Guidelines and Recommendations from Scientific Societies

Aldo Clerico*, Martina Zaninotto, Alberto Aimo, Ruggero Dittadi, Domenico Cosseddu, Marco Perrone, Andrea Padoan, Silvia Masotti, Lucia Belloni, Marco Migliardi, Antonio Fortunato, Tommaso Trenti, Lucia Malloggi, Piero Cappelletti, Gianni Antonio Galli, Sergio Bernardini, Laura Sciacovelli and Mario Plebani

Use of high-sensitivity cardiac troponins in the emergency department for the early rule-in and rule-out of acute myocardial infarction without persistent ST-segment elevation (NSTEMI) in Italy

Expert opinion document from the Study Group of Cardiac Biomarkers associated to the Italian Societies ELAS (European Ligand Assay Society, Italy Section), SIBIoC (Società Italiana di Biochimica Clinica), and SIPMeL (Società Italiana di Patologia Clinica e Medicina di Laboratorio)

https://doi.org/10.1515/cclm-2021-1085
Received October 7, 2021; accepted November 26, 2021; published online December 20, 2021

Abstract: Serial measurements of cardiac troponin are recommended by international guidelines to diagnose myocardial infarction (MI) since 2000. However, some relevant differences exist between the three different international guidelines published between 2020 and 2021 for the management of patients with chest pain and no ST-segment elevation. In particular, there is no agreement on the cut-offs or absolute change values to diagnose non-ST-segment elevation MI (NSTEMI). Other controversial issues concern the diagnostic accuracy and cost-effectiveness of cut-off values for the most rapid algorithms (0 h/1 h or 0 h/2 h) to rule-in and rule-out NSTEMI. Finally, another important point is the possible differences between demographic and clinical characteristics of patients enrolled in multicenter trials compared to those routinely admitted to the Emergency Department in Italy. The Study Group of Cardiac Biomarkers, supported by the Italian Scientific Societies Società Italiana di Biochimica Clinica, Italian Society of the European Ligand Assay Society, and Società Italiana di Patolgia Clinica e Medicina di Laboratorio decided to revise the document previously published in 2013 about the management of patients with suspected NSTEMI, and to provide some suggestions for the use of these biomarkers in clinical practice, with a particular focus on the Italian setting.

***Corresponding author: Prof. Aldo Clerico**, MD, Laboratory of Cardiovascular Endocrinology and Cell Biology, Department of Laboratory Medicine, Fondazione CNR Toscana G. Monasterio, Scuola Superiore Sant'Anna, Via Trieste 41 56126, Pisa, Italy, E-mail: clerico@ftgm.it
Martina Zaninotto, Department of Laboratory Medicine, University Hospital of Padova, Padova, Italy; and Department of Medicine-DIMED, University of Padova, Padova, Italy
Alberto Aimo and Silvia Masotti, Fondazione CNR Regione Toscana G. Monasterio e Scuola Superiore Sant'Anna, Pisa, Italy
Ruggero Dittadi, Ospedale dell'Angelo ULSS 3 Serenissima, Laboratorio di Analisi Cliniche, Mestre, Italy
Domenico Cosseddu and Marco Migliardi, S.C. Laboratorio Analisi, A.O. Ordine Mauriziano di Torino, Torino, Italy
Marco Perrone and Sergio Bernardini, Division of Cardiology and Clinical Biochemistry, University of Rome Tor Vergata, Rome, Italy
Andrea Padoan, Laura Sciacovelli and Mario Plebani, Department of Laboratory Medicine, University Hospital of Padova, Padova, Italy. https://orcid.org/0000-0003-1284-7885 (A. Padoan). https://orcid.org/0000-0003-3156-1399 (L. Sciacovelli). https://orcid.org/0000-0002-0270-1711 (M. Plebani)
Lucia Belloni, Dipartimento di Medicina di laboratorio, Arcispedale Santa Maria Nuova - IRCCS, Reggio Emilia, Italy
Antonio Fortunato, U.O.C. Patologia Clinica, Ascoli Piceno, Italy
Tommaso Trenti, Azienda Ospedaliero - Universitaria Policlinico di Modena c/o Ospedale Civile di Baggiovara, Modena, Italy
Lucia Malloggi, Laboratorio Analisi, Azienda Ospedaliera-Universitaria di Pisa, Pisa, Italy
Piero Cappelletti, SIPMeL, Castelfranco Veneto, Italy
Gianni Antonio Galli, Estote Misericordes, Borgo San Lorenzo, Firenze, Italy

Keywords: acute coronary syndrome; cardiac troponins; cardiovascular risk; high-sensitivity methods; myocardial infarction; myocardial injury.

Aim of the new document and methodology

In 2013, an intersociety group including experts of Emergency/Urgency Medicine, Cardiology and Clinical Biochemistry published some recommendations on the use of high-sensitivity cardiac troponins (hs-cTn) in the Emergency Department (ED) to diagnose non-ST segment elevation myocardial infarction (NSTEMI) [1]. These recommendations were meant to be tailored on the specific needs of the Italian healthcare system [1], had a wide diffusion and were applied by many institutions across Italy.

