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Evaluation of 99th percentile and reference change values of the hs-cTnI method using ADVIA Centaur XPT platform: A multicenter study☆

Aldo Clerico[a,*], Silvia Masotti[a], Veronica Musetti[a], Andrea Ripoli[a], Rosalia Aloe[b], Martina Di Pietro[b], Sara Rizzardi[c], Ruggero Dittadi[d], Cinzia Carrozza[e], Lucia Belloni[f], Marco Perrone[g], Tommaso Fasano[f], Simone Canovi[f], Antonio de Santis[h], Concetta Prontera[a], Cristina Guiotto[i], Domenico Cosseddu[i], Marco Migliardi[i], Sergio Bernardini[g]

[a] Laboratorio Clinico, Fondazione CNR-Regione Toscana, G. Monasterio e Scuola Superiore Sant'Anna, Pisa, Italy
[b] S. S. Dipartimentale Biochimica ad Elevata Automazione, Dipartimento Diagnostico Azienda Ospedaliero-Universitaria di Parma, Parma, Italy
[c] Laboratorio Analisi Chimico Cliniche e Microbiologiche - ASST di Cremona, Cremona, Italy
[d] UOC Medicina di Laboratorio, Ospedale dell'Angelo ULSS 3 Serenissima, Mestre, Italy
[e] UOC Chimica, Biochimica e Biologia Molecolare Clinica, Fondazione Policlinico Universitario "A. Gemelli", IRCCS, and Università Cattolica del Sacro Cuore, Roma, Italy
[f] Laboratorio di Autoimmunità, Allergologia e Biotecnologie Innovative and Laboratorio Analisi Chimico-Cliniche ed Endocrinologia, Azienda Unità Sanitaria Locale - IRCCS di Reggio Emilia, Italy
[g] Dipartimento di Medicina Sperimentale, Università di Roma Tor Vergata, Roma, Italy
[h] Laboratorio di Analisi Cliniche e Microbiologiche P.O. San Paolo – ASL Bari, Bari, Italy
[i] S.C. Laboratorio Analisi, A.O. Ordine Mauriziano di Torino, Torino, Italy

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ABSTRACT

Background: According to quality specifications required by international guidelines, the evaluation of the 99th URL value is a very difficult task that is usually beyond the capacity of a single laboratory. The aims of this article are to report and discuss the results of a multicenter study concerning the evaluation of the 99th percentile URL and reference change (RCV) of the ADVIA Centaur High-Sensitivity Troponin I (TNIH), recently distributed to the Italian clinical laboratories.

Materials and methods: The reference population evaluated with ADVIA XPT method for the calculation of cTnI reference distribution parameters consisted of 1325 healthy adults subjects (age range from 18 to 86 years), including 653 women (mean age 50.7 years, SD 14.5 years) and 672 men (mean age 50.9 years, SD 13.8 years), well matched for both age ($P = .8112$) and sex (F/M = 0.97).

Results: cTnI distribution values of reference population was highly skewed, while log-transformed cTnI values roughly approximated a log-normal distribution. Men have higher cTnI values than women throughout all the adult lifespan. Moreover, the subjects with age ≤ 55 years had significantly lower cTnI values than those with age > 55 years ($p < .0001$). Of note, 62% of women and 77% of men had equal or higher than cTnI values than the LoD value of the method (i.e., 2.2 ng/L).

Conclusions: The results of the present study demonstrate that the ADVIA Centaur High-Sensitivity Troponin I using the XPT automated platform fits both the criteria and quality specifications required by the most recent international guidelines for high-sensitivity methods for cTnI assay.

1. Introduction

Cardiac troponin I (cTnI) and T (cTnT) are the only biomarkers recommend by international guidelines for the detection of myocardial injury and for the diagnosis of myocardial infarction [1–3]. The 99th URL values is strongly affected by several factors: demographic characteristics (especially age and sex) and physiological variables of individual enrolled (i.e., the criteria for considering the reference population "healthy"), analytical performances of cTnI methods, and even the mathematical algorithms used for calculating the 99th percentile

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* Corresponding author at: Laboratory of Cardiovascular Endocrinology and Cell Biology, Department of Laboratory Medicine, Fondazione CNR Toscana G. Monasterio, Scuola Superiore Sant'Anna, Via Trieste 41, 56126 Pisa, Italy.
E-mail address: clerico@ifc.cnr.it (A. Clerico).

