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REVIEW

High-sensitivity cardiac troponin assays: Answers to frequently asked questions



Troponine dosée avec un test de haute sensibilité : éléments de réponse aux questions fréquemment posées

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Abbreviations: ACS, acute coronary syndrome; AMI, acute myocardial infarction; cTn, cardiac troponin; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CV, coefficient of variation; ECG, electrocardiogram; ESC, European Society of Cardiology; HAMA, heterophilic human antimouse antibody; hs-cTn, high-sensitivity cardiac troponin; LBBB, left bundle branch block; LoB, Limit of Blank; LoD, Limit of Detection; LoQ, Limit of Quantitation; NSTEMI, non-ST-segment elevation myocardial infarction; RCV, reference change value; ROC, receiver operator curve; SFBC, Société française de biologie clinique; SFC, Société française de cardiologie; SFMU, Société française de médecine d'urgence; STEMI, ST-segment elevation myocardial infarction; URL, upper reference limit.

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KEYWORDS

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Acute coronary
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Chest pain

Summary Cardiac troponin (cTn) assays have quickly gained in analytical sensitivity to become what are termed 'high-sensitivity cardiac troponin' (hs-cTn) assays, bringing a flurry of dense yet incomplete literature data. The net result is that cTn assays are not yet standardized and there are still no consensus-built data on how to use and interpret cTn assay results. To address these issues, the authors take cues and clues from multiple disciplines to bring responses to frequently asked questions. In brief, the effective use of hs-cTn hinges on knowing: specific assay characteristics, particularly precision at the 99th percentile of a reference population; factors of variation at the 99th percentile value; and the high-individuality of hs-cTn assays, for which the notion of individual kinetics is more informative than straight reference to 'normal' values. The significance of patterns of change between two assay measurements has not yet been documented for every hs-cTn assay. Clinicians need to work hand-in-hand with medical biologists to better understand how to use hs-cTn assays in routine practice.

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MOTS CLÉS

Troponine ;
Haute sensibilité ;
Infarctus du
myocarde ;
Syndrome coronarien
aigu ;
Douleur thoracique

Résumé L'évolution rapide des méthodes de dosage des troponines cardiaques (cTn) vers une meilleure sensibilité analytique (cTn de haute sensibilité, ou cTn HS) s'accompagne de nombreuses données de la littérature mais encore incomplètes. En l'absence de standardisation des cTn et de données consensuelles sur l'utilisation et l'interprétation des résultats, les auteurs de cette revue proposent, à partir d'une revue de la littérature, et de façon multidisciplinaire, des éléments de réponses aux questions fréquemment posées. En conclusion, le bon usage des cTn HS repose sur la connaissance: des caractéristiques propres de la méthode utilisée, en particulier de la précision obtenue au 99^e percentile d'une population de référence; des facteurs de variation de la valeur du 99^e percentile; de la forte individualité des dosages de cTn HS, pour lesquels la notion de cinétique individuelle est plus informative que la simple référence à des valeurs usuelles. La significativité des variations entre deux dosages, n'est pas encore documentée pour toutes les méthodes HS. La collaboration entre cliniciens et biologistes est nécessaire à une meilleure utilisation des troponines au quotidien.

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Background

International guidelines on myocardial infarction (MI) diagnosis recommend running a cardiac troponin (cTn) assay in suspected MI patients unless they present ST-segment elevation (suspected lone-event non-ST-segment elevation MI [NSTEMI]). The need to observe an increase in troponin over the 99th percentile of a reference population together with the significant assay-to-assay variation make it necessary to use what are dubbed sensitive or hypersensitive cTn assays. cTn assays are rapidly gaining in analytical sensitivity. Published data on this latest generation of more sensitive assays are dense, but are still incomplete. Furthermore, cTn assays are not yet standardized and there are still no consensus-built data on how to use and interpret high-sensitivity cTn (hs-cTn) assay results.

Given this context, three French academic societies – the Société française de médecine d'urgence (SFMU) for emergency medicine, the Société française de cardiologie (SFC) for cardiology and the Société française de biologie clinique (SFBC) for clinical biology – have joined forces to co-propose an integrated French-language document that, through a review of the literature, tackles the issue of how to use troponin assays properly. The document adopts a

'Question and Answer' format to connect with grass-roots practitioners, and is written to provide clinicians and biologists with the most routine-relevant conclusions possible, including a series of boxes headed 'In practice/takeaways', which recap the key messages.

Terms and definitions**What does assay sensitivity mean?**

An assay that qualifies as sensitive or hypersensitive (qualifiers arbitrarily grouped under the term 'high-sensitivity' in this paper) is an assay that demonstrates greater analytical sensitivity and precision than the conventional method it is built on. The word 'sensitive' refers to the assay, not to the biomarker itself.

From an analytical standpoint, analytical sensitivity is the smallest measurable analyte concentration above the limit of detection. Here, sensitivity is determined by the slope of the calibration curve. Higher sensitivity increases the possibility of getting low variations between two assays, as their respective signals will be significantly different (Fig. 1).

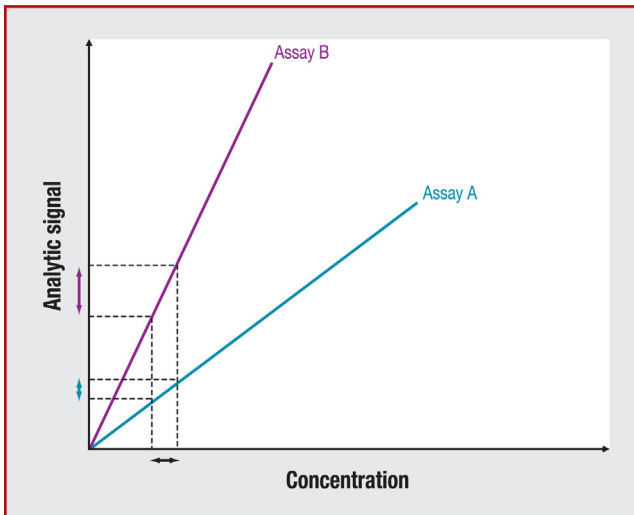


Figure 1. Graphical comparison of the sensitivity of two assays. Assay B sensitivity is higher than assay A sensitivity; it is easier to detect a small difference of concentration between two measures (black arrow) with assay B because the difference between observed signals is higher for assay B (blue arrow) than for assay A (turquoise blue arrow).

In other words, a method's sensitivity is also its ability to precisely and reliably differentiate between two different concentrations.

In practice/takeaways

- The word 'sensitive' refers to the assay, not to the biomarker itself.
- Analytical sensitivity is the smallest difference in concentration measurable by the assay.

What does analytical precision mean?

The analytical precision of an assay is an evaluation of the degree of dispersion in serial test results on a single sample; it is expressed as the analytical coefficient of variation (CV) of the assay, where $CV = \text{mean}/\text{standard deviation} \times 100$, given as a percentage (%). Analytical precision splits into a repeatability strand (intraseres precision) and a reproducibility strand (interseries precision).

A measurement method's lower-range limits are defined by: the Limit of Blank (LoB; the concentration below which 95% of results are expected to be found when replicates [$n > 60$] of a sample containing no analyte [i.e. a biomarker-free sample] are measured); the Limit of Detection (LoD; the lowest detectable analyte concentration likely to be reliably distinguished from the LoB; it is determined based on the LoB and the standard deviation of replicates of a sample containing a low, but non-zero, biomarker concentration); the Limit of Quantitation (LoQ; the smallest value obtained at a predefined CV). In the specific subfield of cTn, and for conventional assay methods, 10% CV is the analytical limit adopted for MI diagnosis [1].

Precision, LoD and LoQ vary from cTn assay to cTn assay, so they need to be verified by the laboratories and, if appropriate, communicated to the clinicians, to optimize the results interpretation process. Within the laboratory competency accreditation framework (standard ISO 15189), the analytical characteristics of hs-cTn assays are to be verified as stipulated in document SH-GTA 04 (see 'What precautions does the laboratory need to take when transitioning to a hs-cTn assay?').

In practice/takeaways

- The analytical precision of an assay is given by the CV at a given analyte concentration.
- Analytical characteristics (sensitivity, precision, LoD, LoQ) are assay specific.

What is an hs-cTn assay and are all hs-cTn assays essentially similar?

An hs-cTn assay possesses better sensitivity and better analytical precision than the 'conventional' assay it is built on. High-sensitivity assays offer 4-fold to 10-fold greater analytical sensitivity than conventional methods.

