Clinica Chimica Acta 496 (2019) 25–34

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Head-to-head comparison of plasma cTnI concentration values measured with three high-sensitivity methods in a large Italian population of healthy volunteers and patients admitted to emergency department with acute coronary syndrome: A multi-center study☆,☆☆

Aldo Clerico[a,*], Andrea Ripoli[a], Martina Zaninotto[b], Silvia Masotti[a], Veronica Musetti[a], Marcello Ciaccio[c], Rosalia Aloe[d], Sara Rizzardi[e], Ruggero Dittadi[f], Cinzia Carrozza[g], Tommaso Fasano[h], Marco Perrone[i], Antonio de Santis[j], Concetta Prontera[a], Daniela Riggio[k], Cristina Guiotto[l], Marco Migliardi[l], Sergio Bernardini[i], Mario Plebani[b]

[a] Laboratorio Clinico, Fondazione CNR-Regione Toscana, G. Monasterio e Scuola Superiore Sant'Anna, Pisa, Italy
[b] Department of Laboratory Medicine, University-Hospital, Padova, Italy
[c] Department of Laboratory Medicine, University-Hospital, Palermo, Italy
[d] S. S. Dipartimentale Biochimica ad Elevata Automazione, Dipartimento Diagnostico Azienda Ospedaliero-Universitaria di Parma, Parma, Italy
[e] Azienda Socio-Sanitaria Territoriale di Cremona, UO Laboratorio analisi chimico cliniche e microbiologiche, Cremona, Italy
[f] Ospedale dell'Angelo ULSS 3 Serenissima, Mestre, Italy
[g] UOC Chimica, Biochimica e Biologia Molecolare Clinica, Fondazione Policlinico Universitario "A. Gemelli", IRCCS, and Università Cattolica del Sacro Cuore, Roma, Italy
[h] Laboratorio Analisi Chimico-Cliniche ed Endocrinologia (LACCE), Azienda Unità Sanitaria Locale - IRCCS di Reggio Emilia, Italy
[i] Università degli Studi Di Roma Tor Vergata, Dipartimento di Medicina, Roma, Italy
[j] Laboratorio di Analisi Cliniche e Microbiologiche P.O. San Paolo – ASL Bari, Bari, Italy
[k] Centro Cardiologico Monzino IRCCS, Milan, Italy
[l] S.C. Laboratorio Analisi, A.O. Ordine Mauriziano di Torino, Torino, Italy

ARTICLE INFO

Keywords:
Cardiac troponins
Reference population values
High-sensitivity methods
Acute coronary syndrome
Myocardial infarction

ABSTRACT

Background: The study aim is to compare cTnI values measured with three high-sensitivity (hs) methods in apparently healthy volunteers and patients admitted to emergency department (ED) with acute coronary syndrome enrolled in a large multicentre study.

Methods: Heparinized plasma samples were collected from 1511 apparently healthy subjects from 8 Italian clinical institutions (mean age: 51.5 years, SD: 14.1 years, range: 18–65 years, F/M ratio:0.95). All volunteers denied chronic or acute diseases and had normal values of routine laboratory tests. Moreover, 1322 heparinized plasma sample were also collected by 9 Italian clinical institutions from patients admitted to ED with clinical symptoms typical of acute coronary syndrome. The reference study laboratory assayed all plasma samples with three hs-methods: Architect hs-cTnI, Access hs-cTnI and ADVIA Centaur XPT methods. Principal Component Analysis (PCA) was also used to analyze the between-method differences among hs-cTnI assays.

Results: On average, a between-method difference of 31.2% CV was found among the results of hs-cTnI immunoassays. ADVIA Centaur XPT method measured higher cTnI values than Architect and Access methods. Moreover, 99th percentile URL values depended not only on age and sex of reference population, but also on the statistical approach used for calculation (robust non-parametric vs bootstrap).

Conclusions: Due to differences in concentrations and reference values, clinicians should be advised that plasma samples of the same patient should be measured for cTnI assay in the same laboratory. Specific clinical studies

☆☆ Study promoted by the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Section of the European Ligand Assay Society (ELAS)

* 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@ftgm.it (A. Clerico).

https://doi.org/10.1016/j.cca.2019.06.012
Received 24 May 2019
Available online 13 June 2019
0009-8981/

are needed to establish the most appropriate statistical approach to calculate the 99th percentile URL values for hs-cTnI methods.

1. Introduction

The 2018 Fourth Universal Definition of Myocardial Infarction [1] states: "the term myocardial injury should be used when there is evidence of elevated cardiac troponin values with at least one value above the 99th percentile upper reference limit (URL)". Myocardial injury is a prerequisite for the diagnosis of myocardial infarction (MI), but also a distinct entity [1–3]. Indeed, several cardiac and systemic pathologies can result in myocardial injury without infarction. Therefore, clinicians should accurately distinguish these clinical conditions from MI [1–3]. The most recent international guidelines recommend that high-sensitivity methods should be preferred for the measurement of cardiac troponin I (cTnI) and T (cTnT) in patients admitted to emergence department with acute coronary syndromes (ACS) [1,4,5].

Recently, several clinical studies, including also three meta-analyses [6–8], demonstrated that the cardiovascular risk tend to increase also in some apparently healthy individuals of both sexes from cTnI and cTnT values below the 99th percentile URL. These studies support the hypothesis that cTn measurement with high-sensitivity (hs) methods may be effective for cardiovascular risk prediction and also for early detection of individuals at the highest risk for progression to symptomatic heart failure in general population [9]. In particular, the North-Trøndelag Health (HUNT) study recently reported that cardiovascular risk in general population seems to increase continuously and progressively from very low cTnI values (i.e., from 4 ng/L for women and 6 ng/L for men), measured with a hs-method [10].

The 2018 Expert Opinion from AACC and IFCC [5] recommends that hs-methods should satisfy two fundamental criteria. First, hs-methods should measure the 99th percentile URL with an imprecision (expressed as CV %) ≤ 10%. Second, these assays should be able to detect cTn concentration at or above the limit of detection (LoD) in at least 50% of healthy men and women. The estimation of 99th percentile URL strongly depends not only on demographic and physiological variables of the reference population, but also on the analytical performances of cTn methods, and the mathematical algorithm used for calculating 99th percentile URL values [5,11,12]. 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 and compare analytical performances and reference values of the hs-cTnI methods commercially available in Italy. According to these aims, the evaluation of the 99th percentile URL and reference change values (RCV) around the 99th URL of three immunoassay methods, which satisfy the two criteria required by international guidelines for hs-methods, have been recently reported by the Study Group of the Italian hs-cTnI Study [13–18]. The aim of this article is to report a head-to-head comparison of cTnI values measured in apparently healthy volunteers and patients admitted to Emergency Department (ED) with ACS with these three hs-cTnI methods.

2. Materials and methods

The Italian hs-cTnI Study is a multicenter clinical study. Heparinized plasma samples were collected from apparently healthy volunteers and patients admitted to ED by some Italian clinical institutions, including both University and Regional Hospitals, which have highly qualified workforce staff in emergency, cardiology and laboratory departments. The geographic distribution of several Italian Clinical Institutions participating to the multicenter study is reported in the Supplementary Fig. S1. These Clinical Institutions contributed to the study by collecting plasma samples, measuring cTnI concentrations and/or analyzing the results.

2.1. Reference healthy population and plasma sample collection

Height Italian clinical laboratories collected from 50 to 150 plasma samples from apparently healthy volunteers from the clinical and laboratory staff or blood donors with age from 18 to 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), according to recommendations of international guidelines [5].

In particular, to more accurately evaluate cTnI concentrations of individuals older than 47 years, plasma samples from 533 adult subjects (35% of overall healthy population) collected in the MEHLP study were also assayed (mean age 63.2 years; SD 8.0 years, minimum 47 years, maximum 85 years). The MEHLP study is a screening study aimed to evaluate the cardiovascular subclinical disease in an asymptomatic general population with age > 45 years from the community of Montignoso (Massa, Italy) [19]. All the subjects of MEHLP study underwent an accurate health investigation by means of a thorough clinical examination and routine laboratory tests (also including NT-proBNP assay) [19]. Furthermore, lifestyle habits and medical history were collected by questionnaires. Participants to the MHELP study underwent also ECG and cardiac imaging analysis (computed tomography scan, carotid echography, echocardiography). 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 [17–19].