The last five years have witnessed a rapid development of high-sensitivity assay methods for cardiac troponin I and T (hs-cTnI and hs-cTnT) [2]. The results obtained with these new assays have allowed a better understanding of the pathophysiology of myocardial injury as well as a more accurate interpretation of circulating levels of cTn in healthy adults, both at rest and during physical exercise [2, 3]. This has warranted an in-depth revision of guidelines published in the previous years by the most important international scientific societies in Cardiology and Laboratory Medicine. These recommendations have revolutioned both the definition of myocardial damage and the diagnostic algorithms for myocardial infarction (MI) [4–9]. In particular, we must remember some recent contributions of Italian researchers, such as the documents published in 2020 by the Study Group on Myocardial Biomarkers of the *Società Italiana di Patologia Clinica e Medicina di Laboratorio* (SIPMeL) on "Myocardial Biomarkers for the Diagnosis and Risk Prediction of NSTEMI", which considered both organizational and clinical issues about the use of hs-cTn assays, particularly in the ED [10–12].

Based on these premises, the Inter-Society Study Group on Cardiac Biomarkers deemed it essential to update 2013 recommendations considering the new evidence. The general scheme of the 2013 document was retained:

(1) Appropriate and correct definitions
(2) Proper indications
(3) Use to rule in MI
(4) Use to rule out MI.

The document was written considering international guidelines published from 2018 to 2021. The focus on the Italian setting is justified by the consideration that international documents are mostly based on results from multicenter clinical studies carried out in highly specialized centers. These results may be not readily applied to the everyday clinical practice in Italy, where elderly patients with multiple comorbidities often represent the majority of patients admitted to ED. Furthermore, as emphasized even recently by guidelines and documents by international scientific societies [5, 8, 10, 13, 14], a close collaboration between clinicians and clinical biochemists is needed to validate the decisional levels and identify the best diagnostic algorithms for each institution. In particular, the results reported in this document refer to studies carried out by the Inter-Society Study Group on Cardiac Biomarkers, which evaluated both the analytical characteristics of hs-cTnI and hs-cTnT assays most used in Italy and the distribution of values of these biomarkers in an Italian population with the calculation of the 99th percentile upper reference level (URL). A draft of the document was revised by a group of external experts belonging to different disciplines: Emergency/Urgency Medicine, Cardiology and Laboratory Medicine. Finally, the approval of the Boards of the Italian scientific societies involved was asked.

Definition, analytical characteristics and clinical results of hs-cTnI and hs-cTnT methods in healthy subjects and in patients with cardiovascular disorders

hs-cTnI and hs-cTnT measurement is regarded by all guidelines as the optimal strategy to detect myocardial injury and to diagnose MI [4–9]. The last 10 years have witnessed a significant and progressive improvement of the analytical performance of immunometric methods for cTnI and cTnT [3, 15]. These hs methods can measure circulating biomarker levels in the majority of healthy subjects of both sexes [2, 3, 15]. The use of hs-cTnI and hs-cTnT methods has changed not only our understanding of MI pathophysiology, but also our approach to the diagnosis and monitoring of MI [1–11]. In particular, the 2018 document by the American Association for Clinical Chemistry (AACC) and International Federation of Clinical Chemistry (IFCC) established two criteria that are crucial to define hs-cTn methods [5]. The first criterion is that the method must measure the threshold value to diagnose MI, defined as the 99th percentile URL, with an imprecision (expressed as coefficient of variation, CV) ≤10% [5]. The second criterion is that a hs-cTn method must measure

biomarker levels above a limit of detection (LoD) in a reference population including apparently healthy subjects of both sexes with at least 300 women and 300 men [3, 5, 15, 16]. The requirement for at least 600 individuals is critical to calculate the 99th percentile URL with an acceptable confidence interval [3, 5, 15, 16]. As women have on average significantly lower hs-cTn than men of the same age, this second criterion basically requires that hs-cTn can be measured in a subgroup of at least 300 apparently healthy women [3, 5, 15, 16].

hs-TnI and hs-TnT methods have LoD values usually ranging from 1 to 3 ng/L (Table 1), corresponding to the troponin content of around 5–8 mg of myocardium, while the 99th percentile URL (i.e., the threshold to diagnose myocardial injury) corresponds to the content of 30–40 mg of myocardium [2, 3, 15–18]. A myocardial damage involving a few mg of tissue is well below the detection limit of echocardiography or other cardiac imaging techniques [2, 3, 15–18].

In 2018, the Fourth Universal Definition of MI has established that a myocardial injury is present when there is at least one hs-cTnI or hs-cTnT value above the 99th percentile URL [6]. This document recommends that hs-cTn methods are used in all subjects with suspected cardiac disease, and not only of acute coronary syndrome (ACS) [6, 11, 18, 19].

Many authors have suggested that hs-cTnI and hs-cTnT levels in a healthy adult subject must be considered a reliable indicator of the physiological daily renewal of myocardial tissue [2, 3, 15–18]. The 99th percentile URL values in healthy adults (17–35 ng/L) (Table 2) are compatible with a daily turnover of about 30–40 mg of myocardial tissue, in agreement with experiments on humans and other mammals [2, 3, 15–18].

Recently, a large number of studies and three meta-analyses have demonstrated that hs-cTnI or hs-cTnT below the 99th percentile URL, but within the third tertile of distribution in the reference population (and then within normal levels), are associated with a significantly higher cardiovascular risk than biomarker levels in the first tertile [20, 21]. The very good performance of hs-cTn for risk stratification is due to their high analytical sensitivity (Table 1) and their small intra-individual variability, which is on average only 9% [22].