Apple and Collinson short-listed two basic criteria for defining whether a cTn assay is 'highly sensitive': precision of a reference population; and proportion of measurable concentrations in healthy individuals above the assay's LoD [2]. For an assay to qualify as 'high-sensitivity', it has to demonstrate $\leq 10\%$ total imprecision at the 99th percentile value and be able to quantitate at least 50% of healthy individuals [2].

cTn assays – whether high-sensitivity or conventional – are still not standardized at this time. Standardization efforts are hampered by a combination of factors, chiefly the heterogeneity of the circulating cTn forms that the assays can recognize, post-translational modifications to cTn isoforms and immunoassay response modifications tied to interferences and autoantibodies.

Compounding the issue, results do not directly correlate from technique to technique. In theory, the assays are equimolar, which means they should identically recognize all circulating forms. In practice, however, the distribution of circulating cTn forms in a given patient at a given time can vary, thus producing different responses in different assays. Consequently, the results given by different assays are not directly transposable from test to test.

The net result is that each assay method has its own characteristics and its own cut-off thresholds (Table 1) [3–5]. Consequently, it is vital that a patient's cTn concentrations are monitored with the same assay. The high-sensitivity assays add to a long list of conventional cTn assays. In most cases, the high-sensitivity method has replaced the conventional assay (e.g. fourth-generation cTnT [cardiac troponin T] upgraded to hs-cTnT at Roche Diagnostics, ARCHITECT cTnI [cardiac troponin I] upgraded to ARCHITECT hs-cTnI at Abbott, etc.). The analytical modifications integrated in high-sensitivity assays are patent protected and are rarely published.

Table 1 Market offer for automated quantitative troponin assays in France: 31 August 2013.

Manufacturer	Analyser	LoB	LoD	LoQ ^a	99th percentile values	99th percentile CV
Conventional assays						
Abbott	AxSYM	0.020 µg/L	NA	0.160 µg/L	0.040 µg/L	14%
ARCHITECT	< 0.010 µg/L	NA	0.032 µg/L	0.028 µg/L	14%	
i-STAT	0.020 µg/L	NA	0.100 µg/L	0.080 µg/L	17%	
Alere	Triage Cardio3 ^b	0.002 µg/L	0.010 µg/L	0.040 µg/L	0.020 µg/L	17%
Beckman	Access Accu	0.010 µg/L	NA	0.060 µg/L	0.040 µg/L	14%
bioMérieux	VIDAS Ultra	< 0.010 µg/L	< 0.010 µg/L	0.110 µg/L	0.010 µg/L	28%
Radiometer	AQT90 TnI ^b	NA	0.008 µg/L	0.026 µg/L	0.017 µg/L	15%
AQT90 TnI ^b	NA	0.010 µg/L	0.039 µg/L	0.023 µg/L	17%	
Response Biomedical	RAMP	0.030 µg/L	0.030 µg/L	0.210 µg/L	0.100 µg/L	20%
Siemens	Dimension RxL	0.040 µg/L	NA	0.140 µg/L	0.070 µg/L	15–22%
IMMULITE	0.100 µg/L	NA	0.220 µg/L	0.190 µg/L	11%	
Tosoh	AIA II 2G	0.060 µg/L	NA	NA	0.060 µg/L	9%
High-sensitivity assays						
Abbott	ARCHITECT ^c	1.3 ng/L	1.9 ng/L	4.7 ng/L	26.2 ng/L	4.0%
Beckman Coulter	Access ^c	2 ng/L	3 ng/L	8.6 ng/L	8.6 ng/L	10%
Mitsubishi	PATHFAST ^b	2 ng/L	8 ng/L	14 ng/L	29 ng/L	5.0%
Ortho Clinical Diagnostics	VITROS ECi ^c	7 ng/L	12 ng/L	34 ng/L	34 ng/L	10%
Roche Diagnostics	Elecsys/Modular E/cobas e	3 ng/L	5 ng/L	13 ng/L	14 ng/L	9%
Siemens Healthcare	ADVIA Centaur	6 ng/L	NA	30 ng/L	40 ng/L	9%
Vista	15 ng/L	NA	45 ng/L	45 ng/L	10%	
Vista HS ^c		0.5 ng/L	3 ng/L	9 ng/L	5%	
Stratus CS ^b	30 ng/L	NA	60 ng/L	70 ng/L	10%	
Dimension EXL	10 ng/L	17 ng/L	50 ng/L	56 ng/L	10%	
Tosoh	AIA 3G	8 ng/L	20 ng/L	35 ng/L	40 ng/L	NA

Manufacturer data, bibliography references [3–5].

CV: coefficient of variation; LoB: Limit of Blank; LoD: Limit of Detection; LoQ: Limit of Quantitation; NA: not available.

^a The smallest concentration of troponin obtained with reliable precision, i.e. with a 10% CV (see 'What does analytical precision mean?').

^b Point-of-care biochemistry analyser.

^c Market release scheduled for 2013 at the earliest.

The gain in analytical sensitivity from conventional to high-sensitivity assays has translated into a change in unit, where high-sensitivity assay results are expressed in ng/L instead of µg/L [5] – a change that simplifies the reporting of results statements.

In practice/takeaways

- An assay qualifies as 'high-sensitivity' if it demonstrates ≤10% total imprecision at the 99th percentile of a reference population and is able to quantitate at least 50% of healthy individuals.
- cTn assays – whether high-sensitivity or conventional – are not standardized.
- Not all cTn assays share the same level of precision at low cTn concentrations.
- Each cTn assay has its own characteristics and cut-off thresholds, which makes it vital to always use the same assay to monitor the same patient.
- The results of hs-cTn assays are expressed in ng/L.

What is an MI?

Defining an MI has always been tricky. The problem is that you have to identify a clinical-anatomical process based on a combination of indirect criteria, as 'hard' anatomopathological findings are rarely used in human subjects. For the academic societies, this means that defining an MI involves determining relevant 'proxy' criteria and their upper-bound/lower-bound thresholds.

Why does the universal definition of an MI combine diagnostic criteria with a series of infarction types?

Since the early 1960s, there have been regular efforts to establish these criteria, to realign them as the technologies emerge and improve, and to establish some kind of consensus. Up until WHO-MONICA, which served as the benchmark until the late 1990s, an MI was defined based on an electrocardiogram (ECG) and pathological Q-waves. The validation of troponins as a reliable specific biomarker of myocardial

injury led first the biochemistry societies then the cardiology societies to refocus the definition onto biomarkers, with troponins leading the way. The European Society of Cardiology (ESC)/American College of Cardiology redefinition of an MI in 2000 marked this turning point.

Initial fuzziness in the redefinition, together with swift and steady progress in the assay techniques, prompted a retweaked 'redefinition' of MI a few years later. In 2007, the biochemistry and cardiology societies joined forces to propose what was dubbed a 'universal definition' of an MI, where 'universal' means that the definition pools all clinical settings qualified as an MI; this has gained very broad consensus. The universal definition gave an even more central role to cTn. The third version – a second update – of the universal definition was published in 2012 [6].

This fairly radical definition of an MI has had two major consequences: a strong jump in the incidence of clinical settings qualified as infarction [7]; and, perhaps of even more concern, mounting confusion over where to draw the line between the concept of an MI and other clinical settings associated with myocardial injury. This second concern only emerged with the improvement in assay procedures. Back in 2000, when MI was 'redefined', the performance quality of cTn assays put them on a par with creatine kinase assays, and only enabled them to pick up the high concentrations typical of relatively large MIs and certain forms of myocarditis.

To get a sharper picture of MI in light of its redefinition, it was necessary to look beyond the first strand of baseline biochemical, clinical, ECG and imaging characteristics used to define an MI. The definition thus integrated a second 'clinical classification' strand, split into five types (numbered 1 to 5).

The definition is only universal if it is taken as a whole - i.e. the criteria plus the clinical types. This makes it crude and, in fact, ultimately wrong to simply say that an individual has had an 'infarction' - you need to say that they have had a 'type-*n* infarction', as it is the type that effectively indicates the immediate severity for triage and treatment strategy.

In practice/takeaways

- Definitions of MI are based on combinations of consensus-based indirect criteria.
- Increase in cTn concentration was first put forward as a pivotal criterion in 2000 and has since taken centre stage.
- The improvement in cTn assays achieved by reducing cTn specificity for infarction compelled the definition to introduce the concept of subcategories or 'types' of infarction on top of the consensus-built clinical criteria for defining myocardial necrosis.

Which criteria were finally selected for identifying an MI?

The universal definition of an MI works up from the general definition of myocardial injury with cell necrosis: 'cardiac biomarker values (preferentially troponin) with at least one

value above the 99th percentile of the upper reference limit (URL)'.

For this myocardial necrosis to qualify as an infarction, it has to be associated with at least two other conditions. First, a compulsory condition of haemodynamic kinetic evidence that the myocardial injury occurred in a clinical setting of acute myocardial ischaemia: 'detection of rise and/or fall'. Second, there must be at least one further criterion designed to help confirm that the clinical setting is consistent with recent myocardial ischaemia: clinical symptoms of ischaemia; development of pathological Q-waves in the ECG; new or presumed-new significant ST-segment changes or new left bundle branch block (LBBB); imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; identification of an intracoronary thrombus by angiography (or autopsy).

