Editorial

The state-of-the-art of "high-sensitivity" immunoassay for measuring cardiac troponin I and T

Aldo Clerico[1], Giuseppe Lippi[2]

[1]Scuola Superiore Sant'Anna and Fondazione CNR Regione Toscana G. Monasterio, Pisa, Italy; [2]Section of Clinical Biochemistry, University of Verona, Verona, Italy

Correspondence to: Prof. Aldo Clerico. Scuola Superiore Sant'Anna and Fondazione CNR Regione Toscana G. Monasterio, Via Moruzzi 1, 56126 Pisa, Italy. Email: clerico@ftgm.it.

Comment on: Wu AHB, Christenson RH, Greene DN, *et al.* Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem 2018;64:645-55.

Received: 16 May 2018; Accepted: 30 May 2018; Published: 14 June 2018.

doi: 10.21037/jlpm.2018.05.04

View this article at: http://dx.doi.org/10.21037/jlpm.2018.05.04

The international guidelines "on the Redefinition of AMI", published in 2000 by the Joint European Society of Cardiology/American College of Cardiology (ESC/ACC) Committee, for the first time recommended that an increase of cardiac troponin I (cTnI) or T (cTnT) values over the 99th percentile upper reference limit (99th URL) should be considered as clinically significant, and they also specifically indicated that this thresholds value should be measured with an imprecision ≤10 coefficient of variation (CV%) (1). More recently, also some guidelines regarding the Universal Definition of Myocardial Infarction, edited by the joint European Society of Cardiology/American College of Cardiology/American Heart Association/World Heart Foundation (ESC/ACCF/AHA/WHF) task force from 2007 (2) to 2012 (3), confirmed that cTnI and cTnT are the preferred biomarkers for differential diagnosis of acute coronary syndrome (ACS), and also that the 99th URL value should be measured with an imprecision ≤10 CV%.

The measurement of the 99th URL of cTnI and cTnT is an analytical challenge due to low biomarker concentrations in healthy subjects (4,5). Considering that the minimum plasma or serum volume needed for measuring cardiac troponins on automated immunochemistry platforms usually spans between 10–100 µL, an immunoassay for cTnI or cTnT should detect with high analytical confidence an amount of 1–5 ng/L (or even less) of protein in order to satisfy the quality specifications endorsed by international guidelines (4,5). Only after the year 2006 some manufacturers commercialized the first new generation of cTnI and cTnT immunoassays characterized by improved analytical sensitivity meeting the quality specifications recommended by international guidelines and consensus documents (4,6). The new generation of immunoassays for cardiac troponin measurement, completely fulfilling these quality specification requirements, were then classified as "high-sensitivity" (HS) techniques.

In 2012, the Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care proposed to label "HS" assay those cTn methods meeting the quality specifications recommended by international guidelines (7). Accordingly, Apple and Collinson (6) pointed out that two basic criteria are needed to label as "HS" a cardiac troponin assay: (I) total imprecision (expressed as CV %) at the 99th percentile value should be ≤10%; (II) measurable concentrations below the 99th percentile should be attainable with an assay at a concentration value above the assay limit of detection for at least 50% (and ideally 95%) of healthy individuals. The amount of measurable values in healthy subjects also contributed to developing a subclassification of HS immunoassays (i.e., from first to fourth generation, with fourth generation immunoassays capable to measure cardiac troponins in virtually all ostensibly healthy subjects). Three authoritative documents have been very recently published on HS cardiac troponin immunoassays (8-10).

Notably, neither US (i.e., Food and Drug Administration;

FDA) nor European (i.e., European Community; CE) institutions have so far identified definitive criteria for labeling cardiac troponin immunoassays with a "HS" designation. In some documents published between 2015 and 2017, the international Federation of Clinical Biochemistry IFCC Task Force on Clinical Applications of Cardiac Bio-Markers (IFCC TF-CB) has endorsed that HS cardiac troponin immunoassays shall aim to measure concentrations at or above the limit of detection (LoD) in ≥50% of an overall group of combined apparently healthy men and women (9,11). In 2018, the expert opinion from the American Association of Clinical Chemistry (AACC) and the IFCC TF-CB (8) suggests to better specify the quality specifications for HS designation. This document recommends that assays unable to detect concentrations at or above the LoD in at least 50% of healthy subjects be labeled as contemporary cardiac troponin immunoassays (8). This recommendation better specifies the second criteria (of the two) previously suggested by Apple and Collinson (6). Indeed, the AACC and IFCC TF-CB document specifically states that two large distinct populations (sample size >300) should be tested, one including women and the other men, rather than a mixed population including both sexes, as in previous documents (6,9,11). For the first time, an international guideline actually stated that HS assays should detect concentrations ≥ LoD in at least 50% of healthy women. This is an important statement, because women usually have lower cTnI and cTnT circulating levels than men of similar age (12). Furthermore, the AACC and IFCC TF-CB document proposed that data to support these claims should be published in peer-reviewed journals, as well as contained in manufacturer's package inserts (8).