Some recent studies reported that within the range from LoD to the 99th percentile URL, a >30% variation between two measurements of hs-cTnI or hs-cTnT in different times in a same individual can be deemed significant [3, 21–25]. Accordingly, the >30% difference can be used to assess changes between two hs-cTn values also in patients with suspected ACS in the ED [3, 22]. Given the widely skewed distribution of hs-cTnI and hs-cTnT in healthy populations, the daily renewal of cardiomyocytes must increase on average of 10–15 times compared to the median of distribution (about 1–4 ng/L) before trespassing the 99th percentile URL (Table 2) [3, 12, 22].

Table 1: Analytical sensitivity parameters of some hs-cTnI and hs-cTnT methods.

Methods	LoB, ng/L	LoD, ng/L	LoQ 20% CV, ng/L	LoQ 10% CV, ng/L	References
hs-cTnI					
ARCHITECT	0.7	1.3	1.8	4.7	[15, 27]
ACCESS DxI	0.6	1.3	2.1	5.3	[15, 24]
ADVIA XPT	1.0	2.2	3.5	8.4	[15, 25]
VITROS	0.3	0.9	1.8	4.7	[29]
hs-cTnT					
ECLIA	3	3–5	6	13	[23, 26]

The analytical sensitivity parameters reported in the Table have been evaluated in the same reference laboratory using standardized protocols, as previously reported in detail refs. [3, 15, 23–27]. LoB, limit of blank; LoD, limit of detection; LoQ 10% CV, limit of quantification at 10% CV; ARCHITECT, Architect Highly Sensitive TnI method for the Architect i1000SR platform (Abbott Diagnostics, Roma, Italy) [15, 27]; CCESS DxI, Access hsTnI method for the DxI platform (Beckman Coulter, Inc. Brea, CA 92821 USA) [15, 24]; ADVIA, ADVIA Centaur High-Sensitivity Troponin I (TNIH) method for the Centaur XPT platform (Siemens Healthineers, Milano, Italy) [15, 25]. VITROS, VITROS High-Sensitivity Troponin I Assay method (Ortho Clinical Diagnostics, Illkirch CEDEX, France) for the platform VITORS 3600 [29]; ECLIA, Elecsys Troponin T-hs method for the Cobas platform (Roche Diagnostics Italia, Monza) [23, 26].

Automated platforms and point-of-care testing (POCT)

Until 2020, the POCT methods for cTn could not be used to diagnose NSTEMI because they did not met the quality requirements specified in international guidelines [7–9]. The introduction of POCT methods to measure hs-cTn represents an important progress given that these tests allow an earlier diagnosis by reducing the turnaround time (TT) [7, 30–34]. Furthermore, POCT methods for hs-cTn could enable an effective screening of patients with suspected ACS at home, in the ambulatory setting or in the ambulance, with a great reduction of the times to diagnosis, treatment and admission to specialized structures [7, 30–34]. A reduction in the ischemic time translates in a smaller area of necrosis, leading to smaller infarct size, less arrhythmias, and a lower likelihood of adverse ventricular remodeling [7–9].

In 2016, the Study Group of Myocardial Biomarkers of the SIPMeL proposed diagnostic and therapeutic

Table 2: Distributions of plasma cardiac troponins I concentrations measured with some commercially available high-sensitivity methods in an Italian reference population (age ranging from 18 to 85 years, mean age 51.5 years).

Population (number of subjects)	25th perc., ng/L	Median, ng/L	75th perc., ng/L	97.5th perc., ng/L	99th perc., ng/L
ARCHITECT					
Women (n=700)	0.9	1.4	2.3	6.5	9.7[a] (6.8–12.4)
Men (n=765)	1.5	2.1	3.3	11.8	17.2[a] (14.2–20.6)
ACCESS DxI					
Women (n=703)	1.6	2.3	3.2	6.4	9.2[a] (7.2–14.2)
Men (n=757)	2.3	3.2	4.6	11.8	14.0[a] (12.4–17.0)
ADVIA XPT					
Women (n=680)	1.1	2.7	3.9	14.7	24.7[a] (16.5–37.8)
Men (n=731)	2.6	3.9	3.9	25.3	41.8[a] (28.7–48.8)

More information on the demographic characteristics of the reference population were previously reported [15, 24, 25, 28]. [a]99th percentile (95% confidence interval) calculated by bootstrap method, as previously described [15, 24, 25, 28].

pathways for patients admitted to the ED with suspected NSTEMI [34]. Clinical studies evaluating POCT methods with analytical performance meeting the requisites for hs-cTn methods have been published from 2019 onwards (Table 3). Sorensen et al. [35] compared the diagnostic accuracy of this method with the hs-cTnI Architect method using both the rapid 0 h/1 h algorithm and the standard 0 h/3 h algorithm in a first group of 669 patients, with a validation cohort of 610 patients. In 2020, Boeddinghaus et al. [36] compared the diagnostic efficiency of a POCT hs-cTnI-TriageTrue method compared to several hs-cTnI and hs-cTnT methods used in different centers, assessing 1,261 patients (178 of whom with MI, 14%). The POCT hs-cTnI-TriageTrue method displayed similar analytical performance (Table 3) and diagnostic accuracy to hs-cTnI or hs-cTnT methods used in each center to diagnose MI [36]. In 2021, Apple et al. [37] evaluated the analytical performance and calculated the 99th percentile URL of the POCT Atellica VTLi hs-cTnI method. This study [37] showed that this method meets the requirements for a hs-cTnI method [5].