Note that neither the scale of the rise (or fall) nor its upper-bound/lower-bound cut-offs are detailed.

In practice/takeaways

- The definition of infarction is centred on the concept of myocardial necrosis/injury, which is itself defined by a cTn assay value above the 99th percentile of a reference population.
- To strengthen the probability that this damage is ischaemic, the definition specifies that myocardial injury has to be combined with conditions based on cTn kinetics (rise and/or fall) plus either clinical, ECG or imaging criteria.

How are the different infarction types defined?

This first strand of the definition is already a source of confusion, as it refers back to a broad and diverse range of clinical infarction settings. To address the issue, the definition goes on to propose five types of MI: two - types 1 and 2 - are clinical; three - types 3, 4 and 5 - are wholly arbitrary.

Clinical definitions: types 1 and 2

Type 1 is defined as spontaneous (sometimes dubbed 'wild') MI related to ischaemia caused by a primary coronary event, such as atheromatous plaque erosion and/or rupture, fissuring or dissection, along with fresh intracoronary thrombus.

Type 2 is defined as instances of myocardial injury with cell necrosis where a condition other than plaque rupture contributes to an imbalance between myocardial oxygen supply and/or demand, e.g. anaemia, respiratory failures, arrhythmias, hypotension or hypertension with or without left ventricular hypertrophy. By extension, coronary artery spasm and coronary embolism not tied to plaque rupture are classified under type 2.

It is abundantly clear that the definitions of type 1 and type 2 have nothing to do with ST-segment elevation or non-ST-segment elevation. ST-segment elevation MI (STEMI) is practically always type 1. NSTEMI will split into either type 1 or type 2 depending on clinical features.

Arbitrary definitions: types 3 to 5

These definitions cover settings where the clinical picture points strongly to infarction, but the classical criteria are missing (type 3), and settings where infarction is clearly identified, but applying the classical criteria strictly is irrelevant (types 4a and 5).

Type 3 is defined as sudden unexpected cardiac death with symptoms suggestive of myocardial ischaemia, accompanied by presumably new ST-segment changes or new LBBB, occurring before biomarkers were obtained or before cardiac biomarker values would be increased.

Type 4a is defined as MI related to percutaneous coronary intervention, arbitrarily defined by an elevation of cTn values over 5-fold the 99th percentile URL, and symptoms suggestive of myocardial ischaemia and/or new ischaemic ECG changes or new LBBB and/or angiographical or imaging-demonstrated criteria.

Type 4b is defined as MI associated with stent thrombosis, as documented by coronary angiography or at autopsy in the setting of myocardial ischaemia. The threshold used is the 99th percentile URL, associated with a rise and/or fall of cardiac biomarker values.

Type 5 is defined as MI associated with coronary artery bypass graft, arbitrarily defined by an elevation of cTn values over 10-fold the 99th percentile URL in a patient with normal baseline values, plus symptoms suggestive of myocardial ischaemia and/or new ischaemic ECG changes or new LBBB and/or angiographical or imaging-demonstrated criteria.

The text accompanying the third version of this universal definition spends more time justifying the selected criteria than clarifying them [6].

In practice/takeaways

- The first strand of the definition is already a source of confusion, as it refers back to a broad and diverse range of clinical infarction settings.
- The second strand of the definition specifies the criteria for five types of MI, with widely different mechanisms, prognoses and treatment options.
- It is the type of infarction that indicates its immediate severity for triage and treatment strategy; MI type must always be stated.
- The two types that are most relevant to clinical practice are type 1 (caused by plaque rupture) and type 2 (caused by an imbalance between myocardial oxygen supply and demand); only type 1 MIs are covered under strategies and treatments recommended in the guidelines.

hs-cTn and MI**How are hs-cTn cut-offs determined and what does the 99th percentile of a normal reference population mean in practice?**

The cut-off is the value at the 99th percentile of a reference population. This reference population should ideally be representative of the general population.

The 99th percentile value of a cardiac biomarker is the cut-off value below, which 99% of values obtained in the normal reference population fall.

The cut-off is tough to establish, as there is no established consensus on the characteristics of a 'normal' population. The demographics of the subjects enrolled in the reference population should be known, and investigations should be completed to confirm the absence of heart disease. Tougher selection criteria tend to result in lower 99th percentile values. The sample has to be large enough, and – ideally – the reference population should be characterized in terms of cardiological health [8,9].

For the so-called conventional cTn assays, if imprecision at the 99th percentile is too high (CV > 10%), the lowest concentration with a CV of 10% should be used as the cut-off [1]. For hs-cTn procedures, the cut-off is the 99th percentile as, by definition, the CV at this level is $\leq 10\%$ [6].

In practice, the 99th percentile URL with its 95% confidence interval is determined from a homogeneous population, following approved guideline procedure, using a non-parametric test [10]. As the proportion of subjects with detectable troponin levels increases, the 99th percentile calculated gains in precision. It takes at least 300 subjects with detectable troponin levels to calculate a 99th percentile to a probability of 95% [11]. European academic society taskforces have singled out this point as one of the critical issues surrounding hs-cTn assays [5]. Age, sex and renal function can all influence the 99th percentile [9,12,13].

It is recommended that the assay reference values of the medical laboratory population are verified (and readapted if appropriate). International medical laboratory accreditation standard ISO 15189 stipulates that the verification of biological analysis reference intervals should be based on at least 100 healthy subjects (which, in this context, means free of chest pain and MI) [14].

In practice/takeaways

- The cut-off value for hs-cTn assays is the 99th percentile.
- The 99th percentile value of a cardiac biomarker is the cut-off value below which 99% of values obtained in the population under study fall.
- The 99th percentile value can vary for a given assay and a given target reference population according to a series of factors that need to be identified.
- Standard ISO 15189 stipulates that the verification of biological analysis reference intervals is to be based on at least 100 healthy subjects.

What is the analytical precision reached at this cut-off?

The cut-off used to diagnose MI has to be clearly specified. According to the ESC 2012 consensus document, optimal analytical precision at the 99th percentile should be defined as a CV $\leq 10\%$ [6].

The manufacturer-stated CV can be biologically verified by a precision profile – 20 measurements made on at least

two different reagent batches and with two different calibrators, over a window of > 20 days. Manufacturer data do not always add up to literature findings [5]; for this reason, local medical biologists are advised to run tests to double check the manufacturer-claimed assay precision (see ‘What precautions does the laboratory need to take when transitioning to a hs-cTn assay?’).

Analytical precision can also be gauged via intermediate precision (intralaboratory reproducibility), which again, in this context, should be regularly verified, particularly for values close to the decision cut-off. Given the potential for interreagent-batch variation, this information needs to be re-evaluated as and when successive batches get used.

Assay accuracy and imprecision need to be cross-verified against the value obtained with an independent quality control test. Biologists should always opt for the cTn concentration that gets closest to the decision limit cut-off.

In practice/takeaways

- The analytical CV at the 99th percentile must be $\leq 10\%$ for hs-cTn assays.
- The 10% CV can be verified by a precision profile.

Are there other decision cut-offs?

In certain specific populations, a decision cut-off for ruling in a diagnosis of MI can be calculated via a receiver operator curve (ROC) [15], which helps to determine an optimal hs-cTn concentration in terms of clinical specificity and sensitivity. Reiter et al. found that the optimal hs-cTnT cut-off decision limit for elderly subjects (aged > 70 years) was 54 ng/L, which is about 4-fold the 99th percentile URL of a younger reference population [16]. Some teams advocate using the 75th percentile of a normal population as a means of increasing the negative predictive value of the cTn assay for diagnostic rule out of MI [17].

In practice/takeaways

- A decision limit cut-off optimized for specificity/sensitivity or designed to find the optimal specificity can be established using a ROC curve in a given population group.

What kind of improvements are hs-cTn assays expected to bring to patients with chest pain?

Despite demonstrating outstanding cardiac specificity, the major criticism levelled at conventional troponin assays was their inability to deliver an early diagnosis of acute MI (AMI), which is why other earlier markers (myoglobin, copeptin) were endorsed. Furthermore, the majority of conventional assays were unable to meet the analytical target precision of < 10% at the academic society taskforce-recommended reference cut-off (99th percentile). High-sensitivity assays were therefore developed with a two-fold objective: earlier detection of troponin release after an ischaemic episode;

and improved precision and analytical sensitivity of the decision limit cut-off [18].

Most of today’s high-sensitivity assays now meet the analytical objective and can factor in any elevation approaching this cut-off with < 10% precision at the 99th percentile decision cut-off with reliably high confidence. Note too that analytical sensitivity has now reached a point where low-level troponin release can be picked up straight from early stage myocardial cell necrosis or from the minute there is any change in cardiomyocyte membrane permeability [19].

