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Analytical characteristics and performance of a new hs-cTnI method: a multicenter-study

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Analytical characteristics and performance of a new hs-cTnI method: a multicenter-study.

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Abstract:	OBJECTIVES: The present multicenter study was designed to evaluate the analytical performance and the 99th percentile value of the reference

	healthy population (i.e., 99th percentile URL) of the MAGLUMI® CLIA hs-cTnI method. METHODS: Analytical performances and the 99th percentile URL value of the CLIA (Chemi-Luminescent-Immuno-Assay) method were evaluated using validated and standardized experimental protocols. Two cohorts including healthy adult individuals were enrolled. The first one included 989 blood donor volunteers (489 women and 500 men) aged 18 to 70 years (mean age 43 years, interquartile range 31-54 years). The second population included 47 healthy individuals (31 women and 16 men, mean age 78 years, interquartile range 73-81 years) aged ≥71 years. RESULTS: The distributions of hs-cTnI levels in both sexes are highly right-skewed, and men show significantly ($p<0.0001$) higher biomarker values than women. Moreover, in both sexes the hs-cTnI levels progressively increase after the 55 years. In the multivariate analysis (n=958), hs-cTnI was found to be significantly associated to NT-proBNP ($p<0.0001$), sex ($p<0.0001$) and BMI ($p=0.0424$). The 99th percentile URL values, calculated using the bootstrap method in the total reference heathy population (age ≥ 18 years), were: Females (n=521): 5.93 ng/L (CI 95% 5.29 – 8.48), Males (n=516): 9.79 ng/L (CI 95% 6.37 – 17.41 ng/L), Total Population (n=1037): 7.18 ng/L (CI 6.08 – 12.20 ng/L). CONCLUSIONS. The MAGLUMI CLIA method met all the criteria for an hs-cTnI assay recommended by international guidelines. The hs-cTnI values measured with the CLIA method are higher in men compared to women at the same age, and also progressively increase after the age >55 years.

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Analytical characteristics and performance of a new hs-cTnI method: a multicenter-study

On behalf of the Study Group on Cardiac Biomarkers of the Italian Societies of Laboratory Medicine SIBioC and ELAS

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Running Head: Evaluation of a new hs-cTnI method

Key words: cardiac troponins, acute myocardial infarction, myocardial injury, high-sensitivity immunoassay methods, cardiac biomarkers, cardiovascular risk

ABSTRACT

OBJECTIVES: The present multicenter study was designed to evaluate the analytical performance and the 99th percentile value of the reference healthy population (i.e., 99th percentile upper reference limit (URLI) of the MAGLUMI® CLIA high-sensitivity cardiac troponin I (hs-cTnI) method.

METHODS: Analytical performances and the 99th percentile URL value of the CLIA *(*Chemi-Luminescent-Immuno-Assay*)* method were evaluated using validated and standardized experimental protocols. Two cohorts including healthy adult individuals were enrolled. The first one included 989

blood donor volunteers (489 women and 500 men) aged 18 to 70 years (mean age 43 years, interquartile range 31-54 years). The second population included 47 healthy individuals (31 women and 16 men, mean age 78 years, interquartile range 73-81 years) aged ≥71 years.

RESULTS: The distributions of hs-cTnI levels in both sexes are highly right-skewed, and men show significantly ($p<0.0001$) higher biomarker values than women. Moreover, in both sexes the hs-cTnI levels progressively increase after the 55 years. In the multivariate analysis (n=958), hs-cTnI was found to be significantly associated to NT-proBNP ($p<0.0001$), sex ($p<0.0001$) and BMI ($p=0.0424$). The 99th percentile URL values, calculated using the bootstrap method in the total reference heathy population (age ≥ 18 years), were: Females (n=521): 5.93 ng/L (CI 95% 5.29 – 8.48), Males (n=516): 9.79 ng/L (CI 95% 6.37 – 17.41 ng/L), Total Population (n=1037): 7.18 ng/L (CI 6.08 – 12.20 ng/L).

CONCLUSIONS. The MAGLUMI CLIA method met all the criteria for an hs-cTnI assay recommended by international guidelines. The hs-cTnI values measured with the CLIA method are higher in men compared to women at the same age, and also progressively increase after the age >55 years.

