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Multi-centre evaluation of recent troponin assays for the diagnosis of NSTEMI

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ABSTRACT

Objectives: We aimed to compare the use of nine different cardiac troponin (cTn) assays (2 cTnT and 7 cTnI) for the diagnosis of NSTEMI in a single multi-centre population.
Design and methods: One hundred and fifty-eight patients were included (mean age 60 years, SD 17 years), including 23 patients (14%) with NSTEMI.
Results: The analytical comparison highlighted a large heterogeneity of cTn assays, as reflected by percentages of patients with detectable cTn, correlation coefficients, Passing-Bablok comparisons and concordance coefficients. Correlations within cTnI assays were good and correlation within cTnT assays was excellent. Diagnostic performances demonstrated that each cTn assay has specific threshold values. Furthermore, some assays (HS-cTnI and T, cTnI-Pathfast and cTnI-Centaur) indicated high sensitivity and negative predictive value using the limit of detection (LoD) diagnostic strategy. For the latter assays, a significant increase in specificity was found when using the 99th percentile or the H0-H3 strategies, in comparison to the LoD strategy. When applying the European Society of Cardiology H0-H3 algorithm, comparable diagnostic performances were obtained.
Conclusion: All 9 cTn assays indicated overall good diagnostic performances for the diagnosis of NSTEMI in emergency departments when the recommended algorithm based on the variation of cTn value between two measurements at admission and 3 h later was used.

1. Introduction

Since the European Society of Cardiology (ESC) recommendations about myocardial infarction in 2012 and 2015, the main role of cardiac troponin (cTn) results has been confirmed in the diagnosis of non-ST elevation acute myocardial infarction (NSTEMI) [1,2]. In 2012, Thygesen et al. recommended a way in which to use high-sensitive cTn (HS-cTn) assays, based on two blood samples (one at admission or H0, and one 3 h later or H3), and the use of a specific delta change (either relative or absolute) between H0 and H3 [3]. Recently, this H0-H3 strategy was confirmed as a 'universal' algorithm for rule-in or rule-out of NSTEMI, for all cTn assays [2]; however, the delta change value was not specifically indicated for each cTn assay. Recent guidelines further suggest the use of a rule-out rapid algorithm, based on a single cTn measurement at admission (H0) and using low threshold values (the limit of detection (LoD) of the assay) [2]. However, this rapid exclusion algorithm is not recommended for all cTn assays [2].

In hospital laboratories and in point-of-care testing, cardiac troponin measurements are achieved by various assays, including "contemporary", "sensitive" and "highly-sensitive" assays [4]. Briefly, the adjectives "contemporary", "sensitive" and "highly-sensitive" are used when the analytical precision of the assay (calculated as the coefficient of variation [CV]) at the 99th percentile value is above, equal to or less than 10%, respectively. Due to the absence of standardisation, troponin results cannot be transferred from one assay to another, and individual cut-offs must be strictly used in the context of the troponin assay for which they were determined [4]. Few studies indicate delta change values for cTn assays in a single population, but most are reported for HS-cTn methods [3,5–7].

We thus aimed to compare, from both an analytical and a clinical view, the use of different cTn assays in a routine setting for the diagnosis of NSTEMI in emergency departments, in a single multi-centre population, in order to determine the diagnostic characteristics of each cTn assay following the recommended algorithms.

2. Materials and methods

2.1. Study population

The study was conducted in 13 French hospitals: 9 centres were involved in patient inclusion and sample collection, with 4 additional centres involved in the measurement of troponin only. Inclusions were performed between April 2014 and November 2015.

Our study complied with all of the relevant national regulations and institutional policies, was in accordance with the tenets of the Helsinki Declaration, and was approved by the local ethics committee (Comité de Protection des Personnes [CPP] Ile-de-France III: study reference SC-3122; Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé [CCTIRS], from the Direction Générale pour la Recherche et l'Innovation [DGRI]: study reference DGRI CCTIRS MG/CP 2014.297). All patients gave informed consent.

We followed the recommendations on reporting diagnostic studies set forth by the Standards for Reporting of Diagnostic Accuracy (STARD) initiative [8]. We enrolled consecutive patients (> 18 years of age) who presented to the ED with chest pain suggestive of AMI with the onset or peak occurring within the previous 6 h. Patients with ST elevation myocardial infarction (STEMI) and patients with acute or chronic kidney failure requiring dialysis were excluded, but no upper age limit was applied. Patients without 2 cardiac troponin measurements (on admission, H0, and 3-h later, H3) were excluded. Patients with haemolysed plasma samples were also not included (sample haemolysis evaluation was performed routinely by the instrument concomitant with cTn measurements).

