Opinion Paper

Magdalena Krintus* and Mauro Panteghini

Judging the clinical suitability of analytical performance of cardiac troponin assays

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Abstract: New millennium diagnostic criteria for acute myocardial infarction precipitated a revolutionary shift from an approach based primarily on electrocardiography and clinical symptoms to a strategy based on biomarkers, and preferably cardiac troponins (cTn) I and T. In the last 20 years, clinical recommendations have strengthened the role of cTn and led to the development of highly sensitive (hs-cTn) assays, which are now leading players in all current clinical practice guidelines. To optimize the clinical use of these hs-cTn assays, focus on their analytical aspects has become increasingly important, emphasizing the need for the establishment of suitable analytical performance by the definition and implementation of appropriate specifications. An accurate estimate of measurement uncertainty, together with the acquisition of the highest analytical quality when very low concentrations of hs-cTn are measured, are essential requirements and should represent a practical laboratory standard in assuring optimal clinical use. Additional goals for further improving the quality of laboratory information should be the establishment of robust data concerning biological variation of cTn and the resolution of practical challenges opposed to the harmonization of cTn I results obtained by differing commercial measuring systems.

Keywords: analytical performance specifications; cardiac troponin; highly sensitive assays.

***Corresponding author: Magdalena Krintus**, Department of Laboratory Medicine, Collegium Medicum, Nicolaus Copernicus University, 9 Sklodowskiej-Curie Street, 85-094 Bydgoszcz, Torun, Poland, Phone: +48 52 585 40 23, Fax: +48 52 585 40 24, E-mail: magdalena.krintus@wp.pl
Mauro Panteghini, Department of Biomedical and Clinical Sciences, University of Milan, Milano, Italy

Transition to cardiac troponin testing: from revolution to evolution

New millennium diagnostic criteria for acute, evolving, or recent myocardial infarction precipitated a revolutionary shift from an approach based primarily on electrocardiogram (ECG) and clinical symptoms to a strategy based on biomarkers, and preferably cardiac troponins (cTn) [1]. Paralleling this revolution, *in vitro* diagnostic (IVD) manufacturers concentrated on improving existing assays to increase precision and achieve higher sensitivity in cTn measurements [2]. Developmental steps and innovation culminated in the introduction of highly sensitive troponin (hs-cTn) assays. *Clinical Chemistry and Laboratory Medicine (CCLM)* devoted a great deal of interest to the impact of novel hs-cTn assays in clinical practice [3–5].

The implementation of these analytically improved assays was initially met with reluctance by clinicians in their application in clinical practice, due to the challenging modifications brought about in clinical protocols and workflows [6, 7]. However, in the decade since these introductions, scientific evidence, multidisciplinary education, and extensive training have led to a wider acceptance of hs-cTn assays, which, when used in the right clinical context, can improve the management of patients with suspected acute coronary syndrome (ACS). hs-cTn measurements could then be incorporated in accelerated clinical pathways using fast track protocols for ruling out and ruling in non-ST elevation ACS [8, 9].

What do current clinical practice guidelines say about troponin?

In 2018, the *expert consensus* document on the “Fourth Universal Definition of Myocardial Infarction” proposed a conceptual model to facilitate a clinical distinction between acute ischemic myocardial injury and chronic conditions of myocardial damage [10]. Further, to consolidate the use of the 99th percentile upper reference limit (URL) as a cut-off to

detect myocardial necrosis, serial testing was proposed to allow for the discrimination of acute from chronic pathophysiological mechanisms of cTn release: where an increasing (or decreasing) cTn pattern indicates an acute disease process, whereas an unchanged stable cTn course indicates a chronic condition [10]. This approach definitively endorsed that proposed over a number of years by several authors [11, 12]. Here, the novelty lies in the definition of 'stable' denoting a ≤20% variation of cTn values [10]. When discussing the biochemical approach for diagnosing myocardial injury and infarction, these authors highlighted the importance of the biological variation of cTn to correctly establish the clinical significance of the biomarker variation, stating that a change of 50–60% is suggested in most studies. However, based again on *expert consensus*, they recommend serial changes >20% as indicative of acute myocardial injury where baseline hs-cTn values are >99th URL [13]. These document also discusses analytical issues, dividing the imprecision performance (as CV) at the 99th URL cTn concentrations in three categories: a) a CV ≤10% as mandatory for hs-cTn assays, b) a CV between 10 and 20% as acceptable for clinical use, while c) assays with a CV >20% should be discarded [10]. They relied on indications published earlier, suggesting that cTn assays with an imprecision of up to 20% CV at the 99th URL may reasonably be used in clinical practice, even if a CV <10% is preferable [14].