In conclusion, taking into considerations the most recent international documents and guidelines (6-9,11), two basic criteria are needed to label as HS a cardiac troponin immunoassay: (I) total imprecision (expressed as CV %) at the 99th percentile value should be ≤10%; (II) measurable concentrations below the 99th percentile URL should be attainable at a concentration value above the assay LoD for at least 50% of adult healthy men and women.

The IFCC TF-CB recommended the LoD as the lowest reportable limit for determining the HS immunoassay designation, according to both International Union of Pure and Applied Chemistry (IUPAC) and International Organization for Standardization (ISO) documents (11). However, the US FDA currently considers the limit of quantitation (LoQ; typically the 20% CV concentration) as the analytical sensitivity parameter for cardiac troponin immunoassays instead of LoD (8). Accordingly, the most recent AACC and IFCC TF-CB document (8) recommends that "during initiation of hs-cTn testing, clinical laboratories should validate the limit of blank (LoB), LoD outside the US, or LoQ as applicable per FDA regulations in the US. These analytical parameters should be validated minimally on an annual basis or more frequently as deemed necessary".

In *Table 1*, we report the LoB, LoD, LoQ values of the last generation of the most popular immunoassays adapted on fully automated platforms, which have been more recently marketed in Europe for both cTnI and cTnT immunoassay. The data reported (except for one method) (13-17) were generated in a single laboratory using the same international standardized protocols (18,19) to allow results comparability. This data indicates that the analytical performances of cTnI and cTnT immunoassays are now greatly improved. Some cTnI methods actually measure the 99th URL values with an error (i.e., between 4 CV% and 6 CV%) about half of that required by international guidelines (12,14,16). These results demonstrate that some cTnI immunoassays currently commercially available in Europe are able to fully satisfy the first criteria endorsed by international guidelines for a HS technique (6-9,11).

Only for the immunoassay system based on ARCHITECT platform (STAT Architect HS cTnI, Abbott Diagnostics, Ref. B3P250) statistically robust evidences exists demonstrating that this method detects the 99th URL with a value above the assay LoD in >50% of healthy adult women (12). Consequently, only this method should be currently considered an HS assay according to the two criteria proposed by the AACC and IFCC TF-CB document (8). For other cTnI immunoassays described in *Table 1*, larger population studies are needed to demonstrate they also fulfill the second criterion required by international guidelines, albeit the results of some separate investigations are seemingly attesting that this target may be met, at least using another immunoassay (20).

According to the most recent guidelines (8), evaluation and comparison of the analytical performance of cTnI and cTnT immunoassays, especially within the normal concentrations, is critically important for both laboratory professionals and clinicians. Unfortunately, the accurate analytical evaluation of cardiac troponin immunoassays is challenging, due to very low cardiac troponin concentrations in healthy subjects (especially in adult women) (4) (*Table 1*), and also because performing an evaluation according to international standardized protocols is expensive and time-

Table 1 Comparison of analytical sensitivity parameters of the most recent immunoassay methods for cTni and cTnT assay

Method	LoB (ng/L)	LoD (ng/L)	LoQ 20% CV (ng/L)	LoQ 10%CV (ng/L)	Ratio*	Reference
cTnI						
Architect	0.7	1.3	1.8	4.7	5	(13,14)
Vidas	0–1.9	1.3–3.2	2.9–4.9	13.1	1.5	Manufacturer's data
Access DxI	0.6	1.3	2.1	5.3	4	(13)
ADVIA	1.0	2.2	3.5	8.4	5	(15)
AIA	1.1	2.1	15.0	30.9	1	(16)
cTnT						
ECLIA	3	3–5	6	13	1.3	(17)

All data reported in this Table have been obtained in the Clinical Laboratory of the Fondazione CNR and Regione Toscana G. Monasterio, Pisa, Italy (with the exclusion of data regarding Vidas method). *, Ratio between 99th URL value, suggested by the manufacturer, and LoQ 10% CV value. Architect, STAT Architect high sensitivity TnI method using the ARCHITECT i1000SR platform (Ref. B3P250, Abbott Diagnostics, Abbott Park, USA); Access DxI, access hsTnI using DXi 800 platform (Ref. B52699, Beckman Coulter, Inc. Brea, CA 92821 USA); Vidas, high sensitive troponin I, using VIDAS platform (Biomérieux, Marcy l'Etoile, France); ADVIA, ADVIA Centaur TNIH using Centaur XPT platform (Siemens Healthineers Diagnostics, Erlangen, Germany); AIA, ST AIA-PACK cTnI 3rd-Gen (Ref. 0025215, TOSOH CORPORATION, Tokyo, Japan).

consuming (18,19).

