Clinica Chimica Acta 492 (2019) 26–28

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Brief reports

Assessment of Access hsTnI 99th percentiles upper reference limits following IFCC recommendations

Martina Di Pietro[a,*], Mariella Dipalo[a], Marco Bruno Luigi Rocchi[b], Roberta Musa[a], Paola Avanzini[a], Cecilia Gnocchi[a], Maria Chiara Anelli[c], Rosalia Aloe[a]

[a] SSD Biochimica ad Elevata Automazione, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci 14, 43126 Parma, Italy
[b] Dipartimento di Scienze Biomolecolari, Università degli studi di Urbino "Carlo Bo", Via Ca' Le Suore, 2–4, 61029 Urbino, Italy
[c] Beckman Coulter srl, Via Roma 108, 20060 Cassina De' Pecchi (MI), Italy

ARTICLE INFO

Keywords:
High-sensitivity Troponin I
IFCC recommendations
99th percentile
Limit of Blank
Limit of Detection
Limit of Quantitation

ABSTRACT

Background: The detection of an increase and/or decrease of cardiac troponin (cTnI) values, with at least one value above the 99th percentile of the upper reference limit (URL) have a central role in acute myocardial infarction (AMI) diagnosis. The employment of sex specific 99th percentile URLs and High-sensitivity (Hs) assays are recommended. We assessed sex specific 99th percentile URL for Access Hs-cTnI and AccuTnI3 + (Beckman Coulter) using European donor reference population following recent International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommendations.

Methods: 300 males and 300 females plasma samples were collected. Both chemiluminescent immunoenzymatic assays were performed on UniCel DxI 800 platform (Beckman Coulter).

Results: For Access hsTnI, the observed sex-specific 99th percentile URLs were 5.5 (90% CI: 4.4–7.6) for females and 13.9 ng/L (90% CI: 7.4–17.4) for males. For AccuTnI + 3 we could not establish them because the assay couldn't report detectable values of troponin for most of the analyzed samples.

Conclusion: The sex-specific 99th percentile URLs established for Access hsTnI assay were significantly lower than those declared by the manufacturer caused by the different choice of population selection, age groups and sample types: for those reasons, we maintain the 99th URLs provided by manufacturer.

The fourth universal definition of myocardial infarction defines an acute myocardial infarction (AMI) as evidence of acute myocardial injury in a patient with clinical features of acute myocardial ischemia, and the detection of an increase and/or decrease of cardiac troponin (cTn, I or T) values, with at least one value above the 99th percentile of the upper reference limit (URL) [1]. High-sensitivity (Hs) troponin assays are recommended over less sensitive ones: hs-cTn assays detect earlier troponin with higher sensitivity and precision [2] in 50% to ideally 95% of healthy individuals [3], with an analytical imprecision ≤10% [1]. A Beckman Coulter hs-cTnI assay has been in development for > 8 years [4]; during this time, the company has considerably reformulated and refined the prototype assay, and has recently released a novel version [5,6], Beckman Coulter Access hsTnI (REF B52699) (Beckman Coulter, Inc. Brea, CA, USA). Before the Access hsTnI employment, we used a sensitive Troponin I Assay: AccuTnI + 3 (REF A98264; Beckman Coulter, Inc. Brea, CA, USA) and we adopted the Access hsTnI in April 2018. Both assays are sequential two–step chemiluminescent immunoenzymatic assays [7,8] and were performed on UniCel DxI 800 analytical platform (Beckman Coulter, Inc. Brea, CA, USA).

