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Development of an algorithm for ruling-out non-ST elevation myocardial infarction in the emergency department using high sensitivity troponin T assay

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ABSTRACT

Introduction: Chest pain and its clinical manifestations are the most common reasons for presentation to the emergency department (ED). Given that the prevalence of chest pain due to acute myocardial infarction (AMI) in the ED is modest, clinicians should use cardiac troponins to safely and rapidly rule out AMI, avoiding the delayed release of low risk patients.

The study aims to develop and validate an algorithm to early rule-out of non-ST elevation myocardial infarction (NSTEMI) in subjects admitted to the ED with symptoms of myocardial infarction.

Methods: High sensitivity cardiac Troponin T (hs-cTnT) serial measurements (baseline, T0; after 1 h, T1; after 3 h, T3) were used to develop and validate the algorithm, respectively, in 6403 and 773 consecutive admissions suggestive of AMI.

Results: Patients were classified as having or not having NSTEMI according to clinical assessment, diagnostic imaging, and serial measurements ofhs-cTnT; ROC curve analysis allowed to find changes in consecutive hs-cTnT associated with diagnostic sensitivity close to 100%.

Only patients with hs-cTnTat T0 lower than 14 ng/L resultedto be eligible for the safe rule-out of NSTEMI.

Conclusions: Although some points remain to be improved, the results obtained indicate that algorithms for fast NSTEMI rule-out are feasible and safe.

1. Introduction

Chest pain and its clinical manifestations represent one of the most common reasons for Emergency Department (ED) visits accounting for 5–10% of the total causes of ED presentation [1]. However, the prevalence of AMI in ED patients with chest pain and/or suggestive symptoms of acute coronary syndrome varies quite widely among studies, ranging from a modest percentage of 4% [2] to 15% or more [3,4]. While the high mortality associated with AMI should entail the importance of a rapid diagnosis for proper and prompt treatments, the very consistent number of chest pain patients admitted to the ED with a final diagnosis different from acute coronary syndrome (ACS) should require that clinicians be able to rapidly exclude AMI in low risk patients. According to the most recent revision of the Universal Definition of Myocardial Infarction [5], in a clinical setting consistent with acute myocardial ischemia, diagnosis can be made, with a troponin value exceeding the 99th percentile of the reference population, associated with a rise and/or fall in serial measurements. In this context, the laboratory plays a key role in the diagnostic workup of AMI. In particular, when results from ECG and/or imaging techniques are unclear and a non-ST elevation myocardial infarction (NSTEMI) is suspected, serial troponin measurements and evaluations of changes between consecutive measurements can support clinicians in ruling-in or ruling-out infarction. Due to the increased analytical sensitivity of troponin tests, in the last few years the timing for consecutive sampling has been shortened. Some years ago, basal (T0) and after 6 h (T6) blood drawings were recommended; currently, the guideline of the European Society of Cardiology (ESC) [6] for the management of ACS in patients without persistent ST segment elevation, recommends serial high-sensitivity cardiac troponin T or I measurements (hs-cTnT, hs-cTnI) on admission

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and after 3 h (T3); as an alternative, a faster protocol with T0 and after 1 h (T1) sampling is feasible when a validated algorithm is available; specific changes are suggested for the ruling-in and ruling-out of an AMI according to the kind of troponin measured and to the assay manufacturer.

In the TRAPID-AMI multicentric study Mueller et al. [7], validated on 1282 patients the ESC 1 h AMI rule-out algorithm: hs-cTnT atT0 below 12 ng/L and an increase within 1 h below 3 ng/L. They found a negative predictive value (NPV) and a diagnostic sensitivity (SENS) equal to 99.1% and 96.7%, respectively, indicating the suitability of the ESC algorithm for AMI exclusion. ESC guideline publication encouraged and promoted discussion among experts [8–13]. The algorithm proposed can potentially be useful but some doubts remain about its feasibility in clinical practice [14,15]. To verify the feasibility of ruling-out AMI by close serial troponin measurements, a retrospective study was performed using a very large dataset collected over 5 years. This dataset included all hs-cTnT measurements obtained from patients admitted to the Desio Hospital ED with chest pain or clinical manifestations attributable to AMI. In particular, an algorithm based on changes between consecutive hs-cTnT measurements was defined and validated.

