	61	yes	yes	no	yes	yes	3 hrs		0.03 µg/L (30 ng/L)	19.7 ng/L	27.2 pmol/L
M	59	no	no	no	no	no	2 hrs 45 min		0.04 µg/L (40 ng/L)	10.1 ng/L	241.8 pmol/L

CAD, coronary artery disease; CPO, chest pain onset; M, men; W, women.

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n=557
[ref. 14]: n=363
[ref. 15]: n=194

Exclusions:
No cTnI: n=7
No CPO: n=67
STEMI: n=34

n=449

CPO <2h
n=160
15 STEMI / 145 non STEMI

CPO 2-4h
n=143
22 STEMI / 121 non STEMI

CPO > 4h
n=146
18 STEMI / 128 non STEMI

Figure 1: Flow chart of the studied population.

135x255mm (150 x 150 DPI)

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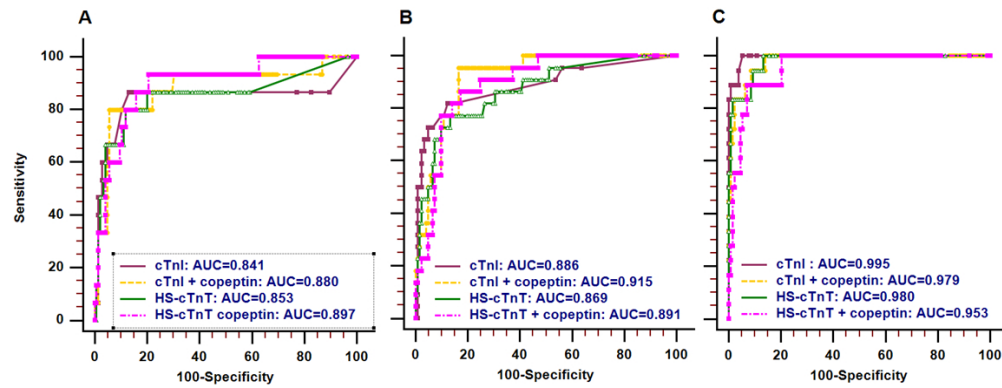53
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Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Page 1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Page 2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Page 4
	4	Study objectives and hypotheses	Pages 4 and 5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Page 6
Participants	**6**	Eligibility criteria	pp6&7 + Table1 p22
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Pages 6-8
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Page 6
	9	Whether participants formed a consecutive, random or convenience series	Page 6
Test methods	**10a**	Index test, in sufficient detail to allow replication	Pages 8 and 9
	10b	Reference standard, in sufficient detail to allow replication	Pages 8 and 9
	11	Rationale for choosing the reference standard (if alternatives exist)	Page 7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Page 9
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Pages 8 and 9
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Pages 6, 8 and 9
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Page 7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	Page 9
	15	How indeterminate index test or reference standard results were handled	Page 7
	16	How missing data on the index test and reference standard were handled	Flow chart (Figure1) + pages 6 and 7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Pages 6 and 7
	18	Intended sample size and how it was determined	NA
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Flow chart (Figure1) + pages 6 and 7
	20	Baseline demographic and clinical characteristics of participants	Table 2, page 23
	21a	Distribution of severity of disease in those with the target condition	Table 2, page 23
	21b	Distribution of alternative diagnoses in those without the target condition	Table 2, page 23
	22	Time interval and any clinical interventions between index test and reference standard	NA
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Tables 3&4, p.24-25
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Table 4, page25
	25	Any adverse events from performing the index test or the reference standard	Table 5 page 26
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Page 15
	27	Implications for practice, including the intended use and clinical role of the index test	Page 16
OTHER INFORMATION			
	28	Registration number and name of registry	Table 1, page22
	29	Where the full study protocol can be accessed	Table 1 page 22
	30	Sources of funding and other support; role of funders	NA

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

High-sensitivity troponin alone or in combination with copeptin seems not sensitive enough for ruling out NSTEMI in very early presenters at admission in Emergency Department: a post-hoc analysis.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023994.R2
Article Type:	Research
Date Submitted by the Author:	14-Feb-2019
Complete List of Authors:	Chenevier-Gobeaux, Camille; Hopital Cochin, SDBA Sebbane, Mustapha; Centre Hospitalier Regional Universitaire de Montpellier, Emergency Departement Meune, Christophe; Avicenne University Hospital, Cardiology; Paris 13 University, Lefebvre, Sophie; Centre Hospitalier Regional Universitaire de Montpellier, Emergency department Dupuy, Anne-Marie; Montpellier University Hospital Centre, Department of Biochemistry Lefevre, Guillaume; Hopital Tenon, Laboratoire de Biochimie et Hormonologie Peschanski, Nicolas; CHU de Rouen, Service des Urgences Adultes; Institute for Biomedical Research and Innovation , INSERM U1096 Ray, Patrick; Hopital Tenon, Emergency Department
Primary Subject Heading:	Emergency medicine
Secondary Subject Heading:	Diagnostics, Cardiovascular medicine, Emergency medicine
Keywords:	high sensitive cardiac troponin, non ST-elevation acute myocardial infarction, copeptin, very early presenters, chest pain onset

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Manuscripts

Revised manuscript Ref: bmjopen-2018-023994_R2

High-sensitivity troponin alone or in combination with copeptin seems not sensitive enough for ruling out NSTEMI in very early presenters at admission in Emergency Department: a post-hoc analysis.

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Abstract

Objectives: Copeptin and high-sensitivity cardiac troponin (HS-cTn) assays improve the early detection of NSTEMI. Their sensitivities may however be reduced in very early presenters. *Setting:* We performed a post-hoc analysis of three prospective studies that included patients who presented to the Emergency Department for chest pain onset (CPO) of less than 6 hours. *Participants:* 449 patients were included, in whom 12% had NSTEMI. CPO occurred <2h from ED presentation in 160, between 2-4h in 143, and >4h in 146 patients. The prevalence of NSTEMI was similar in all groups (9%, 13% and 12%, respectively, p=0.281). *Measures:* Diagnostic performances of HS-cTn and copeptin at presentation were examined according to CPO. The discharge diagnosis was adjudicated by 2 experts, including cTnI. HS-cTn and copeptin were blindly measured. *Results:* Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT (but not HS-cTnT+copeptin) lower through CPO categories. For patients with CPO<2h, the choice of a threshold value of 14 ng/L for HS-cTnT resulted in 3 false negative (Sensitivity 80% [95%CI: 51-95]; specificity 85% [78-90]; 79% of correctly ruled-out patients) and that of 5 ng/L in 2 false negative (Sensitivity 87% [59-98]; specificity 58% [50-66]; 52% of correctly ruled-out patients). The addition of copeptin to HS-cTnT induced a decrease of misclassified patients to 1 in patients with CPO<2h (Sensitivity 93% [66-100]; specificity 41% [33-50]). *Conclusion:* A single measurement of HS-cTn, alone or in combination with copeptin at admission, seems not safe enough for ruling out NSTEMI in very early presenters (with CPO<2h). *Trial registrations:* French Health Ministry (no. DC-2009-1052), French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière).

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Strengths

- Focus on very early chest pain presenters that was not performed before

Limitations of our study

- Small numbers of very early chest pain presenters, although the data grouping of 3 previous studies

- A single measurement of troponin at admission was considered for the analysis, but not its kinetics

- The gold standard diagnosis was based on a non-HS cTn.

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Introduction

The management of patients with acute chest pain at the Emergency Department (ED) is a major health problem and an adequate ruling out process for non-ST elevation acute myocardial infarction (NSTEMI) is crucial. Cardiac troponin (cTn) measurement with conventional assays and more recent high-sensitivity cardiac troponin assays (HS-cTn), are current diagnostic tools for the assessment of these patients (1). The European Society of Cardiology guidelines proposed a rapid rule-out strategy using low HS-cTn values (i.e. values below the Limit of Detection of the assay -LoD-) as decisional threshold for ruling out NSTEMI (1). The accuracy of a rapid 0-hour/1-hour algorithm has also been recently demonstrated in patients with CPO<6h (2) and is endorsed by the latest ESC guidelines (1). In this analysis, the authors indicated that using a single HS-cTnT cutoff value of 14 ng/L at presentation resulted in 88.7% sensitivity and 97.3% negative predictive value (NPV), but that this single assay strategy was less effective than the combination of absolute level of HS-cTnT measured at presentation and again at 1 hour, combined with the absolute difference between the two levels (2).

However, in very early presenters, such rapid and efficient triage at presentation may be uncertain (3). Indeed, very early presenters, defined as having chest pain <2h, are considered by some authors as highly vulnerable as they may not present all symptoms and signs and thus be exposed to a higher risk of misdiagnosis and therefore worse outcome (4); the application of a rapid algorithm to these patients may lead to early discharge within 3 hours from ED admission. Recently, a meta-analysis (based on eleven studies) indicated that a single HS-cTnT concentration below the limit of detection may successfully rule out AMI (5). However, concerns have been raised about the safety of a single measurement rule out protocol performed at presentation in early presenters (6,7).

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Copeptin (in association with cTn) assays improve the early detection of NSTEMI (8–12).

Indeed, a randomized controlled trial demonstrated the safety of early discharge using a single

combination of copeptin + cTn at presentation for patients with CPO <6h (13).

The present study aimed to assess the diagnostic performance of HS-cTn and copeptin as a

single measurement at admission in very early ED presenters with suspected NSTEMI.

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Patients and methods

Study design and population

The study is a *post-hoc* analysis of three French prospective clinical studies (already published) of cardiac biomarker testing, each to explore the usefulness of copeptin and/or HS-cTnT testing in patients who presented to the ED with acute chest pain of less than 6h (14,15). However, none of these studies evaluated the influence of CPO value on diagnostic performances. All three trials had comparable inclusion/exclusion criteria and gathered similar clinical information (Table 1). Patients requiring renal replacement therapy were excluded. All studies received approval from local Institutional Review Board. The study complied with the principles of the Declaration of Helsinki. Recommendations of the Standards for Reporting of Diagnostic Accuracy (STARD) initiative were applied (16).

Patient Involvement

The development of the research question was not informed by patients. Patients were not involved in the design of this study. Patients were not involved in the recruitment to and conduct of the study. There was no results dissemination to study participants.

