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Editorial

How Clinical Laboratories May Improve Their Performance: The "High-Sensitivity" Troponin Paradigm

Mauro Panteghini[1*]

It is well known that a number of assay-related issues can affect the performance of cardiac troponin (cTn)[2] measurement in everyday practice. In this respect, it is vital that all information on the cTn assays is given and that performance characteristics of the 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. Those recent generation assays have improved analytical sensitivity and corresponding performance at low cTn concentrations compared with their predecessors, providing a convincing goal for laboratory medicine by allowing the safe clinical application of international recommendations for the definition of acute myocardial infarction (AMI) *(1)*.

Crucial to the utility of biomarkers is laboratorians' role in closely scrutinizing proposed assays and defining their clinical value according to the available evidence. Analytical, as well as preanalytical and postanalytical, aspects must be documented. Particularly, the introduction of so-called "high-sensitivity" cTn (hs-cTn) measurements should be pursued using only well-validated assays; the use of assays before their robust analytical and clinical validation should be discouraged *(2)*. Laboratory personnel should know their cTn assay performance characteristics and the preanalytical prerequisites for robustness to ensure optimal postanalytical reporting. In this issue of *Clinical Chemistry*, Wu et al. *(3)* highlight many important laboratory aspects of hs-cTn measurement. The document represents a laudable attempt by a group of laboratory experts to inform colleagues about these issues and to offer recommendations for improving current measurement practice.

Accurate calibration of hs-cTn assays in the low range of concentrations is of the upmost importance for clinical applications that rely on a single cTn measurement at admission *(2)*. Even relatively small analytical variations in practice may influence the proportion of patients who could be identified as suitable for discharge. Consequently, there are mandatory tools that laboratories would need to use to check the performance at the low end of measuring ranges of hs-cTn assays. Wu et al. *(3)* are correct in recommending (*a*) a low-level quality control material with cTn concentration close to the 99th percentile upper reference limit (URL) to monitor assay alignment at cutoff, and (*b*) a patient pool with an hs-cTn concentration close to the limit of detection (LoD) to monitor baseline drifts. In addition, long-term monitoring of imprecision across different reagent lots should also be carried out. I would also recommend that the calibration frequency should be based on the imprecision performance and drift characteristics of the specific assay.

According to the consensus established at the 2014 European Federation for Clinical Chemistry and Laboratory Medicine Strategic Conference for setting quality specifications in laboratory medicine, the best scientific approach to define analytical performance specifications (APS) for cTn should rely on data from clinical outcome studies *(4)*. Particularly, cTn APS can be defined in terms of permissible misclassification rates. Performing duplicate cTn measurements, Sheehan et al. *(5)* calculated the frequency with which the result of the second replicate fell in a different diagnostic group, thus defining the percentage of misclassified patients with suspected AMI based on assay imprecision. Recently, Lyon et al. *(6)* have applied a simulation model for estimating the misclassification rate of patients with suspected AMI when an hs-TnI assay in conjunction with its 99th percentile URL is used. A false-positive rate of approximately 1% was obtained when both bias and imprecision (as CV) of measurements were kept around 10%. Accordingly, Wu et al. should have been more decisive in recommending APS for cTn measurement at the concentration corresponding to the assay 99th percentile URL as a CV $<10\%$ and a bias within $\pm 10\%$. If a greater bias than $\pm 10\%$ is detected in the quality control, a readjustment of the measuring system must be undertaken to decrease it.

[1] Department of Biomedical and Clinical Sciences Luigi Sacco, University of Milan Medical School, Milan, Italy.
* Address correspondence to the author at: UOC Patologia Clinica, Ospedale Luigi Sacco, Via GB Grassi 74, 20157 Milano, Italy. Fax +39-02-3564018; e-mail mauro.panteghini@unimi.it.
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[2] Nonstandard abbreviations: cTn, cardiac troponin; AMI, acute myocardial infarction; hs, high sensitivity; URL, upper reference limit; LoD, limit of detection; APS, analytical performance specifications; eGFR, estimated glomerular filtration rate.

Regarding the reporting units, there was a recommendation from the IFCC supporting the use of nanograms per liter and whole numbers to which the authors did not refer *(7)*. It has been demonstrated that the use of decimal numbers may lead to misinterpretation of test results and is a potential source of medical errors *(8)*. Therefore, avoiding the unnecessary use of decimals is a matter of patient safety. Accordingly, it is unclear why the authors still support the unit option of micrograms per liter with 2 decimal points when "contemporary" assays are used. Units for cTn should be harmonized regardless of the analytical sensitivity of the assay used.