2. Materials and methods

2.1. Methods

Blood samples for troponin measurement were collected from November 2011 to October 2016 in all cases of a suspect AMI arriving to the ED of Desio Hospital, Desio, Italy. Serial sampling using EDTA-3K tubes was performed at 3 time points (T0, T1, T3). Analysis was performed on plasma samples obtained after 5 min centrifugation at 3500*g*. Elecsys Troponin T hstest (Roche Diagnostics, Mannheim, Germany; catalogue number: 05092744190) [16] is based on electrochemiluminescence technology and was performed on Roche Cobas 8000 – e 602 module (Roche Diagnostics, Mannheim, Germany). The test is characterized by the following technical specifications: limit of blank (LOB) = 3 ng/L; limit of detection (LOD) = 5 ng/L; limit of quantitation (LOQ) = 13 ng/L. The manufacturer indicates a coefficient of variation near to 5% for measurements around the LOQ and lower than 2% for values above the LOQ. The universally adopted cut-off value, corresponding to the 99% percentile upper reference limit (URL) is equal to 14 ng/L.

The study was conducted in accordance to the Declaration of Helsinki and informed consent was not presented because not applicable.

2.2. Study design and study population

Beginning from 2011 the University Department of Laboratory Medicine of Desio Hospital (suburban setting hospital) adopted hs-cTnT as the biomarker of choice for structural myocardial damage. In all cases of a suspect AMI arriving to the ED within a median time of 3.4 h, serial blood drawing for hs-cTnT was performed. All patients were also clinically assessed through medical history, physical examination, and a 12-lead ECG. Starting from November 2011 allhs-cTnT measurements obtained have been electronically archived generating a dataset which includes also patient information and final diagnoses. Given that, at the time of high-sensitivity test introduction, fast protocols with T1 measurements were very challenging and desirable but still not encouraged, clinicians were not blinded to the T1 results, but were fully aware that they could not rely on it for rapid patient discharge. Final diagnoses were made by the attending ED physician in the case of a subject who was not hospitalized, and by a physician of the specific medical unit in the case of hospitalization, with cardiologist consultations asked when required. AMI diagnostic workup was performed according to best clinical practice using the criteria reported in the *Third Universal Definition of Myocardial Infarction,* that is, the recommendation effective at the time of the study [5]. Based on the codes of the *International Classification of Diseases – Clinical Modification, Ninth Revision* (ICD-9-CM) reported in the medical records, each patient was ultimately classified as belonging to one of the following groups: “NSTEMI”, “STEMI” (ST elevation myocardial infarction) and “non-AMI”. This last category included both individuals discharged from the ED without any further hospitalization, and subjects hospitalized for causes different from AMI. STEMI patients, subjects younger than 18 years of age [17,18], and unclassified AMI (due to rapid transfer to other hospitals or death occurring before AMI classification), were excluded from the study. No other selection criteria were applied in order to provide a realistic picture of a typical emergency setting. A total of 14,170 patients with possible NSTEMI were selected. Data collected from November 2011 to October 2015 (derivation cohort, n = 10,852) was used to define the algorithm, and data obtained from November 2015 to October 2016 (validation cohort, n = 3318) was used to validate it. In both cohorts all patients presented at least one troponin measurement in addition to T0. In the derivation cohort, 6403 subjects had all 3 time points for troponin measurements available (T0, T1, and T3). These patients were evaluated in order to find the threshold of changes in consecutive troponin measurements associated with diagnostic sensitivity and negative predictive value close to 100%. Once the threshold was found, the algorithm was constructed and then verified using the validation cohort (Fig. 1).

2.3. Statistical analysis

Data analysis was performed using MedCalc for Windows, version 17.2 (MedCalc Software, Ostend, Belgium). Absolute and relative changes (Δabs and Δ%, respectively) were calculated between T1 and T0 (ΔT1-T0), and between T3 and T0 (ΔT3-T0). Receiving operating characteristic (ROC) curves were used to evaluate diagnostic performances of absolute and relative changes between consecutive troponin measurements. ROC curve analysis was performed on the derivation

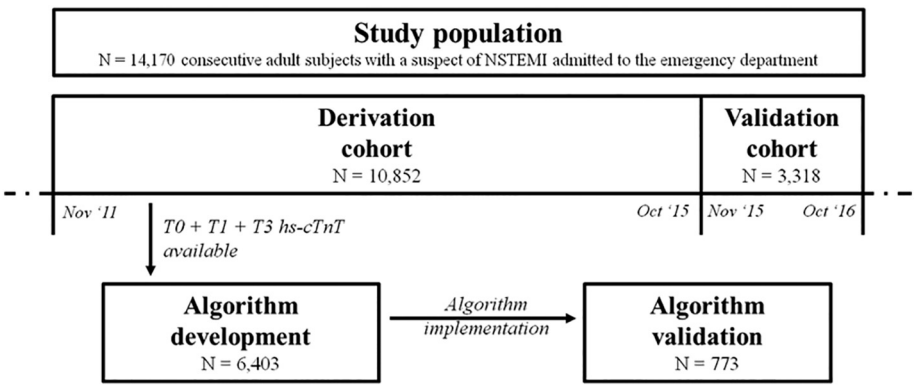

Fig. 1. Graphical description of the study population.
Description of the study population divided in derivation and validation cohort for algorithm development and validation. Abbreviations: NSTEMI, non-ST elevation myocardial infarction; hs-cTnT, high sensitivity cardiac troponin T; T0, T1, T3 mean hs-cTnT measured at baseline, after 1 and 3 h respectively.