Routine assessment

All patients underwent an initial clinical evaluation (including clinical history, physical examination, 12-lead ECG, pulse oximetry, routine blood tests, and chest X-ray). Conventional cardiac Troponin I (cTnI) was measured on a venous blood collection performed at presentation and, if required, repeated after 3 to 9 h, as clinically indicated (17). The onset of chest pain (CPO), defined as the delay from symptom onset to presentation was recorded, based on patient history/declaration. When history was incomplete or inconsistent, CPO was not recorded and patient was excluded of the analysis (see flow chart, Figure 1).

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Based on all clinical, biological (including cTnI value, but not HS-cTnT and copeptin values which were blindly measured) and imaging results, a decision was made by the attending physician to admit or discharge the patient, as well as medical therapy and revascularization if indicated. Attending emergency physicians and cardiologists were blinded to the results of HS-cTnT and copeptin, and biologists were blinded to the suspected diagnosis at presentation. Patients with no cTnI results and/or no recorded CPO value, and patients with a final diagnosis of STEMI, were excluded (see flowchart, Figure 1).

Gold standard diagnosis

The gold standard diagnosis was adjudicated by two independent experts (emergency physician and cardiologist) who reviewed all available medical records (including patient history, physical findings, laboratory results including cTnI value and radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography, summary chart at discharge) pertaining to the patient from the time of ED presentation to 30-day follow-up. Experts were blind to copeptin and HS-cTnT results. In the event of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third expert.

AMI was diagnosed according to the universal definition that was in force at the time of inclusions and adapted to the use of a conventional cTn (18). Thus, patients with a cTnI increase (or a rise/fall pattern) above the 10%CV threshold, associated with at least one of the following: symptoms of myocardial ischemia, new ST-T changes or new Q wave on ECG, imaging of new loss of viable myocardium, or normal cTnI on admission, were classified as having an MI (STEMI with a ST elevation in at least 2 continuous leads on ECG or new onset of left bundle branch block, or NSTEMI). STEMI patients were excluded from the analysis, based on ST elevation observed on the ECG (see Flow chart, Fig. 1). Patients were classified

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according to the CPO, <2h, from 2-4h and >4h; those with a CPO <2 hours were considered as very early presenters.

Unstable angina (UA) diagnosis was adjudicated in patients with history or clinical symptoms consistent with ACS but without ST-T changes on the ECG and without change of cTn on serial testing. Other diagnostic categories beside NSTEMI and UA were non-ACS (e.g., stable angina, myocarditis, arrhythmias, heart failure, pulmonary embolism, and chest pain of unknown origin). UA and non-ACS chest pain were considered as non-NSTEMI in our analysis.

Troponin measurements

Plasma cTnI concentrations were routinely measured on an X-pand® HM analyzer, using the Cardiac Troponin-I immunoassay (Siemens Healthcare Diagnostics Inc., Newark, CT) in two EDs (Cochin and La Pitié Salpêtrière Hospitals). The limit of detection (LoD) was 0.04 μg/L (40 ng/L). The limit of quantitation (LoQ), i.e. the 10% imprecision point (or 10% CV), which is the lowest cTn concentration that can be reproducibly measured with a between-run CV of ≤10%, was 0.14 μg/L (140 ng/L). The 99th percentile of the assay was 0.07 μg/L (70 ng/L), with coefficients of variations (CV) between 15 to 22 %. The measuring range was 0.04 to 40 μg/L (40 to 40,000 ng/L), and the imprecision values across the measuring range were below 10%.

In Bicêtre and in Montpellier hospitals, plasma cTnI concentrations were routinely measured on an Access® analyser (Beckman Coulter, Inc., Brea, CA). According to the manufacturer's data, the limit of detection (LoD) was 0.01 μg/L (10 ng/L), the 20% point on the imprecision curve was 0.02 μg/L (20 ng/L). The LoQ/10%CV was 0.04 μg/L (40 ng/L). The 99th percentile of the assay was 0.04 μg/L (40 ng/L). The measuring range was 0.01 to 100 μg/L (10 to 100,000 ng/L), and the imprecision values across the measuring range were below 10%.

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After routine cTnI measurement, plasma samples were aliquoted and frozen (-40°C) until HS-cTnT and copeptin measurement.

Hs-cTnT was measured in heparinized collected samples, on an Elecsys2010® analyser (Roche Diagnostics, Meylan, France). The limit of blanck (LoB) *was 3 ng/L, the LoD was 5 ng/L, and the 99th percentile was 14 ng/L. The measuring range was 3 to 10,000 ng/L. In our laboratory, CVs obtained in Roche quality controls containing 27.5 and 2,360 ng/L of cTnT were 3.6 and 2.8 % (between-run precision) and 1.4 and 0.4 % (within-run precision). Of note, the LoD is measured with a between-run CV of >10%, while the 99th percentile is a precise concentration (CV<10%) (7). HS-cTnT determinations were performed blinded to the clinical assessment of the emergency physicians.

Copeptin measurement

Copeptin was measured in heparinized blood samples collected on admission. The assay was performed on a KRYPTOR® analyser using ThermoFisher Scientific sandwich immunoluminometric assay (B.R.A.H.M.S Copeptin KRYPTOR, B.R.A.H.M.S Aktiengesellschaft, Hennigsdorf, Germany). The assay principle is based on TRACE technology (Time-Resolved Amplified Cryptate Emission). The lower detection limit is 4.8 pmol/L, and the functional assay sensitivity (20%CV value) is <12 pmol/L (data from manufacturer, recommended threshold value for this method). Copeptin determinations were performed blinded to the clinical assessment of the emergency physicians.

Statistical analysis

Continuous variables are presented as means ± SD, and categorical variables are expressed as numbers (percentage). Continuous variables were compared by using the Kruskal-Wallis test, and categorical variables were assessed using Pearson's χ^2 test. Number of misclassified

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patients and number of correctly ruled-out patients were collected for each threshold strategy, and correspond to the false negative and the true positive patients, respectively.

Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) throughout the concentrations of cTnI, HS-cTnT and copeptin for the diagnosis of NSTEMI, and according to the CPO. cTn and copeptin values were log-transformed before combination for ROC analysis. For cTnI values, as they were obtained from two non-standardized methods, values were normalized by factorizing to the 99th percentile of the method prior to ROC analysis in order to remove any bias due to methodological differences.

Diagnostic thresholds that were used for classification of the data are:

- For cTnI, the LoQ values: 0.04 μg/L (40 ng/L) for Bicêtre and Montpellier hospitals, 0.14 μg/L (140 ng/L) for other sites,

- For HS-cTnT, The LoB (3 ng/L), the LoD (5 ng/L) and the 99th percentile (14 ng/L),

- For copeptin, the manufacturer's recommended threshold at 12 pmol/L.

All data are presented with their 95% confidence intervals [95%CI]. All hypothesis testing was two-tailed, and $p < 0.05$ was considered statistically significant. Statistical analysis was performed using MedCalc (MedCalc Software, Version 12.4.0.0, Mariarkerke, Belgium).

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Results

Characteristics of the studied population

Table 2 shows the characteristics of the studied population. Briefly, in the total cohort mean age was 58 ± 17 years, and included more males. A gold standard diagnosis of NSTEMI was adjudicated in 55 patients (12%). Delay from chest pain onset to ED admission was <2 hours in 160 (36%) patients, was from 2 to 4 hours in 143 (32%) and was >4 hours (and below 6 hours) in 146 (32%) patients. Very early presenters with NSTEMI (n=15) tended to be older than those without NSTEMI (n=145) (65 *vs.* 55 years-old), were more frequently hospitalized (93% *vs.* 58%), and also more frequently underwent diagnostic coronary angiography (73% *vs.* 27%).

Diagnostic performances according to CPO

ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin are presented in Figure 2. Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT are reduced when time to onset of chest pain gets less, as indicated by estimated AUCs and their 95%CI intervals (Table 3). The AUCs of HS-cTnT+copeptin were not different through the CPO categories.

Diagnostic performances of cTn, alone or in combination with copeptin, and using different decisional thresholds, are presented in Table 4.

In very early presenters (CPO <2 hours), a single value of cTnI alone had low sensitivity (73 [73-91] %) but high specificity (97 [92-99]%), and misclassified 4 of 15 NSTEMI patients. However, this strategy could correctly rule-out 141 patients (88%). Combining copeptin with cTnI increases sensitivity, and lowers the number of misdiagnosed patients from 4 to 2, but significantly lowers the rate of ruled-out patients from 88% to 54% (Table 4). Of note, addition of copeptin also significantly lowered specificity. At a threshold of 14 ng/L, HS-

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cTnT had low sensitivity (80 [59-98] %), misclassified 3 NSTEMI patients but could correctly rule-out 123 (79%) patients, which is less than using cTnI. The sensitivity of HS-TnT alone is likely to be suboptimal in early presenters, but can be improved by using a lower threshold for positivity or adding copeptin (but this is accompanied with a marked loss of specificity). The addition of copeptin induced a decrease in misclassified patients from 2 to 1, either with an HS-cTnT threshold at 14 or at 5 ng/L. This ultimate misdiagnosed NSTEMI patient with CPO <2h who presented with all undetectable biomarkers was a 44-years old woman with a history of smoking and no CV risk factors; the CPO was 45 min before hospital admission (Table 5).

In patients with CPO 2-4 hours, results are similar to those observed in very early presenters, although the number of misclassified patients was different (Table 4). Adding copeptin to cTnI induced a decrease of misclassified patients from 5 to 1 for an HS-cTnT threshold at 14 ng/L, and from 2 to 0 for an HS-cTnT threshold at 5 ng/L. In particular, combining copeptin to the LoD (5 ng/L) of HS-cTnT reached 100% sensitivity of the test. Similar performance was observed using the LoB (3ng/L) of HS-cTnT. Indeed, all NSTEMI patients with CPO 2-4 h had a detectable HS-cTnT and/or an elevated copeptin. In this sub-group again, the use of copeptin lowered significantly the specificity of the test.

As expected, in patients with CPO >4 hours, all patients had quantitative cTnI and detectable HS-cTnT. No patient was misclassified. The addition of copeptin in this sub-group had no effect on sensitivity or misclassified patients.

Potential misdiagnosed NSTEMI

Characteristics of potential misdiagnosed NSTEMI patients are detailed in Table 5. All potentially missed NSTEMI in the very early presenters population had a CPO <1 hour. We found no distinguishing characteristics in misclassified patients when comparing to correctly

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diagnosed patients in terms of age, sex, and cardiovascular risks, in each CPO category. Of

note, when STEMI patients were included in our analysis, results were comparable (data not

shown).