Consequently, even a slight rise or fall in cTn can now be detected very early on, from the very start of clinical signs suggestive of acute coronary syndrome (ACS). This means that diagnostic sensitivity is increased from the minute patients get to emergency medical services. Early studies by Keller et al. [20] and Reichlin et al. [21], which have since been confirmed in several more recent studies, showed that using hs-cTn (I or T) in combination with a 99th percentile decision cut-off can improve MI detection from as early as 2 hours after chest pain starts (increase in area under the ROC curve from 0.7 to > 0.9). Note, however, that at this time, the diagnostic value of hs-cTn has only been validated in chest pain settings.

Nevertheless, low troponin concentrations in many clinical settings involving non-ischaemic aetiology still end up decreasing marker specificity for MI diagnosis. This means that to improve diagnostic specificity, repeat assay and analysis of dynamic cTn kinetics remain essential back-ups to clinical features and ECG findings. Given how early cTn elevation can be picked up with high-sensitivity assays, in 2012 the cardiology society taskforce proposed new algorithms to track and trend cTn kinetics ‘live’ (see ‘What kind of time interval has to be left between two hs-cTn assays?’) [6].

In practice/takeaways

- High-sensitivity assays make it possible to:
 - reach high precision (10% CV) at the 99th percentile decision cut-off,
 - rapidly detect troponin very early on,
 - improve the diagnostic performances of cTn for ACS screening;
- The increased sensitivity comes at the cost of decreased specificity for a single cTn measure.

Why is it necessary to run a second assay, even with hs-cTn?

The definition of an MI hinges on detecting a rise and/or fall of circulating cTn values and so requires at least two measurements, performed on at least two blood samples – ideally one drawn at admission and another drawn 3–6 hours post-admission [6] (see ‘Which criteria were finally selected for identifying an MI?’).

Repeat hs-cTn assays prove doubly valuable because they can rule out an MI with maximal safety and certainty, and they can rule in a diagnosis of type 1 MI with adequate certainty.

Patients with suspected MI swiftly get triaged to emergency medical wards, with most getting management in the

first 2 hours after onset of symptoms. Even with the high-sensitivity assays, these short time windows do not always leave enough time to demonstrate elevated cTn. Consequently, a sub-cut-off troponin value at admission is not enough to safely rule out an MI diagnosis, so a second repeat assay at 3 hours post-admission proves necessary.

ESC guidelines on the management of non-ST-segment elevation ACS do, however, authorize a single assay in cases where patients report at least 6 hours of evolving chest pain [22]; in which case, if hs-cTn stays below the cut-off and there is no other aetiology warranting hospitalization, patients can then be sent back home provided they no longer present chest pain, but do present a GRACE score < 140. Note that there is a heavily suspected diagnosis, and if the second repeat assay turns up a sub-cut-off value, it may be advisable to run a third cTn assay to safely and reliably rule out MI.

If cTn is elevated to above the cut-off at the first cTn assay AND there is a heavily suspected diagnosis, the recommendations on non-ST-segment elevation ACS leave it open to the clinician whether or not to run the second assay [22]. A recent opinion paper recommended performing the second repeat hs-cTn assay regardless of the value of the first cTn assay [5]. The rationale is that the increased sensitivity of cTn assays has come at the cost of decreased specificity, and there are several settings that can still drive the hs-cTn value up and over the decision cut-off (see 'In what kind of setting can hs-cTn increase, outside of type 1 MI?' and 'Does the level of hs-cTn elevation point to aetiology?'): root causes of type 2 MI, such as anaemia, tachyarrhythmia/bradyarrhythmia, hypotension/hypertension, hypoxia [6]; multifactorial or indeterminate causes (acute neurological injury, heart failure, catecholamine-induced cardiomyopathy and others) [6]; chronic causes, for which the cut-off limit in new-generation hs-cTn assays is above the URL of a 'healthy adult' reference population, such as advanced age, heart failure, prior history of coronary heart disease or renal failure [6,13,23].

In these settings, assay-to-assay variation in cTn value will not be significant and, associated with evaluation of clinical features, cannot be used as grounds for diagnosing a definitive type 1 MI and, consequently, setting up an invasive strategy.

In practice/takeaways

- It is recommended to run a second cTn assay to rule in or rule out a diagnosis of non-STEMI in patients presenting early within the first hours of onset of chest pain symptoms.

What kind of time interval has to be left between two hs-cTn assays?

ESC guidelines from 2012 on the definition of MI advocate repeating a second cTn assay 3–6 hours later if using a conventional cTn assay and at 3 hours if working with hs-cTn [6]. ESC guidelines from 2011 on the management of non-ST-segment elevation ACS advocate a second assay 3 hours

later, and even a third assay 6 hours later if there is a strong likelihood of MI [22]. It is equally possible to work to the template given by Thygesen et al. in a review paper on how to use hs-cTn measurements (Fig. 2, borrowed from [5]). This algorithm is essentially built from research work with hs-cTnT. Consequently, other high-sensitivity assay methods will need to be validated in prospective clinical trials to benchmark their performances and verify the diagnostic efficiency of the patterns of cTn change recommended in 2012 [6].

A study recently tested the benefits of a second assay at 1 hour post-admission, using hs-cTnT at different hour 0 to hour 1 changes and baseline cut-offs, depending on whether the aim was to rule out or rule in MI. The study yielded highly promising results, but without further confirmation, it cannot be taken as grounds for recommending a shorter time interval to the second assay [24].

In practice/takeaways

- A 3-hour interval between two serially tested cTn samples is sufficient when using an hs-assay in the setting of chest pain with suspected ACS.
- New algorithms with shorter time intervals have been proposed, but they need to be corroborated by further studies.

When does a change in hs-cTn become significant?

The total variation in medical test results stems from a combination of two types of variation: individual subject variation (biological variation, annotated CVi); and analytical variation (CVa). The limit variation between two results in a healthy and/or stable subject can now be evaluated through the so-called reference change value (RCV).

$RCV = 2^{1/2} \times Z \times (CVa^2 + CVi^2)^{1/2}$, where Z is a probability score (Z = 1.96 for significance with 95% confidence level) [25]. This calculation is considered negligible for analytical variation. The RCV is the acceptable limit of result-on-result variation in a normal clinically stable subject. Any variation greater than the RCV can be considered clinically significant.

With the first-generation conventional assays, cTn concentration was only quantifiable in a minority of healthy subjects, and so biological variation was considered negligible because the baseline cTn value was non-quantifiable (i.e. it did not differ far enough from the LoD). However, with high-sensitivity assays, biological variation now takes on significance. Wu et al. studied short-term (within-day) and long-term (between-day) biological variation in hs-cTnI (Singulex® assay) in healthy subjects [26], and found that cTnI demonstrates low intraindividual variation, but high interindividual variation – in other words, cTnI shows high-individuality. Troponin T responded the same way with the hs-cTnT assay [27]. The short-term RCVs measured by ARCHITECT (Abbott), Vista (Siemens), Access 2 (Beckmann) and Centaur (Siemens) assays are around the 50% range [27]. The RCV of hs-cTnT varies in the 38–58% range, depending on the study [27,28]. Note that the RCV calculation can be

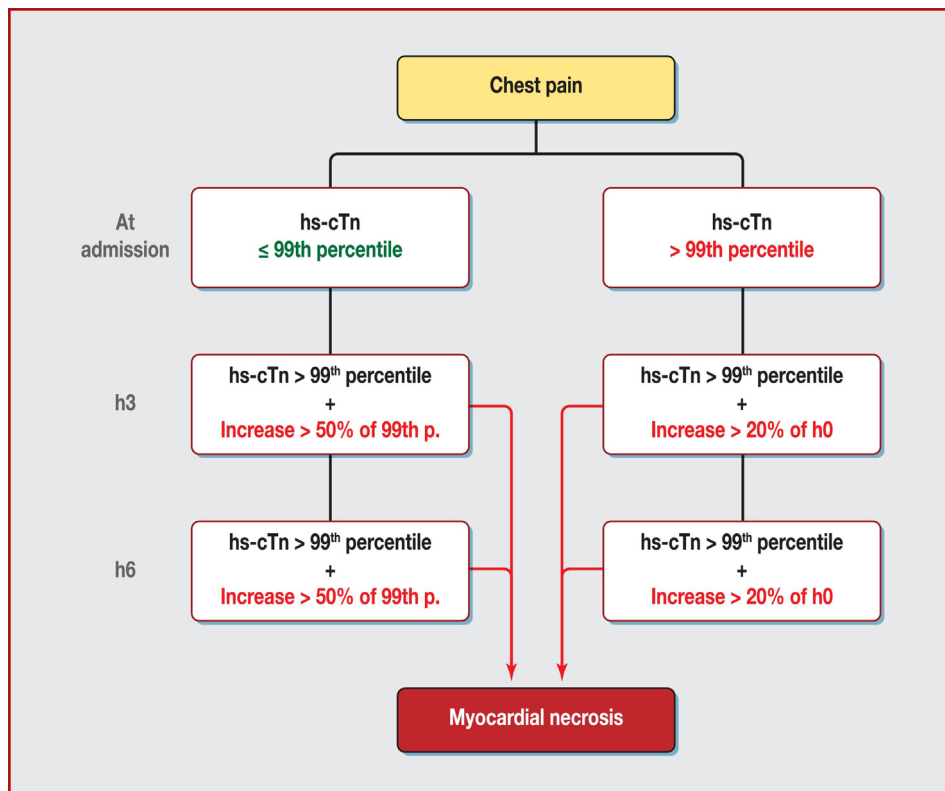


Figure 2. Algorithm for the diagnosis of acute coronary syndrome. hs-cTn: high-sensitivity cardiac troponin; p.: percentile. According to [5].