Table 1
Derivation cohort characteristics.

	All suspected NSTEMI			NSTEMI			Non-AMI		
	M + F	M	F	M + F	M	F	M + F	M	F
No. of cases:	6403	3548	2855	475	286	189	5928	3262	2666
No. of cases with:									
T0 hs-cTnT < 14 ng/L	2925	1689	1236	47	27	20	2878	1662	1216
T0 hs-cTnT ≥ 14 ng/L	3478	1859	1619	428	259	169	3050	1600	1450
Age, years	73 (59–82)	70 (55–79)	76 (63–84)	75 (64–82)	73 (63–80)	77 (68–84)	73 (58–82)	69 (54–79)	76 (630–84)
T0 hs-cTnT, ng/L	15 (6–32)	14 (6–30)	16 (6–32)	53 (23 − 132)	47 (22–107)	66 (27–180)	14 (6–28)	13 (6–27)	15 (6–30)
T1 hs-cTnT, ng/L	16 (6–37)	15 (6–35)	17 (6–38)	103 (49–221)	95 (45–177)	127 (57–269)	14 (6–30)	13 (6–29)	16 (6–32)
T3 hs-cTnT, ng/L	16 (6–40)	15 (6–38)	18 (6–41)	160 (79–343)	142 (74–324)	172 (86–364)	14 (6–32)	13 (6–30)	16 (6–34)

Data are presented in its entirety, for NSTEMI, and non-AMI subgroups. Males and females are considered on a whole and separately for each aforementioned category. Age and hs-cTnT values at T0, T1 and T3 are shown as median and 25–75%iles on parenthesis; for T0 hs-cTnT the frequency of measurements lower and higher than or equal to the upper reference limit of 14 ng/L is reported. Abbreviations: NSTEMI, non-ST elevation myocardial infarction; AMI, acute myocardial infarction; M, males; F, females; hs-cTnT, high sensitivity cardiac troponin T; T0, T1, T3 mean hs-cTnT measured at baseline, after 1 and 3 h respectively.

cohort as a whole, and after subdivision on the basis of sex and hs-cTnT value at presentation (lower than 14 ng/L and greater than or equal to 14 ng/L). SENS, specificity (SPEC), NPV and positive predictive value (PPV) were estimated at different Δ thresholds (absolute and relative) considering both T1-T0 and T3-T0 changes. AMI prevalences used to calculate the predictive values are those observed in each specific group (by sex and hs-cTnT at presentation) taking into account the whole study population from November 2011 to October 2016.

3. Results

3.1. Derivation cohort

Baseline characteristics of the derivation cohort patients are shown in Table 1.

Out of 10,852 patients included in the derivation cohort, 6403 had T0, T1, and T3 hs-cTnT measurements available. In particular, NSTEMI was diagnosed in 475 subjects while 5928 resulted to not have AMI (4718 were discharged from the ED without any admission to other medical units within Desio Hospital, and 1210 were hospitalized for reasons other than AMI).

Among the 475 NSTEMI patients, 428 (90.1%, 259 males and 169 females) presented T0 hs-cTnT greater than or equal to 14 ng/L, whereas 47 (9.9%, 27 males and 20 females) had T0 hs-cTnT lower than 14 ng/L. In the non-AMI group, 3050 (51.4%, 1600 males and 1450 females) had T0 greater than or equal to 14 ng/L and 2878 (48.6%, 1662 males and 1216 females) had T0 lower than 14 ng/L.

ROC curve analysis performed on the whole population revealed that better diagnostic performances, as AUCs, were obtained when taking into consideration Δabs respect to Δ%, both for Δ (T1-T0) (0.902 vs 0.790) and Δ (T3-T0) (0.933 vs 0.848). The analysis, divided according to the T0 value, showed that the group in which T0 was lower than 14 ng/L had higher AUCs than those in which T0 was greater than or equal to 14 ng/L, both for Δabs (T1-T0) (0.948 vs 0.854) and Δabs (T3-T0) (0.983 vs 0.892). Within the group of subjects characterized by T0 lower than 14 ng/L, males presented different AUCs considering Δabs (T1-T0) respect to Δabs (T3-T0) (0.919 vs 0.976) whereas females did not (0.983 vs 0.991). (Table 2, Supplementary Fig. 1, Supplementary Table 1).