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URL [4,5]. According to quality specifications and exacting criteria required by international guidelines, the evaluation of the 99th URL value is a very difficult task, that is usually beyond the capacity of a single laboratory.

On the other hand, the 2016 guidelines of European Society of Cardiology (ESC) on the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation (NSTEMI) recommend the use of the absolute change (i.e., expressed as difference in cTn concentrations, ng/L) rather than percentage variation for the assessment of the rise and/or fall of cTn values in patients admitted to emergency department with diagnosis of Acute Coronary Syndrome without Persistent ST-Segment Elevation (ACS-NSTEMI) [1]. The 99th URL values not only strongly depend on demographic and physiological variables of populations (i.e., the criteria for considering the reference population "healthy"), but also on the analytical performances of cTnI methods, as well as on the mathematical algorithms used for calculating the 99th percentile URL [4,5]. According to quality specifications and exacting criteria required by international guidelines, the evaluation of the 99th URL value is a very difficult task that is usually beyond the capacity of a single laboratory.

Considering these difficulties, the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Section of the European Ligand Assay Society (ELAS) have recently promoted a multicenter study (named Italian hs-cTnI Study) with the aim to accurately evaluate analytical performances and reference values of the most popular cTnI methods commercially available in Italy. The most important aims of this article are to report and discuss the results of the Italian hs-cTnI Study concerning the evaluation of the 99th percentile URL and reference change (RCV) values around the 99th URL of the of the new immunoassay method, named ADVIA Centaur High-Sensitivity Troponin I (TNIH), recently distributed to the Italian clinical laboratories.

2. Materials and methods

2.1. Reference population and plasma sample collection

The Italian hs-cTnI Study is a multicenter clinical study. Heparinized plasma samples were collected from healthy adult volunteers by 8 Italian clinical institutions, including both University and Regional Hospitals, which have highly qualified workforce staff in emergency, cardiology and laboratory departments. Every center collected from 50 to 150 plasma samples from apparently healthy adult subjects enrolled from the clinical and laboratory staff or volunteers blood donors with age from 18 to 86 years. Every laboratory participating to the study stored at −80 °C two aliquots of about 1 mL of plasma samples collected from healthy subjects in tubes identified by alphanumeric barcodes. The stored tubes were sent to the reference laboratory of the study (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 adult 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 cTnI immunoassay. The informed consent was obtained by all subjects enrolled in the study in accordance with the guidelines recommended by the respective local ethical committees.

Authors divided the study population in two parts. The first population includes the samples collected by 7 clinical Italian Institutions, while the second population includes only the healthy subjects collected in the MEHLP study.

Study population 1. The first population subgroup includes 918 blood donors or apparently healthy volunteers, collected from the laboratory and clinical staff, enrolled by 7 different clinical Italian Institutions (mean age: 45.1 years, SD: 12.5 years, interquartile range: 36–54 years, minimum value 18 years, maximum value 86 years). All apparently healthy volunteers enrolled in this study denied the presence of chronic or acute diseases. In particular, the blood donor volunteers, representing > 80% of this study population, were evaluated by means of a thorough clinical examination, routine laboratory tests (including creatinine, electrolytes, glucose and blood counts) and ECG.