The 99th percentile URL is universally endorsed as the clinical cut-off to aid in the diagnosis of AMI. The CLSI (Clinical & Laboratory Standards Institute) document "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory" EP28-A3c provides guidance on how to validate laboratory test reference intervals [9]. However, for hs-cTn assays, the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) Committee on Clinical Applications of Cardiac Bio-Markers (C-CB) has proposed to determine the 99th percentile URLs using minimum 300 healthy male and 300 healthy female samples with one-tailed non-parametric statistical method [10]. URL studies have been published for various cTn assays available in the marketplace, reporting different URL values between cTn assays, as expected, and different study producers also. Despite of the IFCC recommendations, to date, there is poor consistency in how to enroll healthy subjects in the URL studies. The definition of a cardiac healthy individual is a topic of debate for troponins. Sex and age are

* Corresponding author.
E-mail address: martinadipietro@hotmail.it (M. Di Pietro).

https://doi.org/10.1016/j.cca.2019.01.028
Received 31 October 2018; Received in revised form 30 January 2019; Accepted 30 January 2019
Available online 31 January 2019

Table 1
LoB, LoD and LoQ of Access hsTnI vs contemporary AccuTnI + 3.

Parameter (ng/L)	Access hsTnI			AccuTnI + 3			
	Parma Laboratory	IFU [8]	Lippi [5]	Masotti [15]	IFU [7]	Lippi [16]	Moretti [17]
LoB	0.8	1.7	0.1	0.6	< 10	9	2.6
LoD	1.1	2.3	0.3	1.3	10	13	12
LoQ 10%CV	7.1	5.6 [18]	ND	5.3	40	37	18
LoQ 20%CV	2.9	2.3	Functional sensitivity 1.3	2.1	20	ND	ND

both important factors that could influence the 99th percentile [11]. Moreover, the 99th percentile URLs are impacted by the lack of standardization and harmonization between cTn assays and the adoption of different statistical method and specimen type.

The manufacturer conducted URL studies for both Access hsTnI and AccuTnI + 3 assays. For Access hsTnI assay, the 99th percentile URLs were 11.6 ng/L (95% CI: 8.4–18.3, $N = 595$) in female, 19.8 ng/L (95% CI: 14.0–42.9, $N = 494$) in male, and 17.5 ng/L (95% CI: 12.6–20.7, $N = 1089$) in overall population, respectively. For AccuTnI + 3, the URL study reported overall 99th percentile URL at 20 ng/L (95% CI: 10–40, $N = 527$). In the literature search, we found a couple of publications where the authors reported the sex-specific 99th percentile URLs for AccuTnI + 3 assay [12,13]. As few experiences are published in terms of troponin 99th percentile assessment, in 2017 we did a study to establish the sex-specific 99th percentile URLs for Access AccuTnI + 3 assay, using European donor reference population, but we could not establish them because the assay couldn't report detectable values of troponin for most of the samples from the reference population. After analysis, residual samples were dispensed into aliquot tubes and stored at −80 °C. Then, in February 2018, we established our gender-specific cut off for Access hsTnI assay using stored aliquot tubes. Samples (plasma K2-EDTA; Becton Dickinson, Franklin Lakes New Jersey) were collected from active donors of both genders (300 males, 300 females), aged between 18 and 68 years old, after the donors provided the proper informed consent. The study was performed in accordance with the Declaration of Helsinki, under the terms of relevant local legislation. In this study, we also verified for Access hsTnI Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) values according to CLSI guidelines EP17 A2 [14].[1]

On one single instrument we run 4 blank samples (LoB, 2 different lots of diluent, one calibrator zero and one negative patient) and 4 low measurand content samples (LoD), 5 replicates for 3 days with 2 different reagent lot (120 replicates in total).

LoQ at 10% and 20% coefficient of variation (CV) were derived by imprecision profile determined running 5 replicates for 5 days with 2 different reagent lot (500 replicates in total) on the following 10 samples pool: 1.1(A); 1.8 (B); 2.3 (C); 2.8 (D); 5.5 (E); 7.0 (F); 9.5 (G); 16.1 (H); 20.4 (I) and 30.4 (J) ng/L.

LoB, LoD and LoQ were calculated as follow: LoB by non-parametric method (due to the presence of negative data converted to 0); LoD by adjusted Currie method; LoQ by estimation of concentration corresponding to 10% and 20% CV obtained by linearized hyperbolic regression; they resulted 0.8, 1.1, 7.1 and 2.9 ng/L respectively.