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Discussion

Our results indicate that diagnostic performances of HS-cTnT values at admission, alone or combined with copeptin, are reduced in the subgroup of patients with shorter CPO. Emergency physicians may not rule out NSTEMI in very early presenters with a single low value of HS-cTnT and/or copeptin at presentation.

Our studied population included one third of early presenters, and this proportion is similar to that found by Boeddinghaus et al (26% of patients presented within 2h from CPO) (4), by Keller et al (37% and 38% of patients with CPO<3h, (8,19)), and by Reichlin et al. (222 patients with CPO<3h out of 718, i.e. 31%) (20). More recently, Stallone et al reported 519 (26%) patients that arrived within 2 hours of symptom onset to the ED (6), and Twerenbold et al. reported the largest subgroup investigated so far with 1,322 early presenters (with CPO<3h) out of 4,368, i.e. 30% (21). This may reflect that our study was performed in large urban areas equipped with pre-hospital emergency ambulances. A more prolonged delay might be expected in more rural regions. The exact definition of very early presenters is still a matter of debate. Authors nevertheless agree to define them as presenting before 2h (20). Very few studies examined the specific groups of early/very early presenters (4,6,8,20) In the ESC guidelines, a different strategy is recommended for patients with versus without CPO<6h (0/3h-algorithm) (1). Earlier presenters are taken into account in the 0/1h-algorithm, but in this strategy the rapid exclusion with a unique measurement at admission (H0) is only applicable if CPO>3h (1). We believe that this proportion is not negligible and the impact of this very early population might be underestimated in studies were the CPO is not evaluated.

We observed that AUCs of cTnI, cTnI+copeptin and HS-cTnT were significantly lower when CPO<4h. The NPVs were not significantly impacted by the CPO, but a suboptimal sensitivity and a non-negligible proportion of misclassified NSTEMI was observed in patients with CPO<2h for all tested thresholds and combinations, even if cTnI and HS-cTnT performances

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can be improved by using a lower threshold for positivity or adding copeptin. The same was true for patients with CPO 2-4h, except when using the combination of HS-cTnT<5 ng/L and copeptin <12 µmol/L. We reported the number of misclassified patients in addition to sensitivity and NPV, because NPV is known to be dependent of the prevalence, thus its value might be biased. The absolute number of misdiagnosed patients might be more clinically pertinent than NPV (22).

As previously suggested (1), we found that sensitivity and NPV of a single measurement of HS-cTn at admission seems not safe enough to exclude a NSTEMI in very early presenters. We here show that lowering HS-cTn decisional threshold to LoD or to LoB is not sufficient to detect all NSTEMI among very early chest pain presenters. Although combining copeptin to HS-cTnT increased sensitivity and lowered number of misclassified patients, none of the tested strategies allowed for identification of all NSTEMI. Results obtained in very early presenters were very similar to patients with CPO 2-4h, except that in this later sub-group combining copeptin with HS-cTnT succeeded in significantly increasing sensitivity (all NSTEMI patients had a detectable HS-cTnT and/or an elevated copeptin).

Our results can be compared to those of Boeddinghaus et al. who found that sensitivity of a single HS-cTn measurement is lower in very early presenters, in comparison to all patients (4). These authors indicated that using a single cut-off approach, 61% of the very early presenters were ruled-out, which resulted in a sensitivity of 94% and a NPV of 98%. They conclude that the single cutoff strategy should not be applied in early presenters. However, the authors did not evaluate the impact of copeptin across CPO categories. In another study, the same authors indicated that the additional use of copeptin did not sufficiently improve diagnostic accuracy in early presenters (23). Here again, our results are in accordance with those of Boeddinghaus indicating that copeptin did not improve diagnostic accuracy of hs-cTnI at presentation in early presenters (23). Mueller et al showed, using the rapid 0-hour/1-hour algorithm that 63%

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patients with CPO<6h were classified as rule-out. However, 7 patients were missed (0.9% rate), in whom 3 had HS-cTnT<LoD at presentation and at 1 hour (2). Moreover, a single cutoff value for HS-cTnT at 14 ng/L at presentation resulted in a sensitivity of 88.7% and a NPV of 97.3%, and performed less adequately than the combination of HS-cTnT at presentation with 1-hour level and 1-hour absolute change (2). In a more recent study, Mokhtari et al. evaluated a rapid 0H/1H protocol for discharge chest pain patients based on a single value <LoD at admission (24). These authors found 2 missed patients in their population, and recognize that the safety of such rapid protocol is not clear in very early presenters. They further recommend additional HS-cTnT testing at 3 hours for very early presenters (24).

Performance of cardiac troponins, although measured using HS assays, might be limited in very early presenters because of their kinetics of release into the blood circulation (8). The release of cTn into the circulation following cardiomyocyte damage is a time-dependent phenomenon (25), and a single measurement approach may fail at identifying AMI very early after the onset of symptoms (26). Indeed we, like other authors, found very early presenters with undetectable HS-cTnT at admission. Time-dependent release of copeptin during AMI has been described earlier, and this biomarker has been considered as an early biomarker (8). Copeptin increases immediately after induction of ischemia, and peaks 90 min after (27). However, some authors recently indicated that copeptin kinetics might be different in NSTEMI in comparison to STEMI, and that if copeptin is increased at first medical contact in the ambulance, the circulating concentrations may rapidly decrease down to normal ranges at the time of hospital admission (28). Our results are in accordance with this observation, as we found one misdiagnosed NSTEMI very early presenter with non-elevated copeptin.

Lastly, our results are reinforced by those of Stallone et al who found that the additional use of copeptin did not increase diagnostic accuracy in very early presenters (6). Furthermore, the

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NPV for the combination of HS-cTnT and copeptin was lower in patients arriving in the first 2 hours than in those arriving after 2 hours (6). However, these authors did not evaluate the LoD nor LoB of HS-cTnT in their work. We here report that even when lowering the cutoff of HS-cTnT, the combination of HS-cTnT and copeptin seems not enough to detect all NSTEMI among all very early presenters. Our study and the one of Stallone et al. (6) are in accordance with previous studies that have shown that there is no or marginal benefit when adding copeptin to HS-cTn assays; indeed, Wildi et al. indicated that copeptin provides no significant increase in AUC when combined to HS-cTn (29), either in their all population or in patients with CPO<4h. These authors found an incremental value in sensitivities, NPV and calculating the integrated discrimination improvement index (IDI), but they did not evaluated low HS-cTn thresholds such as LoB and LoD values (29).

Of note, all patients with CPO >4 hours had detectable cTnI and HS-cTnT, i.e. the use of copeptin in this situation added no gain. Other studies reported that copeptin testing for the rule out of NSTEMI should be limited to CPO< 6 hours (1,13). According to our data, the added value of copeptin might be more restricted, but further studies are needed to confirm our findings.

The current ESC guidelines incorporate an additional criterion for direct rule-out of patients that are not very early presenters; indeed, the rapid rule-out using a single measurement at admission is possible only if CPO is >3h (1). Furthermore, this rapid algorithm can be used only for 3 HS-cTn assays, including HS-cTnT. Considering our data about patients with CPO >4h, we note that our conclusions are in line with the recommendations. Therefore, the use of a single measurement at admission might be used for a safe rule-out in patients that are not very early presenters. The alternative rule-out criteria, combining baseline concentration and 1h-change, should be used in early presenters.

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Limitations of our study

First, it is a post-hoc analysis of three previously published studies, and some data are missing (vital signs at admission, details in ECG findings for example). Second, only a single measurement of troponin at admission was considered for this analysis, and we did not evaluate its kinetics; we therefore cannot comment on the accuracy of the recent 1h algorithm in our population (1,2). Third, different cTn assays were used across the different centers (11–13) and we had to normalize cTnI values before analysis in order to minimize bias. However, all centers evaluated the same HS-cTnT and copeptin. Fourth, as the gold standard diagnosis was based on a non-HS cTn, we recognize that this could result in underdiagnosis of myocardial injury. Previous studies have shown that an early rule-out of NSTEMI using hs-cTn alone, also in the vulnerable subgroup of early presenters, is safe (with high AUC, sensitivities and NPV in patients presenting with CPO<3h) (20).In our study, the combined biomarkers are not safe-enough for early rule-out of NSTEMI in patients presenting very early (CPO<2h). The fact that gold standard diagnoses were adjudicated by the use of a conventional but not a hs-cTn assay, and that different assays where used, may have led to underdiagnose patients. This point limits the generalizability of our findings and explains why sensitivities and NPVs were much lower as compared to previous studies. However, our results are comparable to those of Stallone et al how recently used an HS-cTn, as suggested by our AUC (0.85[95%CI : 0.79-0.90]) in comparison to those of Stallone (0.86 [95%CI:0.82-.090]) (6). However, the aim of our work is not to evaluate another time global HS-cTn accuracy, but to highlight CPO effects on diagnostic accuracy of HS-cTn combined or not to copeptin. Fifth, we examined 3 subgroups with CPO<2h, 2-4h and 4h and defined very early presenters as those having CPO <2 hours, as based on the accepted definition of early presenters (20). Even if very early presenters represent more than one third of the studied population, the number of very early presenters (CPO<2h) is relatively small,

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although data from three cohorts was used. This explains why false rule-out of 3 patients results in a significant drop in sensitivity and NPV. Many previous studies investigated the rule-out performance in early presenters using e.g. the LoD of hs-cTnT and hs-cTnI and found much higher sensitivities and NPVs. This can be explained due to a larger number of patients (4, 20).

Conclusion

A single measurement of HS-cTnT alone or in combination with copeptin at admission seems not sensitive enough to safely rule out NSTEMI in very early presenters (chest pain onset < 2 hours from ED admission). If other studies confirm our findings, another strategy to safely exclude NSTEMI in this specific population that represents one third of chest pain patients is warranted.

Acknowledgments

The authors would like to thank again the nursing, technical and medical staff at the emergency departments and biochemistry laboratories for their assistance throughout the original studies.

Reagents for the Kryptor ® Compact Plus and HS-cTnT assays used for the 3 studies were kindly provided by ThermoFisher Scientific and Roche Diagnostic, France, respectively.

Figure legends:

Figure 1: Flow chart of the studied population.

Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

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Contributorship statement:

PR, CM, MS, and NP contributed to the design and inclusions. CCG, SL, AMD and GL realized sample collections and biomarker measurements. CCG and PR realized statistical analysis. All authors approved the final manuscript. ThermoFisher Scientific and Roche Diagnostic provided reagents.

Competing interests:

CCG, PR and CM received honorarias and lecture fees from Roche Diagnostics and Thermofisher Scientific.

Funding:

None

Data sharing statement:

No data sharing

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Table 1: Main characteristics of original study designs

	Sebbane et al. (14)	**Chenevier-Gobeaux et al. (15)**
Inclusion Criteria	Prospective cohort of ED patients with CPO <12h	Consecutive patients, >18 years old, admitted to the ED or to the ICU by pre-hospital emergency ambulances
Exclusion criteria	Patients with traumatic causes of chest pain	Patients <18 years old Acute or chronic renal failure requiring dialysis
Plasma sampling and storage	Heparinized and EDTA blood collection. Storage at -80°C for later analysis	Heparinized blood collection after routine cTnI measurement. Storage at -40°C until HS-cTnT and copeptin measurement
Registration number/name	French Health Ministry (no. DC-2009-1052)	French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière)
Consent	Written informed consent	Cochin Hospital: waiver of informed consent was authorized. Pitié-Salpêtrière Hospital: informed consent was granted.

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Table 2: Main Characteristics of the studied population

	All patients	**CPO <2h (very early presenters)**	**CPO 2-4h**	**CPO 4-6h**
n	449	160	143	146
Age (years)	58 ± 17	57 ± 16	59 ± 17	58 ± 17
Men	281 (63)	101 (63)	96 (67)	84 (58)
Past medical history:				
Familial history of CAD, present/total (%) *	104/301 (35)	63/147 (43)	26/90 (29)	15/64 (23)
Personal history of CAD	120 (27)	38 (24)	42 (29)	40 (27)
Dyslipidaemia	168 (37)	61 (38)	57 (40)	50 (34)
Diabetes	67 (15)	20 (13)	22 (15)	25 (17)
Smoking	176 (39)	72 (45)	51 (37)	53 (36)
Hypertension	158 (35)	52 (33)	58 (41)	48 (33)
Outcome:				
Coronary angiography	131 (29)	49 (31)	46 (32)	36 (25)
Admission	256 (57)	98 (61)	91 (63)	67 (46)
Final Diagnostic:				
NSTEMI	55 (12)	15 (9)	22 (13)	18 (12)
other	394 (88)	145 (91)	121 (85)	128 (88)

CAD, coronary artery disease; CPO, chest pain onset; NSTEMI, non ST-elevation myocardial infarction.

Results are expressed in mean ± SD or in number (percentage)

*, missing data exist for this variable.

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Table 3: AUC values according to CPO category

	Biomarker	AUC	95% CI
CPO <2h (very early presenters)	cTnI	0.841	0.775 to 0.894
	cTnI + copeptin	0.880	0.819 to 0.926
	HS-cTnT	0.853	0.789 to 0.904
	HS-cTnT + copeptin	0.897	0.840 to 0.940
CPO 2-4h	cTnI	0.886	0.823 to 0.933
	cTnI + copeptin	0.915	0.857 to 0.955
	HS-cTnT	0.869	0.802 to 0.919
	HS-cTnT + copeptin	0.891	0.829 to 0.937
CPO >4h	cTnI	0.995	0.965 to 1.000
	cTnI + copeptin	0.979	0.940 to 0.995
	HS-cTnT	0.980	0.942 to 0.996
	HS-cTnT + copeptin	0.953	0.905 to 0.981

AUC, area under the ROC curve; CPO, chest pain onset

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Table 4: Diagnostic performances for NSTEMI according to onset chest pain

CPO	Biomarker	Threshold	Sensitivity (%, [95%CI])	Specificity (%, [95%CI])	Negative predictive value (%, [95%CI])	Positive predictive value (%, [95%CI])	Misdiagnosed (**) (n)	Correctly ruled-out, n (%)
<2h (very early presenters) **(n=160)**	cTnI	LoQ (*)	73 [45-91]	97 [92-99]	97 [92-99]	73 [45-91]	4	141 (88)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	87 [59-98]	60 [52-68] ε	98 [92-100]	18 [7-24] ε	2	87 (54) ε
	HScTnT	14 ng/L	80 [51-95]	85 [78-90] ε	98 [93-100]	35 [20-53]	3	123 (79) ε
		5 ng/L	87 [59-98]	58 [50-66] ε ζ	98 [92-100]	18 [10-29] ε	2	84 (52) ε ζ
		3 ng/L	87[58-98]	40 [32-49] ε ζ	97 [88-99]	13 [7-22] ε	2	58 (36) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	93 [66-100]	54 [46-62] ε ζ	99 [93-100]	18 [10-29] ε	1	79 (49) ε ζ
		5 ng/L and 12 pmol/L	93 [66-100]	41 [33-50] ε ζ	98 [89-100]	14 [8-23] ε	1	59 (37) ε ζ
		3 ng/L and 12 pmol/L	93 [66-100]	25 [19-34] ε ζ	97 [85-100]	12 [7-19] ε	1	37 (23) ε ζ
2-4h **(n=143)**	cTnI	LoQ (*)	73 [50-89]	95 [89-98]	95 [89-98] ψ	73 [50-89]	6	115 (80)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	95 [75-100]	57 [48-66] ε	99 [92-100]	29 [19-41] ε	1	69 (48) ε
	HScTnT	14 ng/L	77 [54-91]	79 [71-86] ε	95 [88-98] ψ	40 [26-56]	5	96 (67) ε μ
		5 ng/L	91 [70-98]	55 [46-64] ε ζ	97 [89-100]	27 [18-39] ε	2	67 (47) ε ζ
		3 ng/L	96 [75-100]	45 [36-54] ε ζ	98 [89-100]	24 [16-34] ε	1	54 (38) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	95 [75-100]	50 [41-59] ε ζ	99 [91-100]	25 [17-36] ε	1	58 (41) ε ζ
		5 ng/L and 12 pmol/L	100 [82-100]	35 [27-44] ε ζ	100 [90-100]	22 [15-32] ε	0	42 (29) ε ζ
		3 ng/L and 12 pmol/L	100 [82-100]	29 [21-38] ε ζ	100 [88-100]	20 [14-30] ε	0	35 (25) ε ζ
>4h **(n=146)**	cTnI	LoQ (*)	100 [77-100]	96 [91-99]	100 [96-100]	77 [54-91]	0	122 (85)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	100 [77-100]	59 [50-68] ε	100 [94-100]	25 [16-37] ε	0	75 (54) ε
	HScTnT	14 ng/L	100 [78-100]	87 [80-92] ε	100 [96-100]	51 [34-68]	0	111 (77)
		5 ng/L	100 [78-100]	60 [51-68] ε ζ	100 [94-100]	35 [23-50] ε	0	77 (53) ε μ ζ
		3 ng/L	100 [78-100]	52 [43-61] ε ζ	100 [96-100]	23 [14-34] ε	0	67 (47) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	100 [78-100]	55 [46-64] ε ζ	100 [94-100]	24 [15-35] ε	0	70 (49) ε ζ
		5 ng/L and 12 pmol/L	100 [78-100]	40 [32-49] ε ζ	100 [91-100]	19 [12-29] ε ζ	0	51 (35) ε ζ
		3 ng/L and 12 pmol/L	100 [78-100]	34 [26-43] ε ζ	100 [96-100]	18 [11-27] ε ζ	0	43 (30) ε ζ

(*) 0.04 µg/L (40 ng/L) for Bicêtre and Montpellier hospitals, 0.14 µg/L (140 ng/L) for other sites; (**), false negative patients

ε p<0.05 versus cTnI alone; ζ p<0.05 versus HS-cTnT <14 ng/L alone; ψ p<0.05 versus delay >4h; μ, p<0.05 versus delay <2h

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Table 5: Potentially misdiagnosed patients with low cTn thresholds and copeptin

Sex	Age	Dyslipidaemia	Smoke	Diabetes	Hypertension	Personal history of CAD	Chest pain since :	CPO` category	cTnI	HScTnT	Copeptin
M	89	no	no	no	yes	yes	1 hr 15 min	<2h (very early presenters)	0.04 µg/L (40 ng/L)	44.9 ng/L	208.7 pmol/L
M	86	yes	no	no	yes	yes	30 min		0.06 µg/L (60 ng/L)	11.3 ng/L	77.2 pmol/L
W	35	no	no	no	no	no	50 min		0.01 µg/L (10 ng/L)	<3 ng/L	54.7 pmol/L
W	44	no	yes	no	no	no	45 min		0.01 µg/L (10 ng/L)	<3 ng/L	10.7 pmol/L
W	74	yes	no	no	yes	yes	3 hrs	2-4h	0.04 µg/L (40 ng/L)	8.8 ng/L	10.7 pmol/L
M	34,2	yes	yes	no	no	no	2 hrs 35 min		0.04 µg/L (40 ng/L)	<3 ng/L	23.5 pmol/L
M	55	yes	no	no	no	yes	3 hrs 50 min		0.02 µg/L (20 ng/L)	6 ng/L	25.9 pmol/L
M	34	no	yes	no	no	no	3 hrs 15 min		0.01 µg/L (10 ng/L)	4 ng/L	52.4 pmol/L
M	61	yes	yes	no	yes	yes	3 hrs		0.03 µg/L (30 ng/L)	19.7 ng/L	27.2 pmol/L
M	59	no	no	no	no	no	2 hrs 45 min		0.04 µg/L (40 ng/L)	10.1 ng/L	241.8 pmol/L

CAD, coronary artery disease; CPO, chest pain onset; M, men; W, women.

n=557
[ref. 14]: n=363
[ref. 15]: n=194

Exclusions:
No cTnI: n=7
No CPO: n=67
STEMI: n=34

n=449

CPO <2h
n=160
15 STEMI / 145 non STEMI

CPO 2-4h
n=143
22 STEMI / 121 non STEMI

CPO > 4h
n=146
18 STEMI / 128 non STEMI

Figure 1: Flow chart of the studied population.