applied to relative change variations (in ng/L) or absolute change variations (in %) (see ‘Elevated hs-cTn values and infarct size’). Apple and Collinson [2] proposed a short recap detailing the first work on the RCVs of hs-cTn assays – work that started to shape the key concepts for interpreting time-course patterns of hs-cTn with a variation-based template.

In practice/takeaways

- The RCV indicates the URL of acceptable variation between two sample measurements in a healthy subject; it varies with the hs-cTn assay used.
- The RCV applies to hs-cTn values \leq 99th percentile.
- The global RCV, all hs-cTn assays included, is around the 50% mark; a relative variation $>$ 50% of concentrations observed at the 99th percentile is to be considered clinically significant; an absolute change variation cut-off can prove an advantage in terms of classifying patients.
- hs-cTn assays are high-individuality tests, for which the notion of individual kinetics is more informative than straight reference to ‘normal’ values.

Is it best to use absolute change or relative change variation in hs-cTn? Does zero change rule out a type 1 MI?

The guidelines to using cTn advocate using the 99th percentile decision cut-off and the evaluated kinetics cTn changes (rising or falling variation) [6]. Level of change has

not been evaluated with all the hs-cTn assays on the market. The change calculation can be expressed either as an absolute change value (cTn hour 3 – cTn hour 0, expressed in ng/L) or as a relative change value ($\Delta\%$ = [cTn hour 3 – cTn hour 0]/cTn hour 0, expressed in %).

Opting to work with absolute change value variation makes it possible to modulate the interpretation according to initial hs-cTn concentration. The rationale is that in absolute values, the variation can often prove considerably greater than the RCV (% of change tied to biological and analytical variability) if the initial hs-cTn concentration is particularly low, yet well below the RCV if the initial hs-cTn concentration is already high (Fig. 3). It is always going to be difficult to obviate \geq 50% increases in areas under the curve, where initial concentrations are comfortably over the 99th percentile URL.

Reichlin et al. showed, using repeat cTn assays 2 hours post-admission, that the absolute changes were diagnostically superior to relative (percentage) changes with the Roche hs-cTnT assay (absolute change decision cut-off = 7 ng/L) and the Siemens cTnI-ultra assay (absolute change decision cut-off = 20 ng/L) [29]. These findings have since been confirmed by Mueller et al. with the hs-cTnT assay and a 6-hour delay between the two samples (absolute change decision cut-off = 7 ng/L) [30]. Similarly, Wildi et al. showed that absolute changes were diagnostically superior to relative changes with the Vista and Beckman hs-cTnI assays, especially in patients presenting early in response to chest pain [31]. However, with other assays, relative change can prove efficient. Keller et al. showed with the ARCHITECT hs-cTnI assay that a 30% relative change associated

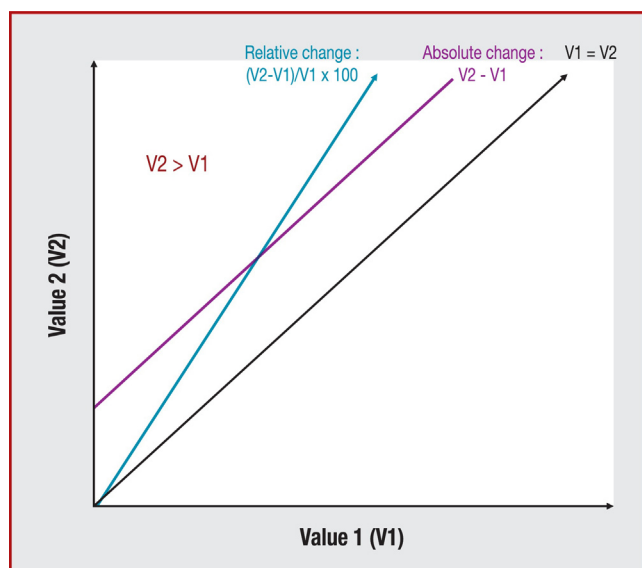


Figure 3. Graphical representation of relative and absolute changes, calculated with two values (V1 and V2). There is no equivalence between the two calculations in the lowest values (absolute change > relative change) or in the highest values (relative change > absolute change).

with a hs-cTnI value above the 99th percentile holds practically perfect specificity (98%) despite very modest sensitivity (56%) for the diagnosis of ACS [32].

For hs-cTn assays, a low first-line troponin value < 99th percentile and a relative change < RCV can rule out acute cardiac injury. Nevertheless, the evidence suggests that clinicians are more comfortable working in terms of absolute change. A study recently showed that an hs-cTnT assay repeated at 3 or 6 hours performs equally efficiently for rule in or rule out of non-STEMI (absolute change decision cut-off = 7 ng/L at 3 hours and 9 ng/L at 6 hours) [33].

Clearly then, the diagnostic performance and calculation of cTn change ($\Delta\%$ and absolute figures) will need to be estimated separately for each hs-cTn assay. Furthermore, as there are still no standardized protocols, clinical validation studies need to be carried out to optimize timing of the change interval and interpretation of the change patterns.

There is a whole series of conditions where heart damage translates into a cTn value above the 99th percentile cut-off value. This situation, already well described with conventional troponins [34], has become even more common with the advent of hs-cTn assays because of their zoom-in effect on the lower values and their gain in precision. The differentiation between an acute change (tending to suggest an acute coronary episode) and a chronic elevation (tending to suggest chronic myocardial injury) has not been definitively validated. It is important to note that troponin change profiles similar to those seen in MI can also be found in other different disease settings, as reported by Wu [35], which makes it vital to interpret any dynamic change in cTn value in relation to clinical presentation (Fig. 4).

In practice/takeaways

- The significance of change between two cTn measurements is assay dependent.
- The cTn change-range cut-offs published in academic society guidelines are grounded in scattered literature data.
- The change-from-baseline cut-offs need to be validated for each high-sensitivity assay.

Elevated hs-cTn values and infarct size

Several studies have demonstrated that hs-cTnT concentrations are directly related to infarct size and extent of coronary lesions, whether in STEMI patients [36] or NSTEMI patients [37]. The same team found a similar relationship with hs-cTnI, where the correlation was significant ($r = 0.67$; $P < 0.001$) [38]. Twenty-four-hours hs-cTnT was significantly correlated to myocardial necrosis size, as obviated by 48-hour cumulative creatine kinase release ($r = 0.86$; $P < 0.001$) [36].

Although hs-cTn assays detect cTn release earlier than their older conventional cTn assays, time-to-peak blood levels (around 18–24 hours) and fall pattern are still the same.

In practice/takeaways

- hs-cTn values are correlated to infarct size.
- cTn fall kinetics follow the same pattern in a high-sensitivity assay as in its older original conventional protocol.

hs-cTn elevation outside of type 1 MI

What does an elevation in hs-cTn signify in terms of pathophysiology?

The cardiomyocyte has two troponin pools. The first is a cytosolic pool composed of free troponin I (minority component) and troponin T (majority component, accounting for around 8% of the total troponin). The second pool, composed of the three troponin subunits (I, T and C) is integral to the muscle contraction complex (the actin-myosin-troponin protein complex). To recap, myocardial tissue only contains the cTn isoforms I and T.

In myocardial ischaemia, the free cytosolic troponin may be first to be released. If the ischaemia is intense and/or prolonged, the cell starts to necrotize, all free troponin gets released, the contractile system collapses and/or is destroyed and the complexed troponin is then second to get released.

On top of the many forms of troponin (binary and ternary complexes) already found in circulating blood, the action of various proteases can add further heterogeneity [39]. In practice, only the free or complexed cTn isoforms I and T are effectively quantifiable.