Every laboratory participating to the study stored two aliquots of about 1 mL of plasma samples at temperatures ranging from -25°C to -80 °C in tubes identified by alphanumeric barcodes. The stored tubes were sent to the study reference laboratory (Fondazione CNR Regione Toscana G. Monasterio, Pisa Italy) from January to March 2019. Only age and sex of apparently healthy volunteers were known by the reference laboratory staff members. In the reference laboratory the clinical samples were immediately stored at −80° and then samples measured within three months with the all three hs-cTnI methods [17,18].

The informed consent was obtained by all volunteers enrolled in the study in accordance with the respective local ethical committee guidelines.

2.2. Patients admitted to Emergency Department (ED) for Acute Coronary Syndromes (ACS)

Nine Italian clinical laboratories collected 1322 blood samples (from 37 to 193 samples for each institution) of patients admitted to ED with suspect of ACS. The study population included 570 women and 752 men with a mean age of 66.7 ± 16.5 years (range from 18 to 101 years). The plasma samples were measured by clinical laboratories with the cTnI or cTnT immunoassay method used in routine practice. The stored tubes (identified by alphanumeric barcodes) were sent to the study reference laboratory (Fondazione CNR Regione Toscana G. Monasterio, Pisa Italy) within one month. Only age, sex, and time of blood collection of patients were known by the staff of the reference laboratory. In the reference laboratory the samples were immediately stored at −80° and then measured within three months with the three hs-cTnI methods.

The informed consent was obtained by all patients enrolled in the study in accordance with the guidelines recommended by the respective local ethical committees.

Table 1
Analytical parameters of hs-cTnI methods tested in the study.

Methods	LoB (ng/L)	LoD (ng/L)	LoQ 20% CV (ng/L)	LoQ 10% CV (ng/L)	Ratio[a]	Reference
Architect	0.7	1.3	1.8	4.7	5	[13,16]
Access DxI	0.6	1.3	2.1	5.3	4	[14,17]
ADVIA XPT	1.0	2.2	3.5	8.4	5.6	[15,18]

[a] Ratio: this value is the ratio between the 99th percentile URL value (ng/L) suggested by the manufacturer and the respective LoQ 10% CV value (ng/L) calculated by the reference laboratory of the study.

2.3. cTnI immunoassay methods

Three hs-cTnI methods were tested in the Italian Multicenter Study: ARCHITECT STAT High Sensitive Troponin-I, the Access hsTnI using DxI platforms, and ADVIA Centaur High-Sensitivity Troponin I using the XPT automated platform. For each of the three hs-cTnI methods, limits of blank (LoB), detection (LoD), and quantitation (LoQ) at both 10% and 20% CV values were calculated according to international standardized protocols [20,21], as previously described in details [13-18]. These results [13-18] demonstrated that the three immunoassays are able to satisfy the quality specifications required by international guidelines [5] to be considered as hs-cTnI assays.

The hs-cTnI method (REF 3P25-27) using the i1000SR platform (ARCHITECT STAT High Sensitive Troponin-I, Abbott Diagnostics Division, Ireland) is a two-site immunometric assay method. In particular, the Architect hs-cTnI method uses two monoclonal antibodies against the epitopes 24–40 (capture antibody) and 41–49 (detection antibody) of the human cTnI. The Internal Reference Standard (IRS), used for method calibration, is a human recombinant cTnI, traceable with the NIST SRM (Standard Reference Material). The analytical parameters for the Architect hs-cTnI method, previously evaluated by the study reference laboratory, were reported in Table 1 [13,16]. The 99th percentile URL values (reference values), suggested by the manufacturer, for women, men and total population are 15.6 ng/L, 34.2 ng/L, and 26.2 ng/L, respectively.

The Access hsTnI (REF B52699) assay is a two-site immunoenzymatic sandwich assay method (Beckman Coulter, Inc. Brea, CA 92821 USA). In particular, the Access hs-cTnI method uses two monoclonal antibodies against the epitopes 41–49 (capture antibody) and 80-90 (detection antibody) of the human cTnI. A recombinant troponin complex is used as standard for calibration of immunoassay system. The analytical parameters for the Access hs-cTnI method, previously measured by the study reference laboratory, were reported in Table 1 [14,17]. The reference values, suggested by the manufacturer, for women, men and total population are 11.6 ng/L, 19.8 ng/L, and 17.5 ng/L, respectively.

The ADVIA Centaur High-Sensitivity Troponin I using the XPT automated platform (Ref. 10994774-5, Siemens Healthineers Diagnostics) is a 3-site sandwich immunoassay using direct chemiluminometric technology. Two capture monoclonal antibodies are conjugated with streptavidin, while another recombinant anti-human cTnI sheep Fab is covalently attached to bovine serum albumin (BSA) for chemiluminescent detection. The two capture antibodies bind epitopes in the N-Terminal region and in the C-Terminal region, respectively, while the detection antibody recognizes an epitope in the N-terminal region of cTnI. The analytical parameters for the ADVIA XPT method hs-cTnI method, previously measured by the study reference laboratory, were reported in Table 1 [15,18]. The reference values, suggested by the manufacturer, for women, men and total population are 37.0 ng/L, 57.3 ng/L, and 47.3 ng/L, respectively.

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., 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 performed by means of Tukey's test [22], using the following formula: outlier cTnI value > Q_3 + 3 IQR; where, Q_3 and IQR are the third quartile and interquartile range ($Q_3 - Q_1$) of cTnI distribution, respectively.

The calculation of cTnI distribution and 99th percentile URL values was performed with the JMP program using nonparametric method, as recommended by international guidelines [2]. Lognormal distribution using a robust method was also calculated for comparison. The 99th percentile and the respective 95% and 99% confidence interval (CI) values were also calculated with adjusted bootstrap percentile method according to Carpenter & Bithelll, using random replacement of 68.27% of overall reference population and 100,000 repetitions [23].

Principal Component Analysis (PCA) was also used to evaluate and compare the cTnI values measured with the three hs-cTnI methods [24,25]. PCA is a statistical procedure that uses an orthogonal transformation to convert a set of several observations of correlated variables into a set of values of uncorrelated variables called principal components. The central idea of PCA is to reduce data set dimensionality including a large number of inter-related variables, while retaining data set variation as much as possible [24,25]. Log-transformed values were used for the PCA, as cTnI concentrations distribution is highly skewed both in apparently volunteers and patients.

4. Results

4.1. Comparison between hs-cTnI values measured by clinical center and reference laboratories

In order to evaluate the possible degradation of cTnI in plasma samples during the experimental study, the results of 692 plasma samples, collected from both apparently healthy volunteers and patients admitted to ED, were evaluated. These plasma samples were measured with the Architect hs-cTnI method in the clinical center as soon as after blood collection (mean 2219.5 ng/L, SD 9415.3 ng/L, median 7.9 ng/L, interquartile interval 2.3–77.9 ng/L), and also in the reference laboratory after shipping and storage at −80 °C (mean

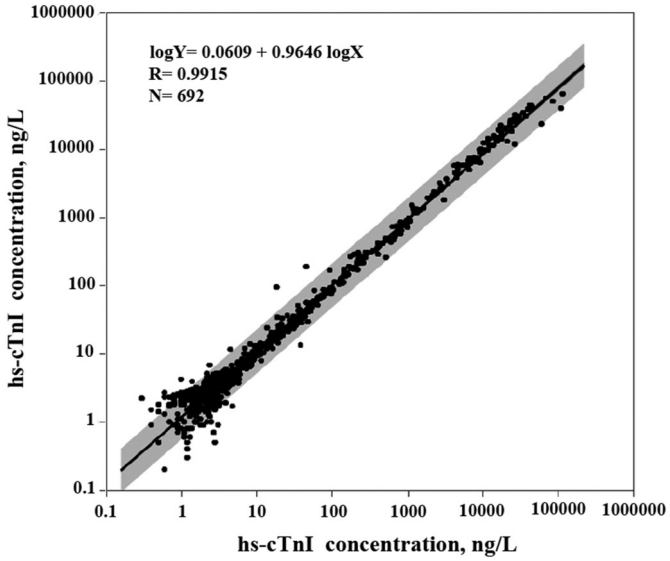

Fig. 1. Linear regression between hs-cTnI values of 692 plasma samples of healthy volunteers and patients admitted to ED, measured with Architect method in the clinical center as soon as after blood collection (X-axis) and in the reference laboratory after shipping and storage at −80 °C (Y-axis). The linear regression equation is also reported in the Figure. The 95% prediction interval (grey zone) is also indicated.