INTRODUCTION

The measurement of cardiac troponin I (cTnI) and cTnT is currently recommended by international guidelines for the detection of myocardial injury and the diagnosis of acute myocardial infarction (AMI) in patients admitted to the emergency department (ED) with acute chest pain [1–8]. From a pathophysiological point of view, the finding of a dynamic variation of abnormal high-sensitivity cardiac troponin I (hs-cTnI) or hs-cTnT concentrations is not necessarily related to a myocardial ischemic necrosis [1-9]. Other mechanisms beyond ischemic necrosis (such as apoptosis or exocytosis) can produce an acute release of cardiac troponins from cardiomyocytes [10-12]. Accordingly, the finding of a hs-cTnI and hs-cTnT concentration above the cut-value is necessary to diagnose myocardial injury, but it is not sufficient to diagnose AMI [1-8,10-13].

Both analytical and pathophysiological issues regarding the hs-cTnI and hs-cTnT assay must be taken into consideration for a correct diagnosis of AMI. Firstly, variations of circulating levels depend not only on the analytical error of hs-cTnI and hs-cTnT methods, but also on the intra-individual variability of the biomarker [12,14-16]. Second, the cut-off value to diagnose myocardial injury is defined as the 99th percentile value of hs-cTnI and hs-cTnT concentrations measured in the reference healthy population (i.e., the 99th URL value) [1-4,14,17-22]. The 99th upper reference limit (URL) value depends on method, age and sex, and, probably, on ethnic characteristics of individuals enrolled in the reference population [1,4,14,17-22]. Because of the importance of these points for proper evaluation of serial measurements, all the expert documents and guidelines emphasize the importance

of using validated and standardized experimental protocols for the evaluation of relative change value (RCV) and 99th URL values of hs-cTnI and hs-cTnT methods [1,4,14,17-22]. The most recent international guidelines recommend that only the immunoassays for cTnI and cTnT measurement, able to completely satisfy these quality specifications, should be defined "high-sensitivity" methods (hs-cTnI and hs-cTnT) [2-8,19,21]. Moreover, some expert documents and international guidelines recommend that the hs-cTnI and hs-cTnT methods should be preferred not only for early diagnosis of AMI [2-8,19,21], but also for cardiovascular risk stratification both in general population and cardiac patients [23,24]. Therefore, knowledge of the analytical performance of these improved immunoassay methods, especially at the low normal concentration range, is a relevant issue important for laboratory professionals and clinicians [2,6,12,14,15,19,21].

The present multicenter study, promoted by the Italian Study Group on Cardiac Biomarkers, was designed to evaluate the analytical performance of a new hs-cTnI method, named MAGLUMI® CLIA hs-cTnI (SNIBE Diagnostics, Shenzhen New Industries Biomedical Engineering Co., Ltd.No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China). The imprecision profile and 99th percentile URL of this new CLIA (Chemi-Luminescent-Immuno-Assay) method were evaluated and compared with those found in previous studies and using validated and standardized experimental protocols for hs-cTnT and hs-cTnT methods [4,12,14,17,18,25-33].

MATERIALS AND METHODS

Analytical characteristics of the CLIA method

The MAGLUMI® CLIA hs-cTnI is an *in vitro* CLIA method used to measure hs-cTnI in human serum and plasma using the Maglumi^{TM} X8 fully-automated analyzer (SNIBE Diagnostics). The magnetic microbeads coated with cTnI monoclonal antibody, the N-(4-aminobutyl)-N-ethylisoluminol (ABEI) label conjugated to another cTnI monoclonal antibody and buffer are mixed and incubated, reacting to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and a wash cycle is performed. A starter reagent is then added to initiate a chemiluminescent reaction. Finally, the light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of the cTnI present in the sample. The manufacturer reported in the instruction manual that only serum and plasma (K_2-EDTA, Li-heparin) specimens were tested and found acceptable for the MAGLUMI® CLIA hs-cTnI method.

The analytical characteristics and performance of the CLIA method were assessed in agreement with the standard international protocols [14,34,35], as previously discussed in details in other experimental and clinical studies supported by the Italian Study Group on Cardiac Biomarkers [22,25-33]. In particular, Table 1 reports the parameters of analytical sensitivity, including: limit of blank

(LoB), limit of detection (LoD), and limits of quantitation (LoQ) at 10% and 20% CV. These analytical parameters were calculated as previously reported in detail [14,30-32].