In 2020, the European Society of Cardiology (ESC) released *guidelines* for the management of ACS in patients presenting without persistent ST-segment elevation in ECG [15]. In this document, the use of the 0 h/1 h algorithm (best option) or the 0 h/2 h algorithm (second best option) is recommended, without mentioning the 99th URL as a cut-off. These guidelines endorse the evidence that very low concentrations of hs-cTn determined at the time of patient admission (0 h), and not exceeding assay-specific thresholds, may safely and efficiently exclude acute myocardial infarction (AMI) without persistent ST-segment elevation (NSTEMI). In addition, recommendations for 0 h/1 h delta hs-cTn value use, using assay-specific cut-offs expressed as absolute numbers, were included both for rule-out and rule-in of suspected NSTEMI.

A *guideline* report of the American College of Cardiology (ACC)/American Heart Association Joint Committee on Clinical Practice Guidelines for the evaluation and diagnosis of chest pain was published in 2021 [16]. In different parts, it combines elements of the two aforementioned documents. In the 'Recommendations for biomarkers', the "Fourth Universal Definition of Myocardial Infarction" consensus is endorsed (except for the 20% delta cut-off value which is not made explicit), including the indication that the CV at the 99th URL for each assay should be ≤10%. On the other hand, in the chapter 'Recommendations for patients with acute chest pain and suspected ACS', a 0 h to 1–3 h protocol for hs-cTn (as similarly proposed by the ESC) is endorsed, though not clearly described. Furthermore, the guideline recommendation on using the hs-cTn limit of detection (LOD) as a threshold for excluding myocardial necrosis at time 0 does not resolve the issue that in the United States (US), according to Food and Drug Administration (FDA) regulations, the use of a LOD-based strategy is not applicable. Quite recently, the ACC also released an *expert consensus* on decision pathways focused on the evaluation and disposition of patients with acute chest pain in the emergency department (ED) [17]. In this document, there is nothing particularly novel, though a couple of aspects are noteworthy. First, the authors revise the application of the ESC-recommended 0 h/1 h protocol by partially modifying the list of hs-cTn assays (and some recommended cut-offs, by adapting these values to the FDA's indications for use), removing assays which have not been fully validated. In addition to this change, the 2022 ACC document defines myocardial injury "as at least 1 ng/L [of cTn] above the 99th URL" (Table 5 in ref. [17]), as the phrasing used by the "Fourth Universal Definition of Myocardial Infarction" is "with at least one value above the 99th URL" [10].

1st interlude: biological variation of cardiac troponins

The importance of understanding the knowledge of biological variation of laboratory measurands for their correct interpretation has been widely demonstrated [18]. Accordingly, *CCLM* has focused much attention on this topic (e.g., recently, an entire journal issue [2022; vol. 60, issue 4] featured this subject). cTn is not exceptional with numerous studies having sought to estimate its biological variation [19–22]. Nonetheless, the vast majority of studies which have tried to assess biological variation of cTn (with the very few exceptions discussed below) provided data which was unworkable as a significant number of results for selected individuals were lower than the assay's LOD, even when hs-cTn assays were employed, preventing accurate measurement of random physiological fluctuations around the homeostatic set-point of this analyte [23]. Data recently published by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group on Biological Variation strongly supported these concerns, highlighting enormous differences in biological variation estimates observed in both short-term and long-term protocols and raising some perplexing questions [24]. To permit a meta-analysis approach, the authors attempted to homogenize available findings by excluding

