Review

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Laboratory-related issues in the measurement of cardiac troponins with highly sensitive assays

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Abstract: A number of assay-related issues can affect the performance of cardiac troponin (cTn) measurement in everyday practice. In this respect, it is vital that all information on cTn assays is known and that the performance characteristics of assays are objectively assessed and adequately described. The advent of the latest generation of more sensitive cTn assays has heralded a new wave of information about low concentrations of cTn in blood. These recent generation assays have improved analytical sensitivity and corresponding performance at low cTn concentrations when compared to their predecessors, providing a convincing goal for laboratory medicine in helping clinicians in the diagnosis of acute myocardial infarction. Crucial to the clinical utility of highly sensitive cTn assays is the laboratorians' role in closely scrutinizing proposed assays and defining their value in relation to available evidence. Analytical, as well as pre-analytical and post-analytical, aspects must be documented. In this review, we describe what laboratory professionals should know about their cTn assay performance characteristics and the pre-analytical prerequisites for robustness to ensure optimal post-analytical reporting.

Keywords: cardiac troponin; highly sensitive assays; laboratory issues.

Introduction

The implementation of cardiac troponin (cTn) assays has played a groundbreaking role in the diagnosis, risk stratification and management of patients with suspected acute myocardial infarction (AMI), stimulating a rapid evolution in cTn testing and concomitant innovations in clinical guidelines. Despite the performance of more highly sensitive cTn (hs-cTn) assays being infinitely superior to their predecessors, the issues relating to their introduction cannot be depreciated. In this regard, the role of medical laboratories is central as a number of assay-related issues can markedly affect the performance of cTn measurement in everyday practice [1]. Following the introduction of hs-cTn, it is therefore of vital importance to ensure that all analytical characteristics are specifically verified and that their performance is objectively assessed and adequately described. In this review, we discuss the fundamentals which laboratory professionals should know of hs-cTn assay performance characteristics and the pre-analytical prerequisites for robustness to ensure optimal post-analytical reporting.

Using hs-cTn assays: a way of no return

Due to their superior diagnostic accuracy, cTn tests have largely replaced other non-specific biomarkers, such as creatine kinase MB or myoglobin, for diagnosing suspected AMI [2]. The new millennium criteria for AMI and subsequent updates to the "universal definition of myocardial infarction" have strengthened the role of cTn as the preferred biomarker for the evaluation of AMI [3–5]. Afterward, the intensive efforts of in vitro diagnostics (IVD) manufacturers, aimed at improving assays for cTn measurement, resulted in the advent of hs-cTn assays, leading to the attendant laboratory scenario where several different assays are now available on the market. Paradoxically, the rapid evolution of assays has created an uncertainty among cardiologists regarding the clinical interpretation of cTn elevations [6]. Hence, many clinicians did not enthusiastically welcome more sensitive assays for cTn measurement and voiced concerns have appeared in clinical practice. The reaction of clinicians to hs-cTn assays is perfectly illustrated in the Robert Jesse's statement: "when troponin was a lousy assay it was a great test, but

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now that it's becoming a great assay, it's getting to be a lousy test" [7]. According to data from a survey carried out in 2016 involving ~1900 medical centers in 23 countries evenly distributed across five continents, 41% of them adopted hs-cTn assays, varying from 60% in Europe to only 7% in North America [8]. More recently, the fourth universal definition of AMI, in providing practical updates to the previous 2012 version, has for the first time explicitly recommended the introduction of hs-cTn assays for their ability to detect even very small amounts of myocardial necrosis, expanding the use of cTn testing from the classical AMI diagnosis to the broad detection of myocardial injury of any cause [9]. This recommendation should theoretically have a global practice-changing impact, impelling medical laboratories to a rapid adoption of hs-cTn assays worldwide. The recent, as yet unpublished, update of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) initiative on the Cardiac Marker Guideline Uptake in Europe (CARMAGUE) confirms this trend, as approximately two-thirds of 439 surveyed European laboratories declared to use cTn as a first-line test for suspected acute coronary syndrome (ACS), with most using hs-cTn assays (Päivi Laitinen, presented at the 23th IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine, May 2019).