All blood samples related to the adult reference population, collected in the 3 different clinical laboratories involved in the multi-center-study, were centrifuged at high speed for 5-10 min in order to reach a centrifugal force of 3000 × g, as soon as after blood collection. These serum samples were then employed to evaluate the analytical characteristics and performance of the MAGLUMI® CLIA hs-cTnI method in the Department of Clinical and Molecular Sciences (Università Politecnica delle Marche, Ancona, Italy) and the Clinic of Laboratory and Precision Medicine, of the IRCCS INRCA (Ancona, Italy). To evaluate the agreement between methods, 204 blood serum samples, collected from both heathy subjects and patients with cardiovascular diseases were evaluated in the Clinical Laboratory of the Ascoli Piceno Hospital. These serum samples were also assayed with the ACCESS DXi hs-cTnI for between-methods (Beckman Coulter Life Sciences Inc., Indianapolis, USA). The analytical characteristics and performance of the ACCESS DXi hs-cTnI method were previously assessed [19,21].

Studied populations

In this multicenter study, two different Italian populations, including apparently healthy individuals, were enrolled for the evaluation of the age- and sex-matched 99th percentile values (99th URL value). The first population included 989 blood donor volunteers (489 women and 500 men) aged from 18 to 70 years (mean age 43 years, interquartile rage 31-54 years). These healthy individuals were enrolled in collaboration with the Transfusion Medicine/Blood Banking Unit of the AUSL Romagna (Cesena, Italy). The second population, including 47 apparently healthy individuals (31 women and 16 men, mean age 78 years, interquartile range 73-81 years) aged ≥71 years, was enrolled in collaboration with the Department of Clinical and Molecular Sciences (Università Politecnica delle Marche, Ancona, Italy) and the Clinic of Laboratory and Precision Medicine, of the IRCCS INRCA (Ancona, Italy). Finally, 204 serum samples of healthy subjects and patients with cardiovascular diseases have been collected by the Clinical Laboratory of the Department of Clinical Pathology (Ascoli-Piceno Marche, Italy) to compare the hs-cTnI results obtained with different hs-cTnI methods.

Informed consent was obtained from all the subjects enrolled. All apparently healthy individuals enrolled in this study denied the presence of chronic or acute diseases and had normal values for all routine laboratory tests, including: glycated hemoglobin, creatinine, electrolytes, glucose and blood counts, and N-terminal pro-B-type natriuretic peptide (NT-proBNP). The cut-off values recommended by expert documents and international guidelines for the routine laboratory tests and

NT-proBNP assay were considered [3,6,19-23,34,35]. Moreover, in order to better screen healthy individuals of the reference population the presence of subclinical cardiac diseases was excluded by clinical examination, including electrocardiogram (ECG) examination. Finally, the Tukey test was used for excluding from the study the apparently healthy individuals showing outlier biomarker values, measured with the hs-cTnI methods, currently used in the clinical laboratories participating in the study.

Statistical analysis

Standard statistical analyses were carried out using the JMP-17 program (JMP Statistical Discovery LLC, 920 SAS Camp Drive Cary, NC 27513). Because cTnI circulating levels are not normally distributed, the statistical analyses were performed using log10 transformation of the original data.

The distribution of hs-cTnI circulation levels of two reference Italian populations, including the 99th percentiles with their 95% confidence interval (CI) values, were calculated in accordance with the international guidelines [6,19-21] and previous studies supported by the Italian Study Group on Cardiac Biomarkers [18,22,25]. Two statistical software programs were used for the evaluation of the 99th percentile URL value: the JMP-17 program and the package "boot" in R programming [38-40]. In particular, the JMP-17 program calculates the 99th percentile value by using the quantile exponential smoothing method, according to the Harrell-Davis distribution-free quantile estimator [36,39], while the package "boot" in R programming uses the bootstrap method [25,40].

RESULTS

Analytical characteristics and performance

The results concerning the analytical characteristics and performance of the CLIA method are reported in Table 1, together with the analytical performance declared by the manufacturer. The imprecision profile of the CLIA hs-cTnI method is reported in Figure 1; 14 serum samples with hs-cTnI concentrations from 0.67 ng/L to 426 ng/L were considered in this analysis. Moreover, the imprecision results of the CLIA hs-cTnI, calculated from the imprecision profile reported in Figure 1, are reported in Table 2. According to the results reported in Table 1 and Figure 1, the MAGLUMI® CLIA hs-cTnI method meets the requirements for hs-cTnI assays [1-3,6,19,21].