MEHLP population. To more accurately evaluate cTnI concentrations of healthy subjects older than 47 years, plasma samples from 407 adult subjects collected in the MEHLP study were also assayed (mean age 63.5 years; SD 8.2 years, interquartile range 57.0–69.0 years, minimum value 47 years, maximum value 85 years). The MEHLP study is a screening study aimed to evaluate the cardiovascular subclinical disease in an asymptomatic general population with age > 47 years, enrolled from the community of Montignoso (Massa, Italy). All the subjects enrolled in the MEHLP study were accurately evaluated to exclude the presence of asymptomatic cardiac disease by means of thorough clinical examination, laboratory tests, complete echocardiography investigation (including the evaluation of diastolic dysfunction), and, if necessary, even cardiac tomographic imaging. 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 [6]. Echocardiographic and other cardiac imaging techniques was used to exclude the presence of any myocardial disease, including the presence of heart failure with preserved ejection fraction (HFpEF), ventricular hypertrophy and cardiac valvular dysfunction according to the 2016 ESC guidelines [7]. Moreover, laboratory test investigation also included NT-proBNP assay, as previously suggested by Sandoval and Apple [4] and 2018 IFCC guidelines [2]. The cut-off values of 125 ng/L and 450 ng/L were used for individuals with age ≤ and > 75 years, respectively, as suggested by 2018 IFCC guidelines [2]. NT-proBNP distribution values in the MEHLP population included in this study were: mean 59.6 ng/L; SD: 46.5 ng/L, median 50.4 ng/L; interquartile range: 30.1–743.6 ng/L; minimum value: 5.0 ng/L; maximum value: 339 ng/L.

2.2. cTnI immunoassay method

The ADVIA Centaur High-Sensitivity Troponin I using the XPT automated platform (Ref. 10994774-5), distributed in Italy by Siemens Healthineers S.r.l. (Via Vipiteno, Milano, Italy), is an immunometric assay method for the cTnI assay. The analytical characteristics and performances of the ADVIA XPT method were previously analyzed by the reference laboratory of the study [8]. The plasma samples were assayed according to the analytical procedures suggested by the manufacturer. Limits of blank (LoB), detection (LoD), and quantitation (LoQ) at 10% CV and 20% CV were calculated according to international standardized protocols [9,10], as previously described in details [8]. The imprecision profile was estimated by repeatedly ($N = 40$) measuring 10 heparinized plasma pools collected from healthy subjects and patients with cardiovascular diseases (cTnI ranging from 1 ng/L to 50 ng/L) [8].

The reference change values (RCV) were evaluated according to Callum G. Fraser [11]. The bidirectional *Z*-score RCV between two results (CI 95%) were calculated by considering both the analytical variability of the method (CV_A) and the intra-individual variability (CV_I), using the formula: $RCV = 1.96\ [2(CV_A\ [2]\ +\ CV_I^2)]^{1/2}$.

2.3. Statistical analysis

For the evaluation and comparison of the analytical performance of tested cTnI immunoassay methods, standard statistical analyses were carried out using the JMP program (version 12.1.0, SAS Institute Inc.,

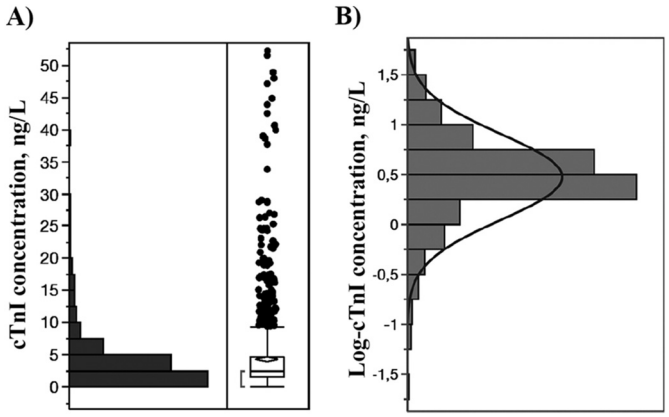

Fig. 1. Distribution of cTnI concentration values in 1325 apparently healthy subjects.
Part A – Distribution of original cTnI values.
Part B – Distribution of log_{10}-transformed cTnI values. The log-normal distribution is indicated in red color.
In the figures are also reported the median, the interquartile range (i.e., 25 th and 75th percentiles as box), and the 10th and 90th percentiles. The values below the 10th percentiles and above the 90th percentiles are reported with black circles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

SAS Campus Drive, Cary, NC, USA). As cTnI circulating levels are not normally distributed, both non-parametric and parametric tests after logarithmic transformation (log-10) of data were used for statistical analysis. The identification of outlier values was based on the interquartile range (IQR) assuming a log-normal distribution (Tukey's test) [12]. An outlier value was evaluated by 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 nonparametric method (Fig. 1A), as recommended by international guidelines [2]. Lognormal distribution using a robust method was also calculated for comparison (Fig. 1B). The 99th percentile and the respective confidence interval (CI) values (at 90%, 95% and 99%) were also calculated with adjusted bootstrap percentile method according to Carpenter & Bithell using random replacement and 100,000 repetitions [13].