As part of the routine assessment, all patients underwent an initial clinical evaluation that included vital signs, clinical history, physical examination, electrocardiography (ECG), pulse oximetry, routine blood tests and chest X-rays. Patients were managed at the discretion of the treating physicians based on all available data, including the local cTn measured at presentation and repeated at 3 h [2], using the in-house assay (56% of included patients were diagnosed using HS-cTnT, 28% using HS-cTnI, and 16% using two different sensitive cTnIs; see Table 1 Suppl. for distribution across centres). The final adjudicated diagnosis was determined by two independent physicians at each centre, based on all medical charts up to 30 days after presentation, and including the local troponin test results only. AMI was diagnosed according to the joint European Society of Cardiology/American College of Cardiology/American Heart Association/World Heart Federation Task Force redefinition of MI guidelines [1]. The diagnosis of AMI required a cTn value above the 99th percentile value together with at least one of the following: symptoms of ischemia, new ST-T changes or a new Q wave on an electrocardiogram, imaging of new loss of viable myocardium despite normal cTn on admission. Predefined further diagnostic categories included cardiac symptoms without infarction, non-cardiac symptoms and chest pain of unknown origin. If the diagnosis remained uncertain despite detailed investigations, and no adverse cardiac event occurred up to 1 month of follow-up, the index event was classified as non-cardiac.

Distribution of inclusions across study centres is presented in Table 1 Suppl.

2.2. Troponin measurements

Centres that included patients in the study were all using local HS or sensitive cTn (Table 1 Suppl.). After local cTn measurement, and within 24 h, surplus plasma was aliquotted in triplicate and stored at −40 °C until further measurements were made. All centres involved in patient inclusion operated in the same way. Samples were stored locally all patients had been included and were then shipped frozen to Cochin hospital. When all samples were centralised at Cochin, 3 identical sample cohorts (each containing one series of aliquots) were prepared simultaneously. Each cohort was sent to one of the 4 additional centres involved for the measurement of troponin only; one cohort was further sent to the last analysing centre. Each aliquot was used twice for testing other cTn assays, during the same episode if both assays were in the same lab, or separated by one supplemental freeze-thaw cycle if not. Since in a few cases, aliquots were not sufficient to perform all assays, some cTn results are missing. Finally, samples were stored at −40 °C for up to 18 months.

The analytical characteristics, type and thresholds of assays are given by the manufacturers and in the literature [4,9] (Table 1).

The absolute cTn change between H0 and H3 (hereafter referred to as H0-H3 [Abs]) was calculated as follows: cTn value (H3) – cTn value (H0), in ng/L. The relative cTn change between H0 and H3 (hereafter referred to as H0-H3 [Rel]) was calculated as follows: [cTn value (H3) – cTn value (H0)]/[cTn value (H0)], and expressed in percent. For change calculations, cTn values below the LoD were considered as equal to the LoD. Furthermore, we also applied the 'Guidelines-recommended' change value [3]: cTn value (H0) < 99th percentile and cTn value (H3) increase > 50% of 99th percentile, cTn value (H0) ≥ 99th percentile and cTn value (H3) increase > 20% of H0 value. The latter criterion was applied only if the 99th percentile assay was sufficiently precise (thus, not applicable to AQT assays, see Table 1).

2.3. Statistical analysis

Continuous variables are presented as median (25th – 75th percentile), and categorical variables are expressed as numbers and percentages. Continuous variables were compared by using the Kruskal-Wallis test, and categorical variables were assessed using Pearson's χ^2 test. A receiver operating characteristic (ROC) curve was constructed to assess the sensitivity and specificity, positive

Table 1
Characteristics of cTn assays studied.

Type of assay	Name of the assay	Analyser	Manufacturer (see legend)	Limit of blank (LoB)	Limit of detection (LoD)	99th percentile	10% coefficient of variation (10% CV) (manufacturer's data)
Hs-assay	HS-cTnI	Architect	Abbott	0.7–1.3 ng/L	2 ng/L	34 ng/L (men) 16 ng/L (women)	4.7 ng/L
Hs-assay	HS-cTnT	Cobas	Roche	3 ng/L	5 ng/L	14 ng/L	13 ng/L
POC assay	cTnI-Pathfast	Pathfast	Mitsubishi	ND	1 ng/L	20 ng/L	3.1 ng/L
Sensitive assay	cTnI-Ultra	Centaur	Siemens	6 ng/L	6 ng/L	40 ng/L	30 ng/L
Sensitive assay	cTnI-Vista	Vista	Siemens	15 ng/L	20 ng/L	45 ng/L	40 ng/L
Sensitive assay	cTnI-Access	Access	Beckman	< 10 ng/L	10 ng/L	40 ng/L	40 ng/L
Sensitive assay	cTnI-Vitros	Vitros	Ortho Clinical Diagnostics (OCD)	7 ng/L	12 ng/L	34 ng/L	34 ng/L
POC assay	cTnI-AQT	AQT	Radiometer	ND	9.5 ng/L	23 ng/L	27 ng/L
POC assay	cTnT-AQT	AQT	Radiometer	ND	8 ng/L	17 ng/L	26 ng/L

ND: not declared.
Abbott: Abbott Diagnostics, Abbott Park, IL, USA. Roche: Roche Diagnostics, Rotkreuz, Switzerland. Mitsubishi: Mitsubishi Chemical Europe, Dusseldorf, Germany. Siemens: Siemens Healthineers, Erlangen, Germany. Beckman: Beckman Coulter, Brea, CA, USA. Ortho Clinical Diagnostics: Ortho Clinical Diagnostics, Raritan, NJ, USA. Radiometer: Radiometer, Copenhagen, Denmark.

predictive value (PPV) and negative predictive value (NPV) throughout the concentrations cTn in the diagnosis of NSTEMI. For each assay, we reported the sensitivity, specificity, NPV, and PPV at different thresholds: the LoD, the 99th percentile, the optimal calculated delta change in absolute value or in relative value given by the ROC analysis, and the threshold proposed by the literature [3]. All data are presented with their 95% confidence intervals [95%CI]. A $p < .05$ was considered statistically significant. Statistical analysis was performed using MedCalc software for ROC analysis (MedCalc Statistical Software version 15.8, MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015).