The manufacturer reported that the MAGLUMI® CLIA hs-cTnI method was tested for several potential endogenous and exogenous interferents using the standardized protocol (EP7-A2) of the Clinical and Laboratory Standards Institute (CLSI), including: hemoglobin (up to 1000 mg/100 mL), bilirubin (up to 60 mg/100 mL), intralipid (up to 3 g/100 mL), Human Anti-Mouse Antibody (HAMA,

up to 40 ng/mL), biotin (up to 0.5 mg/100 mL), digoxin (up to 7.5 μg/mL), acetaminophen (up to 500 μg/mL), and nifedipine (up to 60 μg/mL).

hs-cTnI circulating levels in age- and sex-matched adult populations

The distribution data analyses related to MAGLUMI® CLIA hs-cTnI levels, measured in the Italian healthy population (age >18 years) according to sex and age, are reported in the Figures 2 and 3. The distribution of hs-cTnI levels in both sexes are very skewed to the right. Men (mean=1.92 ng/L, SD=2.10 ng/L; median=1.495 ng/L, interquartile range= 1.22-1.87 ng/L; n=516) have significantly higher biomarker values (p=0.0028, n=1037) than women (mean=1.61 ng/L, SD=1.14 ng/L; median=1.28 ng/L, interquartile range=1.07-1.625 ng/L; n=521). In both sexes the biomarker concentrations increase progressively after 55 years (Figure 2). The age and sex distribution of the total study population is detailed in the Figure 3, while the 99th percentile URL values with their corresponding 95% CI, divided according to sex and age, are reported in Table 3. In particular, hs-cTnI values higher than the LoD value estimated in this study (i.e., 1.46 ng/L) were found in the 49% of healthy women, while in more than 70% if the LoD value suggested by the manufacturer (i.e., 1.00 ng/L) is considered (Table 1). Moreover, 53% of men have values hs-cTnI ≥ LoD value estimated in this study, while ≥75%, when the LoD value suggested by the manufacturer is taken into account.

At univariable linear regression analysis, hs-cTnI concentrations (log10-transformation values) were found to be significantly correlated to NT-proBNP concentrations (log10-transformed, p=0.0125, n=979), age (p=0.0034, n=989), and Body Massa Index (BMI) values (p=0.0018, n=967). Considering a multivariable linear regression model (number of observations, n=958), cTnI (i.e., considered as the independent log-transformed variable) was found to be very significantly correlated to NT-proBNP (i.e., as continuous log-transformed variable, p<0.0001), and sex (i.e., as dichotomous variable, p<0001), but only weakly to BMI (i.e., as continuous variable, p=0.0541), and not to age (i.e., as continuous variable, p=0.6820). Excluding from the multivariate model the continuous variable age, the results of the multivariate analysis (number of observations, n=958), including the hs-cTnI (log10-transformation) as independent variable, and NT-proBNP (log10-transformed), sex and BMI values as dependent variables are reported in Table 4.

Comparison between hs-cTnI methods

To evaluate the agreement between methods, 204 samples collected from both heathy subjects and patients with cardiovascular diseases, already analyzed for routine activities with the ACCESS DXi hs-cTnI methods and the measured values, were evaluated through the MAGLUMI® CLIA method. The linear regression between the hs-cTnI values measured with the ACCESS DXi (X-axis, log-

transformed data) and MAGLUMI CLIA (Y-axis, log-transformed data) is reported in Figure 4. In particular, the Figure 4A reports the linear regression analysis, while the Figure 4B the Passing and Bablok regression analysis, respectively (analyses performed using the program JMP-17, SAS Institute). There was a close agreement between the two hs-cTnI methods across a wide range of biomarker concentrations (i.e., of about 5 orders of magnitude).

DISCUSSION

At present, overdiagnosis, rather than underdiagnosis, may be the dominant form of AMI misdiagnosis in patients admitted to ED with thoracic pain [9]. This is an important problem for healthcare systems, because the individuals with an incorrect diagnosis of AMI may undergo unnecessary, costly and possibly invasive procedures [9]. AMI overdiagnosis may be due to the common elevations in hs-cTnI and hs-cTnT values among individuals with chest pain, who tend to be older and with a high burden of comorbidities [1-12].