Depending on available resources and capability, it may not be practically feasible for a laboratory to determine its own cTn reference interval. It is easier to validate previously established reference limits that are appropriate for the laboratory. The validation can be done according to the Clinical and Laboratory Standards Institute (document C28-A3c, paragraph 11.2), by examining 20 apparently healthy individuals from a laboratory's own subject population *(9)*. In selecting these subjects, the exclusion criteria are of utmost importance. Among the surrogate biomarkers to be used, Wu et al. suggest relying on natriuretic peptides, glycohemoglobin, and (estimated) glomerular filtration rate (eGFR) *(3)*. However, they contradict the notion of recommending the selection of healthy individuals when individuals with prediabetes and/or eGFR until 60 mL/min/1.73 m^2 will be included. According to KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease *(10)*, the 60-mL/min/1.73 m^2 limit identifies persons with already mildly decreased GFR and a possible initial stage of chronic kidney disease. More importantly, the authors seem to ignore previously published data in which a slight decrease of eGFR was associated with a significant increase of hs-cTn. For instance, Martens et al. *(11)* showed that eGFR of 60 to 90 mL/min/1.73 m^2 compared with >90 mL/min/1.73 m^2 was associated with a 1.21 and 1.14 times higher hs-cTnT and hs-cTnI, respectively. These findings confirmed the previously published observations of Bjurman et al. *(12)*.

The improved analytical sensitivity of hs-cTn assays has reinforced the evidence that the 99th percentile decision limit, if applied to only 1 result, is not functional to the diagnosis of AMI, and only serial testing allows for the discrimination of acute from chronic pathophysiological mechanisms of cTn release *(1)*. This is supported by the evidence of high interindividual biological variability of cTn, which limits the clinical application of any fixed cutoff value, and by the wide disagreement between hs-cTn methods to identify patients above the 99th percentile cutoff *(13)*. In contrast with this evidence, Wu et al. seem to put too much emphasis on the concept of URL, including the long discussion about the need (or not) of sex-partitioned thresholds, leaving the central issue of the high individuality of the biomarker confined to a short sentence in the last paragraph of the document.

The authors' definition of hs-cTn assays (see recommendation 5) is subjective. Too many factors are influencing this definition (e.g., experimental definition of LoD, selection of population). Approaches using evidence-based information instead of those based on the number of healthy subjects with cTn > LoD are preferable. The UK National Institute for Health and Care Excellence guideline is a nice example, considering the available literature supporting the presumed high clinical sensitivity of the evaluated assays in a clinical setting (i.e., the ability to rule out AMI at hospital admission) *(14)*. The National Institute for Health and Care Excellence guideline showed that the Beckman Coulter assay, even if fulfilling the Wu et al. criteria of ≥50% of healthy values above LoD, could not be recommended at the time of guide release because robust scientific clinical data were lacking. On the other hand, according to the approach of Wu et al. *(3)*, an assay like the Roche Diagnostics Gen 5 cTnT, whose clinical performance is supported by an enormous amount of data *(14)*, would not be able to meet their recommendation *(15)*. Overall, the need of a definition of "high-sensitivity" cTn assays is scientifically questionable. In the near future, even more analytically sensitive cTn assays will certainly be developed, thus making any absolute high sensitivity designation obsolete. In perfect agreement with this view, the US Food and Drug Administration decided not to use the high sensitivity terminology when cTnT Gen 5 received clearance in January 2017.

During the past decade, the analytical performance of cTn assays has been continuously improving: Comparison of different generation assays clearly shows that there has been a marked improvement in the quality of the measurement offered by the newer assays. With some exceptions discussed in this editorial, the suggestions reported by Wu et al., if correctly applied, can contribute to alleviate inconsistencies and confusion that may exist for hs-cTn assays. To avoid the possibility for misinterpretation of a cTn result for patient care, one always has to keep in mind that the performance characteristics of the assays used should be adequately described. Clinical stakeholders who rely heavily on cTn measurement in medical decision-making will be substantially impacted by the quality of information regarding the assays used in clinical laboratories.

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