These results, as a whole, indicate that specific Δ thresholds, corresponding to those suggested by ROC curve analysis as Youden Index, have to be adopted for a correct classification of NSTEMI or non-AMI and therefore as a safe rule-out strategy. In particular: in females with T0 lower than 14 ng/L a threshold of 3 ng/L as Δabs (T1-T0) is associated with SENS = 100% (true positives = 20, false negatives = 0), SPEC = 91.2% (true negatives = 1108, false positives = 108), NPV = 100%, and PPV = 3.1%; in males a threshold of 4 ng/L as Δabs (T3-T0) is associated with SENS = 96.3% (true positives = 26, false negatives = 1), SPEC = 93.4% (true negatives = 1552, false positives = 110), NPV = 100%, and PPV = 5.5% (Fig. 2).

ROC curve analysis revealed that the subjects with T0 hs-cTnT higher than or equal to 14 ng/L could not be considered as candidates for early rule-out because of inadequate SENS and NPV, both for T3-T0 (SENS lower than 85%) and T1-T0 (SENS lower than 77%).

3.2. Algorithm creation

The results found allowed to develop the algorithm illustrated in Fig. 3.

This is based on a multi-step approach that must be applied to all adult subjects presenting to the ED and for whom a suspect diagnosis of NSTEMI is established. The algorithm proceeds as follows:

1) Is NSTEMI suspected? Evaluate troponin T at T0.
2) Is the T0 hs-cTnT lower than 14 ng/mL? If so, both females and males will be candidates for early NSTEMI rule-out. If female, perform the troponin T measurement at T1; if male, perform the troponin T measurement at T3.
3) In subjects with hs-cTnT < 14 ng/L, do Δabs (T1-T0) not exceed the threshold of 3 ng/L and Δabs (T3-T0) not exceed the threshold of 4 ng/L, for females and males, respectively? If so, in accordance to the clinical assessment, NSTEMI may be reasonably ruled-out. In all other cases, patients should continue to be monitored with additional troponin measurements and further investigations, in order to define whether the clinical presentation is compatible or not with acute myocardial infarction.

3.3. Validation cohort

The validation process involved 3318 patients. Since the algorithm developed proved to be suitable for NSTEMI exclusion only for subjects with T0 lower than 14 ng/L, 773 (549 females and 224 males) satisfied the applicability criteria (Fig. 4).

Among the 549 females, 500 had Δabs (T1-T0) not exceeding 3 ng/L. All of these 500 patients were not diagnosed as having NSTEMI. Therefore, they represented the true negatives and confirmed the 100% NPV of the algorithm applied to females. In the remaining 49 cases, Δabs (T1–T0) exceeded the threshold, of these, 6 were NSTEMI whereas 43 had increased troponin values that were not related to NSTEMI.

Considering the males, in 192 out of 224 subjects, the Δabs (T3-T0) did not exceed the threshold of 4 ng/L, and of these, 189 were non-AMI (true negatives), and 3 were NSTEMI (false negatives) thus leading to a NPV of the algorithm applied to men equal to 99.9%. Thirty-two patients had Δabs exceeding the cut-off, 12 times in case of NSTEMI and 20 times in patients with final diagnosis different from NSTEMI.

The attention was focused on the 3 NSTEMI falsely identified by the

Table 2
Diagnostic performances of changes between consecutive hs-cTnT measurements.

T0 hs-cTnT	Sex	Δ changes							
		(T1-T0)				(T3-T0)			
		Δabs		Δ%		Δabs		Δ%	
		AUC (95% CI)	Youden index	AUC (95% CI)	Youden index	AUC (95% CI)	Youden index	AUC (95% CI)	Youden index
All	M + F	0.902 (0.895–0.910)	4	0.790 (0.780–0.800)	29	0.933 (0.926–0.939)	9	0.848 (0.839–0.856)	32
***n* = 6403**	*n* = 6403								
	M	0.895 (0.884–0.905)	4	0.792 (0.778–0.805)	27	0.929 (0.920–0.937)	10	0.854 (0.842–0.865)	31
	n = 3548								
	F	0.917 (0.906–0.927)	4	0.790 (0.774–0.804)	29	0.940 (0.931–0.948)	9	0.840 (0.826–0.853)	32
	n = 2855								
< 14 ng/L	M + F	0.948 (0.939–0.956)	3	0.938 (0.929–0.947)	30	0.983 (0.978–0.987)	7	0.977 (0.971–0.982)	77
***n* = 2925**	n = 2925								
	M	0.919 (0.905–0.931)	3	0.904 (0.889–0.918)	30	0.976 (0.967–0.983)	4	0.967 (0.957–0.975)	77
	n = 1689								
	F	0.983 (0.974–0.989)	3	0.980 (0.970–0.987)	32	0.991 (0.984–0.996)	9	0.991 (0.984–0.995)	84
	n = 1236								
≥14 ng/L	M + F	0.854 (0.842–0.866)	10	0.764 (0.750–0.778)	21	0.892 (0.881–0.902)	17	0.819 (0.806–0.832)	32
***n* = 3478**	n = 3478								
	M	0.848 (0.831–0.864)	9	0.781 (0.761–0.799)	21	0.888 (0.873–0.902)	17	0.834 (0.817–0.851)	34
	n = 1859								
	F	0.869 (0.852–0.885)	10	0.746 (0.724–0.767)	22	0.901 (0.886–0.916)	17	0.800 (0.780–0.820)	32
	n = 1619								