studies in which results below the declared LOD of the employed hs-cTn assay had been included as the basis for biological variation estimates, but included studies where only cTn results <LOD were excluded from calculation, thereby introducing a selection bias as they clearly showed how the within-subject CV (CV_I) is being influenced by cTn absolute concentrations, with higher variability at the lowest concentrations (Figure 1) [24]. Importantly, this aspect now becomes a serious limitation in obtaining an accurate determination of biological variation for cTn, as only studies utilizing assays able to measure hs-cTn *in all samples of all enrolled subjects* will deliver robust information on biological variation of this biomarker without any result selection bias. To date, only two studies using the Singulex single-photon assay methodology for measuring hs-cTn I have met this requirement (it should be noted, however, that Singulex ceased operations in 2019) [25, 26]. The study by Wu et al., published more than a decade ago, provided conceptually correct estimates for biological variation of cTn I, both on short-term and long-term protocols, employing a commercially unavailable prototype Singulex Erenna [25]. Given the inclusion of hs-cTn in accelerated algorithms (e.g., 0 h/1 h), it seems reasonable to consider biological variation derived from the short-term (0–4 h) sampling protocol in that study, which yielded an average CV_I of 9.7% [25]. More recently, Ceriotti et al. partially replicated that study by using a newer version of the Singulex assay undertaken on a Clarity platform. Unfortunately, they only evaluated long-term biological variation of cTn (with blood sample drawing weekly, for 10 consecutive weeks), information of little use when fast track protocols are applied [26]. Although, their results for long-term protocol CV_I from this study (16.6%) essentially confirmed those of Wu et al. (14.0%). In addition, it should be noted that in both studies the reference change value (RCV) estimated for establishing significance in cTn I increases ranged from 46% (Wu, short-term) to 60% (Ceriotti, long-term) [25, 26]. Finally, we should be aware that the two cTn's represent totally different measurands which may behave differently in blood, so their individual biological variation can differ and results for cTn I cannot be directly transferred to cTn T.

Performance requirements for recommended troponin use: facts or illusion?

Although the international clinical guidelines outlined above unanimously recommended the use of hs-cTn assays, the analytical requirements essential to ensure their proper implementation in clinical practice are not well defined. Even so, we can use simple simulations to calculate the level of analytical quality in terms of measurement uncertainty (MU) required to fulfil these recommendations. According to the basic statistics [27], for two cTn results for the same individual to be different, the inherent difference (RCV) should be higher than: $\sqrt{2} \times Z \times \sqrt{(MU^2 + CV_I^2)}$, where MU is the standard MU needed to obtain a certain RCV, the CV_I is 9.7% (data from cTn I short-term variation as previously discussed), and Z is the Z-score. In defining the Z-score, a unidirectional variation, i.e., a rise in cTn values, and a certain probability against change for cTn can be considered [28]. In particular, the probability against change can be modulated as 'likely' (p>0.80), 'more likely' (p>0.90), and 'very likely' (p>0.95), which imply increasing strength against the null hypothesis. Table 1 reports two simulations based on: a) the "Fourth Universal Definition of Myocardial Infarction" recommendation of 20% cTn serial change in diagnosing acute myocardial injury when the admission value is >99th URL [10], and b) the ESC algorithm for ruling

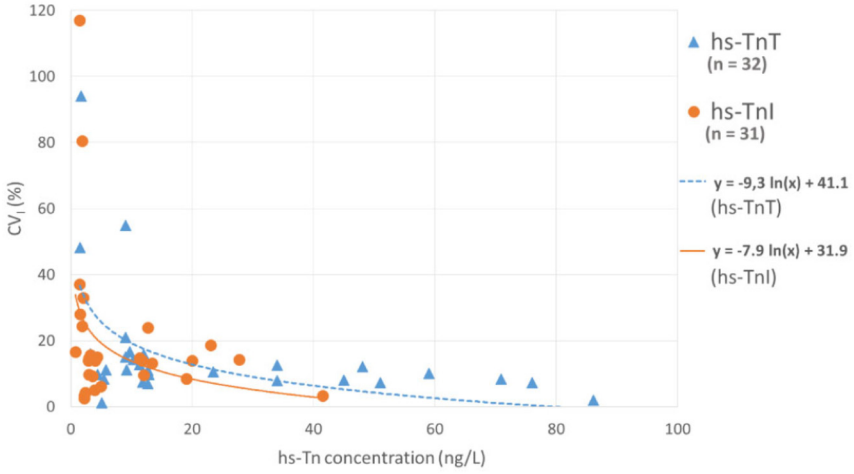

Figure 1: The relationship between within-subject biological CV (CV_I) estimate and cardiac troponin concentrations measured with highly sensitive assays (hs-Tn) as reported in literature studies. Note the inverse correlation that fits a logarithmic (ln) function. This indicates that CV_I estimates are influenced by the absolute troponin concentration level. Reproduced with permission from ref. [24].