1852.8 ng/L, median 7.7 ng/L, interquartile interval 2.6–76.7 ng/L). A very close linear regression was found between the these two hs-cTnI measurements (Fig. 1) with a significant mean difference of 18.0% ($p < .001$ by Wilcoxon test). Considering that cTnI values were measured in three different laboratories, in different periods of time, with different lot of reagents, this significant difference in measured cTnI values may be due to analytical causes rather than cTnI degradation in stored plasma samples.

4.2. Reference population

The study reference laboratory stored 1526 plasma samples of apparently healthy subjects collected by 8 Italian clinical institutions. After statistical analysis of data set, 15 cTnI values (corresponding to 0.98% of total amount, including 5 women and 10 men) were excluded because as outliers. Consequently, the data sample of the study reference population included 1511 cTnI results measured with the three immunoassay methods, including plasma samples of 734 women and 777 men, respectively (whole population mean age: 51.5 years, SD: 14.1 years, range: 18–65 years, F/M ratio: 0.95). However, the plasma volume of some samples was not sufficient for cTnI assay with all the three immunoassay methods, and so different numbers of cTnI results were obtained for each hs-cTnI method (Table 2).

The descriptive statistics of cTnI distribution values (ng/L) of reference population measured with the three methods are reported in Table 2. A between-method difference of 31.2% (expressed as % CV) was found; the Architect method showed on average the lowest, while the ADVIA method the highest cTnI values (Table 2 and Fig. 2A). However, all the three methods showed a similar non-normal distribution, highly skewed on the right, approximating a log-normal distribution. The 99th percentile values for both overall and sex-related apparently healthy populations, calculated with the non-parametric robust method, were always higher (on average 24.8%) than respective values calculated with the nonparametric bootstrap method (26.3 ± 14.6 ng/L vs 19.7 ± 11.3 ng/L, $N = 9$, $p = .0018$) (Table 2). However, the 99th percentile values calculated with the non-parametric robust method were always within the 99% confidence interval of values calculated with the nonparametric bootstrap method. Finally, the 99th percentile values calculated with the non-parametric robust method for both overall and sex-related populations were on average slightly lower than those suggested by manufacturers (26.3 ± 14.6 ng/L vs 29.6 ± 15.5 ng/L; N = 9, $p = .0442$ by paired t-test) (Table 2).

cTnI values, measured with the three immunoassay methods, were significantly higher in men than in women ($p < .0001$) (Table 2), and they tended to progressively increase with age after the 55 year (Fig. 3). In particular, the median values of the cTnI distributions found in healthy women (Table 2) were respectively higher than LoD values of methods, previously evaluated in the study reference laboratory (Table 1) [14–18]. These data confirm previous results [14–18], indicating that Architect, Access and ADVIA XPT immunoassays actually satisfy both the criteria required to be considered high-sensitivity methods for cTnI assay [5].

The correlation matrix between the cTnI values measured with the three immunoassay methods in healthy subjects is reported in Table 3A, while the linear regressions between the measured values of the ha-cTnI immunoassay methods are reported in Fig. 4A. These data, taken as a whole, indicate that the results of hs-cTnI methods in apparently healthy subjects are poorly correlated (correlation coefficients ranging from 0.4868 to 0.6060).

Table 2
cTnI distribution values (ng/L) measured by immunoassay methods in the reference population.

Population groups	Mean ± SD	Median	25th percentile	75th percentile	97.5th percentile	99th percentile	99th perc. BS[a] (95% CI) (99% CI)
Architect							
Whole Population ($N = 1463$)	2.5 ± 2.6	1.8	1.2	2.8	9.6	18.9	14.4 (12.0–17.5) (11.4–19.2)
Women ($N = 699$)	1.8 ± 1.7	1.4	0.9	2.3	6.5	11.5	9.7 (6.8–12.4) (6.7–12.5)
Men ($N = 764$)	3.1 ± 3.1	2.1	1.5	3.4	12.3	21.2	17.2 (14.2–20.6) (13.4–23.9)
Access							
Whole Population ($N = 1460$)	3.3 ± 2.5	2.7	1.9	4.0	10.0	16.8	13.1 (11.8–15.2) (11.7–16.8)
Women ($N = 703$)	2.7 ± 1.9	2.3	1.6	3.2	6.4	15.5	9.2 (7.2–14.2) (6.6–16.8)
Men ($N = 757$)	3.9 ± 2.8	3.2	2.3	4.6	11.8	17.5	14.0 (12.4–17) (12.1–19.5)
ADVIA							
Whole Population ($N = 1411$)	4.6 ± 6.1	3.3	1.8	4.9	22.1	46.9	33.5 (26.2–42.8) (25.2–47.1)
Women ($N = 680$)	3.5 ± 4.8	2.7	1.1	3.9	14.7	38.1	24.7 (16.3–37.8.) (15.8–40.2)
Men ($N = 731$)	5.7 ± 7.0	3.9	2.6	5.6	26.0	50.0	41.8 (28.7–48.8) (26.6–52.2)

[a] BS: Bootstrap method.

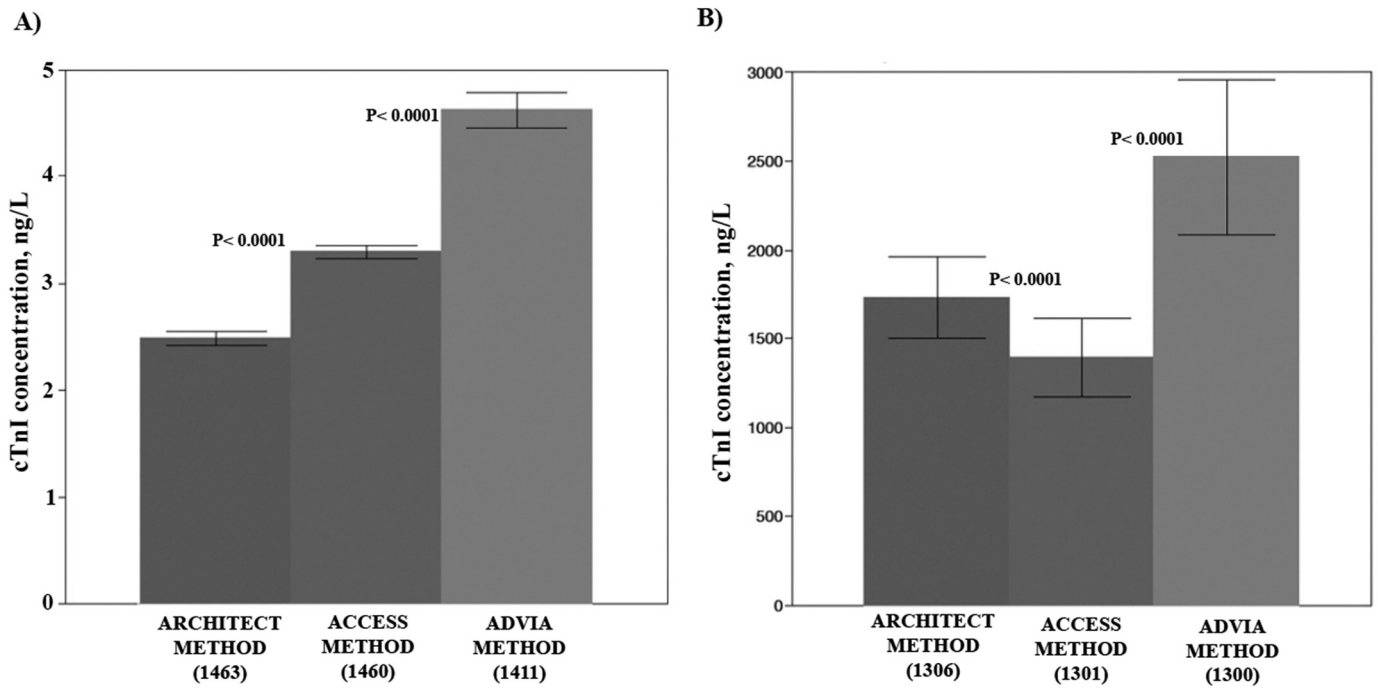

Fig. 2. The column plot reports the mean (± standard error) of cTnI values measured with the three immunoassay methods in samples of apparently healthy volunteers (Part A) and patients admitted to ED (part B), respectively. The *P* values of paired *t*-test between the log-transformed cTnI values are also reported in the Figure.