The Fourth Universal Definition of Myocardial Infarction [1] states that the term AMI should be used when there is acute myocardial injury with clinical evidence of acute myocardial ischaemia and with detection of a rise and/or fall of hs-cTnI or hs-TnT values with at least one value above the 99th percentile URL. Accordingly, together with the pathophysiological conditions, also some analytical issues should be accurately considered for the demonstration and clinical interpretations of a rise and/or fall of hs-cTnI and hs-cTnT variations in patients arriving in the ED with suspected AMI. In particular, two specific analytical issues should be considered for the diagnosis of AMI in patients with suspected AMI: 1) the significant rise and/or fall (i.e., intra-individual variation) of biomarker circulating levels; 2) the significant increment above the 99th percentile URL value (i.e., the cut-off for the diagnosis of myocardial injury) of hs-cTnI and hs-cTnT methods.

The intra-individual variation of circulating levels strictly depends not only on the analytical error of hs-cTnI and hs-cTnT methods, but also on the intra-individual biological variability of the biomarker [12,14-16]. Several studies have clearly stated that the intra-individual biological variation of cardiac troponins is about 8-10% and similar in healthy adult subjects and patients with cardiac or non-cardiac diseases [12,16,42-53]. On the other hand, some recent experimental and clinical studies have demonstrated that all the most common hs-cTnI and hs-cTnT methods show similar RCV values [12,14-18]. As recently proposed [4,12,15,16], to evaluate the kinetics of hs-cTnI and hs-cTnT values in patients admitted to the ED with suspected NSTEMI, it may be easier to use the RCV values expressed as a percentage (using a threshold value ≥30%) rather than method-specific delta (i.e., as an absolute difference), as recommended by the 2020 ESC guidelines [8].

The results reported in Figure 1 and Table 2 demonstrate that the imprecision profile of the MAGLUMI CLIA hs-cTnI is similar to those previously reported for the most common hs-cTnI methods [14,15,22,28-34]. Similar to all the other hs-cTnI and hs-cTnT methods, it is reasonable to assume, also for the MAGLUMI CLIA hs-cTnI method, that an increase ≥30% between two subsequent samples collected from the same individual/patient is significant, suggesting a real dynamic elevation of biomarker levels.

The Fourth Universal Definition of Myocardial Infarction also established that the threshold value for myocardial injury is the 99th percentile of biomarker values estimated in a reference population (99th percentile URL) [1]. In 2018, the document from the American Association for Clinical Chemistry (AACC) and International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine stated the quality specifications required for all hs-cTnI and hs-cTnT methods [19]. The first criterion is that these assays must be able to measure the 99th percentile URL with a coefficient of variation (CV) ≤10%. The second criterion is that these assays must be able to measure biomarker levels above the LoD value in the majority of healthy adult subjects of both sexes. More recently, the Educational Recommendations from the IFCC Committee on Clinical Application of Cardiac Bio-Markers stated that the 99th percentile URL must be calculated in a reference population including at least 400 men and 400 women considered in good health with an age range from 18 to 80 years [54].

Other important issues on the AMI diagnosis using cardiac biomarkers concern the better statistical approach to choose for the calculation of the 99th percentile URL value of hs-cTnI and hs-cTnT methods, and also the use of sex specific cut-off values for the diagnosis of myocardial injury and AMI [1-3,6,18-20,25,52,54]. Furthermore, it is very difficult to enroll a large number of healthy individuals >80 years [17,20,21,25]. Considering these statistical and demographic issues, it is a very hard challenge of measuring with confidence the cut-off values of hs-cTnI and hs-cTnT in healthy populations, because outlier individuals can markedly affect the calculation of 99th URL, especially in individuals aged >60 years [17,20,21,25,54]. Accordingly, the most important issue regarding the calculation of the 99th URL is the identification of possible outlier values [17,20,21,25,54].

To reduce the impact of these problems related to analytical and demographic issues, in this study, a robust statistical approach [38-40] was used to calculate the 99th percentile URL according to sex (Table 3). Furthermore, two reference Italian populations with different ages (i.e., from 18 to 70 years and ≥71years, respectively) were also taken into consideration for the calculation of both sex and age specific 99th percentile URL.