out NSTEMI, using Abbott Architect hs-Tn I recommended admission concentrations of <4, or <5 ng/L with a delta of <2 ng/L at 0 h/1 h sampling [15]. Note that in the latter setting, the actual lowest detectable cTn variation should be 1 ng/L, corresponding to a 25% change at 4 ng/L and 20% change at 5 ng/L, respectively. From the resultant data, it is evident that, even when an optimal level of MU is obtained, e.g., 5% at 99th URL or 10% at very low cTn concentrations for ruling out NSTEMI, the maximum probability against change does not exceed 0.90. If one wishes to reach a 0.95 probability in terms of clinical information, the required discriminating changes should be increased to at least 25% in example a) (providing that a standard MU of 5% at the 99th URL cTn concentration is achieved), and to 2 ng/L in example b) (providing that a standard MU of 10% at very low cTn concentrations is achieved). Employing imprecision profiles for different hs-cTn assays, some recent studies have estimated an average RCV of 32% for hs-cTn assays at the 99th URL [22]. Though two aspects should be highlighted, firstly, that the authors used bidirectional Z-score values in calculating RCV, which is inappropriate if one is focusing solely on cTn increases, as the guidelines do. Secondly, as discussed in detail later, imprecision is not the only source of MU, even if it is probably the predominant one when cTn is measured [29].

A further discussion point concerning performance requirements was recently raised by the 2022 ACC consensus statement, where authors defined myocardial injury "as at least 1 ng/L of cTn above the 99th URL" [17]. This recommendation represents an indirect performance requirement, which becomes erroneously assay-dependent, when the absolute value of 1 ng/L is related to 99th URL concentrations, which are method specific. For instance, for hs-cTn T, using the established 99th URL of 14 ng/L [30], we can estimate that this will require a MU of 7.14% (1/14) to distinguish a 1 ng/L variation. For Abbott hs-cTn I, the required MU would be two-times lower (3.7%), considering the published 99th URL of 27 ng/L (1/27) [31]. Notwithstanding, these differences in performance requirements, they have nothing to do with the clinical use of cTn measurements ot the impact of test variability on patient outcomes. Indeed, the essential question here is to what degree of quality is requisite and to what MU is tolerable without jeopardizing patient safety [32–36].

According to the consensus statement from the 1st EFLM Strategic Conference, analytical performance specifications (APS) for cTn measurements should be defined using the outcome-based model [37–39]. This model, based on the effect of analytical performance on clinical outcomes, should be applied to the measurands which have a central and well-defined role in decision making regarding specific disease or a given clinical situation, and test results should be interpreted through established criteria [39]. Possibly no other laboratory test than cTn possesses these characteristics and has the authority to alter a patient's clinical course and costs of care so broadly. Sheehan et al. first evaluated the effect of analytical performance of cTn measurements on diagnostic misclassification [40]. Performing duplicate cTn measurements, they calculated the frequency at which the result of the second replicate fell into a different diagnostic category according to a predefined cut-off, thus defining the percentage of

Table 1: Target standard measurement uncertainty (MU) for using the 20% cardiac troponin (cTn) change criterion when the admission value is greater than the 99th percentile upper reference limit (URL) as per the "Fourth Universal Acute Myocardial Infarction Definition" (UAMID) (upper part), and in applying the ESC rule-out algorithm for NSTEMI using Abbott Architect hs-Tn I recommended cut-offs [with admission concentrations of <4, or <5 ng/L and a delta of <2 ng/L at 0 h/1 h samples] (lower part), with a stated probability. Note that the starting p-value of 0.80 was considered the minimum for clinical relevance.