Pools detailed CV% were: 51.8 (A); 25.5 (B); 23.9 (C); 18.7 (D); 13.7 (E); 9.3 (F); 8.4 (G); 6.9 (H); 5.4 (I) and 4.9 (J).

Our values are in line with those shown in Masotti's study [15], higher than the LoB, LoD and LoQ reported in Lippi's work [5] and better than the manufacturer's claims in their IFU. In 2014, Lippi [16] and Moretti [17] evaluated AccuTnI + 3 performance reporting much higher LoB and LoD values. The established and reported LoB, LoD and LoQ values are summarized in Table 1.

Even AccuTnI + 3 assay has an imprecision of 10%CV at the 99th percentile URL which is “clinically acceptable” in Apple's troponin scorecard [19], but this assay could not measure troponin above the limit of detection in almost of healthy subjects. For Access hsTnI, the observed sex-specific 99th percentile URLs were 5.5 ng/L (90% CI: 4.4–7.6) for females and 13.9 (90% CI: 7.4–17.4) for males, giving values above LoD in 60.7% and 85.7% respectively. The sex-specific 99th percentile URLs we established for Access hsTnI assay were significantly lower than those declared by the manufacturer. In our laboratory we used EDTA plasma samples, and it is recommended to convert from lithium/heparin values to EDTA plasma values using a proper conversion factor of 0.90 [20]. Another reason why we obtained low sex-specific URLs were possibly due to the composition of our reference population. The subjects enrolled in our study were intentionally per-selected as “healthier”, which might not represent the true patient population presenting to the Emergency departments, either in terms of age as blood donors are not accepted over 65 years old. The 99th URLs established by the manufacturer can still be appropriate for the use in patients presenting to Emergency departments.

Compared to contemporary troponin assays, hs-cTn assays demonstrate significantly improved precision at and below the 99th percentile URL [21]. Better determination of the 99th percentile URL will allow laboratories to report distinct reference ranges for male and female subjects.

Declarations of interest

Maria Chiara Anelli is Beckman Coulter srl Italy employee.

Authorship

The authors take responsibility for all the contents of the manuscript. Every single author has contributed to the planning and realization of the study.

Acknowledgments

We thank Beckman Coulter srl Italy for providing free of charge reagents for this study and Dr. Fabio Stifani, Beckman Coulter Field Application Specialist, for application support.

References

[1] K. Thygesen, J.S. Alpert, A.S. Jaffe, B.R. Chaitman, J.J. Bax, D.A. Morrow, H.D. White, Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction, fourth Universal Definition of Myocardial Infarction (2018), J. Am. Coll. Cardiol. 72 (2018) 2231–2264.
[2] T. Reichlin, W. Hochholzer, S. Bassetti, S. Steuer, C. Stelzig, S. Hartwiger, S. Biedert, N. Schaub, C. Buerge, M. Potocki, N. Noveanu, T. Breidthardt, R. Twerenbold, K. Winkler, R. Bingisser, C. Mueller, Early diagnosis of myocardial infarction with sensitive cardiac troponin assays, N. Engl. J. Med. 361 (2009) 858–867.
[3] F.S. Apple, R. Ler, M.M. Murakami, Determination of 19 cardiac troponin I and T

[1] *Abbreviations:* AMI, acute myocardial infarction; cTnI, cardiac troponin I; Hs, High-sensitivity; C-CB, Committee on Clinical Applications of Cardiac Bio-Markers; URL, upper reference limit; LoB, Limit of Blank; LoD, Limit of Detection; LoQ, Limit of Quantitation; CLSI, Clinical & Laboratory Standards Institute; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; CV, Coefficient of Variation.