3. Results

3.1. Analytical parameters and RCV of cTnI immunoassays

The analytical parameters of the ADVIA XPT method evaluated by the reference laboratory of the study using international standardized protocols [9,10] and those suggested by the manufacturer are reported in Table 1. The RCV and absolute critical change (Δ change) values of ADVIA XPT method in the range of cTnI concentrations from 5 ng/L to 40 ng/L are reported in Table 2. These change values were calculated assuming an intra-individual variability (CV_I) in healthy adult subjects of 9%, according to Van der Linden et al. [14].

3.2. cTnI distribution values of reference population

The total population with measured cTnI concentrations included 1332 individuals. According to the result of Tukey's test, 7 cTnI values (i.e., 5 men and 2 women) were excluded as outliers from the reference population. As a result, the reference population evaluated for the calculation of cTnI reference distribution parameters consisted of 1325 healthy adults subjects (age range from 18 to 86 years), including 653 women (mean age 50.7 years, SD 14.5 years) and 672 men (mean age 50.9 years, SD 13.8 years), well matched for both age (P = .8112 by

Table 1
Comparison of analytical sensitivity parameters of ADVIA cTnI method previously evaluated in the reference laboratory of the study and those suggested by the manufacturer[a].

LoB	LoD	LoQ 20 CV%	LoQ 10 CV%	Ratio	References
(ng/L)	(ng/L)	(ng/L)	(ng/L)		
1.0	2.2	3.5	8.0	5.9	[8]
0.9	2.2	2.5	9.0	5.2	Manufacturer

Ratio: ratio between the 99th percentile of the reference population distribution of cTnI, suggested by the manufacturer for general population (47.34 ng/L), and the LoQ 10% CV value evaluated in the reference laboratory of the study [8].
[a] The data suggested by the manufacturer were reported from the instruction bulletin 11200076-EN Rev A,2017-03.

Table 2
Reference change values (RCV) and absolute critical changes (Δ change) of the cTnI method using ADVIA XPT platform in the range of cTnI concentrations from 5 ng/L to 40 ng/L.

cTnI concentration	CV_A	RCV 95% CI	Δ change 95% CI	RCV 99% CI	Δ change 99% CI
(ng/L)	(%)	(%)	(ng/L)	(%)	(ng/L)
5	13.0	43.8	2.2	57.6	2.8
10	8.9	35.1	3.5	46.1	4.6
15	6.8	31.3	4.7	45.3	6.8
20	5.7	29.5	5.9	38.8	7.8
40	4.1	27.4	11.0	36.0	14.4

CV_A: Analytical variability evaluated according to the imprecision profile, as previously reported [8].
RCV 95% and 99%: reference change values calculated at the probability of 95% and 99% CI, respectively.
Δ Change 95% and 99%: Absolute critical changes calculated at the probability of 95% and 99%, respectively. These change values were calculated assuming an intra-individual variability (CV_I) in healthy adult subjects of 9%, as reported by Van der Linden et al. [14].

student t-test) and sex (F/M = 0.97). The distribution of cTnI values of the reference population was highly skewed (Fig. 1A), while log-transformed cTnI values roughly approximated a log-normal distribution (Fig. 1B).

Mean, median and percentile values of cTnI values of the reference population, according to age and sex, evaluated by nonparametric method, are reported in Table 3. The values of 99th percentiles, calculated with bootstrap method were also reported in Table 3. The values of distribution parameters separated for women and men with age > 55 year were not calculated because the number of enrolled individuals was lower than 300, which is the needed number of cases required by international guidelines for an accurate evaluation of 99th percentile URL values [2,4,5].

