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Letter to the editor

Evaluation of 99th percentile value of a chemiluminescence enzyme immunoassay (CLEIA) for cTnI using the automated AIA-CL2400 platform☆

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Letter to the Editor

According to the document Fourth Universal Definition of Myocardial Infarction, an increase in cardiac troponins (cTn) levels over the 99th percentile upper reference limit (URL) should be considered as clinically relevant for definition of myocardial injury and diagnosis of myocardial infarction [1]. Furthermore, the international guidelines recommend that this cut off value should be measured with an imprecision ≤10 CV% [1,2]. The 99th percentile URL value not only strongly depends on demographic and physiological variables of populations, but also on the analytical performances of cTnI methods, as well as on mathematical algorithms used for 99th percentile calculation [3,4].

In 2018, the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Section of the European Ligand Assay Society (ELAS) promoted a multicenter study (i.e., the Italian hs-cTnI Study) with the aim to evaluate the analytical performances and reference values of commercially available cTnI methods in Italy [5–9].

In the present article, Authors report the evaluation of the 99th percentile URL of a new chemiluminescence enzyme immunoassay (CLEIA) for cTnI measurement (CL AIA-PACK cTnI TEST) using the automated AIA-CL2400 platform (TOSOH BIOSCIENCE, Tessenderlo, Belgium). This method is a two-step CLEIA immunoassay using a combination of two monoclonal antibodies. The calibration curve is standardized against the human cardiac troponin complex SRM 2921 material, certified by NIST (National Institute of Standards and Technology). The cTnI value of the 99th percentile for the reference Caucasian population, suggested by the manufacturer, is 31 ng/L (bulletin A0008871001-116B Rev. 11/16, TOSOH Europe N.V., Tessenderlo, Belgium). Analytical sensitivity parameters of the CLEIA method, previously evaluated by the study reference laboratory, are: limit of detection (LoD) 2.1 ng/L, limit of quantitation (LoQ) at 20% CV 15.0 ng/L and LoQ at 10% CV 30.9 ng/L, respectively [6].

According to the experimental protocol of the Italian hs-cTnI Study, 8 Italian clinical laboratories collected heparinized plasma samples from apparently healthy volunteers of both sexes (age range 18–86 years). All volunteers denied the presence of chronic or acute diseases and had normal values of routine laboratory tests (including creatinine, electrolytes, glucose and blood counts). To more accurately evaluate cTnI concentrations of apparently healthy subjects older than 47 years, plasma samples from some volunteers (corresponding to about 35% of overall healthy population) collected in the MEHLP study were also assayed (mean age 63 years, age range 47–85 years), as previously described in details elsewhere [8–10]. Compared to other apparently healthy volunteers enrolled in this study, participants to the MEHLP study underwent also NT-proBNP assay, ECG and cardiac imaging analysis (computed tomography scan, carotid echography, echocardiography) in order to exclude the presence of any cardiovascular subclinical disease [8–10]. Exclusion criteria were: presence of cardiac or systemic acute or chronic diseases, such as myocardial infarction, heart failure, coronary heart disease, hypertension, diabetes, kidney disease, obesity, tumour, hepatitis, BPCO, and use of drugs [8–10].

Every laboratory participating to the study stored two aliquots of about 1 mL of plasma samples collected from the apparently healthy volunteers in tubes identified by alphanumeric barcodes (the storage temperature range varied from −25 °C to −80 °C). The stored tubes were sent to the reference study laboratory (Fondazione CNR Regione Toscana G. Monasterio, Pisa Italy) using a pack with dry ice within one months after the blood collection. Only age and the sex of apparently healthy volunteers were known by the staff of the reference laboratory. In the reference laboratory the clinical samples were immediately stored at −80° and then the samples were measured within three months with the CLEIA cTnI immunoassay. The informed consent was obtained by all volunteers enrolled in the study in accordance with the guidelines recommended by the respective local ethical committees.

For the evaluation and comparison of analytical performances of tested cTnI immunoassay methods, standard statistical analyses were carried out using the JMP program (version 12.1.0, SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). As an occasional outlier can

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Table 1
cTnI distribution values (ng/L) measured by CLEIA method in the reference population divided in subgroups according to age and sex.

Population groups	Mean ± SD	Median	25th percentile	75th percentile	97.5th percentile	99th percentile
Whole population (N = 1391)	6.8 ± 4.2	6.0	4.0	8.0	17.2	27.0
Women (N = 668)	6.3 ± 3.7	6.0	4.0	8.0	15.3	23.3
Men (N = 723)	7.2 ± 4.5	6.0	5.0	9.0	19.0	27.0
Whole population Age ≤ 55 years (N = 820)	6.3 ± 3.9	5.0	4.0	8.0	16.0	26.8
Whole population Age > 55 years (N = 571)	7.5 ± 4.4	7.0	5.0	9.0	18.7	28.0
Women Age ≤ 55 years (N = 396)	6.0 ± 3.7	5.0	4.0	7.0	15.1	24.1
Women Age > 55 years (N = 272)	6.9 ± 3.6	6.0	5.0	9.0	17.0	24.1
Men Age ≤ 55 years (N = 424)	6.5 ± 4.1	6.0	4.0	8.0	17.4	27.0
Men Age > 55 years (N = 299)	8.1 ± 4.9	7.0	5.0	10.0	21.0	36.0