135x255mm (150 x 150 DPI)

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Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

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Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Page 1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Page 2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Page 4
	4	Study objectives and hypotheses	Pages 4 and 5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Page 6
Participants	**6**	Eligibility criteria	pp6&7 + Table1 p22
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Pages 6-8
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Page 6
	9	Whether participants formed a consecutive, random or convenience series	Page 6
Test methods	**10a**	Index test, in sufficient detail to allow replication	Pages 8 and 9
	10b	Reference standard, in sufficient detail to allow replication	Pages 8 and 9
	11	Rationale for choosing the reference standard (if alternatives exist)	Page 7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Page 9
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Pages 8 and 9
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Pages 6, 8 and 9
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Page 7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	Page 9
	15	How indeterminate index test or reference standard results were handled	Page 7
	16	How missing data on the index test and reference standard were handled	Flow chart (Figure1) + pages 6 and 7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Pages 6 and 7
	18	Intended sample size and how it was determined	NA
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Flow chart (Figure1) + pages 6 and 7
	20	Baseline demographic and clinical characteristics of participants	Table 2, page 23
	21a	Distribution of severity of disease in those with the target condition	Table 2, page 23
	21b	Distribution of alternative diagnoses in those without the target condition	Table 2, page 23
	22	Time interval and any clinical interventions between index test and reference standard	NA
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Tables 3&4, p.24-25
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Table 4, page25
	25	Any adverse events from performing the index test or the reference standard	Table 5 page 26
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Page 15
	27	Implications for practice, including the intended use and clinical role of the index test	Page 16
OTHER INFORMATION			
	28	Registration number and name of registry	Table 1, page22
	29	Where the full study protocol can be accessed	Table 1 page 22
	30	Sources of funding and other support; role of funders	NA

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

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Is high-sensitivity troponin, alone or in combination with copeptin, sensitive enough for ruling out NSTEMI in very early presenters at admission ? A post-hoc analysis performed in emergency departments.

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Is high-sensitivity troponin, alone or in combination with copeptin, sensitive enough for ruling out NSTEMI in very early presenters at admission ? A post-hoc analysis performed in emergency departments.

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Abstract

Objectives: Copeptin and high-sensitivity cardiac troponin (HS-cTn) assays improve the early detection of NSTEMI. Their sensitivities may however be reduced in very early presenters.

Setting: We performed a post-hoc analysis of three prospective studies that included patients who presented to the Emergency Department for chest pain onset (CPO) of less than 6 hours.

Participants: 449 patients were included, in whom 12% had NSTEMI. CPO occurred <2h from ED presentation in 160, between 2-4h in 143, and >4h in 146 patients. The prevalence of NSTEMI was similar in all groups (9%, 13% and 12%, respectively, p=0.281). *Measures:* Diagnostic performances of HS-cTn and copeptin at presentation were examined according to CPO. The discharge diagnosis was adjudicated by 2 experts, including cTnI. HS-cTn and copeptin were blindly measured. *Results:* Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT (but not HS-cTnT+copeptin) lower through CPO categories. For patients with CPO<2h, the choice of a threshold value of 14 ng/L for HS-cTnT resulted in 3 false negative (Sensitivity 80% [95%CI: 51-95]; specificity 85% [78-90]; 79% of correctly ruled-out patients) and that of 5 ng/L in 2 false negative (Sensitivity 87% [59-98]; specificity 58% [50-66]; 52% of correctly ruled-out patients). The addition of copeptin to HS-cTnT induced a decrease of misclassified patients to 1 in patients with CPO<2h (Sensitivity 93% [66-100]; specificity 41% [33-50]). *Conclusion:* A single measurement of HS-cTn, alone or in combination with copeptin at admission, seems not safe enough for ruling out NSTEMI in very early presenters (with CPO<2h). *Trial registrations:* French Health Ministry (no. DC-2009-1052), French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière).

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Strengths

- Focus on very early chest pain presenters that was not performed before

Limitations of our study

- Small numbers of very early chest pain presenters, although the data grouping of 3 previous studies

- A single measurement of troponin at admission was considered for the analysis, but not its kinetics

- The gold standard diagnosis was based on a non-HS cTn.

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Introduction

The management of patients with acute chest pain at the Emergency Department (ED) is a major health problem and an adequate ruling out process for non-ST elevation acute myocardial infarction (NSTEMI) is crucial. Cardiac troponin (cTn) measurement with conventional assays and more recent high-sensitivity cardiac troponin assays (HS-cTn), are current diagnostic tools for the assessment of these patients (1). The European Society of Cardiology guidelines proposed a rapid rule-out strategy using low HS-cTn values (i.e. values below the Limit of Detection of the assay -LoD-) as decisional threshold for ruling out NSTEMI (1). The accuracy of a rapid 0-hour/1-hour algorithm has also been recently demonstrated in patients with CPO<6h (2) and is endorsed by the latest ESC guidelines (1). In this analysis, the authors indicated that using a single HS-cTnT cutoff value of 14 ng/L at presentation resulted in 88.7% sensitivity and 97.3% negative predictive value (NPV), but that this single assay strategy was less effective than the combination of absolute level of HS-cTnT measured at presentation and again at 1 hour, combined with the absolute difference between the two levels (2).

However, in very early presenters, such rapid and efficient triage at presentation may be uncertain (3). Indeed, very early presenters, defined as having chest pain <2h, are considered by some authors as highly vulnerable as they may not present all symptoms and signs and thus be exposed to a higher risk of misdiagnosis and therefore worse outcome (4); the application of a rapid algorithm to these patients may lead to early discharge within 3 hours from ED admission. Recently, a meta-analysis (based on eleven studies) indicated that a single HS-cTnT concentration below the limit of detection may successfully rule out AMI (5). However, concerns have been raised about the safety of a single measurement rule out protocol performed at presentation in early presenters (6,7).

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Copeptin (in association with cTn) assays improve the early detection of NSTEMI (8–12).

Indeed, a randomized controlled trial demonstrated the safety of early discharge using a single

combination of copeptin + cTn at presentation for patients with CPO <6h (13).

The present study aimed to assess the diagnostic performance of HS-cTn and copeptin as a

single measurement at admission in very early ED presenters with suspected NSTEMI.

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Patients and methods

Study design and population

The study is a *post-hoc* analysis of three French prospective clinical studies (already published) of cardiac biomarker testing, each to explore the usefulness of copeptin and/or HS-cTnT testing in patients who presented to the ED with acute chest pain of less than 6h (14,15). However, none of these studies evaluated the influence of CPO value on diagnostic performances. All three trials had comparable inclusion/exclusion criteria and gathered similar clinical information (Table 1). Patients requiring renal replacement therapy were excluded. All studies received approval from local Institutional Review Board. The study complied with the principles of the Declaration of Helsinki. Recommendations of the Standards for Reporting of Diagnostic Accuracy (STARD) initiative were applied (16).

Patient Involvement

The development of the research question was not informed by patients. Patients were not involved in the design of this study. Patients were not involved in the recruitment to and conduct of the study. There was no results dissemination to study participants.

Routine assessment

All patients underwent an initial clinical evaluation (including clinical history, physical examination, 12-lead ECG, pulse oximetry, routine blood tests, and chest X-ray). Conventional cardiac Troponin I (cTnI) was measured on a venous blood collection performed at presentation and, if required, repeated after 3 to 9 h, as clinically indicated (17). The onset of chest pain (CPO), defined as the delay from symptom onset to presentation was recorded, based on patient history/declaration. When history was incomplete or inconsistent, CPO was not recorded and patient was excluded of the analysis (see flow chart, Figure 1).

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Based on all clinical, biological (including cTnI value, but not HS-cTnT and copeptin values which were blindly measured) and imaging results, a decision was made by the attending physician to admit or discharge the patient, as well as medical therapy and revascularization if indicated. Attending emergency physicians and cardiologists were blinded to the results of HS-cTnT and copeptin, and biologists were blinded to the suspected diagnosis at presentation. Patients with no cTnI results and/or no recorded CPO value, and patients with a final diagnosis of STEMI, were excluded (see flowchart, Figure 1).

Gold standard diagnosis

The gold standard diagnosis was adjudicated by two independent experts (emergency physician and cardiologist) who reviewed all available medical records (including patient history, physical findings, laboratory results including cTnI value and radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography, summary chart at discharge) pertaining to the patient from the time of ED presentation to 30-day follow-up. Experts were blind to copeptin and HS-cTnT results. In the event of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third expert.

AMI was diagnosed according to the universal definition that was in force at the time of inclusions and adapted to the use of a conventional cTn (18). Thus, patients with a cTnI increase (or a rise/fall pattern) above the 10%CV threshold, associated with at least one of the following: symptoms of myocardial ischemia, new ST-T changes or new Q wave on ECG, imaging of new loss of viable myocardium, or normal cTnI on admission, were classified as having an MI (STEMI with a ST elevation in at least 2 continuous leads on ECG or new onset of left bundle branch block, or NSTEMI). STEMI patients were excluded from the analysis, based on ST elevation observed on the ECG (see Flow chart, Fig. 1). Patients were classified

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according to the CPO, <2h, from 2-4h and >4h; those with a CPO <2 hours were considered as very early presenters.

Unstable angina (UA) diagnosis was adjudicated in patients with history or clinical symptoms consistent with ACS but without ST-T changes on the ECG and without change of cTn on serial testing. Other diagnostic categories beside NSTEMI and UA were non-ACS (e.g., stable angina, myocarditis, arrhythmias, heart failure, pulmonary embolism, and chest pain of unknown origin). UA and non-ACS chest pain were considered as non-NSTEMI in our analysis.

Troponin measurements

Plasma cTnI concentrations were routinely measured on an X-pand® HM analyzer, using the Cardiac Troponin-I immunoassay (Siemens Healthcare Diagnostics Inc., Newark, CT) in two EDs (Cochin and La Pitié Salpêtrière Hospitals). The limit of detection (LoD) was 0.04 µg/L (40 ng/L). The limit of quantitation (LoQ), i.e. the 10% imprecision point (or 10% CV), which is the lowest cTn concentration that can be reproducibly measured with a between-run CV of ≤10%, was 0.14 µg/L (140 ng/L). The 99th percentile of the assay was 0.07 µg/L (70 ng/L), with coefficients of variations (CV) between 15 to 22 %. The measuring range was 0.04 to 40 µg/L (40 to 40,000 ng/L), and the imprecision values across the measuring range were below 10%.