The methods above for cTnI meet the analytical requirements for a hs assay (Table 3). They allow a rapid time to response and have excellent sensitivity and reproducibility, without a reduction in specificity. Nonetheless, the validation of POCT methods for hs-cTnI has not been completed. As recently remarked by the National Institute for Health and Care Excellence (NICE) guidelines [9], the most important limitation of these studies is that POCT methods were employed on plasma samples stored at very low temperatures for months to years [35–37]. Therefore, values measured could not correspond to those in fresh plasma samples. More recently, Gopi et al. [38] analyzed the analytical performance and results of the POCT PATHFAST hs-cTnI method in 224 samples of plasma from 191 patients admitted to the ED with suspected NSTEMI. This study demonstrated that whole-blood samples can be used interchangeably with plasma [38].

In agreement with NICE guidelines [9], we can conclude that, at the present time, some of the most recent POCT methods for hs-cTnI measurement have a similar analytical performance than hs-cTn methods using completely automated platforms. Nevertheless, further studies are needed to

Table 3: Parameters of analytical sensitivity and 99th percentile values of some POCT methods for cTnI assays.

Author (refs.)	Method	LoD, ng/L	LoQ 10% CV, ng/L	99th percentile URL, ng/L
Sorensen et al. [35]	PATHFAST cTnI-II assay (LSI Medience Corporation; Mitsubishi Chemical Europe, Düsseldorf, Germany)	2.9	11.0	W 21.1 (13.4–25.3)[a] M 27.0 (18.5–27.7)[a]
Boeddinghaus et al. [36]	TriageTrue high sensitivity troponin I test, Quidel Corporation, San Diego, California)	0.7–1.6	4.4–8.4	W 14.4 (13.1–28.7)[b] M 25.7 (18.3–37.6)[b]
Apple et al. [37]	POC Atellica VTLi immunoassay (Siemens Healthineers, Eindhoven,The Netherlands)	1.2		W 18.0 (9.0–78.0)[b] M 27.0 (21.0–37.0)[b]

LoD, limit of detection; LoQ 10% CV, limit of quantification at 10% CV; W, women; M, men. [a]95% confidence interval, [b]90% confidence interval.

establish the accuracy of the cut-offs used and particularly if the cost/benefit ratio of these new POCT methods can be compared to hs-cTnI and hs-cTnT assays currently used in clinical laboratories.

Take-home messages

- International guidelines consider mostly hs methods.
- Clinical studies are generally performed in centers with a high degree of specialization, and patient selection often differs across studies.
- Results from these studies cannot be readily translated to the real situation of Italian institutions, therefore a close collaboration between clinicians and Laboratory Medicine specialists is needed to identify decisional levels and the most effective diagnostic pathways for each institutions.
- Increasing circulating levels and above the critical difference (>30%) of hs-cTnI or hs-cTnT over hours, days or months, even within the normal range, are associated with a worse outcome, not only in patients with heart failure or ACS, but also in apparently healthy, asymptomatic subjects.
- Although some recent POCT methods for the measurement of hs-cTnI have an analytical performance comparable to hs-cTnI methods currently used in clinical laboratories and extensively validated, there is currently no multicenter study assessing the cost/benefit ratio of these new methods in patients with suspected NSTEMI.

Recommendations

- hs assays for the measurement of cTnI and cTnT are immunometric methods able to measure the 99th percentile URL of a reference population with a CV≤10.
- hs methods can also detect circulating cTn in at least one half of a reference population including at least 300 men and 300 women.
- Methods that measure the 99th percentile URL in the reference population between 10 and 20% can still be used in the clinical practice. They must be defined as contemporary sensitivity methods, and cannot be defined as hs. Guidelines strongly recommend that clinical laboratories adopt as soon as possible hs-cTn methods.
- Methods measuring the 99th percentile URL with a CV≥20% should not be used anymore to diagnose MI, but only for a first screening when hs-cTn methods are not available.

Algorithms for the diagnosis of NSTEMI: what the most recent international guidelines say

The 2018 Fourth Universal Definition of MI [6] recommends the use of sex-specific cut-offs to diagnose MI and stresses that significant differences exist between 99th percentile URL values of different hs-cTnI methods. The diagnosis of MI relies on the kinetics of hs-cTnI and hs-cTnT circulating levels, evaluated through standardized diagnostic algorithms, and should be made when there are increasing or decreasing biomarker levels, together with evidence of myocardial ischemia based on signs, symptoms, imaging data or autopsy [6].

The NICE guidelines [9] published in August 2020 specified the analytical requirements (including the sex-specific 99th percentile URL) and results of clinical studies using 9 hs-cTnI and 2 hs-cTnT methods that can be used to diagnose MI [9]. These guidelines also identified two challenging scenarios. The first one is the finding of a hs-cTnI or hs-cTnT value lower than or equal to the LoD on admission to the ED to exclude MI (rule-out). The second one is a strategy with repeated measurements to confirm or exclude the diagnosis of MI. NICE guidelines recommend mostly the 0/3 h algorithm for both rule-in and rule-out [9]. Recommended threshold values for rule-in and rule-out and 99th percentile URL values should be differentiated based on the specific method and patient sex. Strategies based on multiple measurements have usually a better diagnostic accuracy than those based on a single measurement [9]. Furthermore, these guidelines recommend that biomarker values be interpreted according to the clinical presentation. Most notably, a patient with negative hs-cTn measurement should not be hastily discharged without further clinical exams, particularly when symptoms last from less than 2 h, which could account for the lack of a significant increase of the biomarker [9]. NICE guidelines also emphasize that the diagnostic accuracy of the ECLIA Elecsys hs-cTnT and the Architect hs-cTnI methods have been evaluated by multiple studies (30 and 9, respectively), while all other hs-cTnI methods have been validated in few or no studies [9]. The same guidelines notice that limited evidence is available on the comparison between the diagnostic accuracy of different methods and particularly the cost/benefit ratios of the

different diagnostic algorithms employing different hs-cTnI and hs-cTnT methods [9]. The differences between different methods in risk stratification of patients with possible ACS have been specifically evaluated [39].