Since cTnI and cTnT assays are based on the detection of different antigens, they were compared separately. We first compared the assays using linear regression, and correlation matrices were built to present the obtained correlation coefficients. For a cTn test, a discrepant result was arbitrarily defined when the ratio of the observed value to the corresponding 99th percentile was above 2.0, while at the same time, the ratio for the other result was below 1. The matrix table shows the correlation coefficients for paired assays compared by linear regression.

Secondly, Passing-Bablok regression was performed when possible (i.e. sufficient number of detectable samples). The inter-assay absolute (intercept) and relative (slope) differences were obtained by calculating the Passing-Bablok equation [10]. The intercepts were considered non-significant when the zero value fell within the 95% CI and the slopes were considered non-different from 1 when the value was within the 95% CI. Bland-Altman analysis was performed in side-by-side comparison for all assays using absolute difference and relative inter-assay difference. Bland-Altman plots allow checking a possible non-systematic bias between the two methods tested. Accuracy is described by the 95% limits of agreement (mean difference ± 1.96 SD).

Lastly, the strength of agreement between cTn assays was determined by calculating the Pearson concordance correlation coefficient ρ_c that evaluates the degree to which pairs of observations fall on the 45° line through the origin [11]. It contains a measurement of precision ρ and accuracy C_b: $\rho_c = \rho * C_b$, where ρ is the Pearson correlation coefficient (which measures how far each observation deviates from the best-fit line, and is a measure of precision), and C_b is a bias correction factor (that measures how far the best-fit line deviates from the 45° line through the origin, and is a measure of accuracy); a ρ_c result of > 0.99 indicating "almost perfect" and < 0.90 as poor agreement between assays [12].

3. Results

3.1. Characteristics of the population studied

Baseline characteristics of the population studied are presented in Table 2. Briefly, one hundred and fifty-eight patients were included (mean age 60 years, SD 17 years). Patients with NSTEMI (n = 23, 14%) were more frequently men, had higher blood

Table 2
Baseline characteristics of the population studied.

	Non-NSTEMI	NSTEMI	*p*
n	135	23	
Age (years)	56 (45–71)	64 (51–78)	0.282
Men	85 (63)	21 (91)	0.015
Median systolic BP (mmHg)	138 (124–153)	150 (134–165)	0.076
Median diastolic BP (mmHg)	80 (70–90)	88 (80–99)	0.001
Median cardiac rate	74 (63–86)	79 (68–98)	0.027
Median SpO_2 (%)	98 (97–99)	98 (96–99)	0.408
TIMI score	1.0 (2.0–4.0)	3.0 (2.5–4.0)	< 0.0001
Familial history of CAD	19 (14%)	5 (22%)	0.777
Personal history of CAD	26 (19%)	3 (13%)	0.499
Dyslipidaemia	41 (30%)	9 (39%)	0.830
Smoking	43 (32%)	8 (35%)	0.910
Diabetes	20 (15%)	5 (22%)	0.801
Hypertension	54 (40%)	10 (43%)	0.873
History of heart failure	9 (7%)	1 (4%)	0.923
Typical thoracic pain	60 (44%)	15 (65%)	0.283
Mean chest pain onset (h)	3.0 (1.5–4.0)	3.0 (1.6–4.7)	0.829
Mean chest pain length (h)	1.0 (0.5–2.0)	1.5 (0.5–3.5)	0.871
Coronarography	9 (7)	13 (56)	< 0.0001
Delay H0-H3	3.0 (2.8–3.5)	3.0 (2.8–3.5)	0.144
Hospital-admission	51 (38%)	20 (87%)	< 0.0001
Admission in ICU	11 (8%)	16 (69%)	< 0.0001
Median serum creatinine concentration (μM)	79 (68–93)	79 (72–87)	0.633
Final diagnosis of:			
NSTEMI	0 (0%)	23 (100%)	
Unstable Angina	9 (7%)	0 (0%)	
Other diagnosis[a]	126 (93%)	0 (0%)	

BP, blood pressure; CAD, coronary artery disease; ICU, Intensive care unit; STEMI, ST elevated myocardial infarction. Results are presented as median (25th – 75th percentile), or number (percentage).

[a] including: stable angina (n = 3), cardiac but non-coronary causes (n = 30), non-cardiac cause (n = 93).

pressure, higher cardiac rate, and higher TIMI score than those without NSTEMI. They also more frequently had a coronarography, and were more frequently admitted to hospital and into intensive care units. Of note, there was no difference in the H0-H3 interval across inclusion centres (p = .777).