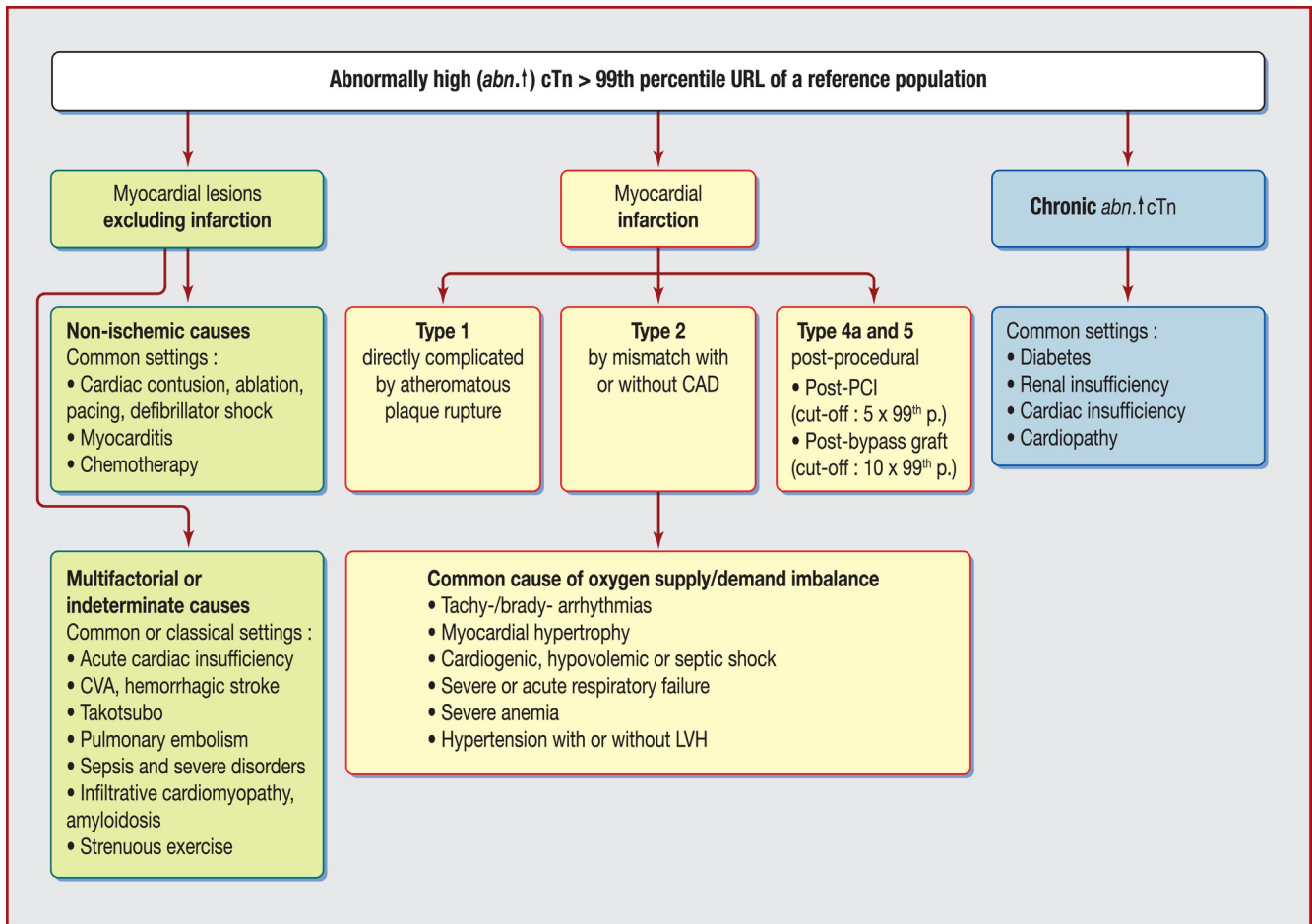


Figure 4. Classification of abnormally high cardiac troponin (cTn) concentrations. CAD: coronary artery disease; CVA: cerebrovascular accident; LVH: left ventricular hypertrophy; p.: percentile; PCI: percutaneous coronary intervention; URL: upper reference limit.

Conventional cTn assays were only able to quantify the strong elevations that signalled cardiomyocyte necrosis, giving no indication as to the type (see MI classification scheme) involved. The advent of high-sensitivity assays made it possible to quantify the release of strictly cytoplasmic troponin [6] (see ‘Can hs-cTn elevation reflect myocardial distress without necrosis?’).

Although the theory posits that that only cTn isoforms are measured and that there is no interference from muscle isoforms, the real story can turn out to be a lot more complicated. Indeed, a study just published has reported the unthinkable: the in-muscle re-expression, against a background of congenital myopathy, of an atypical isoform of troponin T interfering with the hs-cTnT assay [40]. This does, however, remain an exceptional condition – and one that is easily clinically identifiable.

In practice/takeaways

- The cardiomyocyte has two troponin pools: a cytosolic pool and a pool integral to the muscle contraction complex.
- There is strong heterogeneity in the blood-circulating cTn forms.

Can hs-cTn elevation reflect myocardial distress without necrosis?

High-sensitivity methods now open the way to detecting not just strong elevations, but also low releases of (potentially cytoplasmic) troponin occurring in tandem with myocardial distress without necrosis. As things stand, the prevailing opinion is that increases in cTn reflect necrosis of cardiomyocytes. The hypothesis of troponin release without myocardial necrosis in myocardial ischaemia has been suggested in experimental trials [41] in patients with chronic exertional angina stabilized under treatment, but also in healthy subjects [42]. Once again, the pain could be tied to coronary heart disease, non-coronary heart disease or non-heart disease. In all these settings, the elevated cTn is associated with poor prognosis.

Among non-coronary diagnoses, the latest ESC guidelines list renal failure, heart failure, extreme tachyarrhythmias/bradyarrhythmias, pulmonary embolism and myocarditis as frequently responsible for elevated hs-cTn [6].

Rounding up, recent studies have reported a possible rise in cTn in settings other than suspected MI—patients at risk of atherosclerosis are an example. Here again, the rise in cTn carries a bleak prognosis, and the post-admission care

Table 2 The four families of 'acute' increases in blood cardiac troponin concentrations.

Type 1 myocardial ischaemia
Plaque rupture
Intraluminal coronary artery thrombus formation
Type 2 myocardial ischaemia related to oxygen supply/demand imbalance
Tachyarrhythmias/bradyarrhythmias
Cardiogenic, hypovolemic or septic shock
Acute respiratory failure
Severe anaemia
Severe hypertension with or without LVH
Hypertrophic cardiomyopathy
Aortic dissection or decompensated severe aortic valve disease (AI, AR)
Injury not related to myocardial ischaemia
Cardiac contusion, ablation, pacing or defibrillator shock
Cardiotoxic agents (e.g. Herceptin®, anthracycline antibiotics)
Myocarditis
Rhabdomyolysis with cardiac involvement
Multifactorial or indeterminate myocardial injury
Heart failure
Stress (takotsubo) cardiomyopathy
Severe pulmonary embolism
Sepsis and any distress syndrome justifying intensive care
Renal insufficiency
Severe acute neurological disease (e.g. stroke, subarachnoid haemorrhage)
Infiltrative disease (e.g. amyloidosis)
Strenuous exercise

AI: aortic insufficiency; AR: aortic regurgitation; LVH: left ventricular hypertrophy.

period comes with an increased risk of mortality, not just because of AMI, but also heart failure [43].

In practice/takeaways

- An elevation in hs-cTn may point to cardiomyocyte disease or necrosis.
- This elevation carries a bleak prognosis.
- It opens up another potential field of application – prognostic stratification in at risk populations.

In what kind of setting can hs-cTn increase, outside of type 1 MI?

Outside of type 1 MI, elevated cTn concentrations can be grouped into four broad settings (Fig. 4, Table 2).

Setting 1

Type 2 MI relates to an imbalance between myocardial oxygen supply and/or demand (a 'mismatch') at a given point in time. This mismatch can prove relatively modest if the cardiac muscle has already been damaged by chronic pathology (ischaemic, diabetic, hypertensive, valvular or other cardiomyopathy). The main causes are clinical settings involving severe anaemia, hypotension or hypertension, hypoxia, irregular heartbeat (fast or very slow) or a sudden sharp increase in myocardial oxygen demand – factors that will generally be associated (e.g. in extreme physical exertion, sepsis, pulmonary oedema or shock). Clearly, then, most cases of increasing cTn in the wake of noncardiac surgery will fall into the category of type 2 MI. The universal definition of MI clearly indicates that a mismatch type 2 MI is possible even with normal coronary arteries.