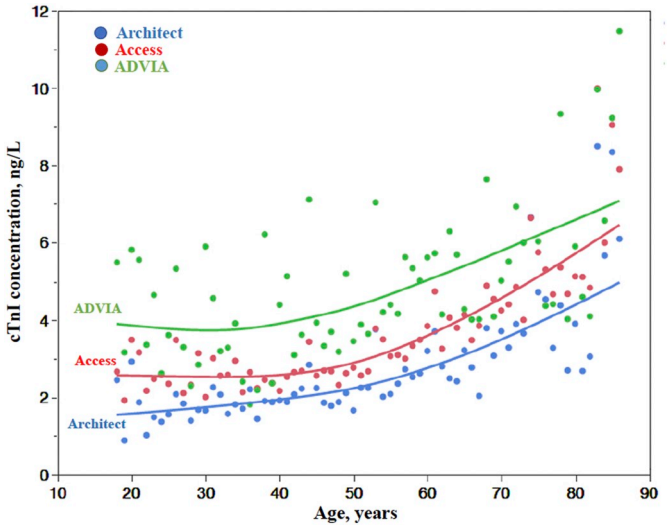

Fig. 3. Relationships between age (X-axis) and cTnI values measured with the three immunoassay methods in plasma samples of apparently healthy volunteers, respectively. The non-linear trend between age and cTnI was evaluated by means of regression spline analyses (indicated by color lines). The results of the three methods are reported with different colors.

4.3. Patients admitted to Emergence Department (ED)

Considering the 1322 heparinized plasma sample collected by 9 Italian clinical institutions from patients admitted to ED with clinical suspect of ACS, the plasma volume collected was not sufficient for cTnI assay with all the 3 immunoassay methods, so the 3 immunoassay methods gave different numbers of cTnI results also for patients admitted to ED.

A between-method difference of 29.2% (expressed as % CV) was found among the cTnI values measured with the three hs-cTnI methods. The ADVIA XPT method on average showed significantly higher cTnI values than Architect and Access methods, while Architect higher values than Access (Fig. 2 B). The correlation matrix between the cTnI values measured with the three immunoassay methods in patients admitted to ED is reported in Table 3B, while the linear regressions between the measured values of the hs-cTnI immunoassay methods are reported in Fig. 4B. The comparison of data reported in Table 3 and Fig. 4 demonstrates that cTnI values measured with the three hs-methods in plasma samples of patients admitted to ED are better correlated than those found in healthy volunteers.

4.4. Principal Component Analysis (PCA)

The loading plots (also known as variable correlation plot) of PCA related to both apparently healthy subjects (Part A) and patients admitted to ED (part B) are reported in Fig. 5. Considering the data related to apparently healthy volunteers (Fig. 5 A), the PCA allowed three principal components respectively explaining 69.0%, 17.9% and 13.1% of total variability of cTnI results (log-transformed values). Architect and Access methods showed more similar results than ADVIA PTX method. Considering the data related to patients admitted to ED (Fig. 5 B), the PCA allowed three principal components respectively explaining 98.5%, 0.9% and 0.5% of total variability of cTnI results (log-transformed values). Moreover, the three hs-cTnI methods showed more similar results in patinets admitted to ED (Part B), than in apparently healthy subjects (Part A).

Finally, considering the results of linear regression analyses (Fig. 4) and PCA (Fig. 5) as a whole, cTnI values observed in patients were on average better inter-correlated compared to those found in apparently healthy volunteers (mean R value in patients: 0.978 ± 0.005 SD; mean R in healthy subjects: 0.536 ± 0.06 SD; $N = 3$, $p = .0058$ by paired *t*-test). Furthermore, PCA data confirm that the hs-cTnI methods, tested in this study, show different results when respectively tested in apparently healthy volunteers and patients admitted to ED.

Table 3
Correlation matrix of cTnI values measured with the immunoassay methods respectively measured in healthy volunteers (part A) and patients admitted to ED (part B).

Methods	Architect	Access	ADVIA XPT
Part A[a]			
Architect	1.0000	0.6060	0.5114
Access	0.6060	1.0000	0.4868
ADVIA XPT	0.5114	0.4868	1.0000

Methods	logArchitect	logAccess	logADVIA XPT
Part B[a]			
logArchitect	1.0000	0.9826	0.9788
logAccess	0.9826	1.0000	0.9728
logADVIA XPT	0.9788	0.9728	1.0000

[a] Log_{10}-transformation of original cTnI data were used for the calculation of correlation matrix.

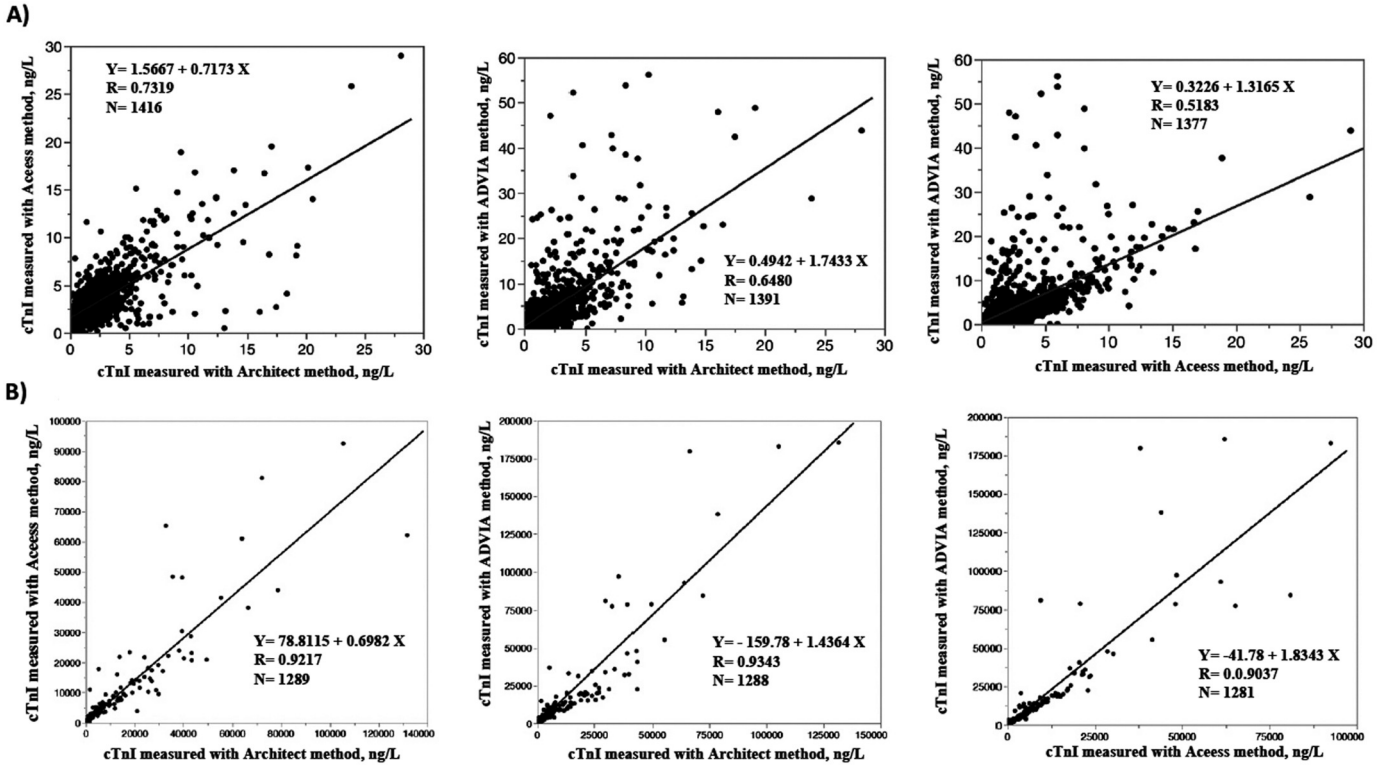

Fig. 4. Linear regression analyses between cTnI values measured with three immunoassay methods in plasma samples of apparently healthy volunteers (part A) and patients admitted to ED (part B). The linear regression equations are reported in the Figure.