3.2. Analytical comparison of cTn results

The proportion of patients with detectable cTn on admission, is presented in Table 3. The highest proportions were reported by the HS-cTn assays, but also by the POC cTnI-Pathfast; the lowest proportions were observed for the cTnT-AQT and cTnI-AQT assays.

Correlation matrices are presented in Table 4. Data collected indicated that cTnI assays are well correlated (coefficient > 0.750), except for HS-cTnI Abbott versus cTnI-Access (coefficients < 0.750). The correlation between HS-cTnT Cobas and cTnT AQT was excellent.

Passing Bablok and Bland Altman graphs were constructed when possible i.e. when there were sufficient numbers of detectable cTn results. Thus, this analysis could not be performed for cTnI-AQT, cTnT AQT, cTnI-Vista and cTnI-Vitros. The results are presented in Supplementary Fig. 1 and show substantial differences in values between assays.

Lastly, we analysed the strength of agreement between cTn assays by calculating the Pearson concordance correlation coefficient (Table 5). The results indicate perfect concordance between cTnI-Vitros and cTnI-Access, substantial concordance between HS-cTnT and cTnT-AQT, and moderate concordance between cTnI-Vitros and cTnI-Vista and between HS-cTnI and cTnI-Vista. All other concordances were poor.

3.3. Diagnostic performances

ROC curves for each assay, at H0 and H3, are presented in Fig. 1.

The diagnostic performances of each cTn assay for the diagnosis of NSTEMI are presented in Table 6. Each assay was tested for a different threshold strategy. Our results demonstrate that each cTn assay has specific threshold values, regardless of the threshold strategy used. Furthermore, some assays (Abbott and Roche HS-cTn assays, Mitsubishi Pathfast and Siemens Centaur cTn assays) indicated high sensitivity and NPV with the LoD strategy. They further showed a significant increase in specificity when using the 99th percentile or the H0-H3 strategies, in comparison to the LoD strategy. These results are in contrast with those observed for the other assays, which did not indicate a high sensitivity for the LoD strategy but showed a constant high specificity across threshold strategies. When applying the recommended H0-H3 algorithm, equivalent diagnostic performances were obtained; all HS-cTn assays demonstrated high specificity with this algorithm and allowed a good rate of rule-out patients.

4. Discussion

We aimed to compare different cTn assays for the diagnosis of NSTEMI in emergency departments, in a single multi-centre population. Our results provide a large analytical comparison of 9 cTn assays in a single population. It shows that (1) each cTn assay has its own analytical characteristics that are not fully transposable from one assay to another, (2) the diagnostic performances of each cTn assay are dependent on specific thresholds, and (3) H0-H3 strategies have a high specificity for the diagnostic of NSTEMI for all evaluated assays.

The analytical comparison of cTn results in our population highlighted large heterogeneity of cTn assays, as reflected by percentages of patients with detectable cTn, correlation coefficients, and Passing-Bablok and Bland-Altman graphs and concordance coefficients. This heterogeneity has been described previously [9], but our study is the first to undertake a large comparison in a single population of chest pain patients. Usually the proportion of patients with detectable cTn is described in a reference population. To define this proportion in a population of chest pain in emergency departments provides additional information that may be useful for practitioners. Furthermore, although cTnI assays are well correlated, concordance between assays is often poor. Only 3 concordance coefficients out of 21 were > 0.90. Agreement between assays is good only for the following pairs: cTnI-Vista/HS-cTnI, cTnI-Vista/cTnI-Vitros, cTnI-Vitros/cTnI-Access. The strength-of-agreement takes into account the imprecision of each assay, which

Table 3
Proportion of patients with detectable cTn on admission.

Assay	LoD	Proportion of patients with detectable cTn on admission
Mitsubishi cTnI-Pathfast	1 ng/L	97.8%
Abbott HS-cTnI	2 ng/L	80.7%
Roche HS-cTnT	5 ng/L	69.5%
Siemens cTnI-Ultra	6 ng/L	35.1%
Beckman cTnI-Access	10 ng/L	29.5%
OCD cTnI-Vitros	12 ng/L	19.8%
Radiometer cTnT-AQT	8 ng/L	16.4%
Siemens cTnI-Vista	20 ng/L	14.9%
Radiometer cTnI-AQT	9.5 ng/L	14.5%

Order of presentation is based on descending proportion.

Table 4
Linear correlation coefficients and number of observations of cTnI assays (comparison A) and cTnT assays (comparison B).