Setting 2

The second setting is myocardial necrosis in which myocardial ischaemia has been definitively ruled out. In this setting, the term infarction gives way to 'myocardial damage'. The setting spans cardiac trauma, myocardial inflammation (myocarditis) and chemotherapy-induced cardiotoxicity.

Setting 3

A third setting covers multifactorial or indeterminate clinical settings, as myocardial ischaemia has the potential to play a role in necrosis indirectly (Table 2). The boundary line with MI type 2 is often hazy [6].

Setting 4

The fourth and final setting involves stable baseline concentrations and abnormal troponin elevation. These cases involve few patients at general population scale (0.04% of people in the 30–65 years age bracket according to the Dallas Heart Study [44,45]), but a far higher proportion in patients presenting at emergency medical services. This is a rare yet challenging population with numerous risk factors. The prevalence of circulating forms, which is very low with conventional cTn assays [45], increases sharply once hs-cTn assays are used [44]. There is a typically high prevalence of left ventricular hypertrophy, diabetes and coronary calcium scan score > 100. Increased baseline cTn in certain chronic clinical conditions, chiefly renal failure and heart failure, reflect the severity of the cardiomyopathy or the fragility of the underlying cardiovascular structure. In these patients, elevated cTn is a biomarker of very high risk of medium-term cardiovascular accident or mortality.

In practice/takeaways

- Elevated hs-cTn concentration can be grouped into five broad diagnostic settings:
 - Type 1 MI,
 - Type 2 MI,
 - Non-ischaemic increases in cTn,
 - Multifactorial or indeterminate clinical settings,
 - Chronic stabilized rise in cTn.

Does level of hs-cTn elevation point to aetiology?

There are several classic clinical settings associated with an increase in hs-cTn. In contrast to conventional cTn assays, which can be interpreted semi-quantitatively, hs-cTn assays have to be interpreted fully quantitatively, as cTn concentration and measure-to-measure change can differ with different aetiologies.

An international team reported hs-cTnT concentrations and patterns of change in repeat assays in 887 patients (mean age 64 years) presenting with chest pain [46]. Patients with type 1 AMI had higher biomarker concentrations on admission: median hs-cTnT was 113 ng/L vs. 12 ng/L in patients with cardiac non-coronary chest pain and 7 ng/L in patients with noncardiac chest pain. Furthermore, hs-cTnT concentration increased in type 1 AMI patients at repeat assay compared with in other-cause patients: increase in hs-cTnT from first to second assay was 19 ng/L within 1 hour and 63 ng/L within 6 hours in AMI patients versus 1 ng/L at a 1-hour interval and 2 ng/L at a 6-hour interval in patients with cardiac non-coronary chest pain. Finally, analysis of biomarker concentrations stratified for aetiology showed that only myocarditis presented a similar hs-cTn profile to the pattern found in AMI (strong elevation at admission rising even higher at repeat assays).

The scale of the increase in hs-cTn in patients presenting with chest pain can guide diagnosis towards different disease settings, which we can schematize as shown below.

Very high hs-cTn concentrations

In the vast majority of cases (>90%), these will point to AMI (without stratifying between type 1 or type 2). The main differential diagnoses are myocarditis and takotsubo cardiomyopathy. Repeat assay could further refine the diagnosis: further elevation from first to second measure points to a type 1 MI (or myocarditis).

Low hs-cTn concentrations on admission

With further elevation from first to second measure

This pattern points to the same profile as very high hs-cTn concentrations on admission followed by further elevation from first to second repeat.

Stable from first to second measure

This setting points to a cardiac non-coronary cause. The particular case of only slight low elevations showing no significant variation over time is often associated with non-decompensated heart failure.

In practice/takeaways

- Blood-circulating cTn concentrations and change from first to second measure differ with different aetiologies; analysis of these two variables is critical to diagnostic thinking in any chest pain patient.

- The highest concentrations and measure-on-measure increases are observed in type 1 MI; against this background, the main differential diagnoses are myocarditis and takotsubo cardiomyopathy.
- Low change in hs-cTn at admission with no significant further increase from first to second measure points first-line treatment towards a cardiac non-coronary cause.

Medical biology practice

Are there hs-cTn assay protocol-related false-positives or false-negatives?

High-sensitivity assay-related analytical false-positive or false-negative elevations of cTn do potentially exist, but there have been few attempts to describe and study them at this time. Every analytical factor takes on a critical dimension when dealing with hs-cTn. The fact is that hs-cTn assays are immunoassays, which means that they may well be exposed to the same analytical interferences as described previously for the older conventional assays [3]. Consequently, the effects of microclotting, heterophilic antibodies or autoantibodies will all need to be estimated separately for each hs-cTn assay. To recap, the heterophilic antibodies encompass rheumatoid factor, natural and non-specific anti-idiotypic antibodies, and human antianimal antibodies (including heterophilic human antimouse antibodies [HAMAs]).

In the high-sensitivity protocol development process, some manufacturers reported modifications able to decrease assay responsiveness against HAMAs when using chimeric antibodies in their immunoassay [47]. Furthermore, analytical limits have already been described—chiefly haemolysis interference potentially inducing over- or under-estimation, depending on the assay [48]. Visually examining the specimen tube can provide an evaluation of sample quality, especially for samples drawn through the intravenous catheter lines used in intensive care units.

The assay result thus needs to be interpreted, not just in relation to clinical presentation and ECG patterns, but also in relation to the analytical 'environment' (presence of haemolyzed samples for instance), especially when none of the cardiac and noncardiac causes of elevated cTn can be ruled in. Only HAMAs are easy to screen for, using specific patent protected commercially available tubes. Interferences from heterophilic antibodies can have varying degrees of impact depending on the assay protocol used, on much the same scale as in other immunoassays.

Note too that reports have shown that antitroponin antibodies can generate false-positives with conventional assays [49], and high-sensitivity assays may be exposed to this same scenario.

In practice/takeaways

- Analytical false-positives (heterophilic antibodies and haemolysis) have been reported for certain high-sensitivity assays.
- Analytical false-negatives (essentially haemolysis) have been reported for hs-cTnT.
- hs-cTn assays may well be exposed to the same analytical interferences as described previously for the older conventional assays.

What precautions does the laboratory need to take when transitioning to a hs-cTn assay?

Any change in assay method is going to prove tricky. To maintain firm control over the transition process, it is crucial that physicians/clinicians work hand-in-hand with medical biologists.

In the laboratory

The assay needs to be verified in accordance with good laboratory practice and in line with international medical laboratory accreditation standard ISO 15189 [14]. First and foremost, the analytical criteria on which the definition of high-sensitivity assay is based (99th percentile, % measurable concentrations in patients) will need to be verified, along with repeatability, intermediate precision, accuracy and LoD and LoQ (both of which can be verified using a precision profile with cross-verification of the 10% CV). Ideally, verification of the 'normal' values (determination of the 99th percentile URL, % of detectable subjects) is also a requisite.

In clinical services

In clinical services, particularly emergency services and the cardiology ward, a review of the literature and decision algorithm scenario can be integrated alongside a more practice-oriented biomarker evidence-based approach, such as a comparative assay analysis. This process could include cross-verification against the literature-validated cTn rise patterns and cut-off limits. To evaluate cTn change from first to second measure, in the absence of relevant literature data, analysts can fall back on a 50% RCV (see 'When does a change in hs-cTn become significant?'). This strategy is needed to enable clinicians to get a better grasp of the analytical characteristics of the biomarker in their day-to-day practice, as the transition to a hs-cTn assay is liable to shake up their clinical practice routines as it entails a repeat assay at a shorter (3-hour) interval and a jump in the frequency of non-ACS cTn elevation syndromes [3,6].

Troponin taskforce

Significant change patterns and cut-off values for ACS have not been sharply detailed for a number of high-sensitivity assays. This gap prompted the SFBC to coordinate the creation of a taskforce pairing up medical biologists with clinicians. The mission given to this national taskforce on the 'Use and interpretation of results with the latest hs-cTn assays in Emergency Cardiology' will be to compare the

performances of the troponin assays on the European market and, for each assay, to validate the recommended cut-offs, the interval to re-analysis and the value signalling significant change (Δ or %). The deliverables assigned to the taskforce will be: to validate manufacturer-claimed diagnostic efficiency of each assay; to propose individualized recommendations for the use of each assay; and to enable optimal use of appropriate cTn assays in routine clinical practice.

In practice/takeaways

- The hs-cTn assay needs to be verified in accordance with good laboratory practice and in line with medical laboratory accreditation standards.
- Decision algorithm scenarios can be used alongside a more practice-oriented biomarker evidence-based approach via a comparative assay analysis.
- Significant change patterns and cut-off values for ACS have not been precisely detailed for a number of high-sensitivity assays.
- To evaluate cTn change from first to second measure, in the absence of relevant literature data, analysts can fall back on a 50% RCV.