The 2020 European Society of Cardiology (ESC) guidelines [7] recommend the most rapid algorithms for the diagnosis of MI in patients admitted to the ED with suspected ACS. The first option is the 0 h/1 h algorithm, which involves blood sampling on admission and after 1 h. As a second option, the 0 h/2 h algorithm is proposed, with blood sampling on admission and after 2 h [7]. ESC guidelines also propose algorithms with different cut-offs for each method. The assessment of biomarker kinetics in these guidelines is based on cut-offs that express the absolute difference between concentrations at baseline and after 1 or 2 h, defined as " delta (Δ) change" and expressed as ng/L.

The preference for more rapid algorithms is justified by the consideration that reducing the time to diagnosis promotes an earlier anti-ischemic treatment and then the salvage of larger areas of myocardium still reversibly damaged [7]. Furthermore, ESC guidelines emphasize that cut-off values to rule out or rule in MI were validated in many multicenter studies [40–50]. hs-cTn concentrations recommended as critical thresholds to exclude MI generally correspond to a minimal sensitivity value associated with a ≥99% negative predictive value. These guidelines [7] also state that rapid algorithms have demonstrated in many clinical studies a balance between efficacy and safety that is not significantly different from the standard 0/3 h algorithm recommended by 2015 ESC guidelines [4].

In February 2021, the document on the diagnosis of NSTEMI by the International Federation of Clinical Chemistry (IFCC) Committee on Clinical Application of Cardiac Biomarkers was published [8]. This document critically reappraised some recommendations by 2020 ESC guidelines [7]. The first controversial point was that 2020 ESC guidelines do not recommend the use of sex-specific cut-offs, particularly regarding the 99th percentile URL [7], as a threshold for myocardial damage and to diagnose MI, as explicitly requested by the Fourth Universal Definition of MI [6]. Both the IFCC document [8] and NICE guidelines [9] recommend sex- and method-specific cut-offs because many studies have demonstrated that differentiation based on sex allows a more accurate diagnosis, particularly for hs-cTnI methods and in women [51–56]. On the contrary, the utility of sex-specific cut-offs has not been definitely demonstrated for hs-cTnT methods, given that the mean difference between values in men and women in the different reference populations is around 5 ng/L, which approaches the LoD of the method (Table 1), while for example this difference is 11 ng/L for the hs-cTnI Architect method [16].

Another relevant aspect is the time from onset of ischemic symptoms. The Fourth Universal Definition of MI [6] identifies a special group of patients presenting late to the ED ("late presenters"). These patients could be evaluated when the peak concentration has already been reached and so the circulating levels of biomarker are decreasing [6, 8, 57]. cTn decrease is much lower than the rapid increase during the first hours of ischemia. Therefore, variations in biomarker values can be difficult to detect across a few hours (as in the 0 h/1 and 0 h/2 h algorithms), especially when infarcts areas are small [6, 8, 57]. Therefore, clinicians must be careful of these "late presenters", who may also present elevated hs-cTnI or hs-cTnT (and then a confirmed myocardial damage), but small variations in biomarker levels during the observation period (from 1 to 3 h) after admission to the ED, possibly leading to an underdiagnosis of MI [8, 10, 57]. According to some studies, this group of patients could account for 26% of cases of MI [8].

Importantly, the cut-off values based on absolute differences (Δ change) recommended by 2020 ESC guidelines [7] for use in the rapid algorithms (0 h/1 h or 0 h/2 h) for ECLIA hs-cTnT method were validated in multicenter clinical trials. However, both NICE guidelines [9] and the IFCC document [8] report that there are still insufficient data on the cut-off values based on absolute differences (Δ change) for some hs-cTnI methods.

Considerations on guideline recommendations

Application of rapid diagnostic algorithms in the clinical practice in Italy

Only about 30% of patients admitted to the ED with chest pain receives a final diagnosis of MI [6, 7]. A rapid evaluation of patients with a low probability of MI allows a more efficient and rapid management of patients admitted to the ED, also reducing costs, and decreases the time to diagnosis, enabling an early and specific treatment that improves outcomes [7, 58, 59]. Accordingly, 2020 ESC guidelines strongly recommend rapid diagnostic algorithms to promptly identify patients at lowest risk, who can be monitored on an outpatient basis or outside of cardiac intensive care units [7]. Nonetheless, some considerations about the organization of clinical activity are warranted to better understand some obstacles to the introduction of rapid diagnostic algorithms in clinical practice. While the diagnostic and prognostic utility of the 0/3 h algorithm has been extensively validated, and this algorithm has also a favorable cost/benefit ratio

compared to previous longer algorithms (6–12 h) [1, 4, 6, 9], the rapid algorithms have not been accurately validated using all hs-cTnI methods [8, 9]. Additionally, as also observed in the document by the IFCC [8], the rapid algorithms are difficult to implement in the majority of hospitals around the world. In particular, a 2019 study including 1,902 centers in 23 countries across five continents reported that in Europe only 60% of hospitals had adopted hs-cTn methods, and could then potentially employ rapid algorithms [60]. While specific data for Italy are not available, even the implementation of the 0/3 h algorithm rather than longer algorithms has required a profound reorganization of ED activity in many centers [1, 61–63]. Considering that even the most recent hs-cTnI and hs-cTnT methods using automated platforms have a mean time to analysis of 15–25 min, obtaining the result and sending it to the ED within 60 min seems almost impossible in almost all clinical laboratories in Italy, including those located in close proximity of the ED and that can devote a specific section to biomarker measurement to this analysis.