In Bicêtre and in Montpellier hospitals, plasma cTnI concentrations were routinely measured on an Access® analyser (Beckman Coulter, Inc., Brea, CA). According to the manufacturer's data, the limit of detection (LoD) was 0.01 µg/L (10 ng/L), the 20% point on the imprecision curve was 0.02 µg/L (20 ng/L). The LoQ/10%CV was 0.04 µg/L (40 ng/L). The 99th percentile of the assay was 0.04 µg/L (40 ng/L). The measuring range was 0.01 to 100 µg/L (10 to 100,000 ng/L), and the imprecision values across the measuring range were below 10%.

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After routine cTnI measurement, plasma samples were aliquoted and frozen (-40°C) until HS-cTnT and copeptin measurement.

Hs-cTnT was measured in heparinized collected samples, on an Elecsys2010® analyser (Roche Diagnostics, Meylan, France). The limit of blanck (LoB) *was 3 ng/L, the LoD was 5 ng/L, and the 99th percentile was 14 ng/L. The measuring range was 3 to 10,000 ng/L. In our laboratory, CVs obtained in Roche quality controls containing 27.5 and 2,360 ng/L of cTnT were 3.6 and 2.8 % (between-run precision) and 1.4 and 0.4 % (within-run precision). Of note, the LoD is measured with a between-run CV of >10%, while the 99th percentile is a precise concentration (CV<10%) (7). HS-cTnT determinations were performed blinded to the clinical assessment of the emergency physicians.

Copeptin measurement

Copeptin was measured in heparinized blood samples collected on admission. The assay was performed on a KRYPTOR® analyser using ThermoFisher Scientific sandwich immunoluminometric assay (B.R.A.H.M.S Copeptin KRYPTOR, B.R.A.H.M.S Aktiengesellschaft, Hennigsdorf, Germany). The assay principle is based on TRACE technology (Time-Resolved Amplified Cryptate Emission). The lower detection limit is 4.8 pmol/L, and the functional assay sensitivity (20%CV value) is <12 pmol/L (data from manufacturer, recommended threshold value for this method). Copeptin determinations were performed blinded to the clinical assessment of the emergency physicians.

Statistical analysis

Continuous variables are presented as means ± SD, and categorical variables are expressed as numbers (percentage). Continuous variables were compared by using the Kruskal-Wallis test, and categorical variables were assessed using Pearson's χ^2 test. Number of misclassified

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patients and number of correctly ruled-out patients were collected for each threshold strategy, and correspond to the false negative and the true positive patients, respectively.

Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) throughout the concentrations of cTnI, HS-cTnT and copeptin for the diagnosis of NSTEMI, and according to the CPO. cTn and copeptin values were log-transformed before combination for ROC analysis. For cTnI values, as they were obtained from two non-standardized methods, values were normalized by factorizing to the 99th percentile of the method prior to ROC analysis in order to remove any bias due to methodological differences.

Diagnostic thresholds that were used for classification of the data are:

- For cTnI, the LoQ values: 0.04 μg/L (40 ng/L) for Bicêtre and Montpellier hospitals, 0.14 μg/L (140 ng/L) for other sites,

- For HS-cTnT, The LoB (3 ng/L), the LoD (5 ng/L) and the 99th percentile (14 ng/L),

- For copeptin, the manufacturer's recommended threshold at 12 pmol/L.

All data are presented with their 95% confidence intervals [95%CI]. All hypothesis testing was two-tailed, and $p < 0.05$ was considered statistically significant. Statistical analysis was performed using MedCalc (MedCalc Software, Version 12.4.0.0, Mariarkerke, Belgium).

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Results

Characteristics of the studied population

Table 2 shows the characteristics of the studied population. Briefly, in the total cohort mean age was 58 ± 17 years, and included more males. A gold standard diagnosis of NSTEMI was adjudicated in 55 patients (12%). Delay from chest pain onset to ED admission was <2 hours in 160 (36%) patients, was from 2 to 4 hours in 143 (32%) and was >4 hours (and below 6 hours) in 146 (32%) patients. Very early presenters with NSTEMI (n=15) tended to be older than those without NSTEMI (n=145) (65 *vs.* 55 years-old), were more frequently hospitalized (93% *vs.* 58%), and also more frequently underwent diagnostic coronary angiography (73% *vs.* 27%).

Diagnostic performances according to CPO

ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin are presented in Figure 2. Diagnostic accuracies of cTnI, cTnI+copeptin and HS-cTnT are reduced when time to onset of chest pain gets less, as indicated by estimated AUCs and their 95%CI intervals (Table 3). The AUCs of HS-cTnT+copeptin were not different through the CPO categories.

Diagnostic performances of cTn, alone or in combination with copeptin, and using different decisional thresholds, are presented in Table 4.

In very early presenters (CPO <2 hours), a single value of cTnI alone had low sensitivity (73 [73-91] %) but high specificity (97 [92-99]%), and misclassified 4 of 15 NSTEMI patients. However, this strategy could correctly rule-out 141 patients (88%). Combining copeptin with cTnI increases sensitivity, and lowers the number of misdiagnosed patients from 4 to 2, but significantly lowers the rate of ruled-out patients from 88% to 54% (Table 4). Of note, addition of copeptin also significantly lowered specificity. At a threshold of 14 ng/L, HS-

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cTnT had low sensitivity (80 [59-98] %), misclassified 3 NSTEMI patients but could correctly rule-out 123 (79%) patients, which is less than using cTnI. The sensitivity of HS-TnT alone is likely to be suboptimal in early presenters, but can be improved by using a lower threshold for positivity or adding copeptin (but this is accompanied with a marked loss of specificity). The addition of copeptin induced a decrease in misclassified patients from 2 to 1, either with an HS-cTnT threshold at 14 or at 5 ng/L. This ultimate misdiagnosed NSTEMI patient with CPO <2h who presented with all undetectable biomarkers was a 44-years old woman with a history of smoking and no CV risk factors; the CPO was 45 min before hospital admission (Table 5).

In patients with CPO 2-4 hours, results are similar to those observed in very early presenters, although the number of misclassified patients was different (Table 4). Adding copeptin to cTnI induced a decrease of misclassified patients from 5 to 1 for an HS-cTnT threshold at 14 ng/L, and from 2 to 0 for an HS-cTnT threshold at 5 ng/L. In particular, combining copeptin to the LoD (5 ng/L) of HS-cTnT reached 100% sensitivity of the test. Similar performance was observed using the LoB (3ng/L) of HS-cTnT. Indeed, all NSTEMI patients with CPO 2-4 h had a detectable HS-cTnT and/or an elevated copeptin. In this sub-group again, the use of copeptin lowered significantly the specificity of the test.

As expected, in patients with CPO >4 hours, all patients had quantitative cTnI and detectable HS-cTnT. No patient was misclassified. The addition of copeptin in this sub-group had no effect on sensitivity or misclassified patients.

Potential misdiagnosed NSTEMI

Characteristics of potential misdiagnosed NSTEMI patients are detailed in Table 5. All potentially missed NSTEMI in the very early presenters population had a CPO <1 hour. We found no distinguishing characteristics in misclassified patients when comparing to correctly

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diagnosed patients in terms of age, sex, and cardiovascular risks, in each CPO category. Of note, when STEMI patients were included in our analysis, results were comparable (data not shown).

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Discussion

Our results indicate that diagnostic performances of HS-cTnT values at admission, alone or combined with copeptin, are reduced in the subgroup of patients with shorter CPO. Emergency physicians may not rule out NSTEMI in very early presenters with a single low value of HS-cTnT and/or copeptin at presentation.

Our studied population included one third of early presenters, and this proportion is similar to that found by Boeddinghaus et al (26% of patients presented within 2h from CPO) (4), by Keller et al (37% and 38% of patients with CPO<3h, (8,19)), and by Reichlin et al. (222 patients with CPO<3h out of 718, i.e. 31%) (20). More recently, Stallone et al reported 519 (26%) patients that arrived within 2 hours of symptom onset to the ED (6), and Twerenbold et al. reported the largest subgroup investigated so far with 1,322 early presenters (with CPO<3h) out of 4,368, i.e. 30% (21). This may reflect that our study was performed in large urban areas equipped with pre-hospital emergency ambulances. A more prolonged delay might be expected in more rural regions. The exact definition of very early presenters is still a matter of debate. Authors nevertheless agree to define them as presenting before 2h (20). Very few studies examined the specific groups of early/very early presenters (4,6,8,20) In the ESC guidelines, a different strategy is recommended for patients with versus without CPO<6h (0/3h-algorithm) (1). Earlier presenters are taken into account in the 0/1h-algorithm, but in this strategy the rapid exclusion with a unique measurement at admission (H0) is only applicable if CPO>3h (1). We believe that this proportion is not negligible and the impact of this very early population might be underestimated in studies were the CPO is not evaluated.

We observed that AUCs of cTnI, cTnI+copeptin and HS-cTnT were significantly lower when CPO<4h. The NPVs were not significantly impacted by the CPO, but a suboptimal sensitivity and a non-negligible proportion of misclassified NSTEMI was observed in patients with CPO<2h for all tested thresholds and combinations, even if cTnI and HS-cTnT performances

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can be improved by using a lower threshold for positivity or adding copeptin. The same was true for patients with CPO 2-4h, except when using the combination of HS-cTnT<5 ng/L and copeptin <12 μmol/L. We reported the number of misclassified patients in addition to sensitivity and NPV, because NPV is known to be dependent of the prevalence, thus its value might be biased. The absolute number of misdiagnosed patients might be more clinically pertinent than NPV (22).

As previously suggested (1), we found that sensitivity and NPV of a single measurement of HS-cTn at admission seems not safe enough to exclude a NSTEMI in very early presenters. We here show that lowering HS-cTn decisional threshold to LoD or to LoB is not sufficient to detect all NSTEMI among very early chest pain presenters. Although combining copeptin to HS-cTnT increased sensitivity and lowered number of misclassified patients, none of the tested strategies allowed for identification of all NSTEMI. Results obtained in very early presenters were very similar to patients with CPO 2-4h, except that in this later sub-group combining copeptin with HS-cTnT succeeded in significantly increasing sensitivity (all NSTEMI patients had a detectable HS-cTnT and/or an elevated copeptin).