Have any hs-cTn assays been validated for point-of-care biochemistry testing? Is prehospital hs-cTn screening reliable?

The use of cTn assays outside the laboratory (point-of-care testing) comes with a host of challenges, and so needs to be tightly framed under a three-way contract between the medical biologist, the clinician and the administrative authority. This requires healthcare staff and ambulance crews to be trained up on the technical procedures involved, and – of course – is governed by good laboratory practice guidelines (referenced to accreditation standard ISO 22870).

Furthermore, the medical biologist retains sole responsibility for the entire end-to-end analytical protocol – the clinician interpreting the result at the patient's bedside must not lose sight of the fact that this 'technical' result cannot be deemed valid without medical biologist approval.

As of 2013, there is no validated hs-cTn assay available for prehospital point-of-care in France: the only point-of-care and triage assays on the market lack sensitivity, which means a negative test result is not necessarily negative – largely negating much of the benefit brought by the faster diagnostic speed of the assay (Roche Cardiac Reader h232, Inverness Triage Meter, Samsung Labgeo IB10). That said, 'high-sensitivity' assays have found their way on board a select few point-of-care testers – the Siemens Stratus and the Mitsubishi PATHFAST (Table 1). However, there are watch points.

The results of point-of-care testing or screening are neither transposable nor comparable to results produced in centralized labs, which means the result of a prehospital test result must never be employed as an elevation test 0-hour start time if the subsequent assay measures are performed with other reagents back at the hospital.

Point-of-care assays are generally run on whole blood, which means the test will not pick up any sample haemolysis

or dilution effects if the result is very high. Furthermore, the detection and measurement ranges are narrow compared with assays done on central laboratory-based analysers [50].

In practice/takeaways

- There is no hs-cTn assay available for point-of-care testing or screening.
- The results of point-of-care testing or screening are neither transposable nor comparable to results produced in centralized laboratories, which means they must be interpreted strictly within the bounds of the point-of-care tester.
- The use of cTn assays outside the laboratory (point-of-care testing) needs a tight control frame, procedural training for healthcare staff and ambulance crews, and compliance with good laboratory practice (international accreditation standard ISO 22870).

Prognostic value and therapeutic strategies directed by hs-cTn

Do all hs-cTn elevation levels in type 1 MI have the same prognostic value?

Troponin concentration is tightly correlated to left ventricular function. In type 1 NSTEMI, it has long been recognized that cTn is a powerful short-term indicator of mortality or large MI. Very low cTn concentrations below the 99th percentile URL have a similarly strong prognostic value.

In practice/takeaways

- cTn concentrations are tightly correlated to risk of fatality in type 1 STEMI and NSTEMI.

Is hs-cTn elevation a prognostic marker outside of type 1 MI?

In other clinical settings (type 2, non-ischaemic, multifactorial, chronic), cTn elevations reflect the severity of cardiac stress and/or cardiac muscle fragility.

For type 2 MI and myocardial lesions qualified as multifactorial or indeterminate, the increased risk of mortality or cardiovascular accident with proportionally higher cTn elevation is stronger in the mid- to long-term than in the short-term. This is because, in these settings, the increase in cTn concentrations indicates the intensity of cardiac stress as a factor driving necrosis, but more important to the bigger picture, the vulnerability of the cardiac muscle, regardless of its underlying cause (coronary disease, cardiopathy, impaired coronary microcirculation, etc.). The same thinking applies to elevated baseline cTn above the 99th percentile URL in the general population in patients presenting cardiopathy or clinical factors (renal failure,

arterial disease, etc.) that point to increased risk of cardiovascular accident. For the general population, mortality and cardiovascular event-free survival curves for individuals with chronic troponin elevation start to slide right from the first few weeks and continue to diverge over several years. This risk is more than doubled at 3 years. This divergence in survival curves is even sharper when there is a background of chronic disease, such as renal failure or heart failure.

Elevated cTn in non-ischaemic disease settings (pulmonary embolism, cardiac trauma, aortic dissection, myocarditis, anthracycline cardiotoxicity, sepsis, etc.) is also a risk marker, at least of extended hospital stay and often of mid-term cardiovascular events. In these patients, cTn concentrations can be used for risk stratification and choice of monitoring regimen, but with little influence on treatment. However, at this time, there is still no consensus on monitoring regimen for these patients, which (a priori) do not need to be triaged to the cardiac intensive care unit, and still no clinical studies or firm recommendations on optimal management strategy – whether to discharge early or admit to hospital, which department to admit to and with what objectives.

In practice/takeaways

- Outside of type 1 MI, elevated cTn concentrations are a marker of sufficiently intense systemic or cardiac stress to cause myocardial lesions or of particularly labile and structurally vulnerable cardiac muscle.
- Consequently, these cTn elevations are a powerful predictive marker of mortality or mid-to-long-term risk of cardiovascular accident.
- However, in a clinical context of non-ischaemic increases in cTn, elevated cTn alone is not sufficient to determine/direct/decide any specific management strategy.

Diagnostic thinking and management policy for chest pain patients

Where we are sure

When type 1 ACS is highly likely (based on clinical chest pain data and cardiovascular risk factors, ECG readings and troponin elevation), an empirical treatment following the latest 2011 and 2012 ESC guidelines should be started (aspirin, antiplatelet, anticoagulant), regardless of cTn concentration [6,22].

Continued uncertainty

There is still no perfect clinical scoring scheme (even counting the recently proposed Thrombolysis in MI [TIMI] score) able to state with certainty whether a troponin elevation stems from developing infarction or from a non-coronary or noncardiac cause. There is also no study demonstrating that starting a specific therapy (such as the one described above) for non-ST-segment elevation ACS is an absolute emergency, and that holding back for minutes or even hours will reduce the patient's chances. Finally, there is no study demonstrating that starting a specific therapy (aspirin

and/or antiplatelet and/or anticoagulant) in response to a non-coronary cause of elevated troponin is in any way useful – indeed, there is a belief that it will expose patients to undue risks of haemorrhagic complications.

In practice

The logical path forward, then, would be to propose a pragmatic evidence-based approach to patient management in response to elevated cTn.

If the clinical probability of type 1 non-ST-segment elevation ACS is high, it would be logical to initiate a guideline-recommended treatment [6] and – obviously after conferring with the cardiologist – to transfer the patient to the appropriate cardiac intensive care facility.

Conversely, if the clinical probability of type 1 ACS is low, and especially if the second-reading cTn elevation is < 50% of the first reading and there is no change in ECG patterns, then there is no logical rationale for proposing a treatment that carries risks of haemorrhagic complications.

Finally, when faced with the middle road, where pre-test likelihood is fair, but not high, and/or second-reading cTn elevation is above the selected cut-off (see 'When does a change in hs-cTn become significant?' and 'Is it best to use absolute change or relative change variation in hs-cTn? Does zero change rule out a type 1 MI?'), it would be logical to initiate a treatment given the go-ahead by the cardiologist and/or if there are changing ECG patterns and/or the patient starts to present chest pain.

In practice/takeaways

- When there is strong suspicion of ST-segment elevation ACS, an hs-cTn assay is redundant.
- In the other clinical settings, regardless of hs-cTn start value or pattern of change, the values cannot be interpreted without integrating clinical probability (even if still estimated poorly by present-day scoring schemes).
- It can only be integrated in a clinical diagnosis and therapeutic strategy management algorithm for routine use by interventionists after a three-way discussion with cardiologists and biochemists.

The taskforce proposals for better routine use of cTn

Coordinated by three French societies – the SFMU for emergency medicine, the SFC for cardiology and the SFBC for clinical biology – a taskforce was formed in late 2012 to lead a multidisciplinary investigation on how to use cTn. The taskforce has proposed a two-strand analysis: a review of the literature and the state-of-the-art working practice using cTn, reported here in question and answer format; and hands-on comparative performance testing of hs-cTn assays. The primary objective of this multidisciplinary approach is to provide clinicians and biologists with answers to a series of frequently asked questions on routine practice.

The second strand of this research documents the hour 0 to hour 3 cTn change patterns for each cTn assay tested, in a multicentre non-selectively included cohort of patients admitted to an emergency room for chest pain with onset within the previous 6 hours.

Conclusions

Given the array of non-standardized assays on the market, effective use of hs-cTn hinges on knowing: the assay specific characteristics, particularly precision at the 99th percentile reference population cut-off; the factors of variation at the 99th percentile; and the high-individuality of hs-cTn assays, for which the notion of individual kinetics is more informative than straight reference to 'normal' values.

The published data, although dense, are still incomplete at this time. The significance of variation between two assays, which is method dependent, has not yet been documented for every hs-cTn assay. In this context, efforts should be made to get clinicians working hand-in-hand with medical biologists to extend the scope of knowledge for each assay and help to better understand how to use hs-cTn assays in routine practice.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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