Z-score (unidirectional variation)	Probability against change (p)	CV_I	Target MU at 99th URL cTn conc.	Discriminating serial change as per UAMID
0.84	>0.80 (likely)	9.7%	13.8%	20%
1.28	>0.90 (more likely)		5.4%	
1.65	>0.95 (very likely)		Unfeasible	

Z-score (unidirectional variation)	Probability against change (p)	CV_I	Target MU at very low/low cut-offs for ruling out NSTEMI	Discriminating change as per ESC (1 ng/L)
0.84	>0.80 (likely)	9.7%	18.7%	25% (very low cut-off)
			13.8%	20% (low cut-off & no 1 h delta)
1.28	>0.90 (more likely)		9.9%	25% (very low cut-off)
			5.4%	20% (low cut-off & no 1 h delta)
1.65	>0.95 (very likely)		4.6%	25% (very low cut-off)
			Unfeasible	20% (low cut-off & no 1 h delta)

CV_I, within-subject biological coefficient of variation; NSTEMI, non-ST elevation myocardial infarction; ESC, European Society of Cardiology.

misclassified patients with suspected AMI based on the assay's MU. Diagnostic misclassification rates of 1.4–1.8%, 0.9–1.2%, and 0.5–0.9% corresponded to MU of 13.0, 9.4, and 6.7%, respectively [40]. Lyon et al. performed another simulation study estimating the fraction of the patient misclassification rate as a function of hs-cTn I assay analytical performance at the 99th URL [41]. In this study, the rate of patient misclassification in terms of false positives and false negatives was ~0.3% when the analytical CV was 10% and the bias set to zero, when this measuring system is perfectly aligned. In summary, although indirect, evidence derived from studies simulating the impact of analytical performance of cTn on the dichotomic clinical classification of patients with suspected AMI indicates that a standard MU <10% at the 99th URL may maintain the misclassification rate below 1%. On the other hand, based on simulations previously performed in our manuscript (Table 1), the use of serial cTn testing with cut-offs for a significant change recommended by international clinical guidelines is anticipated to be more analytically demanding, due to cTn biology. As well as this, where a variation is biologically significant, there is no guarantee that it is also clinically relevant. As Clerico et al. rightly showed [22], the clinical relevance of RCV or delta changes of hs-cTn assays in patients with chest pain should be preferentially evaluated using appropriate methods devoted to confirming their diagnostic performance. Taking for example the study by Biener et al. [42], the best diagnostic cut-off for relative kinetic change of hs-cTn T in an unselected ED population was 53%, much higher than the 20% change recommended by the consensus of the "Fourth Universal Definition of Myocardial Infarction".

2nd interlude: MU estimate for cardiac troponin measurements

MU was recently the subject of a specific contribution to this journal and readers should refer to this article for further information [35]. The newly released ISO 20914:2019 Technical Specification provides practical guidance for the estimation and expression of the MU of quantitative measurand values produced by medical laboratories [43]. cTn I and T are among measurands for which reference measurement systems do not currently exist and for which calibrators are value-assigned by assay manufacturers using in-house traceability procedures. Nonetheless, all end-user calibrator assigned values have an MU (u_{cal}) that contributes to the overall MU of measurement results (u_{result}) according to the formula: $\sqrt{(u_{cal}^2 + u_{Rw}^2)}$, where the u_{Rw} is the assay precision under intermediate reproducibility conditions obtained by the individual laboratory using a given cTn measuring system [43]. This should include the analytical variability belonging to a set period, preferably six consecutive months, sufficient to include most changes in local measuring conditions, also capturing systematic sources of MU, such as those caused by different lots of reagents, different calibrations, and variable environmental conditions [35, 43–45]. We have previously emphasized several times in this journal the importance of selecting a suitable control material for estimating u_{Rw} [33, 35, 36, 44, 45]. Importantly, the material should closely resemble authentic patient samples (i.e., be commutable) with concentrations appropriate for the clinical application of the measurand in question: therefore cTn concentrations close to the 99th URL are recommended to monitor assay variability at this decision level recommended in clinical guidelines [36, 46–48].