Notably, measurement of cTn using highly sensitive assays reflects acknowledgment of scientific progress, and a universal acceptance of their use in diagnosing AMI appears inevitable. The difficulty lies in deciding how best to use this high-quality and previously unobtainable information. For sure, the introduction of hs-cTn assays requires changes to diagnostic rules and algorithms compelling laboratory professionals to ensure the safe clinical application of international recommendations on its use [10, 11]. In contrast to a number of clinical guidance documents, there is still an insufficiency of laboratory practice recommendations on the use of hs-cTn assays. A special report recently issued jointly by a panel from the Academy of the American Association for Clinical Chemistry and the IFCC Task Force on the Clinical Application of Cardiac Bio-Markers constitutes substantial progress in this important but still neglected issue [11]. Among others, the group raises the issue of communication with clinicians and the importance of laboratory in educating clinicians about the influence of pre-analytical and analytical problems potentially confounding hs-cTn assay results [11]. To ensure optimal use of hs-cTn assays in clinical practice, the role of the laboratory is crucial indeed and should cover all aspects of the total examination process, from the pre-analytical to the post-analytical phase [12].

The post-analytical phase: improving results' interpretation

A special scrutiny concerns the post-analytical phase as an appropriate interpretation of hs-cTn results may directly influence major clinical decisions involving diagnosis, classification, monitoring and treatment of patients. The improved analytical sensitivity of hs-cTn assays has distinctly reinforced the evidence that the 99th percentile upper reference limit (URL), when applied as decision limit to a single result, is not more practically functional in the diagnosis of AMI [13]. The restricted utility of the 99th percentile URL has been demonstrated in a study by Ungerer et al. [14]. Of 150 contributory samples to the 99th percentile cut-off, only 20% were shared across three hs-cTn assays. These assays revealed pronounced disparities in samples with increased cTn concentrations, thereby precluding an agreement in clinical classification of patients in the evaluated cohort by using respective 99th percentile URLs [14]. Similar results were more recently published by Clerico et al. [15]. Studies on the biological variation of cTns have emphasized a very high individuality of these biomarkers, expressed as a low index of individuality, confirming major limitations for the use of population-based reference limits [16]. Accordingly, only serial testing in the same individual allows for the discrimination of pathophysiological mechanisms of cTn release [17]. Scrutiny of cTn release kinetics in blood is essential to differentiate acute from chronic myocardial damage and may help to understand the characteristics of underlying processes associated with cTn release [13]. Importantly, if we dichotomize typical/atypical cTn curves (based on 2–3 serial measurements) based on their ability to detect acute damage, a decisional criterion, related to the entity of cTn increase (i.e. the cTn delta change), should be defined to characterize cTn patterns indicative of acute myocardial necrosis [13]. However, though 70% of surveyed laboratories in Europe confirmed the correct use of cTn serial testing, the same number of laboratories stated that no criterion was used for the interpretation of biomarker's changes over time [18]. These observations highlight that many laboratories, which have introduced and currently employ hs-cTn assays, are not rigorous in the application of optimal approaches for the management of AMI [19–21]. The laboratory's attention should therefore better focus on how to facilitate the interpretation of hs-cTn results and correctly adhere to current clinical guidelines, for example, helping clinicians to understand significant hs-cTn changes in the same patient using the electronic laboratory reporting system for automated delta change calculation.

Another undervalued but important issue is the correct use of measurement units in reporting cTn test results. A non-harmonized use of measurement units and unnecessary use of decimal numbers are potential sources of medical errors [22]. So, whenever possible, laboratory results should be presented as whole numbers. In avoiding the unnecessary use of decimals, which is a matter of patient safety, the use of ng/L as unit for cTn tests, which is also acceptable to the International System (SI) of measurement, is strongly recommended, regardless of the analytical sensitivity of the assay used [23]. Despite this, 43% of laboratories surveyed by the CARMAGUE initiative are still using µg/L, followed by two or three decimal numbers.