3.3. Influence of age and sex on cTnI values in the reference population

Age and sex significantly influenced cTnI concentrations in healthy adult subjects, as demonstrated by means of a linear regression model between log-transformed cTnI concentration (dependent variable) and age and sex (independent variables) (Fig. 2 and Table 4). Men have higher cTnI values than women throughout all the adult lifespan. Moreover, the subjects with age ≤ 55 years had significantly lower cTnI values than those with age > 55 years (p < .0001 by Wilcoxon/Kruskal-Wallis test) (Table 3). In particular, the 63 apparently healthy individuals with age > 75 years had a mean (SD) cTnI value of 6.5 (6.1) ng/L with an increment of 1.7 folds compared to the 795 individuals with age ≤ 55 years (p < .0001). As far as the cTnI distribution values according to sex is concerned, 62% of women and 77%

Table 3
Mean, median, and percentile values of cTnI distribution values (ng/L) of the reference population.

	Mean ± SD	Median	25th percentile	75th percentile	97.5th percentile	99th percentile	99th perc. BS[a] method (CI%)
Whole population (*N* = 1325)	4.2 ± 5.5	2.7	1.8	4.6	22.0	40.1	28.9 (26.2–38.7)[a] (25.5–39.6)[b] (24.5–40.6)[c]
Women (*N* = 653)	3.2 ± 4.2	2.2	1.1	3.6	13.8	32.4	24.4 (15.8–29.5)[a] (15.4–33.5)[b] (14.6–35.8)[c]
Men (*N* = 672)	5.2 ± 6.4	3.5	2.2	5.4	25.6	43.4	40.0 (28.8–43.0)[a] (28.7–44.0)[b] (26.8–45.7)[c]
WP ≤ 55 years (*N* = 795)	3.8 ± 5.5	2.5	1.1	4.0	22.0	40.6	32.0 (24.9–40.8)[a] (24.6–42.4)[b] (24.3–42.9)[c]
WP > 55 years (*N* = 530)	4.9 ± 5.6	3.4	2.2	5.4	22.3	40.3	28.9 (25.7–38.5)[a] (24.6–42.3)[b] (21.9–43.0)[c]
Women ≤ 55 years (*N* = 398)	2.8 ± 3.9	2.2	0.6	3.0	13.1	27.0	23.1 (14.2–27.0)[a] (13.2–27.4)[b] (12.9–33.7)[c]
Men ≤ 55 years (*N* = 397)	4.8 ± 6.6	3.1	0.5	4.8	25.6	42.8	40.7 (27.1–44.8)[a] (26.3–45.3)[b] (25.5–52.2)[c]

BS: Bootstrap method
[a] CI 90%.
[b] CI 95%.
[c] CI 99%

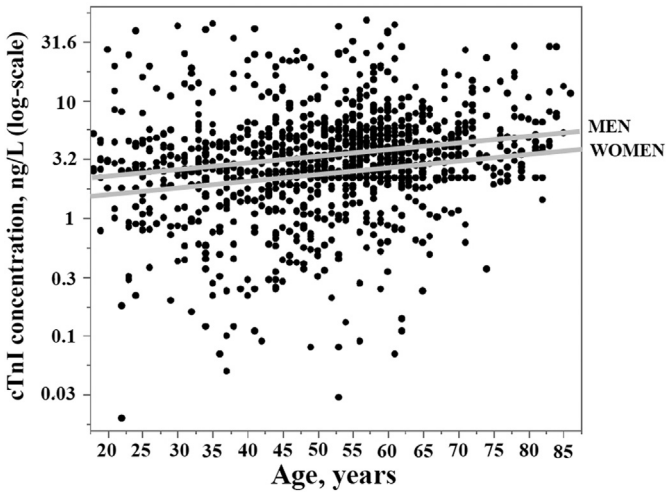

Fig. 2. Inter-relationships between log-transformed plasma cTnI values, considered as the dependent continuous variable, and two independent variables: age (as continuous variable) and sex (as dummy variable). In the figure, the contribution of the dummy variable sex to linear regressions between log-cTnI and age is indicated by two straight lines (grey color). Men showed higher values than women throughout all the range of cTnI concentrations. The statistical results regarding this multivariable regression analysis are detailed in Table 4.

of men had higher cTnI values than the LoD value of the method (i.e., 2.2 ng/L).

4. Discussion

To best of our knowledge, this study reports for the first time the

Table 4
Regression analysis between log-transformed cTnI values (dependent variable) and age and sex (independent variables).