assay 99th percentile values from a common presumably healthy population, Clin. Chem. 58 (2012) 1574–1581.
[4] P.A. Kavsak, A.R. MacRae, M.J. Yerna, A.S. Jaffe, Analytic and clinical utility of a next-generation, highly sensitive cardiac troponin I assay for early detection of myocardial injury, Clin. Chem. 55 (2009) 573–577.
[5] G. Lippi, A. Ferrari, G. Gandini, M. Gelati, C. Lo Cascio, G.L. Salvagno, Analytical evaluation of the New Beckman Coulter Access High sensitivity cardiac troponin I immunoassay, Clin. Chem. Lab. Med. 56 (2017) 157–161.
[6] P.A. Kavsak, A. Worster, S.A. Hill, A.R. MacRae, A.S. Jaffe, Analytical Comparison of three different versions of a high-sensitivity cardiac troponin I assay over 10 years, Clin. Chim. Acta 475 (2017) 51–55.
[7] BC.Inc, Access AccuTnI + 3, https://www.beckmancoulter.com/download/file/phxB00495H-EN_US/B00495H?type=pdf, (2018) ([Vol.Accessed July 2018]).
[8] BC.Inc, Access hsTnI, https://www.beckmancoulter.com/download/file/phxC11140F-EN_US/C11140F?type=pdf, (2018) ([Vol.Accessed July 2018]).
[9] CLSI EP28-A3c protocol, Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition, vol. 28, Wayne, Pennsylvania, 2010 (No. 30).
[10] F.S. Apple, A.S. Jaffe, P. Collinson, M. Mockel, J. Ordonez-Llanos, B. Lindahl, J. Hollander, M. Plebani, M. Than, M.H. Chan, IFCC Task Force on Clinical applications of Cardiac Bio-Markers. IFCC educational materials on selected analytical and clinical applications of high-sensitivity cardiac troponin assays, Clin. Biochem. 48 (2015) 201–203.
[11] P. Venge, B. Lindahl, Cardiac troponin assay classification by both clinical and analytical performance characteristics: a study on outcome prediction, Clin. Chem. 59 (2013) 976–981.
[12] J.P.J. Ungerer, J.R. Tate, C.J. Pretorius, Discordance with 3 cardiac troponin I and T assays: implications for the 99th percentile cutoff, Clin. Chem. 62 (2016) 1106–1114.
[13] D.N. Greene, T.K. Leong, P.O. Collinson, Age, sex and racial influences on the Beckman Coulter Accu TnI + 3 99th percentile, Clin. Chim. Acta 444 (2015) 149–153.
[14] CLSI EP17-A2 protocol, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition, vol. 32, Wayne, Pennsylvania, 2012 (No. 8).
[15] S. Masotti, C. Prontera, V. Musetti, S. Storti, R. Ndreu, G.C. Zucchelli, C. Passino, A. Clerico, Evaluation of analytical performance of a new high-sensitivity immunoassay for cardiac troponin I, Clin. Chem. Lab. Med. (2017) 1–10.
[16] G. Lippi, M. Dipalo, P. Avanzini, A. Formentini, R. Aloe, Analytical assessment of the Beckman Coulter UniCel DxI AccuTnI + 3 immunoassay, Diagnosis 1 (2014) 195–197.
[17] M. Moretti, B. Pieretti, M.B. Rocchi, S. Gasperoni, Analytical performance and clinical decision limit of a new release for cardiac troponin I assay, Ann. Clin. Biochem. 52 (2015) 169–172.
[18] BC.Inc, Access HsTnI Data Sheet, https://www.beckmancoulter.com/en/products/immunoassay/access-hstni#/specifications, (2018) ([Vol.Accessed January 2019]).
[19] F.S. Apple, A new season for cardiac troponin assays: it's time to keep a scorecard, Clin. Chem. 55 (2009) 1303–1306.
[20] A.H. Wu, F.S. Apple, W.B. Gibler, R.L. Jesse, M.M. Warshaw, R. Valdes Jr., National Academy of Clinical Biochemistry Standards of Laboratory Practice: recommendations for the use of cardiac markers in coronary artery disease, Clin. Chem. 45 (1999) 1104–1121.
[21] K. Thygesen, J. Mair, E. Giannitsis, C. Mueller, B. Lindahl, S. Blankenberg, How to use high-sensitivity cardiac troponins in acute cardiac care, Eur. Heart J. 33 (2012) 2252–2257.