Diagnostic performances of Δabs and Δ% for (T1-T0) and (T3-T0) on 6403 cases of the derivation cohort. Data are presented as AUC (95% CI) and the related Youden Index has been reported. The analysis was performed taking into consideration: 1) all cases belonging to the derivation cohort; 2) cases presenting T0 hs-cTnT lower than 14 ng/L; 3) cases presenting T0 hs-cTnT higher than or equal to 14 ng/L. In each category the evaluation was performed on males and females together (M + F) and separately by sex (M, F). Abbreviations: hs-cTnT, high sensitivity cardiac troponin T; T0, T1, T3 mean hs-cTnT measured at baseline, after 1 and 3 h respectively; M, males; F, females; Δabs, absolute delta change between consecutive troponin measurements; Δ%, relative delta change between consecutive hs-cTnT measurements; AUC, area under curve; CI, confidence interval.

	Youden Index	SENS (95% CI)	SPEC (95% CI)	PPV (95% CI)	NPV (95% CI)
T0 hs-cTnT < 14 ng/L F Δabs (T1-T0)	3 ng/L	100 (83.2 - 100)	91.2 (89.5 - 92.7)	3.1 (2.6 - 3.7)	100
hs-cTnT T0 < 14 ng/L M Δabs (T3-T0)	4 ng/L	96.3 (81.0 - 99.9)	93.4 (92.1 - 94.5)	5.5 (4.6 - 6.6)	100 (99.9 - 100)

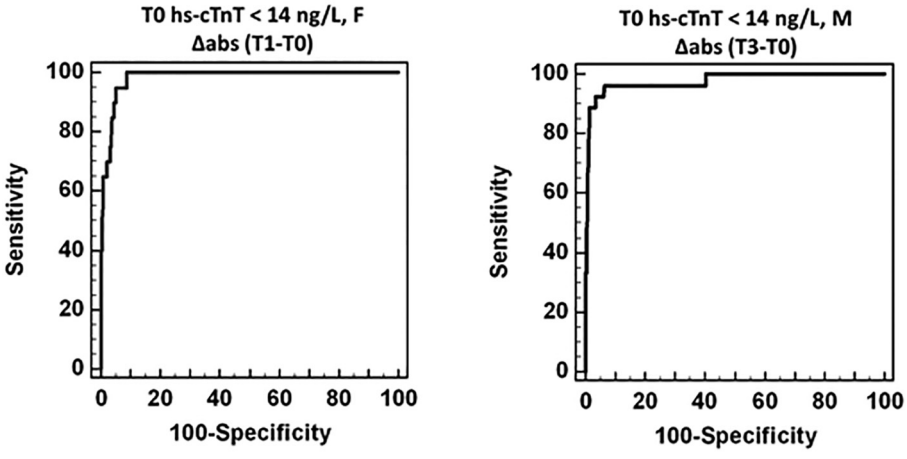

Fig. 2. Optimal changes for NSTEMI rule-out.
Optimal absolute Δ changes considered for ruling out a suspected NSTEMI are different for females (between T1 and T0) and males (between T3 and T0). SENS, SPEC, PPV, NPV are shown. 95% CI are shown in parenthesis when available. Abbreviations: hs-cTnT, high sensitivity troponin T; F, females; M, males; hs-cTnT, high sensitivity cardiac troponin T; T0, T1, T3 mean hs-cTnT measured at baseline, after 1 and 3 h respectively; Δabs, absolute delta change between consecutive hs-cTnT measurements; SENS, diagnostic sensitivity; SPEC, diagnostic specificity; NPV, negative predictive values; PPV, positive predictive values. CI, confidence interval.

algorithm as non-NSTEMI and medical records were analyzed. All of them were characterized by multiple cardiovascular risk factors (familiarity for premature coronary artery disease or sudden cardiac death, smoking, overweight, dyslipidemia, hypertension) in association or not with previous AMI, suggesting that a complete anamnestic evaluation should be considered mandatory for a prompt and accurate medical evaluation beyond the sole troponin measurements.