Optimizing analytical quality

The introduction of hs-cTn assays has substantially increased the accuracy of AMI detection at presentation to the emergency department (ED). The sensitive quantification of cardiomyocyte injury has enabled the development and validation of rapid diagnostic algorithms, which substantially improves the early rule-out or rule-in strategies [24]. In general, in patients admitted to the ED with symptoms suspicious for ACS, the diagnosis of non-ST-segment elevation AMI (NSTEMI) can be ruled out immediately on presentation, with a negative predictive value of >99.5% and a sensitivity of >99%, if the hs-cTn concentration is undetectable [25, 26]. On the contrary, hs-cTn concentration multiples of the assay's 99th percentile URL support a diagnosis of NSTEMI with a high positive predictive value (>85%), when the test results are evaluated in an appropriate clinical context [27, 28]. For hs-cTn values ranging from detectable concentrations to multiples of the 99th percentile URL, decision can be made based on hs-cTn retesting at 1, 2 or 3 h later, depending on the protocol used (Figure 1) [24].

In this clinical scenario, accurate calibration of hs-cTn assays in the low range of concentrations and a low analytical variation are of the utmost importance as even relatively small changes in assay performance in practice may significantly influence the proportion of patients who could be identified as suitable for discharge. It necessitates laboratories to engage in additional activities to ensure proper quality performance for hs-cTn measurements [11]. The tools that laboratories need to check performance at the low end of measuring range of hs-Tn assays are summarized in Table 1 [10, 29].

In general, calibration verification means the assaying of control materials of known concentration to substantiate the acceptability of the measuring system's alignment and, consequently, of patient results [30]. However, control materials offered by the manufacturers together with hs-cTn assays do not usually cover low cTn concentrations, leaving the assay vulnerable to potential drifts that may pass unnoticed. Aloisio et al. recently showed that daily monitoring of an in-house made serum pool with a concentration near the limit of detection (LoD) of the hs-cTn assay, used as an additional control material besides those offered by the manufacturer, improved the measurement accuracy of hs-cTn assays at low but clinically relevant concentrations [31]. Particularly, the laboratory results of the UK NEQAS Cardiac Markers External Quality Assessment (EQA) program, which includes a "low concentration sample" with cTn concentrations lower than the 99th percentile URL, were substantially better after the introduction of this additional quality control tool (the number of

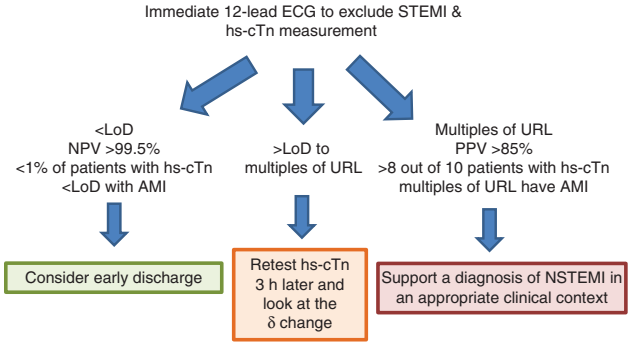

Figure 1: Decisional tree for optimal use of highly sensitive cardiac troponin assays in patients admitted to the emergency department with suspected acute myocardial infarction.
ECG, electrocardiogram; STEMI, ST-elevation myocardial infarction; hs-cTn, highly sensitive cardiac troponin assay; LoD, limit of detection of hs-cTn assay (i.e. the lowest amount of troponin in a biological sample that can be detected by the assay); NPV, negative predictive value; AMI, acute myocardial infarction; URL, upper reference limit defined at the 99th percentile of reference value distribution; PPV, positive predictive value; NSTEMI, non-ST-segment elevation myocardial infarction.