R = 0.2600 (P < .0001)
Intercept = 0.1651 (SE 0.0442) (*P* = .0002)
Age = 0.0057 (SE 0.0009) (*P* < .0001)
Sex (F) = −0.0796 (SE 0.0118) (P < .0001)

SE: standard error

cTnI distribution parameters and RCV and absolute critical change values of the ADVIA XPT method in a large Italian population. The results of the present study confirm that both age and sex affect the measurement of 99th percentile URL value, even when the criteria for selection of reference population are well in accordance with recommendations made by international guidelines [2,4,5]. The sex-related 99th percentile URL values calculated for whole population in this study with the nonparametric method are lower than those suggested by the manufacturer. However, these values are within the 90% confidence limits (CI) suggested by the manufacturer: for whole population (*N* = 2010, age 22–91 years): 47.3 (CI 36.4–64.3) ng/L; for women (*N* = 1012): 37.0 (CI 30.2–72.6) ng/L; for men (*N* = 998): 57.3 (CI 38.6–90.2) ng/L. These discrepancies are probably due to differences in age ranges (present study:18–86 years vs manufacturer: 22–91 years) and in inclusion/exclusion criteria of reference populations.

The results of the present study are well in agreement with previous results, obtained with both cTnI and cTnT methods [4,5,17,18], indicating that both calculation of 99th percentile and method to detect outliers can greatly affect the 99th percentile URL of cTnI and cTnT. In this study both nonparametric bootstrap approach and “Tukey's fences” method were used with the aim to reduce the effect of outliers. The bootstrap method allowed significantly ($p < .0001$) lower 99th percentile values than nonparametric ones for both whole population and subgroups related to age and sex (Table 3). However, the 99th

percentile values calculated with non-parametric approach are within the 99% CI of those obtained with nonparametric bootstrap method (Table 3). From a clinical point of view, the use in the clinical practice of 99th percentile URL values, calculated with nonparametric bootstrap method should theoretically allow a better sensitivity but also a worse specificity in both detection of myocardial injury and diagnosis of myocardial infarction. Of course, diagnostic/prognostic accuracy and relative cost/benefit of these two statistical approaches for the calculation of 99th percentile URL values of the hs-cTnI ADVIA XPT method should be evaluated by specifically designed clinical studies.

The international guidelines suggest that cardiac troponins should be measured with high-sensitivity methods for both detection of myocardial injury and clinical diagnosis of myocardial infarction [1–3]. The 2018 Expert Opinion from the AACC and IFCC [2] states that high-sensitivity methods for cTnI and cTnT assay should satisfy two fundamental criteria. First, high-sensitivity methods should measure the 99th percentile URL value with an imprecision (expressed as CV %) ≤ 10%. Second, high-sensitivity methods should measure cTn concentrations above the LoD of the method at least in 50% healthy subjects enrolled in large populations including > 300 men and 300 women [2].

As previously reported in detail [7], the ADVIA XPT method actually satisfies the first fundamental criterium for high-sensitivity method for cTnI assay. Indeed, the ADVIA XPT method measures the 99th percentile URL with an imprecision < CV 10% (i.e., about 5% CV) (Table 1 and Table 2), as requested by all international guidelines [2]. Furthermore, the results of this study indicate that the ADVIA XPT method for cTnI assay also satisfies the characteristics required by international guidelines for the second criterium [2]. Indeed, > 50% of women enrolled in this study had cTnI values equal to or even higher than the LoD of the method (i.e., 2.2 ng/L). These data indicate that the ADVIA XPT method should be actually considered a high-sensitivity method for the cTnI assay [2].