2020 ESC guidelines suggest that blood sampling for hs-cTn measurement must always be performed at baseline and after 1 h, whether the result of baseline measurement has been reached or not. Furthermore, these guidelines recommend that patients not ruled out nor ruled in after 1 h undergo a third blood sampling 3 h after admission [7]. The 0 h/2 h algorithm seems more reasonable for Italian institutions compared to the 0 h/1 h algorithm. Indeed, in most cases the 1-h sampling would be performed without knowing the result of the baseline measurement, with the possibility of an useless blood sampling (when baseline value already allowed to exclude MI) or alternatively because the 1-h measurement would still not be sufficient to rule out or rule in MI, thus requiring a further sampling at 3 h or later. The 0 h/2 h algorithm should be more accurate than the 0 h/1 h algorithm, particularly to rule in MI. If the 2-h measurement does not show a significant variation of the biomarker, a further measurement at 3 h or later would be required, but the same would have occurred if the 0 h/1 h algorithm were pursued. This possibility could not be infrequent in late presenters, as discussed above.

Pathophysiological and clinical considerations on diagnostic algorithms for NSTEMI

Patients with NSTEMI show different biomarker concentrations and kinetics, even when the same hs-cTn method is used, because circulating levels of hs-cTnI and hs-cTnT depend on sex, age, time from ischemia onset, extent of the ischemic and necrotic area and comorbidities [9, 11, 17, 18, 64–70]. In particular, severe chronic kidney disease can significantly alter biomarker kinetics [9, 17, 18, 67, 70].

The protein chain of cardiac troponins (molecular weight of about 30–40 kDa) is rapidly degraded in the circulation, particularly in their terminal portions, therefore fragments with a lower molecular weight (around 14–20 kDa) can already be detected during the first hours after an MI and can persist in the bloodstream for several days, becoming the prevalent circulating forms of the biomarker at the end of the kinetic curve [3, 11, 17, 18, 64–70]. Furthermore, the three cardiac troponins of the sarcomere complex (TnI, TnC and TnT) can bond with each other and with other plasma proteins [71]. Some patients can also develop autoantibodies against cTn molecules, forming circulating complexes that affect the measurement of cTn with immunometric methods [3, 72–75]. Therefore, the presence of different circulating forms and the formation of complexes with other circulating proteins not only alter cTn kinetics, but also reduce the reliability of cTn measurement, since different immunometric methods show a heterogeneous specificity for degraded forms of troponins and complexes between troponins and other plasma proteins or autoantibodies [3, 12, 72–75].

Árnadóttir et al. [76] recently evaluated the kinetics of hs-cTnI (measured through two different methods) and hs-cTnT over 240 min in 34 humans after myocardial ischemia induced through an intracoronary balloon inflation during an elective coronary angiography. These patients were divided into four groups according to ischemia duration (0, 30, 60 and 90 s). Circulating biomarker levels increased significantly from 15 to 240 min with all hs-cTnI and hs-cTnT methods, but significant differences in biomarker kinetics were observed across the three methods [76]. In particular, after a 60-s ischemia, hs-cTnI (measured through the ADVIA Centaur method) doubled after 45 min, while with the hs-cTnI Architect method after 90 min and with the hs-cTnT methods after 135 min [76]. Furthermore, changes in hs-cTnI over time were greater than those of hs-cTnT and the kinetics were more accurately represented by an exponential than a linear curve [76]. These results support the hypothesis that cardiac troponins can be released also following a reversible myocardial damage, without cardiomyocyte necrosis; indeed, hs-cTnI and hs-cTnT reached values above the 99th percentile URL already with ischemia durations of 30 and 90 s, which according to the Authors should not induce cardiomyocyte necrosis [76].

The results of this study are in agreement with those by Pickering et al. [77], who found that hs-cTnI increases more rapidly than hs-cTnT in patients with NSTEMI and that there are significant differences between patients in biomarker kinetics even when the measure is performed with the same method. Furthermore, the kinetic of both troponins during the first 6 h is log-linear (and then exponential) [77]. Similar findings were reported in a more recent multicenter study that evaluated only the variations within the first 2 h with two hs-cTnI methods (hs-cTnI Architect and ADVIA Centaur) and the hs-cTnT method [78].

Considering the large differences in many studies between hs-cTnI and hs-cTnT levels in patients with NSTEMI during the first hours after admission to the ED [3, 8, 11, 12, 18, 76–78], it is reasonable to expect that Δ changes suggested by 2020 ESC guidelines [7] for rule in and rule out in rapid algorithms, expressed as absolute differences across 1 or 2 h, are characterized by large confidence intervals, which nonetheless are not reported by these guidelines. It is crucial that these cut-offs are accurately validated in multicenter studies based on large patient populations. These studies have not been actually carried out for several hs-cTnI methods, as highlighted by NICE guidelines [9] and the IFCC document [8].