Table 1: Recommended tools which medical laboratories should use to check the analytical performance at the low end of measuring range of their highly sensitive troponin (hs-cTn) assays.

– A control material or patient pool with an hs-cTn concentration near the assay limit of detection to monitor baseline drifts following assay calibration
– A low-level control material with a cTn concentration close to the 99th percentile upper reference limit to monitor assay variability at this decision level
– Calibration frequency to be determined based on the imprecision performance and drift characteristics of the assay

failed results in a 2-year period decreased from 40% to less than 4%) [31]. Other authors also demonstrated that, when using a control material with hs-cTn concentrations close to the LoD, they were able to demonstrate significant differences (up to 100%) in results between similar platforms from the same manufacturer [32]. On the contrary, no differences were seen using control materials with concentrations near to the assay's 99th percentile URL.

On the other hand, it is also important that laboratories evaluate the reproducibility of their hs-cTn assay at concentrations close to the 99th percentile URL to derive the measurement uncertainty at this decision level [33, 34]. This information should be obtained over a period (e.g. 6 consecutive months) sufficient to capture most changes to measuring conditions and systematic sources of uncertainty, such as those caused by different lots of reagents, different calibrations or different environmental conditions [35]. The characteristics of control material for estimating measurement uncertainty have been defined and should be carefully considered, i.e. the material should be different from that used to check the correct alignment of the measuring system, be commutable and with concentration corresponding to the decision cut-point employed in the medical application of the test [36]. The true test of how well measurement uncertainty holds up is therefore when hs-cTn assays are evaluated in daily practice and control materials closely resemble authentic patient samples [37]. Hage-Sleiman et al. [38] have elegantly shown the misleading results obtained in the estimate of hs-cTn precision profile when using a non-commutable control material. The UK Association for Clinical Biochemistry and Laboratory Medicine has endorsed the use of a third-party internal quality control material at or near the 99th percentile URL to verify assay reproducibility. Despite this recommendation, only 7% of surveyed laboratories in the UK were running a quality control sample with these characteristics [39].

Talking about the analytical performance of hs-cTn assays poses the vital question as to the requisite degree of quality needed and to what extent measurement uncertainty is tolerable without jeopardizing patient safety [40]. The basic concept underlying this issue arose from the EFLM Strategic Conference held in Milan in 2014, which defined analytical performance specifications (APSs) according to different models [41, 42]. Notably, cTn should be considered as an analyte for which the model based on the effect of analytical performance on clinical outcomes should be applied in order to define APS [43]. This model should be used when the measurand has a central and well-defined role in decision-making for specific disease or a given clinical situation, and test results should be interpreted through established criteria, and applies to measurands for which results would strongly influence patient's outcome, e.g. in terms of permissible misclassification rates [43]. These measurand's characteristics perfectly applies to cTn and its use in ACS decision-making.

Sheehan et al. [44] first demonstrated the effect of analytical performance of cTn measurement on diagnostic misclassification. Performing duplicate cTn measurements, these authors calculated the frequency at which the result of the second replicate fell in a different diagnostic category according to a predefined cut-off, thus defining the percentage of misclassified patients with suspected AMI based on assay imprecision (assuming unbiased results). As expected, the frequency of misclassification rose together with the assay CV. This approach was fully transcribed into recommendations made in 2012 by the Australasian Association of Clinical Biochemists, where the imprecision goal was modulated for achieving a predefined acceptable rate of misclassification. Assuming as acceptable diagnostic misclassification rates of 1.8%, 1.0% and 0.5%, the corresponding APSs for cTn imprecision (as CV) derived from this outcome-based model were <13%, <10% and <6%, respectively [45]. More recently, Lyon et al. evaluated the combined influence of assay variability and bias on the number of false-positive and false-negative results for AMI diagnosis, using simulation models at hs-cTn 99th percentile URL [46]. A false-positive rate of approximately 1% was obtained when both bias and imprecision (as CV) of measurements were kept around 10% [46]. Table 2 summarizes the current recommendations

Table 2: Analytical performance specifications (APSs) for troponin measurement using highly sensitive assays (hs-cTn) and recommended approaches to optimize their analytical quality.