A	Abbott HS-cTnI					
Mitsubishi cTnI-Pathfast	0.824 (n = 244)	**Mitsubishi cTnI-Pathfast**				
Siemens cTnI-Ultra	0.867 (n = 280)	0.933 (n = 261)	**Siemens cTnI-Ultra**			
Siemens cTnI-Vista	0.954 (n = 246)	0.918 (n = 241)	0.967 (n = 268)	**Siemens cTnI-Vista**		
Beckman cTnI-Access	0.726 (n = 270)	0.937 (n = 262)	0.964 (n = 287)	0.871 (n = 271)	**Beckman cTnI-Access**	
OCD cTnI-Vitros	0.987 (n = 193)	0.964 (n = 183)	0.977 (n = 216)	0.945 (n = 221)	0.999 (n = 218)	**OCD cTnI-Vitros**
Radiometer cTnI-AQT	0.783 (n = 267)	0.853 (n = 268)	0.969 (n = 286)	0.897 (n = 271)	0.992 (n = 289)	0.991 (n = 216)

B	**Radiometer cTnT-AQT**
Roche HS-cTnT	0.993 (n = 293)

The matrix shows the correlation coefficients for paired assays compared by linear regression. Data are obtained from paired comparison of values at H0 and H3. Undetectable values were treated as equal to the LoD of the method. Discrepant results (as described in the Section 2) were excluded before analysis. The number of observations = values at H0 + values at H3 – discrepant results.

Table 5
Pearson concordance correlation coefficient (value of ρ_c and strength of agreement) between cTnI-assays (A) and cTnT assays (B).

A	**Abbott HS-cTnI**					
Mitsubishi cTnI-Pathfast	ρ_c = 0.203 Poor	**Mitsubishi cTnI-Pathfast**				
Siemens cTnI-Ultra	ρ_c = 0.715 Poor	ρ_c = 0.166 Poor	**Siemens cTnI-Ultra**			
Siemens cTnI-Vista	ρ_c = 0.948 Moderate	ρ_c = 0.224 Poor	ρ_c = 0.842 Poor	**Siemens cTnI-Vista**		
Beckman cTnI-Access	ρ_c = 0.717 Poor	ρ_c = 0.493 Poor	ρ_c = 0.720 Poor	ρ_c = 0.841 poor		
OCD cTnI-Vitros	ρ_c = 0.747 Poor	ρ_c = 0.752 Poor	ρ_c = 0.812 Poor	ρ_c = 0.933 Moderate	ρ_c = 0.999 Perfect	**OCD cTnI-Vitros**
Radiometer cTnI-AQT	ρ_c = 0.381 Poor	ρ_c = 0.839 Poor	ρ_c = 0.261 Poor	ρ_c = 0.398 Poor	ρ_c = 0.553 Poor	ρ_c =0.525 Poor

B	**Radiometer cTnT-AQT**
Roche HS-cTnT	0.987 substantial

ρ_c is calculated using the Pearson correlation coefficient and a bias correction factor (see Section 2). $\rho_c < 0.90$, poor strength of agreement; $\rho_c = 0.90 – 0.95$, moderate strength of agreement; $\rho_c = 0.95–0.99$, substantial strength of agreement; $\rho_c > 0.99$, almost perfect strength of agreement.

is not true for linear regression. Thus, it may explain the differences between the two comparisons. The strength-of-agreement is a reliable marker for the global agreement between two assays, but does not provide the number of individual discrepant results [13]. For cTnT assays, the between-assays correlation is excellent, and strength of agreement is substantial. Our results are in accordance with those of the literature, as observed between cTnI-Vista and cTnI-AQT [14]. They highlight the fact that each cTn assay has individual analytical characteristics, and that the results are not fully transferable from one assay to another [15,16]. Consequently, the need for adapted thresholds for each assay is important. This should be taken into account in educational programmes of users/practitioners if several assays are used in the same hospital centre, and/or when there is a POC solution in addition to the central lab assay [15,16].

Considering performances for the diagnosis of NSTEMI, our results highlight the variability in cTn assays. However, using adapted thresholds, diagnostic performances for the rule-in and rule-out of NSTEMI can be optimised for each assay. The literature is scarce concerning studies measuring several cTn assays in a single population. Wildi et al. recently reported data on 4 HS assays and 3 sensitive assays [6], but some assays were in a pre-commercial version. Here, we report data on fully commercial and routinely used