International guidelines recommend that hs-cTnI and hs-cTnT methods should be able to detect the biomarker concentrations at or above the LoD in at least 50% of healthy women [19,20,21,54]. The fraction of healthy women with hs-cTnI levels higher than the LoD value, measured in this study

(i.e., 1.46 ng/L), is 49%. However, the fraction of healthy women with hs-cTnI levels higher than the LoD becomes >70%, if the LoD value suggested by the manufacturer (i.e., 1.00 ng/L) is taken into account. An overall evaluation of data, respectively reported in Table 3 and Figures 2 and 3, clearly confirm the experimental evidence that the number of healthy men and women with hs-cTnI values higher than a certain LoD value strictly depends on age distribution of individual of both sexes, enrolled in the reference population [20,25,54]. From a pathophysiological and clinical point of view, it is important to note that several recent studies have demonstrated that women and men with hs-cTnI and hs-cTnT levels in the first tertile biomarker distribution have a significantly lower cardiovascular risk than those with levels in the third tertile [2,23,24,56]. Considering the present study, the hs-cTnI values corresponding to the third tertile are 4.9 ng/L for women and 6.7 ng/L for men, respectively. Of course, the cut-off values of the MAGLUMI CLIA hs-cTnI method for cardiovascular risk evaluation in general population should be carefully evaluated by means of specifically designed clinical studied, as recommended by some expert documents and guidelines [21,23,24,54,56].

Study strengths and limitations

The most important study strength is that the project, experimental procedures and data evaluation were conducted using the same experimental protocols of other previous studies supported by the Italian Study Group of Cardiac Biomarkers [14,15,22,28-34], in agreement with the most recent national and international guidelines [4-6,19,21,54]. This approach allowed the Authors to utilize the large database acquired with other previous studies on evaluation of analytical and clinical studies on hs-cTnI and hs-cTnT (Table 2).

It is well known that the evaluation of the 99th percentile URL value is strongly influenced by the statistical approach used for the calculation [57]. Accordingly, another important strength of this study is the use of two different robust statistical programs, such as the JMP-17 and the package "boot" in R programming approaches, for calculation of the 99th percentile URL values, according to age and sex (Table 3) [36-40]. In particular, using the Explore Outliers Platform of the JMP-17, it is also possible to identify the individuals enrolled in the reference population with outlier values, measured with the MAGLUMI® CLIA hs-cTnI method, using the Reed/Dixon criteria, as recommended by the IFCC Committee on Clinical Application of Cardiac Bio-Markers [54]. On the other hand, the iterative statistical approach of the bootstrapping method is considered to be resistant to the presence of outliers in the data [58].

A possible study limitation may be the relative low number of healthy subjects (especially those aged >70 years) (Table 3). However, it is important to note that he relationships between the CLIA

hs-cTnI levels according to sex and age are in agreement with data previously reported in other Italian cohorts using different hs-cTnI methods, but similar experimental and statistical protocols [12,17,18,22,25,30-32].

Although the number of healthy women and men enrolled in this study is in accordance with the recommendations made by the most recent international guidelines [54], the accuracy of the statistical analysis critically depends on the number of subjects enrolled in the clinical study. Indeed, the sex- and age-specific values of the 99th percentile URL calculated by the two statistical approaches tend to become equal when the number of individuals considered in the statistical analysis increases, as reported in Table 3.

CONCLUSIONS

Overall, the results found in the present multi-center study indicate that the MAGLUMI CLIA hs-cTnI method fully meets the criteria required by the most recent international documents and guidelines. Moreover, the results of this study confirm that 99th percentile URL values are higher in men compared to women at the same age, and also that these cut-off values progressively increase after 55 years. Finally, using a multivariate regression analysis, the circulating levels of hs-cTnI, measured in the reference population (age >18 years), were found to be significantly to those of NT-proBNP ($p<0.0001$), as also to sex ($p<0.0001$) and BMI ($p=0.0424$).

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Figure Legends

Figure 1. Imprecision profile. Imprecision profile of the MAGLUMI® CLIA hs-cTnI method.

Figure 2. Measured hs-cTnI levels in the reference population. Distribution of hs-cTnI levels measured with the MAGLUMI® CLIA hs-cTnI method in the studied Italian healthy population (age >18 years) according to sex and age.

Figure 3. Age and sex distribution of hs-cTnI levels measured in the studied population. The number of subjects is reported in the Y-axis, while in the X-axis, the age (divided into 5-year groups) and sex of individuals are indicated (F=Females; M=Males; T=F+M).

Figure 4. Methods comparisons using regression analyses. (A) Linear regression analysis. Linear regression between the hs-cTnI values respectively measured with ACCESS DXi (X-axis, log-transformed data) and MAGLUMI CLIA methods (Y-axis, log-transformed data). (B) Passing-Bablok analysis. Passing-Bablok regression analysis (red line) between the hs-cTnI values respectively measured with the ACCESS DXi (X-axis, log-transformed data) and MAGLUMI CLIA methods (Y-axis, log-transformed data). Data analysis: τ(tau) Kendall rank correlation coefficient= 0.8201 ($p<0.0001$); Equation: Y= 0.06356 + 0.86799 X. The dotted line indicates the identity line (Y=X).