markedly change the 99th percentile, Authors used the Tukey test for evaluation of possible outlier values. Assuming a log-normal distribution of cTnI values, the identification of outlier values was performed by means of Tukey test using the formula: cTnI > Q_3 + 3 IQR, as gating parameter; where, Q_3 and IQR respectively are the third quartile and interquartile range (Q_3–Q_1) of cTnI distribution. The calculation of cTnI distribution and 99th percentile URL values was performed with the JMP program using the nonparametric method, as recommended by international guidelines [2].

The assay of all plasma samples collected was performed with the CLEIA cTnI method in the reference laboratory of the study according to the recommendations suggested by the manufacturer. After the exclusion of outlier values, the reference population included 1391 apparently healthy volunteers (668 women and 723 men; mean age: 50.9, median 54 years, interquartile range 42–60 years, age range 18–86 years) (Table 1). The distribution of cTnI values of the reference population was highly skewed, while log-transformed cTnI values roughly approximated a log-normal distribution. Mean, median and some percentile values of cTnI values of the reference population, considered as a whole and also divided in groups according to age (≤55 years or > 55 years) and sex are reported in Table 1. As recommended by international guidelines [1,2], the 99th percentile URL value, suggested by manufacturer (i.e., 31 ng/L) is measured with an imprecision equal to 10% CV, as previously reported in detail [6]. Moreover, considering that both median values of sex-specific reference population values are 6.0 ng/L (Table 1), the CLEIA method is able to measure the most part (> 50%) of apparently healthy men and women with cTnI values higher than LoD value (i.e., 2.1 ng/L), as required by international guidelines [2]. However, considering that the LoQ value at 20% CV is 15 ng/L for the CLEIA method, the major part of apparently healthy subjects of both sexes is measured with an imprecision ≥20% CV [6].

The 2018 AACC/IFCC international guidelines [2] recommend that at least 300 men and 300 women are needed to appropriately define 99th percentile URL values for each sex. In accordance with these recommendations, the results related to men (N = 299) and women (N = 272) with age > 55 years have an insufficient number for calculation of 99th percentile URL value with statistical confidence. Therefore, these cTnI values are only approximate, and so the 99th percentile URL value of CLEIA method for healthy men and women with age > 55 years should be confirmed by means of large population studies.

On average, women showed significantly lower cTnI values than men at the same age ($p < .0001$ by Wilcoxon/Kruskal-Walls test). Moreover, the 820 subjects with age ≤ 55 years had significantly lower cTnI values than the 571 individuals with age > 55 years ($p < .0001$ by Wilcoxon/Kruskal-Wallis test) (Table 1). Finally, both age (as continuous variable $p < .0001$) and sex (as dichotomous variable $p < .0091$) were able to independently affect log-transformed cTnI values as demonstrated with a multivariable model of linear regression analysis ($R = 0.1923$, $p < .0001$) (Supplementary Fig. 1 and Table 1). Therefore, the results of the present study confirm that measurement of 99th percentile URL value can be affected by age and sex distribution of reference population.

A limitation of the present study is that the measurement of NT-proBNP, as surrogate biomarkers, was not measured in all apparently healthy volunteers, as recommended by the AACC/IFCC guidelines [2]. Another possible limitation of this study is that the reference population is constituted for > 95% of Caucasian ethnic individuals. However, the 2018 AACC/IFCC guidelines recommend that each study should enrol sex-group reference individuals that are representative of the patient population observed in their geographic area admitted to emergence department with symptoms suggestive of myocardial injury [2]. Accordingly, the ethnic distribution of the reference population enrolled in this study is representative of the population observed in overall geographic area of Italy. Of course, the 99th percentile URL values, reported in Table 1, should be used with care for populations different in ethnicity, range of age, or men/women ratio to that enrolled in the present study. In particular, the number of elderly individuals enrolled in this study was relatively small (Table 1). Therefore, further studies based on larger reference populations, including apparently healthy individuals over age of 65 years, are needed for a more accurate calculation of 99th percentile URL value of cTnI measured with CLEIA method.

In conclusions, the results of the present study indicate that the CLEIA cTnI method shows a 99th percentile URL value (27.0 ng/L, 99% confidence limits 25.8–28.3 ng/L), evaluated in a large reference population, which is very similar to that suggested by manufacturer (31 ng/L). This slight discrepancy in cTnI concentrations (i.e., 4 ng/L) is probably due to differences in age and sex-ratio between the reference populations enrolled in manufacturer and present studies, respectively. To best of our knowledge, this study is the first reporting the cTnI distribution values measured with the CLEIA method in a large reference population. Therefore, the results of this study will be useful for all clinical laboratories, which uses the CLEIA method with the automated AIA 2400 platform for cTnI assay.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.06.018.

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