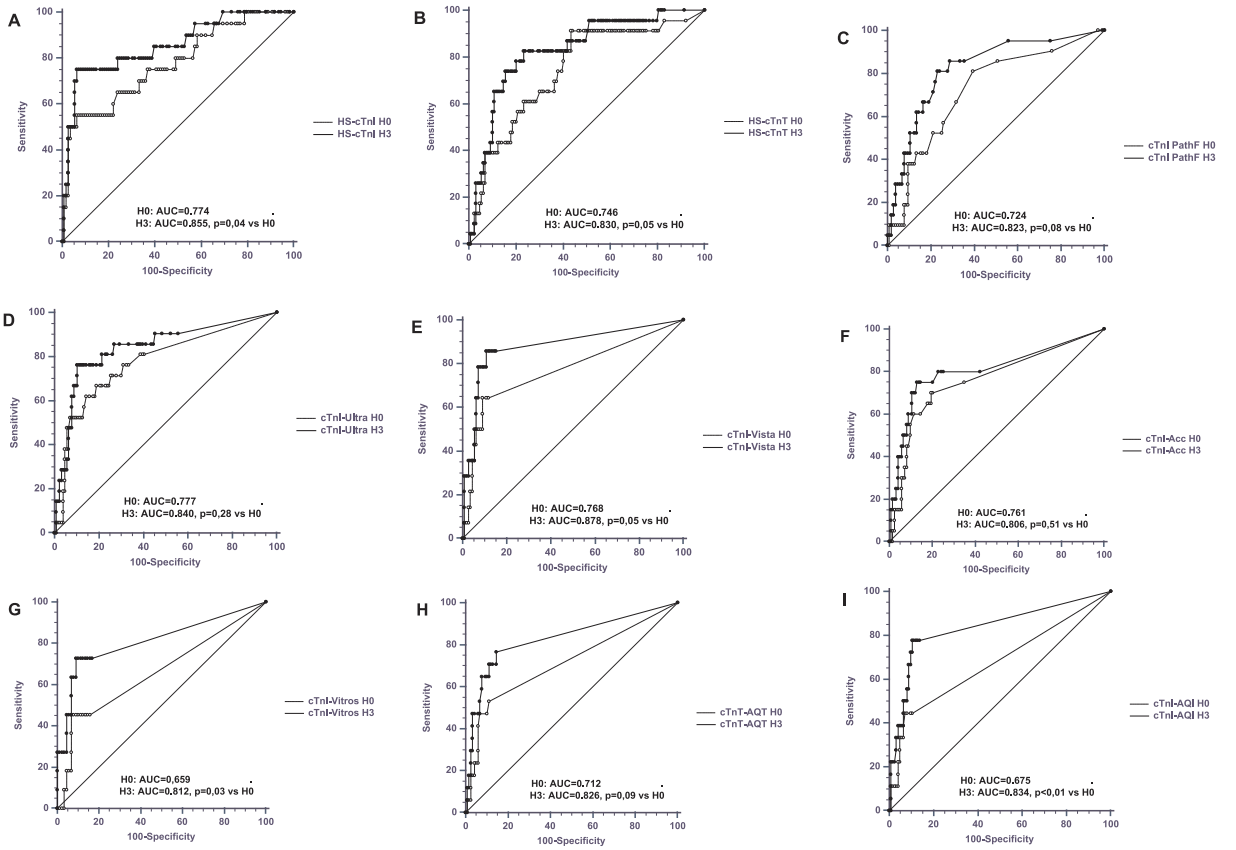

Fig. 1. Receiver operating characteristic (ROC) curves of cTn assays for the diagnosis of NSTEMI at H0 and H3: A, Abbott HS-cTnI; B, Roche HS-cTnT; C, Mitsubishi cTnI-Pathfast; D, Siemens cTnI-Ultra; E, Siemens cTnI-Vista; F, Beckman cTnI-Access; G, OCD cTnI-Vitros; H, Radiometer cTnT-AQT; I, Radiometer cTnI-AQT. Areas under the ROC curve (AUC) values are indicated for each measurement (at H0 and H3).".

HS, sensitive and POC assays, which we compare to the literature.

Regarding the rule-out algorithms using LoD at H0 [2], our study confirms that this strategy gives the highest sensitivity and a high NPV for HS-assays and some other assays (cTnI-Pathfast, cTnI-Access, cTnI-Ultra). Our results are similar to those of the literature [17–23]. However, we question the safety of this algorithm for ruling-out NSTEMI in routine practice because the analytical reliability of the LoD as a cut-off is vulnerable to variation [24–26]. Some authors recently argued that if LoD refers to the capability of the measurement process, it should not be used as a decision limit to categorise results as detected or not [26]. Moreover, laboratories do not have suitable materials for quality control at such low values. This single strategy is not sensitive enough for conventional assays such as cTnI-Vista, cTnI-Vitros, cTnI-AQT and cTnT-AQT.

The use of the 99th percentile is more specific than the LoD for all assays. Our results observed for HS-assays and cTnI-Access are similar to those of the literature [5,17,20]. However, the 99th percentile threshold is not the best strategy (i.e. with the highest specificity) for all assays. Moreover, considering the differences observed by some authors in calculating 99th percentile values [27], it is still recommended that cTn elevation in acute settings should not be defined by a single value but instead by serial measurements, demonstrating significant changes [28]. Indeed, Mueller et al. indicated that a single cut-off value for HS-cTnT at the 99th percentile at presentation performed less adequately than the combination of HS-cTnT at presentation with a 1-h level and 1-h absolute change [20]. Our results obtained with cTnI-Pathfast are similar to those of the literature [29]. Considering POC assays for which the 99th percentile is lower than the 10%CV value (cTnI-AQT and cTnT-AQT), we observed that the diagnostic performances using a threshold at the 10%CV value were similar to those observed when using the 99th percentile value.