The results of our study (Fig. 2) indicate that after the age of 55 years there is a progressive rise in cTnI concentrations in both sexes. It is important to note that cTnI results concerning individuals with age higher than 75 years predominantly depend on the healthy volunteers enrolled in the MEHLP study (52/63, corresponding to 82.5%). The healthy status of these individuals was well characterized using a thorough clinical history, comprehensive laboratory analyses (also including assay of BNP/NT-proBNP) and an accurate cardiac investigation (also including a complete echocardiographic examination). An important question is whether the increase in hs-cTnI values observed in apparently healthy subjects with age > 55 years (Fig. 2) is related to senescence or more probably to the presence or subtle comorbidities. This important pathophysiological and clinical issue was previously discussed in details [4,5,15]. Of note, several recent evidences demonstrated that cTn assay with high-sensitivity methods is a more sensitive biomarker for detection of myocardial injury than cardiac imaging techniques (3,4,5,15,16.19). In particular, Marjot et al. [19] recently reported that the 99th percentile URL of cTnI and cTnT values, measured with high-sensitivity methods, may be exceeded by necrosis of 40 mg of myocardium, which is a volume of myocardial tissue much too small to detect by noninvasive imaging, including the NMR techniques. From a physiological point of view, the circulating levels of cTnI measured with high-sensitivity methods in adult healthy subjects, may be related to physiological renewal of myocardial tissue [15,16,20].

From a clinical perspective, a working hypothesis may be suggested: a progressive increment over some months in circulating hs-cTnI levels of about 10 ng/L, measured with the hs-cTnI ADVIA XPT method (Table 2), should be considered as a hallmark of an adverse myocardial remodelling, ultimately culminating in symptomatic HF [21]. Of course, some clinical studies designed ad hoc to test this hypothesis are needed.

Another important clinical issue is that clinicians are not always aware on limitations related to the clinical use of cut-off and reference change values, calculated in a reference population, but, however, currently applied to a single individual/patient in the routine clinical practice. Indeed, this is the case of 99th percentile URL for cTnI, measured with hs-methods, which has a CV_I greatly lower than between-subject variability (8–10% vs 40–60%) [14]. However, a detailed discussion on all the difficulties related to pathophysiological interpretations and clinical applications of 99th percentile URL of hs-cTnI assay methods is beyond the purpose of the present article, and so interested readers should consult some review articles specifically focused on this important clinical issue [4,5,22,23].

4.1. Strengths and limitations of the study

The strengths of this study are related to the experimental design. Several Authors emphasized the need of both characterized reference populations and standardized statistical approach to calculate cTnI 99th percentiles [2,4,5,17,18]. Our study is a multi-center study which enrolled a well selected healthy reference population according to indications made by international guidelines and expert documents [2,4]. The study reference population consisted of 1325 healthy adult subjects with a large age range (18 to 86 years), and including 653 women and 672 men, well matched for age and sex-ratio. Furthermore, for a better evaluation of the 99th percentile URL values two different statistical approaches were used: the standard nonparametric robust approach, as suggested by international guidelines [2], and also the nonparametric bootstrap method, specifically used for a more accurate evaluation of confidence intervals [13].

A limitation of this study is that a relative low number of individuals with age > 55 years was enrolled (i.e., 530). This low number did not allow an accurate evaluation of the 99th percentile URL values divided for men and women with age > 55 years. However, it is well known that it is very difficult to enroll a great number of well characterized healthy subjects with age > 70 years [4–6,23].

Another limitation of this study regards the calculation of RCV [23]. Indeed, there are no study on intra-individual variability (CV_I) of cTnI values in healthy subjects, measured with the hs-cTnI ADVIA Centaur XPT method. For this reason, in this study for calculation of RCV values (Table 2), the CV_I, estimated by Van der Linden et al. [14] with the Architect hs-cTnI method (by Abbott Diagnostics), was used. Since ADVIA Centaur XPT and Architect methods actually present systematic differences between the measured cTnI values [8], some specific studies are needed to evaluate the actual intra-individual variability of cTnI with the hs-cTnI ADVIA method. Therefore, clinicians should be aware that the RCV values reported in Table 3 are only very preliminary, and so they should be confirmed by specific studies.

5. Conclusions

The results of the present study demonstrate that the ADVIA Centaur High-Sensitivity Troponin I using the Centaur XPT automated platform fits both the criteria and quality specifications required by the most recent international guidelines for high-sensitivity methods for cTnI assay [2]. Furthermore, the results of this study confirm that the calculation of the 99th percentile URL values are greatly affected not only by age and sex of the reference population, but also by the statistical approach used for calculation of cTnI distribution parameters.

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