5. Discussion

5.1. Analytical performances and systematic differences among hs-cTnI methods

The results of this study confirm that Architect, Access and ADVIA XPT methods show analytical performances in accordance with the quality specifications required by international guidelines for hs-cTnI assay [5]. In particular, these hs-cTnI methods on average measure 99th percentile URL values with an imprecision ranging from 4 to 6% CV (Table 1) [13–18], and they are also able to determine cTnI concentrations of the majority of healthy women with values above LoD values of the respective immunoassay methods (Table 2), in accordance with the two fundamental criteria recommended by international guidelines [5].

This study reports for the first time a head-to-head comparison of plasma cTnI concentration values measured with three hs-cTnI methods in a large Italian population of apparently healthy volunteers and patients admitted to ED with ACS. Our results demonstrate that significant systematic differences in measured cTnI values still persist even among the last generation of hs-cTnI immunoassay methods (Fig. 2).

PCA is commonly used to reveal the internal structure of different data sets in such a way that it is possible to better explain the differences (related to variances) among experimental data values [24,25]. In this study, PCA was used to analyze more in detail the between-method differences among hs-cTnI immunometric systems. Indeed, the first principal component, which accounts for as much of the variability in the data as possible, was found to be greatly different when calculated in a population including apparently healthy subjects (i.e., 69.0% of total variability) rather than in a group of patients admitted to ED (i.e., 98.5% of total variability) (Fig. 5). Accordingly, the second and third components were significantly reduced when measured in the data set

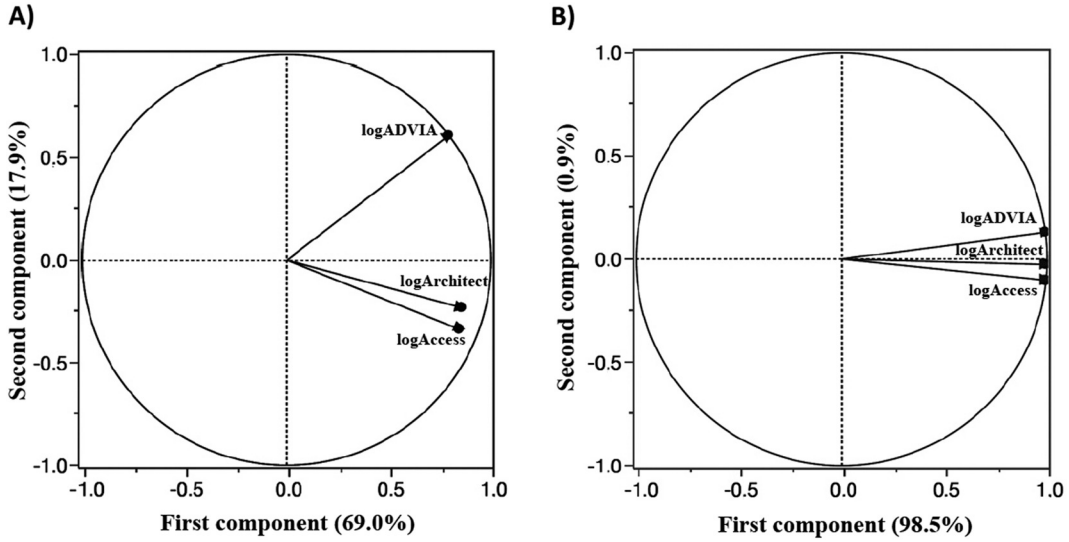

Fig. 5. Loading plots of the Principal Component Analysis (PCA) concerning cTnI values related to apparently healthy volunteers (part A) and patients admitted to ED (part B).

including patients admitted to ED (< 2% of total variability) compared to apparently healthy subjects (about 40% of total variability).

Recent studies demonstrated that there are differences in circulating molecular forms of protein, measured with hs-cTnI methods, in healthy subjects and patients with myocardial necrosis, respectively [26–29]. In healthy subjects, both at rest and after physical exercise, smaller molecular forms of cardiac troponins are usually detected into circulation, which are similar to the cTnI and cTnT degraded forms usually present in cytoplasm of cardiomyocytes [28–31]. On the contrary, higher molecular forms of circulating cTnI and cTnT are predominantly detect in patients with irreversible myocardial injury, especially those with MI [28,29,31].

Although hs-cTnI methods usually use different monoclonal antibodies, they are usually directed against epitopes located between the amino-acids 20 and 110 of human cTnI [32]. Indeed, this central part of protein chain is lesser susceptible to attack by plasma proteolytic enzymes than amino- and carboxyl-terminal parts of cTnI molecule. Furthermore, cTnI is protected from in vivo degradation by its specific binding with Troponin C [32]. In particular, ADVIA XPT method uses two capture monoclonal antibodies, which are specific for the respective N- and C-terminal regions of human cTnI peptide chain. On the contrary, Architect and Access methods use as capture phase of immunometric system only one monoclonal antibody specific for the more stable part of cTnI molecule.

These differences in number and epitope specificity of capture antibodies may explain the major part of systematic differences among the hs-cTnI assays, tested in this study. Indeed, it is theoretically conceivable that ADVIA XPT method, using two capture antibodies specific for the amino- and carboxyl-terminal parts of peptide chain, should theoretically be more specific for the intact or less degraded forms of cTnI molecule than Architect and Access methods. Of note, Architect and Access methods use two monoclonal antibodies specific for epitopes located in central part, more stable, of human cTnI. The utilization of monoclonal antibodies specific for the central part of cTnI explains why Architect and Access methods shows more similar measured values both in apparently healthy volunteers and patients admitted to ED compared to ADVIA XPT method, which uses three different monoclonal antibodies specific for epitopes positioned along all the peptide chain of cTnI.

Cytoplasmic forms are usually reported to represent the predominantly amount of circulating cTnI in adult healthy subjects [27,28,32]. Considering the different epitope specificity of capture antibodies, these degraded forms should affect more the cTnI measurement with Architect and Access than ADVIA XPT method, especially at very low cTnI concentrations, typical of healthy subjects. These interferences may also explain the increase in the second PCA component in samples of apparently healthy volunteers (Fig. 5 A). Indeed, the total variability explained by the second component was 17.9% in apparently healthy volunteers (Fig. 5 A), but only 0.92% in patients (Fig. 5 B). On the contrary, in patients admitted to ED with AMI the predominant forms present in the first 24 h are strictly related to sarcomeric cTnI, which is used (as standard material) for the calibration of immunoassay systems. Accordingly, the variability explained by first PCA component increased from 69.0% for group of apparently healthy volunteers (Fig. 5 A) to 98.5% for group of patients admitted to ED (Fig. 5 B).

Taking into-account the PAC results as a whole, the first PCA component may be considered as an index of harmonization among different hs-cTnI methods, while the other PCA components may be related to some possible analytical characteristics of immunometric assays as well as to some interfering substances present in plasma samples, which are able to produce an increase in systematic differences between hs-cTnI methods. Therefore, the results of the present study are well in accordance with those reported in previous studies suggesting the usefulness of PCA in evaluation and comparison of analytical characteristics and measured values of both cTnI [33] and TSH immunoassay methods [34,35],

5.2. Evaluation of 99th percentile URL values

Both selection criteria of reference population and statistical methods are critical points for a reliable evaluation of the 99th percentile URL value [5,11,12,36,37].