We further compared 3 different delta change evaluations of H0-H3 strategies: two were calculated (absolute cTn variation/delta, relative cTn variation/delta), and the third was derived from the literature [3] and was applied only for assays that have measurable 99th percentiles (thus, inapplicable to AQT assays). An overall evaluation indicated that the three H0-H3 strategies indicated high specificity for the diagnosis of NSTEMI. This specificity was significantly superior to a single measurement strategy for HS-assays, cTnI-Pathfast, cTnI-Ultra and cTnI-Vista. Our results are comparable to those in the literature when considering HS assays [6,17,18,22,30], but also cTnI Access, cTnI-Vista, cTnI-Vitros, cTnI-AQT [5,7,14,30], and cTnT-AQT [19,30], although comparison is limited because of the sample size of the population and study protocols (some data in the literature is reported for H0-H2). Moreover, the percentage of rule-out patients with these H0-H3 strategies is elevated for all assays. Delta change values adapted for each assay are not fully given by the guidelines [2]; only one expert opinion recommends a strategy that was validated for a few HS-

Table 6
Performances of cTn assays for the diagnosis of NSTEMI.

Assay		n	Threshold	Sensitivity, % [95%CI]	Specificity, % [95%CI]	NPV, % [95%CI]	PPV, % [95%CI]	rule-out n (%)
Abbott HS-cTnI	**LoD**	135	2 ng/L	100 [81–100]	23 [16–32]	100 [84–100]	19 [12–28]	26 (19)
	99th p.	135	34(M)/ 16(F) ng/L	48 [27–70] $	90 [83–95] $	90 [83–95]	48 [27–70]	102 (76)
	H0-H3 (Abs)	130	+5.6 ng/L	75 [51–91]	91 [84–96] $ £	95 [88–98]	58 [38–76] $	99 (76)
	H0-H3 (Rel)	130	+39%	75 [51–91]	77 [68–85] $	94 [86–98]	37 [23–54]	83 (64)
	Guidelines (*)	130	(*)	70 [46–87]	95 [89–98] $ £	95 [89–98]	70 [46–87] $	104 (80)
Roche HS-cTnT	**LoD**	154	5 ng/L	91 [70–98]	34 [26–43]	96 [85–99]	20 [13–29]	74 (48)
	99th p.	154	14 ng/L	61 [39–80]	72 [63–79] $	91 [83–96]	28 [17–43]	94 (61)
	H0-H3 (Abs)	152	+14 ng/L	65 [43–84]	90[83–95]$&	93 [87–97]	52 [30–70] $	115 (76)
	H0-H3 (Rel)	152	+73%	61 [39–80]	89[83–94]$&	93 [87–97]	47 [29–66] $	113 (74)
	Guidelines (*)	152	(*)	61 [54–67] $	89[82–94]$&	93 [87–97]	50 [31–69] $	115 (76)
Mitsubishi cTnI-Pathfast	**LoD**	140	1 ng/L	100 [81–100]	3 [1–8]	100 [31–100]	15 [10–23]	3 (2)
	99th p.	140	20 ng/L	38 [19–61] $	89 [82–94] $	89 [82–94]	38 [19–61]	106 (76)
	H0-H3 (Abs)	124	+9 ng/L	67 [43–85]	81 [71–88] $	92 [84–96]	45 [28–64] $	85 (69)
	H0-H3 (Rel)	124	+100%	62 [38–82] $	83 [73–89] $	90 [80–96]	27 [16–42]	66 (53)
	Guidelines (*)	124	(*)	48 [27–70] $	90 [82–95] $	89 [81–94]	50 [28–72] $	93 (75)
Siemens cTnI-Ultra	**LoD**	148	6 ng/L	73 [50–89]	71 [62–79]	94 [87–98]	31 [19–46]	90 (61)
	99th p.	148	40 ng/L	26 [11–49] $	98 [93–100] $	88 [81–93]	67 [31–91]	123 (83)
	H0-H3 (Abs)	148	+40 ng/L	64 [41–83]	91 [85–96] $	93 [87–97]	54 [34–73]	114 (77)
	H0-H3 (Rel)	148	+150%	55 [32–76]	86 [78–91] $&	91 [84–95]	41 [24–61]	109 (74)
	Guidelines (*)	148	(*)	57 [34–77]	91 [84–95] $	93 [87–97]	50 [30–70]	114 (77)
Siemens cTnI-Vista	**LoD**	134	20 ng/L	56 [32–78]	91 [84–95]	93 [86–97]	50 [28–72]	106 (79)
	99th p.	134	45 ng/L	39 [18–64]	93 [86–97]	91 [84–95]	47 [23–73]	108 (81)
	H0-H3 (Abs)	125	+60 ng/L	79 [49–95]	95 [90–99]	97 [91–99]	65 [39–85]	103 (82)
	H0-H3 (Rel)	125	+25%	79 [49–95]	92 [85–96]	97 [91–99]	52 [30–73]	101 (81)
	Guidelines (*)	125	(*)	79 [49–95]	92 [85–96]	97 [91–99]	58 [34–79]	102 (82)
Beckman cTnI-Access	**LoD**	146	10 ng/L	76 [52–91]	78 [70–85]	95 [88–98]	37 [23–53]	98 (67)
	99th p.	146	40 ng/L	52 [30–73]	89 [82–94]	92 [85–96]	44 [25–65]	111 (76)
	H0-H3 (Abs)	144	+36 ng/L	65 [41–85]	90 [84–95] $	94 [88–97]	52 [32–72]	112 (78)
	H0-H3 (Rel)	144	+146%	60 [36–81]	84 [76–90]	93 [86–97]	48 [28–68]	111 (77)
	Guidelines (*)	144	(*)	60 [36–80]	91 [84–95] $	93 [87–97]	52 [31–72]	112 (78)
OCD cTnI-Vitros	**LoD**	106	12 ng/L	46 [20–74]	84 [75–91]	92 [84–97]	29 [13–53]	82 (77)
	99th p.	106	34 ng/L	39 [18–64]	93 [87–97]	91 [84–95]	47 [23–73]	78 (74)
	H0-H3 (Abs)	100	+46 ng/L	73 [39–94]	93 [86–98]	96 [89–99]	44 [22–68]	82 (82)
	H0-H3 (Rel)	100	+33%	73 [39–94]	90 [82–95]	96 [89–99]	53 [27–78]	79 (79)
	Guidelines (*)	100	(*)	73 [40–93]	92 [84–96]	96 [89–99]	53 [27–78]	82 (82)
Radiometer cTnI-AQT	**LoD**	145	9.5 ng/L	44 [22–68]	90 [83–94]	92 [85–96]	38 [19–61]	114 (79)
	99th p.	145	23 ng/L	33 [14–59]	95 [89–98]	91 [85–95]	50 [22–78]	121 (83)
	10%CV	145	27 ng/L	33 [14–59]	95 [89–98]	91 [85–95]	50 [22–78]	121 (83)
	H0-H3 (Abs)	143	+1 ng/L	63 [39–84]	89 [82–94]	96 [90–99]	48 [29–68]	112 (78)
	H0-H3 (Rel)	143	+40%	67 [41–87]	92 [86–96]	95 [89–98]	52 [31–72]	115 (80)
Radiometer cTnT-AQT	**LoD**	140	8 ng/L	53 [29–76]	89 [82–94]	93 [86–97]	16 [5–38]	109 (78)
	99th p.	140	17 ng/L	29 [11–56]	94 [88–97]	91 [84–95]	39 [16–68]	115 (82)
	10%CV	140	26 ng/L	24 [8–51]	94 [88–97]	90 [83–94]	33 [11–64]	115 (82)
	H0-H3 (Abs)	134	+3 ng/L	65 [38–86]	93 [87–97]	95 [89–98]	52 [30–73]	108 (81)
	H0-H3 (Rel)	134	+36%	65 [38–86]	95 [89–98]	95 [89–98]	65 [39–85] $	112 (84)