Table 2 summarizes the key steps in obtaining a correct u_{Rw} estimate of a cTn measuring system using a commutable internal quality control material (IQC) with concentrations near to the 99th URL. The six-month derived CV (i.e., u_{Rw}) is then combined with the corresponding u_{cal} provided by the kit manufacturer, using the formula reported above, to obtain u_{result} of clinical samples. In a practical example from the laboratory of one of the authors using an hs-cTn T assay performed on two Cobas e601 platforms, the average u_{Rw} on the two systems, obtained according to approach described in Table 2, was 4.5% at a mean concentration of 17 ng/L. This was combined with the value of u_{cal} provided by Roche Diagnostics (Troponin T hs STAT CalSet, code no. 05092736), by selecting the calibrator concentration closer to the 99th

Table 2: Steps to obtain u_{Rw} (i.e., assay precision under intermediate reproducibility conditions, according to the ISO/TS 20914:2019 definition) estimate of a cardiac troponin (cTn) measuring system using a commutable control material with concentration near to the 99th upper reference limit.

1. Provide that the cTn measuring system is running properly and is correctly aligned, through the measurement of the manufacturer's control materials.
2. Run the control material, appropriately selected for u_{Rw} estimate, randomly in a routine analytical run (mimicking analytical conditions of clinical samples).
3. Repeat measurements of this material at least daily for six consecutive months, i.e., a period sufficient to capture most changes in measuring conditions and systematic sources of measurement uncertainty.
4. Do not include gross outliers in the u_{Rw} estimate, but check the measuring system performance and explain any outlier results.
5. At the end of the evaluation period, collect all results and revise the data (excluding explicable outliers, separating data obtained from different lots of control material, etc.).
6. Calculate the mean and SD of control replicates.
7. Calculate relative u_{Rw} as SD/mean × 100.

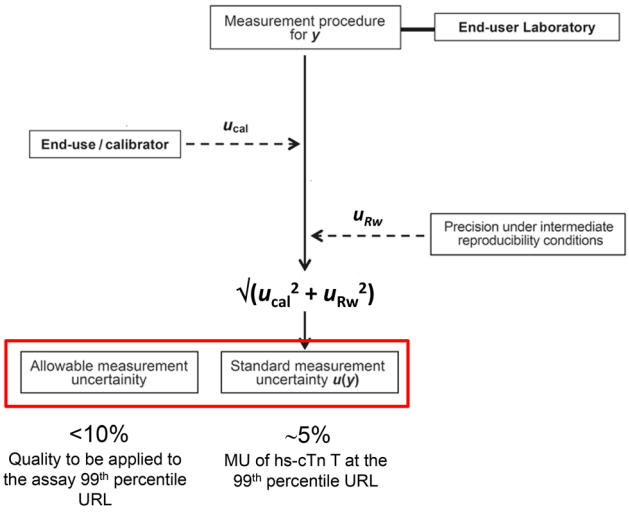

Figure 2: Sources of measurement uncertainty (MU) of cardiac troponin measurements using a highly sensitive assay (hs-cTn). u_{cal}, MU of calibrator; u_{Rw}, assay precision under intermediate reproducibility conditions obtained by an individual laboratory using a given hs-cTn measuring system [ISO/TS 20914]; URL, upper reference limit.

URL (i.e., 1.9% for a calibrator value of 21.6 ng/L), as follows: $\sqrt{(1.9^2 + 4.5^2)}$, to give a u_{result} of 4.9%. This is the MU value which should be compared with the APS for MU derived as previously discussed in order to show if the magnitude of MU of cTn measuring system is suitable for use in medical decision making (Figure 2).