An accurate evaluation of LoB, LoD and LoQ values actually requires the preparation of several plasma samples (usually pools) obtained from both healthy subjects and patients with cardiac disease, which should be evaluated throughout 60 consecutive working days using at least two different list of calibrators and reagents (13-20). Furthermore, some Authors (21,22) have raised concerns about the original definition of LoB and LoD proposed by IUPAC (23**)**. Indeed, there are some theoretical assumptions related to this definition, which are not usually satisfied in laboratory practice (21,22). Considering the particular case of cardiac troponin immunoassays on fully automated platforms, it is difficult to find (from the manufacturer) or prepare a sample (in the laboratory), which should be considered a "blank of the method" for calculation of LoB according to both IUPAC (23) and CLSI documents (17,19). Some Authors proposed that these theoretical and analytical drawbacks may actually limit the use of LoD as an accurate uncertainty paradigm of detection capability, especially for cardiac troponin methods (21,22). The use of LoQ (at 20 CV%) as lower limit of detection for cardiac troponin immunoassays may have advantages compared to LoD. The LoQ is usually calculated without theoretical or analytical problems from imprecision profile by using several plasma samples (or pools), repeatedly measured in different runs (more than 25 replicates), using different lots of calibrators and reagent materials throughout 60 working days (19,24). Moreover, the measurement error of LoQ is set at 20 CV%, whilst the measurement error of LoD is largely heterogeneous between cTnI and cTnT methods, and is much higher than 20 CV%.

Mair *et al.* (10), on behalf of The ESC Study Group on Biomarkers in Cardiology of the Acute Cardiovascular Care Association (ACCA) have very recently discussed some controversial aspects of degradation, tissue release and elimination from the human circulation of cTnI and TnT, reinforcing previous data published by Lippi *et al.* in 2012 (25). Some biomarkers values above the 99th URL, which are difficult to be clinically interpreted, are increasingly seen due to widespread use of HS cardiac troponin immunoassay (10). In individuals with very small myocardial tissue damage, the anatomical alterations responsible of troponin release cannot be found even using high-resolution cardiac imaging techniques, because the currently available imaging technologies are less sensitive than HS cardiac troponin immunoassays (4,5,10). However, different data supporting alternative mechanisms of cardiac troponin release from injured cardiomyocytes have been published, including apoptosis and reversible damage of cytoplasmic membrane (4,5,10,26). Whether cTnI and cTnT (as intact proteins or in form of highly degraded peptides) can be released from reversibly injured cardiomyocytes still remains one of the most controversial

topics in the area of cardiac biomarkers (4,5,10,26). Although myocardial necrosis is the leading cause of cardiac troponin increase in the vast majority of patients, Mair *et al.* (10) suggest that clinicians should be cautious in using biomarker increases as direct proof of myocardial necrosis, particularly when detecting only modest elevations above the URL of cTnI and cTnT, using HS immunoassays. Physical exercise is a paradigmatic example, since the release of cardiac troponins from the intensively contracting myocardium is directly dependent to exercise intensity (27).

Regardless of the mechanisms underlying increased biomarker concentrations, the prognostic significance of increased cardiac troponin concentrations, even below the 99th URL value, has been clearly demonstrated (28). Therefore, accurate clinical work-up is recommended in all patients with increased cTnI and cTnT values over the 99th URL when measure with HS methods (10). Finally, the significance of close communication between laboratory and clinicians is repeatedly recommended by the IFCC TF-CB document (8), especially for troubleshooting pre-analytical and analytical problems (e.g., differences in sensitivities and measurement units may affect the interpretations of results generated with HS cardiac troponin techniques).

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Executive Editor Dr. Zhi-De Hu (Department of Laboratory Medicine, the Affiliated Hospital of Inner Mongolia Medical University, Hohhot, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2018.05.04). Giuseppe Lippi serves as the unpaid Editor-in-Chief of *Journal of Laboratory and Precision Medicine* from November 2016 to October 2021. Aldo Clerico serves as an unpaid editorial board member of *Journal of Laboratory and Precision Medicine* from July 2017 to June 2019. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Alpert JS, Thygesen K, Antman E, et al. Myocardial infarction redefined: a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959-69.
2. Thygesen K, Alpert JS, White HD; Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. J Am Coll Cardiol 2007;50:2173-95.
3. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012;60:1581-98.
4. Giannoni A, Giovannini S, Clerico A. Measurement of circulating concentrations of cardiac troponin I and T in healthy subjects: a tool for monitoring myocardial tissue renewal? Clin Chem Lab Med 2009;47:1167-77.
5. Marjot J, Kaier TE, Martin ED, et al. Quantifying the release of biomarkers of myocardial necrosis from cardiac myocytes and intact myocardium. Clin Chem 2017;63:990-6.
6. Apple FS, Collinson PO. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem 2012;58:54-61.
7. Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J 2012;33;2252-7.
8. Wu AHB, Christenson RH, Greene DN, et al. Clinical laboratory practice recommendations for the use of cardiac troponin in acute coronary syndrome: expert opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Biomarkers of the International Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem

2018;64:645-55.
9. Apple FS, Sandoval Y, Jaffe AS, et al. Cardiac troponin assays: guide to understanding analytical characteristics and their impact on clinical care. Clin Chem 2017;63:73-81.
10. Mair J, Lindahl B, Hammarsten O, et al. How is cardiac troponin released from injured myocardium? Eur Heart J Acute Cardiovasc Care 2017:2048872617748553. [Epub ahead of print].
11. Apple FS, Jaffe AS, Collinson P, et al. IFCC educational materials on selected analytical and clinical applications of high sensitivity cardiac troponin assays. Clin Biochem 2015;48:201-3.
12. Clerico A, Zaninotto M, Ripoli A, et al. The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology and clinical implications. Clin Chem Lab Med 2017;55:1634-51.
13. Masotti S, Prontera C, Musetti V, et al. Evaluation of analytical performance of a new high-sensitivity immunoassay for cardiac troponin I. Clin Chem Lab Med 2018;56:492-501.
14. Caselli C, Cangemi G, Masotti S, et al. Plasma cardiac troponin I concentrations in healthy neonates, children and adolescents measured with a highly sensitive immunoassay method: Highly sensitive troponin I in pediatric age. Clin Chim Acta 2016;458:68-71.
15. Musetti V, Masotti S, Prontera C, et al. Evaluation of analytical performance of a new ADVIA immunoassay using the Centaur XPT platform system for the measurement of cardiac troponin I. Clin Chem LabMed 2018. [Epub ahead of print].
16. Masotti S, Musetti V, Prontera C, et al. Evaluation of analytical performance of a chemiluminescence enzyme immunoassay (CLEIA) for cTnI using the automated AIA-CL2400 platform. Clin Chem Lab Med 2018. [Epub ahead of print].
17. Franzini M, Lorenzoni V, Masotti S, et al. The calculation of the cardiac troponin T 99th percentile of the reference population is affected by age, gender, and population selection: A multicenter study in Italy. Clin Chim Acta 2015;438:376-81.
18. Clinical and Laboratory Standards Institute. CLSI EP17-A protocol. Protocols for determination of limits of detection and limits of quantitation; approved guidelines. 2004, Vol. 24, No. 34. Wayne, Pennsylvania.
19. Clinical and Laboratory Standards Institute. CLSI EP21 protocol. Evaluation of total analytical error for quantitative medical laboratory measurement procedure. Second edition July 2016. Wayne, Pennsylvania.
20. Lippi G, Ferrari A, Gandini G, et al. Analytical evaluation of the new Beckman Coulter Access high sensitivity cardiac troponin I immunoassay. Clin Chem Lab Med 2017;56:157-61.
21. Thompson M, Ellison SLR. Towards an uncertainty paradigm of detection capability. Anal Methods 2013;5:5857-61.
22. Ungerer JPJ, Pretorius CJ. High-sensitivity cardiac troponin: do think twice, it's not all right. Clin Chem Lab Med 2017;55:1669-71.
23. Currie LA. Nomenclature in evaluation of analytical methods including detection and quantitation capabilities. Pure Appl Chem 1995;67:1699-723.
24. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyrotropin (TSH) assays. Clin Chem 1996;42:140-5.
25. Lippi G, Cervellin G. Degradation of troponin I in serum or plasma: mechanisms, and analytical and clinical implications. Semin Thromb Hemost 2012;38:222-9.
26. Hickman PE, Potter JM, Aroney C, et al. Cardiac troponin may be released by ischemia alone, without necrosis. Clin Chim Acta 2010;411:318-23.
27. Lippi G, Cervellin G, Schena F. How much myocardium mass may be injured during endurance physical exercise? Clin Chim Acta 2017;470:29-30.
28. Lippi G, Bonfanti L, Dipalo M, et al. Clinical, organizational and economic analysis of high-sensitivity cardiac troponin testing in the emergency department. J Res Hospl 2017;1:44.

doi: 10.21037/jlpm.2018.05.04
Cite this article as: Clerico A, Lippi G. The state-of-the-art of "high-sensitivity" immunoassay for measuring cardiac troponin I and T. J Lab Precis Med 2018;3:53.