Abs: absolute variation, ACS: acute coronary syndrome, AUC: area under the curve, LoD: limit of detection, NPV; negative predictive value, PPV: positive predictive value, Rel: relative variation. M = Male, F = Female.
H0-H3 (Abs) and H0-H3 (Rel) threshold values are optimal values given by the ROC analysis.
(*) [3]:.
- Access: H0 < 40 ng/L and H3 > 60 ng/L, or H0 ≥ 40 ng/L and H3 + 20%.
- Architect: H0 < 34/16 ng/L and H3 > 51/24 ng/L, or H0 ≥ 34/16 ng/L and H3 + 20%.
- Centaur: H0 < 40 ng/L and H3 > 60 ng/L, or H0 ≥ 40 ng/L and H3 + 20%.
- Cobas: H0 < 14 ng/L and H3 > 21 ng/L, or H0 ≥ 14 ng/L and H3 + 20%.
- Pathfast: H0 < 20 ng/L and H3 > 30 ng/L, or H0 ≥ 20 ng/L and H3 + 20%.
- Vista: H0 < 45 ng/L and H3 > 68 ng/L, or H0 ≥ 45 ng/L and H3 + 20%.
- Vitros: H0 < 34 ng/L and H3 > 51 ng/L, or H0 ≥ 34 ng/L and H3 + 20%.

$, $p < .05$ versus LoD.
£, $p < .05$ versus H0-H3 (Rel).
&, $p < .05$ versus 99th p.

cTn assays [3]. In the present study, we give some new information for each tested cTn assay, regardless of the HS/sensitive/POC type assay, in a single population, applying this recommended H0-H3 algorithm [3]. We also show that a simple calculation of an H0-H3 variation (in absolute or relative change) can be used and gives the same performances to the method proposed in the guidelines. Furthermore, our relative delta change values are in accordance with the RCV limits observed in the literature [31]. Although our

results should be confirmed in a larger population, we believe that this information might be useful for clinical and laboratory practitioners.

Our study has some limitations: first, we included a small number of patients; thus, our study is under-powered to assess differences in diagnostic performance between the assays. Second, the final diagnosis was performed using different cTn assays, as all centres did not use the same method. Third, there are missing data for some cTn assays, as we could not collect sufficient plasma from all patients. Finally, we only assessed the H0-H3 algorithm for this analysis, and we did not evaluate the recent H0-H1 algorithm in our population.

5. Conclusion

In conclusion, our results show that despite analytical differences, all 9 cTn assays have good diagnostic performances for the diagnosis of NSTEMI in emergency departments, with an algorithm based on the variations in cTn value between admission and 3 h later.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.plabm.2018.02.003.

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