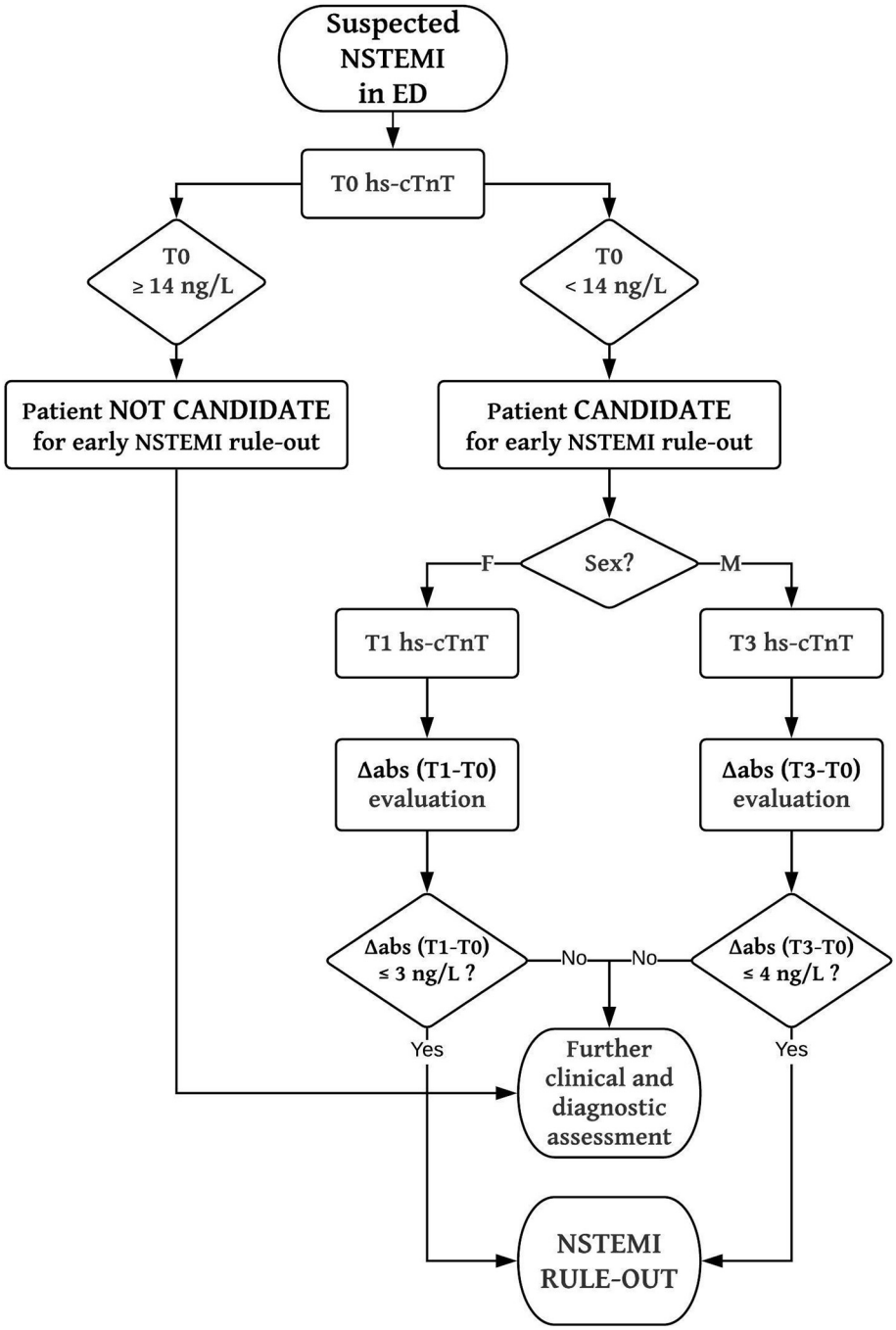

Fig. 3. Algorithm created using data belonging to the derivation cohort.
Abbreviations: NSTEMI, non-ST elevation myocardial infarction; ED, emergency department; F, females; M, males; hs-cTnT, high sensitivity cardiac troponin T; Δabs, absolute delta change between consecutive hs-cTnT measurements; T0, T1, T3 mean hs-cTnT measured at baseline, after 1 and 3 h respectively.

4. Discussion

Given the low prevalence of AMI in the ED, the need to adopt a criterion to rapidly and safely exclude a NSTEMI is clearly desirable by clinicians, so they may address their efforts on really deserving cases [19,20]. Unfortunately, although highly sensitive tests for troponin measurements have been available for some years, it is still not clear if and how the use of close serial measurements can be feasible to safely rule-out a NSTEMI [21,22]. The first formal suggestion about the use of specific changes for an AMI rule-out strategy with T1 measurement was proposed by ESC in the 2015 guideline for the management of ACS in patients without persistent ST-segment elevation at presentation. Briefly, after the guideline publication many doubts were raised on the actual chance to adopt as a very early rule-out strategy. Although the guideline clearly recommended a local clinical validation of any T1-T0 algorithm prior to usage, the debate grew and the criteria suggested appeared to be too challenging and not cautious enough.