- hs-cTn APS must be targeted at the concentration corresponding to the assay's 99th percentile upper reference limit.
- A measurement uncertainty ≤10% at this marker concentration represents the desirable goal. According to the classical Fraser's paradigm for deriving APS for random variability [47], the quality level can be modulated to minimum [10% + ½ 10% = 15%] and optimal [½ 10% = 5%] goals.
- If a bias greater than ±10% is detected in the quality control, a readjustment of the measuring system must be undertaken to correct it. If the bias remains, the manufacturer should be requested to take an immediate investigation and eventually fix the problem with a corrective action, e.g. a process of reassigning the manufacturer's calibrators for correcting the detected bias.

about APS for hs-cTn measurements based on the available information from studies using the outcome-based model and related approaches to optimize their analytical quality. In addition, for result interpretation in EQA, when control materials are measured by participants in singlicate and results include effects of both bias and imprecision errors, a total error specification of maximum ±22.5% is recommended [48].

Understanding the impact of pre-analytical and analytical interferences

It is well known that the sample matrix-related issues, which result from differences between serum and plasma, and the use of different anticoagulants may negatively affect the accuracy of cTn results and should be thoroughly investigated [49]. Plasma is usually the matrix of choice in order to ensure timeliness in patients with suspected AMI in ED. The use of plasma reduces the turnaround time (TAT) of sample treatment by eliminating clotting time and avoids potential problems associated with prolonged clotting time in patients receiving anticoagulation therapy or with coagulation abnormality (e.g. liver failure). The use of serum also has limitations associated with the possible presence of fibrin clots due to insufficient centrifugation of the tube or centrifugation prior to clot retraction.

Studies using previous generations of cTn assays already revealed significant differences in the 99th percentile URLs derived in cardio-healthy reference populations when both serum and heparin plasma were used [50]. When hs-cTn assays were introduced, similar investigations confirmed the frequent presence of some bias when comparing serum with plasma samples. Results from a multicenter analytical evaluation of Roche Diagnostics hs-cTn T assay showed a mean difference of −4% in cTn T values when lithium heparin plasma was compared with serum [51]. The inter-sample bias was also an issue for Abbott Diagnostics hs-cTn I assay, because mean differences in hs-cTn I concentrations of −5.7% and 17.4% were observed between serum and EDTA plasma and serum and lithium heparin plasma, respectively [52]. Both manufacturers in their package inserts list however as acceptable for hs-Tn determination both serum and plasma (heparin and EDTA), simply advising that the sample types are not interchangeable. Interestingly, they report as an acceptability criterion for sample validation a correlation slope ranging from 0.8 to 1.2, corresponding to a systematic bias of ±20% in results, well above the desirable APS recommended in Table 2 [53]. The analytical impact of the use of samples different from those used in the characterization of hs-cTn assays (e.g. serum for Roche hs-cTn T and heparin plasma for Abbott hs-cTn I) may be therefore noticeable and should require appropriate assay (re)validation and, more importantly, the redefinition of clinical thresholds specific for each employed sample. Incidentally, Katrukha et al., using a proteomic analysis, showed a consistent difference in the composition of cTn T forms in simultaneously collected serum and heparin plasma samples from the same patients with AMI [54]. While heparin plasma samples contained full-sized cTn T (~35 kDa), in serum samples cTn T was present in a 29-kDa form as a probable result of thrombin-mediated cleavage of cTn T molecule occurring *in vitro* during the clotting process.