Maintaining analytical quality in daily practice for intended clinical use

In the clinical scenario described in ESC recommendations for early NSTEMI ruling out at patient admission [15], accurate calibration of hs-cTn assays in the very low range of concentrations is of the upmost importance as even relatively small analytical misalignments in practice may influence the proportion of patients identified as suitable for discharge [36, 49]. Suboptimal performance, differences between instruments, reagents and calibrator lots may strongly influence measurements at low cTn concentrations resulting in patient misclassification when using accelerated algorithms in the ED. Aakre et al. showed that the cumulative percentage of hs-cTn T <5 ng/L (corresponding to the LOD value for this assay) ranged from 18 to 31% owing solely to the change of reagent lots, thereby undermining the efficacy of fast-track protocols employed in the ED [50]. It should be emphasized therefore that even when utilizing an hs-cTn assay for which the MU around the 99th URL is acceptable, does not automatically provide acceptable variability at lower cTn concentrations in meeting the requirements set out in clinical recommendations for early rule-out strategies. A calibration verification, which entails the assaying of a control material with cTn concentrations close to the LOD to confirm the accuracy of the measuring system alignment at very low concentration levels and, accordingly, the accuracy of patient results, is therefore highly desirable. However, commercial control materials supplied by manufacturers do not cover such very low cTn concentrations, making the assay susceptible to undetected drifts. The use of a control material or patient pool with cTn concentration near the assay's LOD to monitor baseline drifts following assay calibration, in addition to those usually offered by manufacturers, is therefore strongly recommended [36, 51]. In using this very-low concentration control material additionally, if its results are beyond the range of acceptability (corresponding to APS for MU discussed in the previous paragraphs) immediate corrective action could be undertaken prior to the reporting of biased patient results analyzed in an affected run, and with measurements repeated. Other authors in this journal also agree that medical laboratories should implement more stringent IQC procedures at the relevant low cTn concentration levels when using the fast 0 h/1 h algorithm in daily practice [52].

How to deal with bias in cardiac troponin measurements

In the traceability framework, medical laboratories should rely on IVD manufacturers who must ensure traceability of their measuring systems to the highest available references [32, 53–56]. Accordingly, correct alignment to measuring systems is expected before it goes to market. As discussed above, medical laboratories should simply consider the MU of the value assigned to the calibrator (u_{cal}) and combine it with u_{Rw} to obtain u_{result}, in turn to be compared with the respective APS to assess the suitability of measurements [57]. During daily use the system alignment may undergo change due to systematic sources of MU, such as those caused by different lots of reagents. As this bias is incorporated in the MU of clinical samples through the u_{Rw} estimate, it can be tolerated until the u_{result} fulfills the predefined APS (e.g., for cTn a MU of 10% at 99th URL). The presence of a medically unacceptable measurement bias will be detected when u_{result} exceeds APS or through external quality assessment (EQA) surveillance [45, 58]. In this case, a readjustment of the measuring system by the end-user must be undertaken to correct it. If the bias remains and the calculated u_{result} is still

not meeting the predefined APS, the manufacturer should be requested to take immediate investigation and corrective action and rectify the problem by, e.g., a process of reassigning values to the calibrators for correcting the detected bias [59].