Several authoritative reviews and guidelines recommend that an accurate selection of the reference population is needed for evaluation of cTnI and cTnT distribution values. It is well known that age, sex, physical exercise, and even the presence of asymptomatic cardiovascular disease or co-morbidities can affect cTnI and cTnT circulating levels in apparently healthy individuals of general population [5,11,12,26–29]. The 2018 IFCC guidelines [5] recommend that the 99th percentile URL value for hs-cTnI assays should be reported according to sex-specific cutoffs. However, these guidelines do not currently recommend specific URL values divided by age/decade or by ethnicity [5]. According to Sandoval and Apple [11], an accurate evaluation of "healthy status" of the reference population (especially for volunteers with age > 55 years) was performed in this study.

Large differences (up to 2.7 folds between the Access and ADVIA methods) were observed among the 99th percentile URL values measured both in apparently healthy volunteers and patients admitted to ED with the hs-cTnI methods, tested in the present study (Table 2). A mean difference of 3.3 ng/L (SE 1.7 ng/L, $p = .0442$, corresponding to about 10%) was observed between the 99th percentile URL values calculated with the non-parametric method in this study (Table 2) and those suggested by manufacturers for both overall and sex-specific populations. This slight discrepancy among 99th percentile URL values are probably due to some differences in age ranges and men/women ratios of population enrolled in this multicenter study and that taken into consideration by manufacturers. Considering the Architect method, which is at present time the only hs-cTnI immunoassay with a great number of published clinical results, a recent meta-analysis [12] reported a mean sex-related difference of 10.97 ng/L (95% CI 7.10–14.85 ng/L) among 11 reference populations of different ethnic origins. Of note, in the present study the sex related difference of the reference population with the Architect hs-cTnI method was 9.7 ng/L, a value very similar to the mean sex-difference difference reported in the meta-analysis [12]. Accordingly, the results of the present study support the hypothesis that cTnI distribution values are similar among reference populations with different ethnic origins, and that the slight differences observed in different studies are probably due to differences in age and sex-ratio among apparently healthy subjects enrolled in these studies.

As far as the statistical methods for calculation of cTnI distribution in reference population are considered, parametric and non-parametric methods are usually used to calculate 99th percentile URL values [5,11,12,36,37]. The 2018 AACC/IFCC guidelines [5] recommend the use of non-parametric method for calculation of 99th percentile URL values for cTnI and cTnT assays. However, this document also states that it is critically important to implement an appropriate strategy for removing outliers as the statistical methods used can be differently affected by outliers, leading to different 99th percentile values [5]. Therefore, an accurate detection of possible outlier cTnI values is fundamental, especially for reference populations including several individuals with age > 65 years.

In the present study, a first preliminary screening of outlier values was performed by means of the Tukey's test [22]. In order to further reduce the influence of other possible outlier values, due to the presence of some individuals with asymptomatic cardiac disease, two different robust statistical approaches were used for calculation of cTnI distribution values of reference population. Both non-parametric and bootstrap statistical approaches were used in this study. In particular, the bootstrap non-parametric robust method, using random sampling with replacement (68.27% of overall reference population) and 100,000 repetitions, was used, because this statistical approach should be less affected by possible outlier values than the non-parametric robust method [23].

In this study, identical cTnI values were obtained for median, interquartile range, and 97.5th percentile values, measured with the three hs-cTnI methods, for overall and sex-related populations, with both non-parametric and bootstrap robust methods. On the contrary, significantly lower 99th percentile URL values (on average of about 25%) were obtained when bootstrap was compared to non-parametric approach (Table 2). At present time, there is no consensus about the best statistical approach for calculation of 99th percentile URL values for hs-TnI methods [5,11,12,36]. Sandoval & Apple [11] suggested that the future metric of a gold standard for MI may shift from the 99th percentile to the more conventional 97.5th percentile as used in laboratory medicine for most analytes. In accordance with this suggestion [11], the results of this study indicate that evaluation of 97.5th percentile of cTnI distribution of large reference populations is less sensitive to statistical approach, and so to presence of possible outliers than 99th percentile. Therefore, some specifically designed clinical studies are needed to evaluate the most reliable cut-off values for both clinical detection of myocardial injury and differential diagnosis of acute myocardial infarction.

5.3. Pathophysiological considerations related to circulating cTnI levels measured with hs-methods in healthy subjctes and patients with ACS

The 2018 AACC/IFCC guidelines [5] include three specific recommendations about the great relevance of closer communication and collaboration between clinicians and laboratorians, related to some possible problems in clinical interpretations on analytical aspects, cut-off values and poor harmonization of hs-cTn methods. In particular, considering the large between-method systematic differences in measured cTnI concentrations and cut-off values, clinicians should be advised that plasma samples of the same patient should be measured in the same laboratory for cTnI assay. Furthermore, laboratories and manufacturers should inform clinicians about specific metrics and cut-off values of different hs-cTnI assays [5].

At present time, the physiological mechanisms underlying the presence of measurable cTn levels in healthy adult subjects, both at rest and after physical exercise, are not well understood [12,26–30,38]. cTnI and cTnT may be released in healthy adult subjects due to some physiological mechanisms related to renewal in humans of cardiomyocytes [12,26–30,38]. Cardiomyocyte renewal primarily depends on the maturation/proliferation process of endogenous cardiac stem cells, and death by apoptosis of cardiomyocytes, which have reached the end of their life-span [39–43].

Some recent studies found an age-dependent renewal of human cardiomyocytes with the highest turnover during the first two decades of life, corresponding to rates of approximately 1% per year at the age of 20, declining to lower than 0.5% per year in elderly individuals [41,43]. These data might help explain not only the variability found in relation to the age of circulating cTnI and cTnT, but also the dependence on gender in healthy subjects. The total number of cardiomyocytes renewed per day might strictly depend on myocardial mass that, in men, is usually greater than in women. Indeed, some experimental data indicate that circulating cTn levels are strictly related to both ventricular mass and gender in large populations of healthy subjects [44–47].

There are only few studies regarding the circulating cTnI and cTnT levels in pediatric age, measured with hs-methods [13,48]. In particular, these results [13,48] support the hypothesis that the cardiac renewal is higher in children and adolescents than in adults, and it is probably related to physiological growth. Indeed, these Authors reported that cTnI and TnT levels are higher in neonatal period and infancy and then biomarkers values show a trend to progressively decrease throughout all adolescence until the adult age [13,48]. On the contrary, a huge number of studies demonstrate that cTnI and cTnT levels on average progressively increase in men and women, even in individuals apparently free of cardiac disease, after the age of 55 years (Fig. 3). These evidences suggest the hypothesis that some pathophysiological mechanisms, typical of senescence, are responsible of progressive increase in cTn levels in apparently healthy adults after the age of 65 years [49–51].

5.4. Strengths and limitations of the present study

The most important strength of this study is that several different Italian Research and Clinical Institution, distributed across all the Italian country (Supplementary Fig. S1), contributed to the study by collecting plasma samples of > 1500 healthy volunteers and 1300 patients admitted to ED. This large number of plasma samples allowed a robust statistical evaluation of 99th percentile URL values both for overall and sex-related populations for all the three hs-cTnI methods, using two robust statistical approaches (i.e., non-parametric and bootstrap methods) (Table 2). The very large set of data also allowed an accurate evaluation both of between-method regression analyses in

plasma samples both of healthy volunteers and patients (Fig. 4) and the two principal components of PCA (Fig. 5).

As far as the analytical procedure of experimental study is concerned, the samples of both apparently healthy volunteers and patients were assayed in only one reference laboratories with all the three hs-cTnI methods in order to reduce the analytical imprecision. Finally, the stability of cTnI in plasma samples throughout all the experimental procedure (from blood to assay in the reference laboratory) was also evaluated (Fig. 1).