Apart from sample matrix-related differences, other pre-analytical and analytical interferents can lead to unreliable hs-cTn results. Hemolyzed samples, though very undesirable, are common in daily laboratory practice with a prevalence ranging from 3.3% of all routine samples to more than 12% in samples coming from ED [55, 56]. Significantly decreased results due to a moderate degree of hemolysis (free hemoglobin concentration >1 g/L) have been reported using cTn T assay and, more recently, confirmed for hs-cTn T assays, potentially leading to unrecognized myocardial injury [57–59]. Some evidence suggests that intracellular proteases like cathepsin E, released from erythrocytes when a sample is hemolyzed, may degrade cTn T leading to a significant decrease in marker detection [60]. Laboratories should not only be aware of the possible influence of hemolysis, but also of the issues dealing with its accurate detection and reporting. Unfortunately, practices for identifying and rejecting hemolyzed specimens are highly variable in medical laboratories. According to a recent European survey, hemolysis is still identified using visual detection by 30% of laboratories and only 42% are using automated hemolysis index (HI) detection [61]. It is well demonstrated that visual examination of serum/plasma samples is neither accurate nor sensitive for detecting relatively low amount of hemolysis [62, 63]. By applying a risk management approach, Luksic et al. have elegantly shown that visual inspection for hemolysis detection increased the risk of reporting inaccurate cTn T results, potentially affecting clinical decision-making and patient outcome [64]. This emphasizes the need to introduce automated HI detection for accurately managing the possible impact of hemolysis by the automatic transmission of hemolysis degree to the laboratory

information system and to harmonize hemolysis detection strategies in medical laboratories by overcoming visual inspection and arbitrary judgment of sample quality [65]. The analytical performance of HI determination and the reproducibility of results appear satisfactory, providing that employed platforms are reporting HI results as continuous values [66].

Even when using the automated approach for HI detection, laboratories differ significantly in their practices for dealing with hemolyzed samples in cTn result reporting. According to a UK survey, 40% of laboratories utilizing hs-cTn T assay removed all results automatically when free hemoglobin is >1 g/L, whilst 10% reported annotated hs-cTn T results indicating possible interference [39]. Other authors have proposed tiered hemolysis thresholds suggesting a protocol for reporting or not hs-cTn T results dependent on marker concentrations [67]. For example, considering the significance of markedly elevated hs-cTn T concentrations at patient admission to the ED [27] and the inhibiting effect of hemolysis, values >100 ng/L could be reported even if the HI value is >100 (i.e. the critical limit for interference), while for hs-cTn T concentrations <100 ng/L, the results should be deleted and a repeat sample requested.

In general, interference thresholds for hemolysis are defined as the greatest concentration of free hemoglobin in the sample that does not compromise the accuracy of cTn results. Manufacturers define internally their acceptance criteria when evaluating and validating these interference thresholds. Although the declared hemolysis limit for marketed hs-cTn I assays is usually very high (up to 4–5 g/L of free hemoglobin), the employed acceptance criteria for interference bias are however as high as ±10%, which corresponds to the entire budget of allowable bias for hs-cTn measurements reported in Table 2 [68]. Consuming all the permissible bias in evaluating the hemolysis interference is therefore impractical and unsuitable in the context of the measurement quality. More correctly, the interference bias tolerated in the hemolysis interference studies should be a part of the total allowable bias and not exceeding a fraction of it [69].

As the LoD of hs-cTn assays is significantly lowered, it is necessary to carefully consider potential sources of non-specificity that have not been frequently detected in previous generations of less sensitive assays [70]. Inconsistent profiles of patient's test results, unexplained change in hs-cTn concentrations or disproportionate increases in measured values should suggest investigating interferences in suspected samples. Heterophile antibodies (HAs) remain the most frequently reported confounding factor giving both false-positive and false-negative hs-cTn results [71]. If an HA interference is suspected, the laboratories should implement appropriate strategies for detecting these interferents by: (a) checking linearity following serial dilutions, (b) reanalyzing samples using a different measuring system, or (c) measuring hs-cTn concentrations before and after the use of HA blocking reagents [29]. Although manufacturers have optimized their hs-cTn assays to reduce HA interferences by using chimeric human-mouse antibodies and by the addition of HA blocking agents to assay reagents, the occurrence of this interference has not been completely eliminated [72].