The hs-cTnT assay was introduced at Desio Hospital in 2011 and, in the meantime, when no guidelines were still available, a serial hs-cTnT testing protocol with T0, T1, T3 evaluations for any probable AMI in the ED was already adopted at that time. The aim was to collect data from a number of NSTEMI cases in order to define changes potentially associated with 100% SENS and NPV. The derivation cohort included 4 years of suspected cases of NSTEMI and, except for younger and a

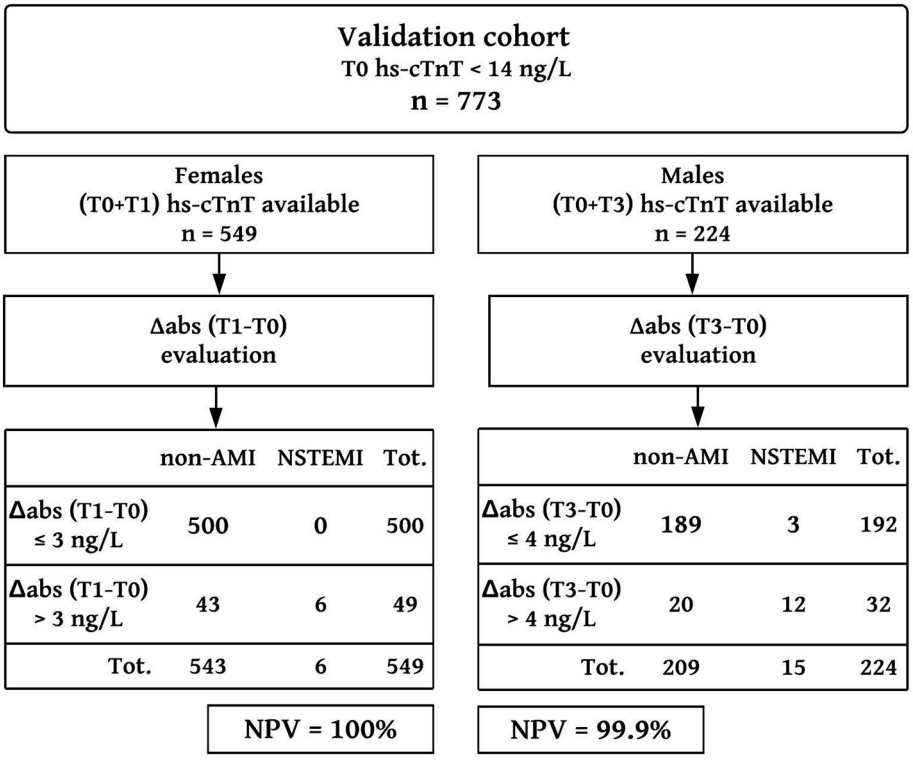

Fig. 4. Algorithm validation.
Abbreviations: F, females; M, males; hs-cTnT, high sensitivity cardiac troponin T; Δabs, absolute delta change between consecutive hs-cTnT measurements; T0, T1, T3 mean hs-cTnT measured at baseline, after 1 and 3 h respectively; NSTEMI, non-ST elevation myocardial infarction; AMI, acute myocardial infarction; NPV, negative predictive values.

final diagnosis of STEMI, no other exclusion criteria were applied. Our will was to conduct an "evidence based study" representing the real ED context, in which the observed troponin variations corresponded to real troponin changes. For this reason also patients with any kind of final diagnosis were taken into consideration, including those with "non-specific chest pain" or any other diagnosis requiring hospitalization.

It was observed that a universal Δ cut-off for NSTEMI exclusion cannot reliably be applied indiscriminately to all suspected infarctions. In other words, only patients with hs-cTnT at presentation lower than 14 ng/L are eligible for a rapid and safe rule-out strategy. Furthermore, the evaluation of males and females requires to be conducted separately, and by adopting different Δ changes for cardiac troponin serial measurements. These findings lead to some considerations concerning sample size, sensitivity of the analytical method, and suitability of the algorithm in clinical practice.