Considering the cTnI reference population, all apparently healthy volunteers were enrolled according to the recommendations made by 2018 AACC/IFCC document [5]. In particular, the reference population included individuals of clinical and laboratory staff or volunteers blood donors with age from 18 to 86 years with men/women ratio approximately equal to 1. These two groups of subjects were periodically screened for the presence of chronic or acute diseases and had normal values of routine laboratory tests (including creatinine, electrolytes, glucose and blood counts). A limitation of this study is that the BNP/NT-porBNP assay was not assessed in all apparently healthy volunteers, but only in 533 adult subjects of the MEHLP study (including the 35% of overall healthy population) [19].

Another relative limitation of this study is that the reference population is constituted for > 95% of Caucasian ethnic individuals. The 2018 AACC/IFCC guidelines recommend that each study should enroll sex-group reference individuals that are representative of the patient population observed in their geographic area for patients admitted to ED with symptoms suggestive of myocardial injury [5]. Accordingly, the ethnic distribution of the reference population enrolled in this study is well representative of the population observed in overall geographic area of Italy. However, the 99th percentile URL values, found in this study, should not be used for populations with different ethnicity, range of age, or men/women ratio compared to the present study.

6. Conclusions

The results of this study demonstrate that systematic differences and cut-off values are still present among high-sensitivity methods for cTnI assay. These differences are wider for cTnI values under the 99th percentile URL value. Therefore, clinicians should be advised that plasma samples of the same patient should be measured in the same laboratory for the cTnI assay.

Furthermore, the results of this study confirm that 99th percentile URL values strongly depend not only on demographic characteristics of the reference population (i.e., age range and sex-ratio) but also on statistical approaches used for calculation of distribution cTnI parameters. Further specific clinical studies are needed to establish what is the most appropriate statistical approach to calculate the 99th percentile URL values for cTnI measured with high-sensitivity methods.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.06.012.

Acknowledgements

All the immunoassay kits used in this study were kindly supported by the manufacturers, namely: Abbott Diagnostics Italia (Rome, Italy), Beckman Coulter S.P.A Italy (Cassina de' Pecchi, Milan, Italy), and Siemens Healthineers Italia srl (Milano, Italy).

References

[1] K. Thygesen, J.S. Alpert, A.S. Jaffe, B.R. Chaitman, J.J. Bax, D.A. Morrow, H.D. White, Fourth universal definition of myocardial infarction, J. Am. Coll. Cardiol. 72 (2018) 2231–2264.
[2] K. Thygesen, What's new in the fourth universal definition of myocardial infarction? Eur. Heart J. 39 (2018) 3757–3765.
[3] C.M. Valentine, J.E. Tcheng, T. Waites, Translating the translation. What clinicians should know about the fourth universal definition of myocardial infarction, J. Am. Coll. Cardio. 72 (2018) 2668–2670.
[4] M. Roffi, C. Patrono, J.P. Collet, C. Mueller, M. Valgimigli, F. Andreotti, J.J. Bax, M.A. Borger, C. Brotons, D.P. Chew, B. Gencer, G. Hasenfuss, K. Kjeldsen, P. Lancellotti, U. Landmesser, J. Mehilli, D. Mukherjee, R.F. Storey, S. Windecker, 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the Management of Acute Coronary Syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC), Eur. Heart J. 37 (2016) 267–315.
[5] A.H.B. Wu, R.H. Christenson, D.N. Greene, A.S. Jaffe, P.A. Kavsak, J. Ordonez-Lianos, F.S. Apple, 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. 64 (2018) 645–655.
[6] N. van der Linden, L.J. Klinkenberg, O. Bekers, L.J. Loon, M.P. Dieijen-Visser, M.P. Zeegers, S.J. Meex, Prognostic value of basal high-sensitive cardiac troponin levels on mortality in the general population: a meta-analysis, Medicine. 95 (2016) e5703.
[7] J. Sze, J. Mooney, F. Barzi, G.S. Hillis, C.K. Chow, Cardiac troponin and its relationship to cardiovascular outcomes in community populations - a systematic review and meta-analysis, Heart Lung Circ. 25 (2016) 217–228.
[8] P. Willeit, P. Welsh, J.D.W. Evans, L. Tschiderer, C. Boachie, W. Jukema, I. Ford, S. Trompet, D.J. Stott, P.M. Kearney, S.P. Mooijaart, S. Kiechl, E. Di Angelantonio, N. Sattar, High-sensitivity cardiac troponin concentration and risk of first-ever cardiovascular outcomes in 154,052 participants, J. Am. Col. Cardiol. 70 (2017) 558–568.
[9] C. Passino, A. Aimo, S. Masotti, V. Musetti, C. Prontera, M. Emdin, A. Clerico, Cardiac troponins as biomarkers for cardiac disease, Biomark. Med (2019 Apr 3), https://doi.org/10.2217/bmm-2019-0039 (Epub ahead of print).
[10] F.D. Sigurdardottir, M.N. Lynbakken, O.L. Holmen, H. Dalen, K. Hyeem, H. Røsjø, T. Omland, Relative prognostic value of cardiac troponin I and C-reactive protein in the general population (from the north-Trøndelag health [HUNT] study), Am. J. Cardiol. 121 (2018) 949–955.
[11] Y. Sandoval, F.S. Apple, The global need to define normality: the 99th percentile value of cardiac troponin, Clin. Chem. 60 (2014) 455–462.
[12] A. Clerico, M. Zanonotto, M. Ripoli, S. Masotti, C. Prontera, C. Passino, M. Plebani, The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology, and clinical implications, Clin. Chem. Lab. Med. 55 (2017) 1634–1651.
[13] C. Caselli, G. Cangemi, S. Masotti, R. Ragusa, I. Gennai, S. Del Ry, C. Prontera, A. Clerico, 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 458 (2016) 68–71.
[14] 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. 56 (2018) 492–501.
[15] V. Musetti, S. Masotti, C. Prontera, S. Storti, R. Ndreu, G.C. Zucchelli, C. Passino, M. Emdin, A. Clerico, Evaluation of the analytical performance of a new ADVIA immunoassay using the Centaur XPT platform system for the measurement of cardiac troponin I, Clin. Chem. Lab. Med. 56 (2018) (e229e231).
[16] V. Musetti, S. Masotti, C. Prontera, R. Ndreu, G.C. Zucchelli, C. Passino, M. Emdin, A. Clerico, Evaluation of reference change values for a hs-cTnI immunoassay using both plasma samples of healthy subjects and patients and quality control samples, Clin. Chem. Lab. Med. (2019), https://doi.org/10.1515/cclm-2019-0032 Mar 20. pii: /j/cclm.ahead-of-print/cclm-2019-0032/cclm-2019-0032.xml. (Epub ahead of print).
[17] A. Clerico, A. Ripoli, S. Masotti, V. Musetti, R. Aloe, M. Dipalo, S. Rizzardi, R. Dittadi, C. Carrozza, S. Storti, L. Belloni, M. Perrone, T. Fasano, S. Canovi, M. Correale, C. Prontera, C. Guiotto, D. Cosseddu, M. Migliardi, S. Bernardini, Evaluation of 99th percentile and reference change values of a high-sensitivity cTnI method: a multicenter study, Clin. Chim. Acta 493 (2019) 156–161.
[18] A. Clerico, S. Masotti, V. Musetti, A. Ripoli, R. Aloe, M. Dipalo, S. Riuzzardi, R. Dittadi, C. Carrozza, S. Storti, L. Belloni, M. Perrone, T. Fasano, S. Canovi, M. Correale, C. Prontera, C. Guiotto, D. Cosseddu, M. Migliardi, S. Bernardini, Evaluation of 99th percentile and reference change values of the hs-cTnI method using ADVIA centaur XTP platform: a multicenter study, Clin. Chim. Acta 495 (2019) 161–166.
[19] M. Franzini, V. Lorenzoni, S. Masotti, C. Prontera, D. Chiappino, D. Della Latta, M. Daves, I. Deluggi, M. Zuin, L. Ferrigno, A. Mele, F. Marcucci, C.A. Caserta, P. Surace, A. Messineo, G. Turchetti, C. Passino, M. Emdin, A. Clerico, 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 438 (2015) 376–381.
[20] CLSI EP17-A protocol, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guidelines, (2004) Vol. 24, No. 34. Wayne, Pennsylvania.
[21] CLSI EP5-A2 protocol, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved guideline, Second edition, (2004) Vol. 24, No. 25. Wayne, Pennsylvania.
[22] W. Tukey, Exploratory Data Analysis, Addison-Wesley, Reading (PA), 1977.
[23] J. Carpenter, J. Bithell, Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians, Stat. Med. 19 (2000) 141–164.
[24] L.R. Fabrigar, D.T. Wegener, Exploratory Factor Analysis, Oxford University Press, Inc., New York, 2001, pp. 1–176.
[25] I.T. Joliffe, Principal Component Analysis, Springer, New York, 2013, pp. 1–520.