False-positive hs-cTn results may also be attributed to the presence of complexes between antibodies and cTn, resulting in a delayed clearance of the macrocomplex from the circulation. Initially recognized by Plebani et al. [73], the presence of macrotroponin I was identified in 5% of patients with elevated hs-cTn I values, when measured with the Abbott Architect assay; but given the biological characteristics of the interferent, the investigated assay may not be the only hs-cTn I assay affected by this problem [74]. A case of macrotroponin T has been also reported [75]. The presence of macrotroponin (I or T) in a suspected sample can be demonstrated by using the classical polyethylene glycol 6000 precipitation [76].

Biotin interference, potentially affecting immunoassays employing streptavidin-biotin binding technology, has been recently widely debated [77]. This assay architecture is used in ~60% of marketed automated immunoassays (bioMérieux, Ortho Clinical Diagnostics, Roche Diagnostics, Siemens Healthcare Diagnostics, etc.) as the highly specific interaction of streptavidin with biotinylated antibodies aids in the detection and targeting of biological measurands [78]. In blood samples from individuals taking biotin supplements, free biotin molecules can compete with the ability of streptavidin-coated microparticles to bind the biotinylated antibodies that have captured the target analyte, leading to inaccurate measurements [79]. Multivitamin formulations (containing biotin in microgram amounts) do not elevate plasma biotin concentrations sufficiently to interfere with immunoassays. However, high-dose biotin supplements or novel treatments with very high biotin doses for patients with multiple sclerosis have increased concerns about biotin interference [80]. In streptavidin-biotin binding immunoassays using a sandwich format, such as hs-cTn assays, the presence of biotin may decrease the signal intensity producing falsely low results that may go undetected and lead to potentially serious clinical implications such as inappropriate patient discharge. However, biotin

interference thresholds between different hs-cTn assays can vary substantially, from 2.5 to 10,000 ng/mL [68, 80]. Frame et al. have experimentally shown a negative interference on hs-cTn T assay at biotin concentrations >30 ng/mL (approximately 10× lower than the fourth-generation cTn T assay), while the Abbott hs-cTn I assay was not affected by biotin concentrations in blood up to 2000 ng/mL, due to a different assay design [81]. Although these findings require caution in considering the impact of biotin intake on some hs-cTn assays, they did not elucidate the real clinical impact of this issue. The magnitude of biotin interference is dependent on: (a) type of assay (design, sample volume), (b) time since last biotin intake (maximum impact at 1–3 h after ingestion), (c) biotin dose and (d) kidney function (delayed clearance in case of renal impairment). Studies quantifying biotin concentrations in the ED setting estimated at 0.4–0.5% the prevalence of patients admitted with vitamin levels >30 ng/mL [82, 83]. Mumma et al. [84] carried out a simulation of the potential negative clinical impact of biotin interference on hs-cTn T in a population of 850 patients with suspected AMI (prevalence 15%). In this cohort, biotin concentrations exceeding 20 ng/mL were found in only one patient, with a prevalence of 0.7% at ED admission. Based on a 42% reduction of hs-cTn T recovery at 100 ng/mL biotin, the authors estimated the range between 19.0 and 45.2 ng/L as the hs-cTn T concentrations that could potentially lead to false-negative AMI prediction. As 25% of patients with AMI had results in this range, the likelihood of false-negative results due to biotin interference was estimated to be 0.026%. This suggests a low clinical impact, equating to less than three patients per 10,000 with AMI potentially being affected. More importantly, the manufacturer has recently worked by modifying the hs-cTn T assay to provide substantially higher tolerance to biotin interference, i.e. an hs-cTn T recovery ≥96% for biotin concentrations of 1250 ng/mL [85].