Our results can be compared to those of Boeddinghaus et al. who found that sensitivity of a single HS-cTn measurement is lower in very early presenters, in comparison to all patients (4). These authors indicated that using a single cut-off approach, 61% of the very early presenters were ruled-out, which resulted in a sensitivity of 94% and a NPV of 98%. They conclude that the single cutoff strategy should not be applied in early presenters. However, the authors did not evaluate the impact of copeptin across CPO categories. In another study, the same authors indicated that the additional use of copeptin did not sufficiently improve diagnostic accuracy in early presenters (23). Here again, our results are in accordance with those of Boeddinghaus indicating that copeptin did not improve diagnostic accuracy of hs-cTnI at presentation in early presenters (23). Mueller et al showed, using the rapid 0-hour/1-hour algorithm that 63%

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patients with CPO<6h were classified as rule-out. However, 7 patients were missed (0.9% rate), in whom 3 had HS-cTnT<LoD at presentation and at 1 hour (2). Moreover, a single cutoff value for HS-cTnT at 14 ng/L at presentation resulted in a sensitivity of 88.7% and a NPV of 97.3%, and performed less adequately than the combination of HS-cTnT at presentation with 1-hour level and 1-hour absolute change (2). In a more recent study, Mokhtari et al. evaluated a rapid 0H/1H protocol for discharge chest pain patients based on a single value <LoD at admission (24). These authors found 2 missed patients in their population, and recognize that the safety of such rapid protocol is not clear in very early presenters. They further recommend additional HS-cTnT testing at 3 hours for very early presenters (24).

Performance of cardiac troponins, although measured using HS assays, might be limited in very early presenters because of their kinetics of release into the blood circulation (8). The release of cTn into the circulation following cardiomyocyte damage is a time-dependent phenomenon (25), and a single measurement approach may fail at identifying AMI very early after the onset of symptoms (26). Indeed we, like other authors, found very early presenters with undetectable HS-cTnT at admission. Time-dependent release of copeptin during AMI has been described earlier, and this biomarker has been considered as an early biomarker (8). Copeptin increases immediately after induction of ischemia, and peaks 90 min after (27). However, some authors recently indicated that copeptin kinetics might be different in NSTEMI in comparison to STEMI, and that if copeptin is increased at first medical contact in the ambulance, the circulating concentrations may rapidly decrease down to normal ranges at the time of hospital admission (28). Our results are in accordance with this observation, as we found one misdiagnosed NSTEMI very early presenter with non-elevated copeptin.

Lastly, our results are reinforced by those of Stallone et al who found that the additional use of copeptin did not increase diagnostic accuracy in very early presenters (6). Furthermore, the

NPV for the combination of HS-cTnT and copeptin was lower in patients arriving in the first 2 hours than in those arriving after 2 hours (6). However, these authors did not evaluate the LoD nor LoB of HS-cTnT in their work. We here report that even when lowering the cutoff of HS-cTnT, the combination of HS-cTnT and copeptin seems not enough to detect all NSTEMI among all very early presenters. Our study and the one of Stallone et al. (6) are in accordance with previous studies that have shown that there is no or marginal benefit when adding copeptin to HS-cTn assays; indeed, Wildi et al. indicated that copeptin provides no significant increase in AUC when combined to HS-cTn (29), either in their all population or in patients with CPO<4h. These authors found an incremental value in sensitivities, NPV and calculating the integrated discrimination improvement index (IDI), but they did not evaluated low HS-cTn thresholds such as LoB and LoD values (29).

Of note, all patients with CPO >4 hours had detectable cTnI and HS-cTnT, i.e. the use of copeptin in this situation added no gain. Other studies reported that copeptin testing for the rule out of NSTEMI should be limited to CPO< 6 hours (1,13). According to our data, the added value of copeptin might be more restricted, but further studies are needed to confirm our findings.

The current ESC guidelines incorporate an additional criterion for direct rule-out of patients that are not very early presenters; indeed, the rapid rule-out using a single measurement at admission is possible only if CPO is >3h (1). Furthermore, this rapid algorithm can be used only for 3 HS-cTn assays, including HS-cTnT. Considering our data about patients with CPO >4h, we note that our conclusions are in line with the recommendations. Therefore, the use of a single measurement at admission might be used for a safe rule-out in patients that are not very early presenters. The alternative rule-out criteria, combining baseline concentration and 1h-change, should be used in early presenters.

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Limitations of our study

First, it is a post-hoc analysis of three previously published studies, and some data are missing (vital signs at admission, details in ECG findings for example). Second, only a single measurement of troponin at admission was considered for this analysis, and we did not evaluate its kinetics; we therefore cannot comment on the accuracy of the recent 1h algorithm in our population (1,2). Third, different cTn assays were used across the different centers (11–13) and we had to normalize cTnI values before analysis in order to minimize bias. However, all centers evaluated the same HS-cTnT and copeptin. Fourth, as the gold standard diagnosis was based on a non-HS cTn, we recognize that this could result in underdiagnosis of myocardial injury. Previous studies have shown that an early rule-out of NSTEMI using hs-cTn alone, also in the vulnerable subgroup of early presenters, is safe (with high AUC, sensitivities and NPV in patients presenting with CPO<3h) (20).In our study, the combined biomarkers are not safe-enough for early rule-out of NSTEMI in patients presenting very early (CPO<2h). The fact that gold standard diagnoses were adjudicated by the use of a conventional but not a hs-cTn assay, and that different assays where used, may have led to underdiagnose patients. This point limits the generalizability of our findings and explains why sensitivities and NPVs were much lower as compared to previous studies. However, our results are comparable to those of Stallone et al how recently used an HS-cTn, as suggested by our AUC (0.85[95%CI : 0.79-0.90]) in comparison to those of Stallone (0.86 [95%CI:0.82-.090]) (6). However, the aim of our work is not to evaluate another time global HS-cTn accuracy, but to highlight CPO effects on diagnostic accuracy of HS-cTn combined or not to copeptin. Fifth, we examined 3 subgroups with CPO<2h, 2-4h and 4h and defined very early presenters as those having CPO <2 hours, as based on the accepted definition of early presenters (20). Even if very early presenters represent more than one third of the studied population, the number of very early presenters (CPO<2h) is relatively small,

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although data from three cohorts was used. This explains why false rule-out of 3 patients results in a significant drop in sensitivity and NPV. Many previous studies investigated the rule-out performance in early presenters using e.g. the LoD of hs-cTnT and hs-cTnI and found much higher sensitivities and NPVs. This can be explained due to a larger number of patients (4, 20).

Conclusion

A single measurement of HS-cTnT alone or in combination with copeptin at admission seems not sensitive enough to safely rule out NSTEMI in very early presenters (chest pain onset < 2 hours from ED admission). If other studies confirm our findings, another strategy to safely exclude NSTEMI in this specific population that represents one third of chest pain patients is warranted.

Acknowledgments

The authors would like to thank again the nursing, technical and medical staff at the emergency departments and biochemistry laboratories for their assistance throughout the original studies.

Reagents for the Kryptor ® Compact Plus and HS-cTnT assays used for the 3 studies were kindly provided by ThermoFisher Scientific and Roche Diagnostic, France, respectively.

Figure legends:

Figure 1: Flow chart of the studied population.

Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

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Contributorship statement:

PR, CM, MS, and NP contributed to the design and inclusions. CCG, SL, AMD and GL realized sample collections and biomarker measurements. CCG and PR realized statistical analysis. All authors approved the final manuscript. ThermoFisher Scientific and Roche Diagnostic provided reagents.

Competing interests:

CCG, PR and CM received honorarias and lecture fees from Roche Diagnostics and Thermofisher Scientific.

Funding:

None

Data sharing statement:

Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi: https://doi.org/10.5061/dryad.8t87571

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Table 1: Main characteristics of original study designs

	Sebbane et al. (14)	**Chenevier-Gobeaux et al. (15)**
Inclusion Criteria	Prospective cohort of ED patients with CPO <12h	Consecutive patients, >18 years old, admitted to the ED or to the ICU by pre-hospital emergency ambulances
Exclusion criteria	Patients with traumatic causes of chest pain	Patients <18 years old Acute or chronic renal failure requiring dialysis
Plasma sampling and storage	Heparinized and EDTA blood collection. Storage at -80°C for later analysis	Heparinized blood collection after routine cTnI measurement. Storage at -40°C until HS-cTnT and copeptin measurement
Registration number/name	French Health Ministry (no. DC-2009-1052)	French Local Ethic comity « Comité de Protection des Personnes Ile-de-France » III (Hôpital Cochin) et VI (CHU Pitié-Salpétrière)
Consent	Written informed consent	Cochin Hospital: waiver of informed consent was authorized. Pitié-Salpêtrière Hospital: informed consent was granted.

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Table 2: Main Characteristics of the studied population

	All patients	CPO <2h (very early presenters)	CPO 2-4h	CPO 4-6h
n	449	160	143	146
Age (years)	58 ± 17	57 ± 16	59 ± 17	58 ± 17
Men	281 (63)	101 (63)	96 (67)	84 (58)
Past medical history:				
Familial history of CAD, present/total (%) *	104/301 (35)	63/147 (43)	26/90 (29)	15/64 (23)
Personal history of CAD	120 (27)	38 (24)	42 (29)	40 (27)
Dyslipidaemia	168 (37)	61 (38)	57 (40)	50 (34)
Diabetes	67 (15)	20 (13)	22 (15)	25 (17)
Smoking	176 (39)	72 (45)	51 (37)	53 (36)
Hypertension	158 (35)	52 (33)	58 (41)	48 (33)
Outcome:				
Coronary angiography	131 (29)	49 (31)	46 (32)	36 (25)
Admission	256 (57)	98 (61)	91 (63)	67 (46)
Final Diagnostic:				
NSTEMI	55 (12)	15 (9)	22 (13)	18 (12)
other	394 (88)	145 (91)	121 (85)	128 (88)

CAD, coronary artery disease; CPO, chest pain onset; NSTEMI, non ST-elevation myocardial infarction.

Results are expressed in mean ± SD or in number (percentage)

*, missing data exist for this variable.