[26] F.S. Apple, Y. Sandoval, A.S. Jaffe, J. Ordonez-Lianos, IFCC Task Force on Clinical Application of Cardiac Bio-Markers. Cardiac troponin assays: guide to understanding analytical characteristics and their impact on clinical care, Clin. Chem. 63 (2017) 73–81.
[27] P.E. Hickman, J.M. Potter, C. Aroney, G. Koerbin, E. Southcott, A.H. Wu, M.S. Roberts, Cardiac troponin may be released by ischemia alone, without necrosis, Clin. Chim. Acta 411 (2010) 318–323.
[28] J. Mair, B. Lindahl, O. Hammarsten, C. Müller, E. Giannitsis, K. Huber, M. Möckel, M. Plebani, K. Thygesen, A.S. Jaffe, How is cardiac troponin released from injured myocardium? Eur. Heart J. Acute Cardiovasc. Care. 7 (2018) 553–560.
[29] T. Gresslien, S. Agewall, Troponin and exercise, Int. J. Cardiol. 221 (2016) 609–621.
[30] W.H.M. Vroemen, S.T.P. Mezger, S. Masotti, A. Clerico, O. Beckers, D. de Boer, A. Mingels, Cardiac troponin T: only small molecules in recreational runned after marathon completion, J Appl. Lab. Med. (2018), https://doi.org/10.1373/jalm.2018.027144 Published September 2018.
[31] E.P. Cardinaels, A.M. Mingels, T. van Rooij, P.O. Collinson, F.W. Prinzen, M.P. van Dieijen-Visser, Time-dependent degradation pattern of cardiac troponin t following myocardial infarction, Clin. Chem. 59 (2013) 1083–1090.
[32] M. Panteghini, Assay-related issues in the measurement of cardiac troponins, Clin. Chim. Acta 402 (2009) 88–93.
[33] Aldo Clerico, Andrea Ripoli, Silvia Masotti, Concetta Prontera, Simona Storti, Antonio Fortunato, Paola Buzzi, Ivo Casagranda, Maria Franzini, Rudina Ndreu, Gian Carlo Zucchelli, Martina Zaninotto, Mario Plebani, Pilot study on harmonization of cardiac troponin I immunoassays using patients and quality control plasma samples. On behalf of the Italian Section of the European Ligand Assay Society (ELAS) and of the Study Group on Cardiovascular Biomarkers of the Società Italiana di Biochimica Clinica (SIBioC), Clin. Chim. Acta 456 (2016) 42–48.
[34] A. Clerico, A. Ripoli, A. Fortunato, A. Alfano, C. Carrozza, M. Correale, R. Dittadi, G. Gessoni, M. Migliardi, S. Rizzardi, C. Prontera, S. Masotti, G. Zucchelli, C. Guiotto, P.A. Iacovazzi, G. Iervasi, Italian Section of the European Ligand Assay Society (ELAS), Harmonization protocols for TSH immunoassays: a multicenter study in Italy, Clin. Chem. Lab. Med. 55 (2017) 1722–1733.
[35] A. Clerico, T. Trenti, R. Aloe, R. Dittadi, S. Rizzardi, M. Migliardi, R. Musa, M. Dipalo, C. Prontera, S. Masotti, V. Musetti, R. Tozzoli, A. Padoan, M. Bagnasco, A multicenter study for the evaluation of the reference interval for TSH in Italy (ELAS TSH Italian study), Clin. Chem. Lab. Med. 57 (2019) 259–267.
[36] P.E. Hickman, G. Koerbin, J.M. Potter, W.P. Abhayaratna, Statistical considerations for determining high sensitivity cardiac troponin reference intervals, Clin. Biochem. 50 (2017) 502–505.
[37] A.H. Reed, R.J. Henry, W.B. Mason, Influence of statistical method used on the resulting estimate of normal range, Clin. Chem. 17 (1) (1971) 275–284.
[38] A. Giannoni, S. Giovannini, A. Clerico, Measurement of circulating concentrations of cardiac troponin I and T in healthy subjects: a tool for monitoring myocardial tissue renewal? Clin. Chem. Lab. Med. 47 (2009) 1167–1177.
[39] G. Olivetti, G. Giordano, D. Corradi, M. Melissari, C. Lagrasta, S.R. Gambert, P. Anversa, Gender differences and aging: effects on the human heart, J. Am. Coll. Cardiol. 26 (1995) 1068–1079.
[40] L.M. Buja, D. Vela, Cardiomyocyte death and renewal in the normal and diseased heart, Cardiovasc. Pathol. 17 (2008) 349–374.
[41] O. Bergmann, R.D. Bhardwaj, S. Bernard, S. Zdunek, F. Barnabé-Heider, S. Walsh, J. Zupicich, K. Alkass, B.A. Buchholz, H. Druid, S. Jovinge, J. Frisén, Evidence for cardiomyocyte renewal in humans, Science. 324 (2009) 98–102.
[42] E. Lázár, H.A. Sadek, O. Bergmann, Cardiomyocyte renewal in the human heart: insights from the fall-out, Eur. Heart J. 38 (2017) 2333–2342.
[43] O. Bergmann, S. Zdunek, A. Felker, M. Salehpour, K. Alkass, S. Bernard, S.L. Sjostrom, M. Szewczykowska, T. Jackowska, C. Dos Remedios, T. Malm, M. Andra, R. Jashari, J.R. Nyengaard, G. Possnert, S. Jovinge, H. Druid, J. Frisen, Dynamics of cell generation and turnover in the human heart, Cell. 161 (2015) 1566–1575.
[44] P.M. McKie, D.M. Heublein, C.G. Scott, M.L. Gantzer, R.A. Mehta, R.J. Rodeheffer, et al., Defining high sensitivity cardiac troponin concentrations in the community, Clin. Chem. 59 (2013) 1099–1107.
[45] K.M. Eggers, L. Lind, H. Ahlström, T. Bjerner, C. Ebeling Barbier, A. Larsson, et al., Prevalence and pathophysiological mechanisms of elevated cardiac troponin I levels in a population-based sample of elderly subjects, Eur. Heart J. 29 (2008) 2252–2258.
[46] C. Sinning, T. Keller, T. Zeller, F. Ojeda, M. Schlüter, R. Schnabel, et al., Association of high-sensitivity assayed troponin I with cardiovascular phenotypes in the general population: the population-based Gutenberg health study, Clin. Res. Cardiol. 103 (2014) 211–222.
[47] M. Bossard, S. Thériault, S. Aeschbacher, S. Aeschbacher, T. Schoen, S. Kunz, M. von Rotz, J. Estis, J. Todd, M. Risch, C. Mueller, L. Risch, G. Paré, D. Conen, Factors independently associated with cardiac troponin I levels in young and healthy adults from the general population, Clin. Res. Cardiol. 106 (2016) 96–104.
[48] M.K. Bohn, V. Higgins, P. Kavsak, B. Hoffman, K. Adeli, High-sensitivity generation 5 cardiac troponin T sex- and age-specific 99th percentile in the caliper cohort of healthy children and adolescents, Clin. Chem. 65 (2019) 589–592.
[49] A. Sheydina, D.R. Riordon, K.R. Boheler, Molecukar mechanisms of cardiomyocyte aging, Clin. Sci. 121 (2011) 315–329.
[50] M. Rota, P. Goichberg, P. Anversa, A. Leri, Aging effects on cardiac progenitor cell physiology, Compr. Physiol. 5 (2015) 1775–1814.
[51] R.H. Henning, B.J.J.M. Brundel, Proteostasis in cardiac health and disease, Nat. Rev. Cardiol. 14 (2017) 637–653.