First, given that SENS should be close to 100% to allow the exclusion of a condition, and given that it is calculated on positive cases, is the number of NSTEMI among patients with T0 hs-cTnT lower than 14 ng/L (n = 47) high enough for a robust rule-out criterion? The 47 NSTEMI of the derivation cohort seem to be significant in number and should reassure physicians about the feasibility of the algorithm, especially considering the following observations. Threshold for infarction rule-out reported in the 2015 ESC guideline derived from the study conducted by Reichlin et al. in 2012 [23] which involved 872 subjects. The algorithm showed 100% SENS and NPV, in the case of patients with T0 lower than 12 ng/L and Δabs (T1-T0) lower than 3 ng/L. However they did not specify how many subjects with AMI resulted in the category with T0 lower than 12 ng/L. The population evaluated in the study corresponded to the same cohort used by the same research group for another cornerstone study published in 2011 [24] in which the number of NSTEMI presenting T0 lower than 14 ng/L were only 7. This observation should allow one to think that also the population on which ESC guidelines are based on, could present a similar sample size. Likely for this reason the 100% SENS and the 100% NPV reported, although extremely interesting, probably did not appear sufficient to make end-user clinicians confident for safe utilization of the algorithm.

Another issue arising from the present study concerns the ability of the analytical method to distinguish very small variations of troponin T. As indicated by the manufacturer, within-run precision evaluation around LOD (CV = 7.8% at 6.5 ng/L) should allow to distinguish small troponin changes such as those considered in the study, also taking into account as a source of variability the biological variability within 1 or 3 h [25,26] since our analysis involve serial measurements. Moreover, the evidence based disposition of the study, its duration (5 years), and the obtained SENS should confirm that analytical method performances are suitable for the purpose.

Finally, is it useful to adopt an algorithm which is able to rule-out an AMI only in patients with hs-cTnT T0 < 14 ng/L? How many NSTEMI could be ruled out using the algorithm? Since the hs-cTnT test implementation, periodic meetings with cardiologists were organized in order to discuss the work in progress. The need for an algorithm to exclude AMI in low-risk patients corresponding to those with basal troponin T values below 14 ng/L was immediately evident. Moreover, according to data obtained in the validation cohort, for patients with T0 hs-cTnT lower than 14 ng/L, the algorithm could have allowed to exclude NSTEMI in 91.1% (500/549) of females and in 85.7% (192/224) of males, within 1 h and within 3 h, respectively. Given that, according to the derivation cohort, among females and males, those with T0 < 14 ng/L are 43.3% and 47.6% respectively, the algorithm should be able to exclude a NSTEMI in a total of 39.4% (91.1% of 43.3%) and 40.8% (85.7% of 47.6%) of females and males presenting to the ED with symptoms suggestive of infarction. With this in mind, the algorithm developed, although limited to patients with baseline troponin levels < 14 ng/L, is surely a suitable measure to safely avoid useless serial blood sampling and prolonged monitoring of patients. Moreover the economic impact of applying a fast AMI rule-out algorithm cannot be neglected, given the evidence in cost savings reported in literature [27,28].

The study presents some limitations: 1) "patient's age" and "evaluation of renal function" were variables not considered in the study because accounting for all of them would have required additional subclassification of NSTEMI subjects, and consequently, a higher number of patients for each sub-category in order to obtain robust estimation of NPVs. 2) The consensus 99% percentile threshold (14 ng/L)

was adopted to subdivide patients in candidates and non-candidates for NSTEMI rule-out in spite of the limitations of using a single cut-off [29], not differentiated for age and sex. 3) Final diagnosis was not adjudicated by an independent cardiologist but corresponded to the outcome of the clinical work-up for each patient. A confirmation of the initial diagnosis of AMI made in the ED by the consulting cardiologist was derived from patient records concerning management performed by the cardiology equipe during the subsequent hospitalization in the Cardiac Care Unit. It can not be excluded that some patients, identified by the algorithm as candidates for NSTEMI rule-out and with non-AMI diagnosis, were later admitted to other hospitals, reducing the sensitivity of the algorithm in this case. Future studies with the aim to increase the number of NSTEMI occurrences, and to obtain patient follow-up information could improve the performances of the algorithm in terms of safety and efficacy, and overcome the limits afore mentioned.

5. Conclusions

Although some points remain to be improved, the results obtained may be considered a strong proof that algorithms for fast NSTEMI rule-out are feasible and safe. A robust algorithm for the safe exclusion of NSTEMI in patients with chest pain and/or symptoms suggestive of an acute myocardial infarction has been developed. The NSTEMI rule-out strategy could successfully be applied to all subjects with baseline hs-cTnT measurements below the cut-off of 14 ng/L, in the case that absolute changes in troponin between T1 and T0 for females do not exceed 3 ng/L, and between T3 and T0 for males do not exceed 4 ng/L. The implementation of such an algorithm could lead to exclude NSTEMI within 3 h in about 40% of the total number of subjects with probable AMI with a NPV of 99.9%.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.03.1625.

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