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Table 3: AUC values according to CPO category

	Biomarker	AUC	95% CI
CPO <2h (very early presenters)	cTnI	0.841	0.775 to 0.894
	cTnI + copeptin	0.880	0.819 to 0.926
	HS-cTnT	0.853	0.789 to 0.904
	HS-cTnT + copeptin	0.897	0.840 to 0.940
CPO 2-4h	cTnI	0.886	0.823 to 0.933
	cTnI + copeptin	0.915	0.857 to 0.955
	HS-cTnT	0.869	0.802 to 0.919
	HS-cTnT + copeptin	0.891	0.829 to 0.937
CPO >4h	cTnI	0.995	0.965 to 1.000
	cTnI + copeptin	0.979	0.940 to 0.995
	HS-cTnT	0.980	0.942 to 0.996
	HS-cTnT + copeptin	0.953	0.905 to 0.981

AUC, area under the ROC curve; CPO, chest pain onset

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Table 4: Diagnostic performances for NSTEMI according to onset chest pain

CPO	Biomarker	Threshold	Sensitivity (%, [95%CI])	Specificity (%, [95%CI])	Negative predictive value (%, [95%CI])	Positive predictive value (%, [95%CI])	Misdiagnosed (**) (n)	Correctly ruled-out, n (%)
<2h (very early presenters) **(n=160)**	cTnI	LoQ (*)	73 [45-91]	97 [92-99]	97 [92-99]	73 [45-91]	4	141 (88)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	87 [59-98]	60 [52-68] ε	98 [92-100]	18 [7-24] ε	2	87 (54) ε
	HScTnT	14 ng/L	80 [51-95]	85 [78-90] ε	98 [93-100]	35 [20-53]	3	123 (79) ε
		5 ng/L	87 [59-98]	58 [50-66] ε ζ	98 [92-100]	18 [10-29] ε	2	84 (52) ε ζ
		3 ng/L	87[58-98]	40 [32-49] ε ζ	97 [88-99]	13 [7-22] ε	2	58 (36) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	93 [66-100]	54 [46-62] ε ζ	99 [93-100]	18 [10-29] ε	1	79 (49) ε ζ
		5 ng/L and 12 pmol/L	93 [66-100]	41 [33-50] ε ζ	98 [89-100]	14 [8-23] ε	1	59 (37) ε ζ
		3 ng/L and 12 pmol/L	93 [66-100]	25 [19-34] ε ζ	97 [85-100]	12 [7-19] ε	1	37 (23) ε ζ
2-4h **(n=143)**	cTnI	LoQ (*)	73 [50-89]	95 [89-98]	95 [89-98] ψ	73 [50-89]	6	115 (80)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	95 [75-100]	57 [48-66] ε	99 [92-100]	29 [19-41] ε	1	69 (48) ε
	HScTnT	14 ng/L	77 [54-91]	79 [71-86] ε	95 [88-98] ψ	40 [26-56]	5	96 (67) ε μ
		5 ng/L	91 [70-98]	55 [46-64] ε ζ	97 [89-100]	27 [18-39] ε	2	67 (47) ε ζ
		3 ng/L	96 [75-100]	45 [36-54] ε ζ	98 [89-100]	24 [16-34] ε	1	54 (38) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	95 [75-100]	50 [41-59] ε ζ	99 [91-100]	25 [17-36] ε	1	58 (41) ε ζ
		5 ng/L and 12 pmol/L	100 [82-100]	35 [27-44] ε ζ	100 [90-100]	22 [15-32] ε	0	42 (29) ε ζ
		3 ng/L and 12 pmol/L	100 [82-100]	29 [21-38] ε ζ	100 [88-100]	20 [14-30] ε	0	35 (25) ε ζ
>4h **(n=146)**	cTnI	LoQ (*)	100 [77-100]	96 [91-99]	100 [96-100]	77 [54-91]	0	122 (85)
	cTnI + copeptin	LoQ (*) and 12 pmol/L	100 [77-100]	59 [50-68] ε	100 [94-100]	25 [16-37] ε	0	75 (54) ε
	HScTnT	14 ng/L	100 [78-100]	87 [80-92] ε	100 [96-100]	51 [34-68]	0	111 (77)
		5 ng/L	100 [78-100]	60 [51-68] ε ζ	100 [94-100]	35 [23-50] ε	0	77 (53) ε μ ζ
		3 ng/L	100 [78-100]	52 [43-61] ε ζ	100 [96-100]	23 [14-34] ε	0	67 (47) ε ζ
	HS-cTnT+copeptin	14 ng/L and 12 pmol/L	100 [78-100]	55 [46-64] ε ζ	100 [94-100]	24 [15-35] ε	0	70 (49) ε ζ
		5 ng/L and 12 pmol/L	100 [78-100]	40 [32-49] ε ζ	100 [91-100]	19 [12-29] ε ζ	0	51 (35) ε ζ
		3 ng/L and 12 pmol/L	100 [78-100]	34 [26-43] ε ζ	100 [96-100]	18 [11-27] ε ζ	0	43 (30) ε ζ

(*) 0.04 µg/L (40 ng/L) for Bicêtre and Montpellier hospitals, 0.14 µg/L (140 ng/L) for other sites; (**), false negative patients

ε $p<0.05$ versus cTnI alone; ζ $p<0.05$ versus HS-cTnT <14 ng/L alone; ψ $p<0.05$ versus delay >4h; μ, $p<0.05$ versus delay <2h

Table 5: Potentially misdiagnosed patients with low cTn thresholds and copeptin

Sex	Age	Dyslipidaemia	Smoke	Diabetes	Hypertension	Personal history of CAD	Chest pain since :	CPO` category	cTnI	HScTnT	Copeptin
M	89	no	no	no	yes	yes	1 hr 15 min	<2h (very early presenters)	0.04 µg/L (40 ng/L)	44.9 ng/L	208.7 pmol/L
M	86	yes	no	no	yes	yes	30 min		0.06 µg/L (60 ng/L)	11.3 ng/L	77.2 pmol/L
W	35	no	no	no	no	no	50 min		0.01 µg/L (10 ng/L)	<3 ng/L	54.7 pmol/L
W	44	no	yes	no	no	no	45 min		0.01 µg/L (10 ng/L)	<3 ng/L	10.7 pmol/L
W	74	yes	no	no	yes	yes	3 hrs	2-4h	0.04 µg/L (40 ng/L)	8.8 ng/L	10.7 pmol/L
M	34,2	yes	yes	no	no	no	2 hrs 35 min		0.04 µg/L (40 ng/L)	<3 ng/L	23.5 pmol/L
M	55	yes	no	no	no	yes	3 hrs 50 min		0.02 µg/L (20 ng/L)	6 ng/L	25.9 pmol/L
M	34	no	yes	no	no	no	3 hrs 15 min		0.01 µg/L (10 ng/L)	4 ng/L	52.4 pmol/L
M	61	yes	yes	no	yes	yes	3 hrs		0.03 µg/L (30 ng/L)	19.7 ng/L	27.2 pmol/L
M	59	no	no	no	no	no	2 hrs 45 min		0.04 µg/L (40 ng/L)	10.1 ng/L	241.8 pmol/L

CAD, coronary artery disease; CPO, chest pain onset; M, men; W, women.

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n=557
[ref. 14]: n=363
[ref. 15]: n=194

Exclusions:
No cTnI: n=7
No CPO: n=67
STEMI: n=34

n=449

CPO <2h
n=160
15 STEMI / 145 non STEMI

CPO 2-4h
n=143
22 STEMI / 121 non STEMI

CPO > 4h
n=146
18 STEMI / 128 non STEMI

Figure 1: Flow chart of the studied population.

135x255mm (150 x 150 DPI)

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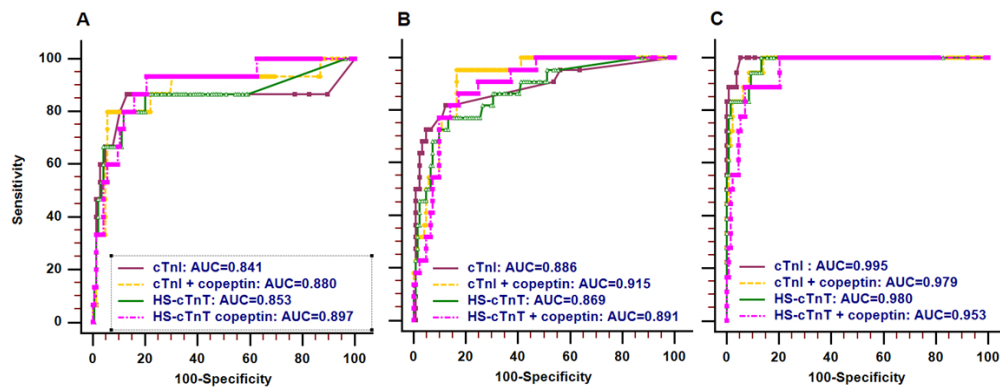53
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Figure 2: ROC curves of cTn for the diagnosis of NSTEMI, alone or in combination with copeptin. A, CPO<2h; B, CPO 2-4h; C. CPO >4h.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Page 1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Page 2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Page 4
	4	Study objectives and hypotheses	Pages 4 and 5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Page 6
Participants	**6**	Eligibility criteria	pp6&7 + Table1 p22
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Pages 6-8
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Page 6
	9	Whether participants formed a consecutive, random or convenience series	Page 6
Test methods	**10a**	Index test, in sufficient detail to allow replication	Pages 8 and 9
	10b	Reference standard, in sufficient detail to allow replication	Pages 8 and 9
	11	Rationale for choosing the reference standard (if alternatives exist)	Page 7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Page 9
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Pages 8 and 9
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Pages 6, 8 and 9
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Page 7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	Page 9
	15	How indeterminate index test or reference standard results were handled	Page 7
	16	How missing data on the index test and reference standard were handled	Flow chart (Figure1) + pages 6 and 7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Pages 6 and 7
	18	Intended sample size and how it was determined	NA
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Flow chart (Figure1) + pages 6 and 7
	20	Baseline demographic and clinical characteristics of participants	Table 2, page 23
	21a	Distribution of severity of disease in those with the target condition	Table 2, page 23
	21b	Distribution of alternative diagnoses in those without the target condition	Table 2, page 23
	22	Time interval and any clinical interventions between index test and reference standard	NA
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Tables 3&4, p.24-25
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Table 4, page25
	25	Any adverse events from performing the index test or the reference standard	Table 5 page 26
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Page 15
	27	Implications for practice, including the intended use and clinical role of the index test	Page 16
OTHER INFORMATION			
	28	Registration number and name of registry	Table 1, page22
	29	Where the full study protocol can be accessed	Table 1 page 22
	30	Sources of funding and other support; role of funders	NA

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.