Another important aspect from an analytical perspective is that the reference change value between two measures of hs-cTnI and hs-cTnT is on average 30% for biomarker concentrations ≥5 ng/L [3, 12, 23–25] (Figure 1). In other words, although the absolute difference between two measures is strictly dependent on demographic and clinical characteristics and the analytical performance of the method, the percentage that denotes a significant change between two measures of hs-cTnI or hs-cTnT in a same individual is on average the same (i.e., >30%). It is then easy to calculate if a biomarker increase or a decrease can be considered as significant, namely if there is a >95% probability that the variation is not due to an analytical error and to the intraindividual biological variability, but more likely to an ongoing disease process such as a myocardial necrosis [3, 12, 23–25].

Another aspect that might be confusing for clinicians who use rapid algorithms is that some patients with suspected NSTEMI can show hs-cTnI and hs-cTnT concentrations higher than cut-off values, calculated based on Δ changes, after 1 or 2 h, and then be ruled in, even though not having any value higher than the 99th percentile URL. According to the Fourth Universal Definition of MI [6], these patients do not display a myocardial damage, which is considered by this document as an essential prerequisite for the diagnosis of MI. According to 2020 ESC guidelines [7], these patients should be ruled in for NSTEMI and then be promptly referred to coronary angiography. The same reasoning can apply to patients who display an increment, after 1 or 2 h, much higher than 30% (such as 40 or 50%), but even in this case do not reach the threshold of 99th percentile URL (considering the sex- and method-specific URL). Contrary to 2020 ESC guidelines [7], NICE guidelines [9] and the IFCC document [8] suggest that these patients be reevaluated with a further sampling at 3 h or later to check if the 99th percentile URL is reached, as requested for the diagnosis of myocardial injury and then MI [6].

Asymptomatic individuals from the general population with hs-cTnI and hs-cTnT values in the third tertile of reference values (and then still below the 99th percentile URL) have a significantly higher risk of death and major cardiovascular events even in the median term (from 6 months to 2 years), compared to subjects in the first tertile [20, 21, 79, 89]. In general, the cardiovascular risk in the general population increases first linearly and then exponentially with increasing values of cardiac-specific biomarkers hs-cTn and also B-type natriuretic peptides [20, 21].

The case of patients with suspected NSTEMI who present a significant change in hs-cTnI or hs-cTnT values, expressed as absolute or percentage change, over 1–3 h, but that still does not reach the threshold of 99th percentile URL, deserves consideration in clinical studies assessing the cost/benefit ratio of the different diagnostic approached based on rapid algorithms for the diagnosis of NSTEMI. At present, considering the results of studies on the cardiovascular risk in the general population [20, 21, 79–89] and in agreement with 2020 ESC guidelines [7], we should consider patients with suspected NSTEMI who present a significant increase in hs-cTnI and hs-cTnT over a few hours as individuals with a higher risk of acute cardiovascular events. Moreover, 2020 ESC guidelines [7] recommend that also Global Registry of Acute Coronary Events (GRACE) risk score should be considered to predict the outcome of patients with a high cardiovascular risk (Class of recommendation I, level of evidence B). The suggestion by 2020 ESC guidelines [7] of starting an antithrombotic treatment and referring these patients as soon as possible to coronary angiography seems than reasonable, although a careful evaluation of clinical, ECG and echocardiographic findings remains essential.

Another important clinical issue is the influence of reduced glomerular filtration rate (GFR) on the kinetics of hs-cTnI and hs-cTnT in patients with AMI. Indeed, patients with renal disease can have increased circulating levels and cut-off values of hs-cTnI and hs-cTnT due to the reduced GFR [90–92]. Accordingly, assay-specific optimal cutoff levels for hs-cTnI and hs-cTnT methods adjusted for GFR values should be considered [90–92]. Furthermore, the diagnostic performance of hs-cTnI and hs-cTnT methods in