TAT: an additional indicator of hs-cTn assay performance

TAT is another important laboratory-related issue in hs-cTn measurements and their clinical use. For hs-cTn testing, a TAT ≤60 min from the time of receipt of blood tubes in the central laboratory to result reporting to clinical wards has been recommended [11]. Meeting appropriate TAT to ensure timeliness in reporting hs-cTn results is a prerequisite for the implementation of fast track algorithms currently recommended in clinical guidelines [86]. Several strategies to improve TAT have been undertaken, involving multidisciplinary teams acting from the pre-analytical to the post-analytical phase of the total examination process [87]. Decreasing TAT may require modifications of existing workflow, including the use of pneumatic tube transportation and computerized order entry management [88]. hs-cTn testing exploiting both these tools, combined with a dedicated path, may enable faster pre-analytical management of patient samples and the quicker availability of hs-cTn results. We demonstrated that this strategy may substantially decrease hs-cTn TAT and permit the achievement of the 60-min goal for almost all assayed samples [89, 90].

Point-of-care (POC) testing has been proposed as an alternative to meet patient care needs in situations when TAT requirements cannot be met with central laboratory systems [91]. However, a performance gap always historically existed between central laboratory systems and POC cTn testing in terms of analytical sensitivity [92–94]. More importantly, studies designed to compare POC technologies with central laboratory testing have shown that the lower clinical sensitivity of POC assays may result in a great number of misclassified patients [95]. Quite recently, novel highly sensitive technologies have been used to develop POC systems meeting hs-cTn assay criteria, providing promising perspectives in delivering accurate and quick hs-cTn results [96].

Definition of “high-sensitivity” cTn assays: do we really need it?

In 2015, an expert opinion conventionally designated as “high sensitivity” those cTn assays able to measure the biomarker at the 99th percentile URL concentration with a CV ≤10% and to detect results above the assay LoD in ≥50% of healthy individuals [97]. More recently, the second criterion became more stringent as the designated “high sensitive” assays should be able to measure cTn in at least 50% of healthy men and women *separately* [11]. This designation of cTn assays as being “high sensitive” has been however criticized because of substantial drawbacks [98]. By definition, the imprecision performance is strongly dependent upon the 99th percentile limit cTn concentration, and this may induce some authors to artificially inflate the 99th percentile value to obtain much favorable data owing to the relationship existing between the concentration of the analyte and the assay imprecision (i.e. the higher the concentration, the better the precision)

[29, 99]. More generally, the aforementioned definition lacks consistency and is affected by too many factors (such as experimental definition of LoD, selection of reference population, etc.) [100, 101]. For these reasons, an approach using evidence-based clinical information instead of one based on the number of healthy subjects with cTn concentrations >LoD should be preferable [10]. Overall, the need for a definition of "high sensitivity" cTn assays appears scientifically questionable. With the ongoing development of cTn assays with still greater analytical precision and lower detection limits, any absolute definition is probably rendered superfluous. Undeniably, cTn measurement is not a static science, but represents a dynamic laboratory and clinical activity, which may be affected by many pre-analytical, analytical and post-analytical factors [102].

Conclusions

Five years ago, in this journal we used the "high-sensitivity" cTn as a paradigm to show how laboratory medicine acts as the science that underpins medicine [13]. Crucial to the application of this biomarker and its recent measurement development is the laboratorians' role in closely scrutinizing proposed assays and defining their clinical application in relation to available evidence. Therefore, education of laboratorians is as important as educating clinicians. For laboratory professionals, a good understanding of hs-cTn assay-related issues and the correct monitoring of its performance are crucial for appropriate use and interpretation of hs-cTn results. Relevant education, ongoing training and proper communication in the use of hs-cTn assays are essential in delivering efficient, high-quality laboratory service and promoting concomitant better patient care.

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Article note: This paper is dedicated to the memory of Jillian R. Tate, a leader in the field of cardiac troponin science and an unforgettable friend.