3rd interlude: harmonization of cardiac troponin results

It is widely known that differences still exist within results in commercially available cTn assays which can impact the clinical classification of patients [60, 61]. The lamented Jill Tate, for many years Associate Editor of *CCLM*, pioneered this issue, publishing some important papers [62–64]. The story of harmonization projects for cTn I measurements began more than 20 years ago [65]. The cTn I Standardization Subcommittee created by the American Association for Clinical Chemistry initiated the work and had the merit to identify and characterize a primary reference material (a purified human troponin ternary complex), which was then released by the US National Institute for Standards and Technology (NIST) as SRM 2921 [66]. However, as this reference material did not always behave equally to patient samples, the need for an intermediate step arose transferring trueness to clinical samples using an SRM 2921-calibrated reference procedure together with a human matrix-matched secondary reference material to be employed as common calibrator of field assays was pointed out [67]. With the aim of developing a complete reference measurement system, the IFCC initiated the Working Group for cTn I Standardization [68]. This group's primary focus was on the development of reference procedure for cTn I [69]. As analytical principles commonly used for implementing reference procedures, such as mass spectrometry, lacked the sensitivity to measure low nanogram-level concentrations of cTn in serum, a candidate immunoassay-based reference procedure was developed, even allowing for the selectivity of the predicated reference procedure to match the selectivity of commercial assays in terms of antibody reactivity. Unfortunately, obtained results in terms of comparability between this candidate procedure and field assays were not encouraging. Consequently, the IFCC group focused on the possibility of harmonizing cTn I results through a recalibration process using suitable secondary reference materials. Results from a pilot study showed that prevailing differences among assays may be removed through a mathematical recalibration using the regression data of each assay against the medians of values obtained from the 16 commercial assays included in the study based on a panel of native serum samples [63]. This justified the notion that preparing serum pools with varying cTn I concentrations, assigned through a protocol transferring SRM 2921 trueness, and using them as common calibrators for field assays could improve measurement harmonization. This notwithstanding, several underlying problems delayed the project. Firstly, the cTn-positive patient samples required to produce pools were only available in limited supply (as only patients with AMI could donate blood posing potential ethical issues and opposing the necessity for large quantities of serum (~5 L) at each cTn concentration level in order to yield a minimum five-year supply of the reference material. Secondly, cTn assays were rapidly developing, even changing internal standards and/or antibody combinations in order to improve their sensitivity. Thirdly, lacking a higher-order reference procedure, the only possible approach in maintaining traceability to the International System of Units (SI) is to use a relatively complex protocol for transferring trueness, involving from commercial assays only those for which SRM 2921 was commutable. Similar to that made for the ERM-DA470/IFCC reference material for plasma proteins, the value transfer protocol should consist of gravimetric dilutions of pure material (SRM 2921) and of candidate serum-based reference pools [70]. If matrix effects are not present (i.e., both SRM 2921 and reference pools are commutable for assays employed in the value transfer), the arithmetic plots of dilutions of the two materials should give two diverging lines, each passing through zero. If one of the two materials is used as the assay calibrator (SRM 2921 for cTn I), a single line of proportionality is obtained which can be used to assign SI-traceable values to the second other.

CCLM and the quality of troponin measurements: a long and fruitful partnership

Over the last two decades, the requirements regarding the clinical suitability of analytical performance in cTn assays have become more stringent in parallel with the assay evolution and the pivotal role assumed by this biomarker in patient care. *CCLM* has paralleled this progress contributing to each stage of development described in Figure 3 with landmark papers. At the start of the new century, the 'IFCC Quality Specifications' paper first elaborated the theory on the issues which need addressing where quality of cTn measurements is considered [71]. This began the decade of the 'experimental age' where discussions about metrics application and statistical approaches became central [62, 72, 73]. As a consequence, in the ensuing decade, models for validating the clinical suitability of cTn assay performance were defined. In so doing, the EFLM made a landmark

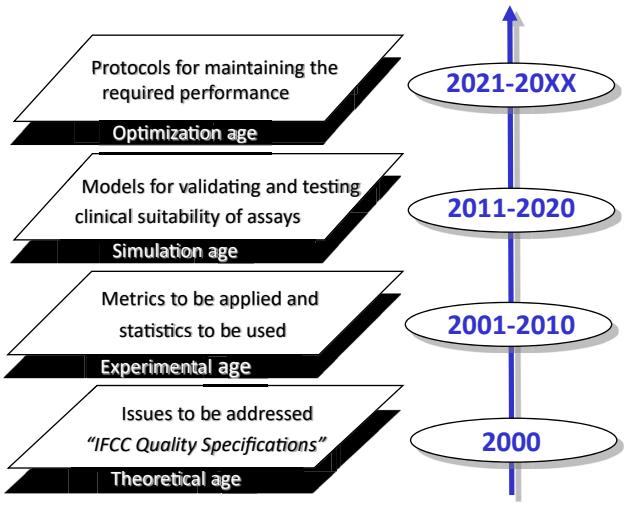

Figure 3: Ages of cardiac troponin analytical performance requirements.

contribution in organizing their 2014 conference in which a consensus was reached in defining models for establishing APS [37, 39]. Concomitantly, some further practical educational assistance was also provided [74, 75]. Finally, in this current paper, we focus on how to best estimate and maintain the required quality performance in daily practice, hopefully bringing greater order to the cosmos of hs-cTn literature. The successful implementation of suggested quality measures as part of standard laboratory practice will permit the delivery of sustained improvements not only in the quality of hs-cTn results but also in ensuring the reliability of clinical pathways.

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