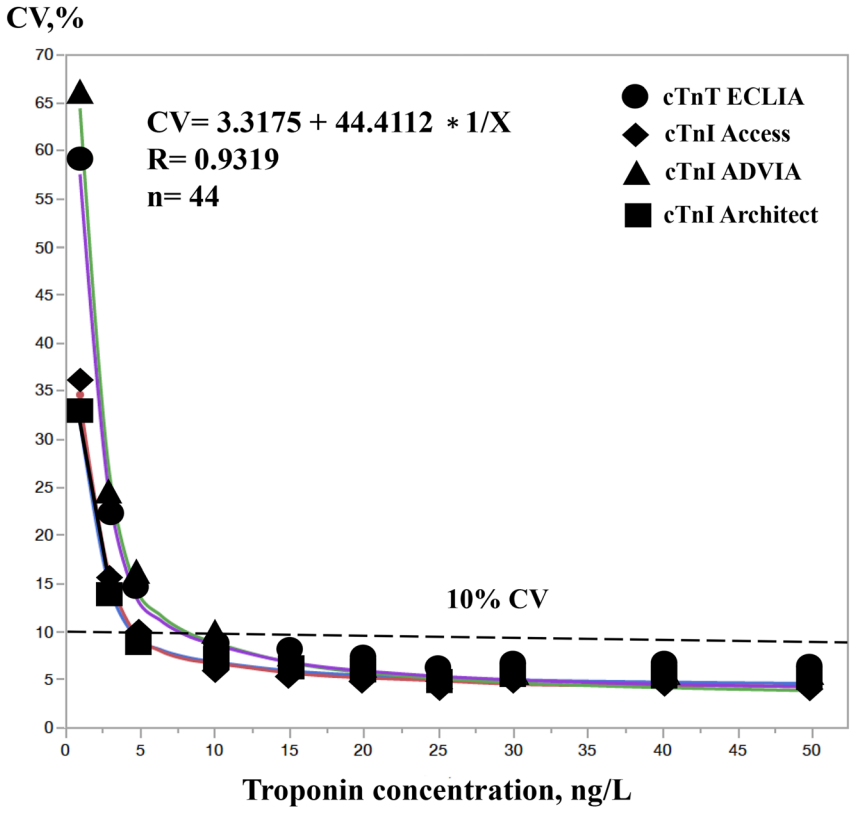

Figure 1: Imprecision profile of 4 hs-cTnI methods and the hs-cTnT method.
The imprecision profile was evaluated in the same laboratory (Fondazione CNR Regione Toscana G. Monasterio, Pisa) using a standardized protocol, as described in details in previous papers [15, 23–29]. Plasma samples (usually 10 to 14 for each method) of healthy adult subjects or patients with cardiac disorder and different biomarker concentrations (from about 2 ng/L to around 50 ng/L) were measured several times over at least 2 months using at least two different lots of the same method. The values from hs-cTnI and hs-cTnT methods are reported with different colors and symbols. The equation best fitting the values from different methods is reported, together with the R coefficient of correlation. The dashed line indicates the critical value of analytical imprecision (10% coefficient of variation) recommended by guidelines for the 99th percentile upper reference limit [5]. The methods evaluated were: (i) cTnT ECLIA: Elecsys Troponin Gen five STAT Immunoassay electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics). (ii) cTnI Access: Access hsTnI with the DxI platform (Beckman Coulter Inc.). (iii) cTnI ADVIA: ADVIA centaur high-sensitivity troponin I, with the Centaur XPT platform (Siemens Healthineers Diagnostics). (iv) cTnI Architect: ARCHITECT STAT High Sensitive Troponin-I, with the ARCHITECT i1000SR platform (Abbott Diagnostics Division).

patients with renal disease and suspected NSTEMI can be improved by use of algorithms taking into account both admission troponin and dynamic changes in biomarker concentrations [90–92].

Proposals for the use of diagnostic algorithms for hs-cTnI and hs-cTnT methods in patients with suspected NSTEMI in the Italian setting

In the 2013 document, the Inter-Society group proposed that the 0/3 h algorithm be adopted as soon as possible in Italian institutions [1]. The same algorithm was then recommended by 2015 ESC guidelines [4]. A further sampling at 6 h could be considered, according to clinical judgment, when no significant change was observed during the first 3 h. Biomarker kinetic was deemed suggestive for myocardial necrosis if circulating levels increased by at least 50% from baseline [1]. MI could be diagnosed in the presence of significant biomarker variations together with signs and/or symptoms suggesting myocardial ischemia [1].

These recommendations seem still valid in the current setting of Italian institutions, given that the transition from algorithms lasting ≥6 h to the 0/3 h algorithm is still ongoing in many hospitals [62, 63]. Moreover, rapid algorithms endorsed by 2020 ESC guidelines [7], particularly the 0 h/1 h algorithm, do not seem to be applicable in almost all Italian centers.

All the most recent guidelines recommend that NSTEMI is ruled out when admission value is lower than or equal to the LOD of the method [4, 7–9]. Similarly, if the admission value is higher than around 5 times the 99th percentile URL, as indicated in a specific table of 2020 ESC guidelines [7], but also by other guidelines [4, 6, 8, 9], we can reasonably rule in NSTEMI. When patients are ruled out or ruled in based on a single sample, given that values ≤LOD or well above the 99th percentile URL are considered, sex- (or even age-) specific cut-offs are not deemed necessary. Considering the rapid 0 h/1 h and 0 h/2 h algorithms, the 2020 ESC guidelines [7] state that optimal thresholds for rule-out were selected to allow for a minimal sensitivity and Negative Predictive Value (NPV) of 99%, while optimal thresholds for rule-in were selected to allow for a minimal positive predictive value (PPV) of 70%.

In May 2021, Westwood et al. [93] performed a meta-analysis on behalf of NICE. The authors evaluated hs-cTn assays for the management of adults presenting with acute chest pain, in particular for the early rule-out of MI using a meta-analysis of 36 studies. The results of this meta-analysis confirmed that hs-cTnI and hs-cTnT methods may be cost-effective compared with standard troponin testing using algorithms lasting more than 3–6 hours [93].

Recommendations

(1) Algorithms for the rapid rule-out or rule-in of NSTEMI using a single sample

- NSTEMI can be rapidly ruled out if the hs-cTnI or hs-cTnT value on admission is lower than or equal to the LOD of the method [7].
- NSTEMI can be rapidly ruled in if patients have on admission a hs-cTnI or hs-cTnT value higher than around 5 times the 99th percentile URL of the method [7].

(2) Algorithms for the rule-out or rule-in of NSTEMI using serial samples

- The 0/3 h algorithm has been evaluated in terms of diagnostic accuracy and cost/benefit in many multicenter studies [93], and is currently adopted in the majority of Italian centers [62, 63].
- Rapid algorithms (0 h/1 h e 0 h/2 h) certainly allow to reduce the observation time and to start more rapidly a specific antithrombotic treatment in patients with NSTEMI, but the cost/benefit ratio of this approach has not been formally evaluated for all hs-Tn methods [93]. Considering the large differences in organization and patient admission to ED in Italy [1, 10–12, 61–63, 94, 95], the rapid algorithms could be mostly easily implemented in highly specialized cardiovascular centers where there is a laboratory dedicated to urgencies and a well-organized network for the management of ACS [94, 95].

Research funding: None declared.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Informed consent: Not applicable.
Ethical approval: Not applicable.

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