Figure 1

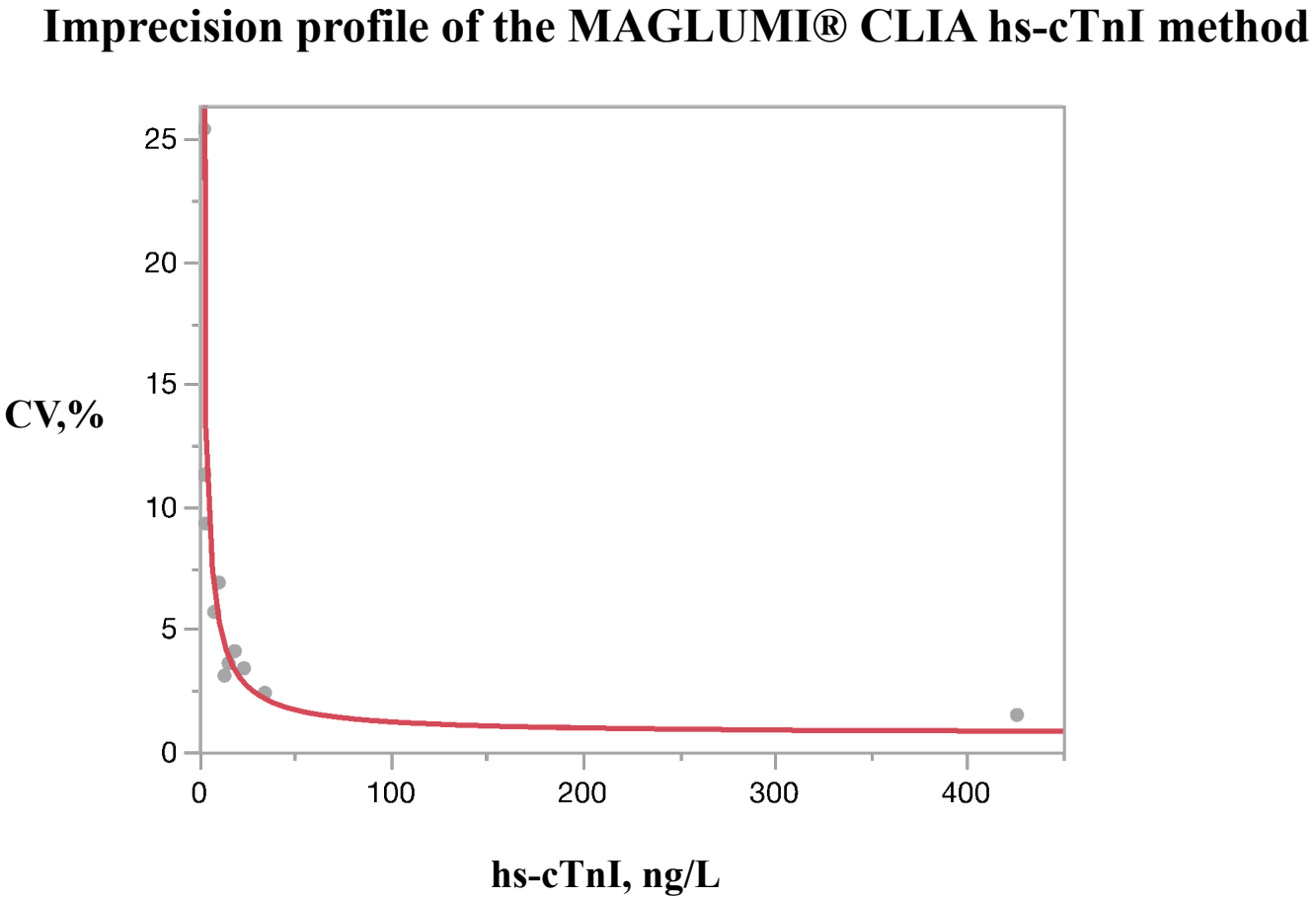

Imprecision profile of the MAGLUMI® CLIA hs-cTnI method
CV,%
25
20
15
10
5
0
0
100
200
300
400
hs-cTnI, ng/L

Figure 2

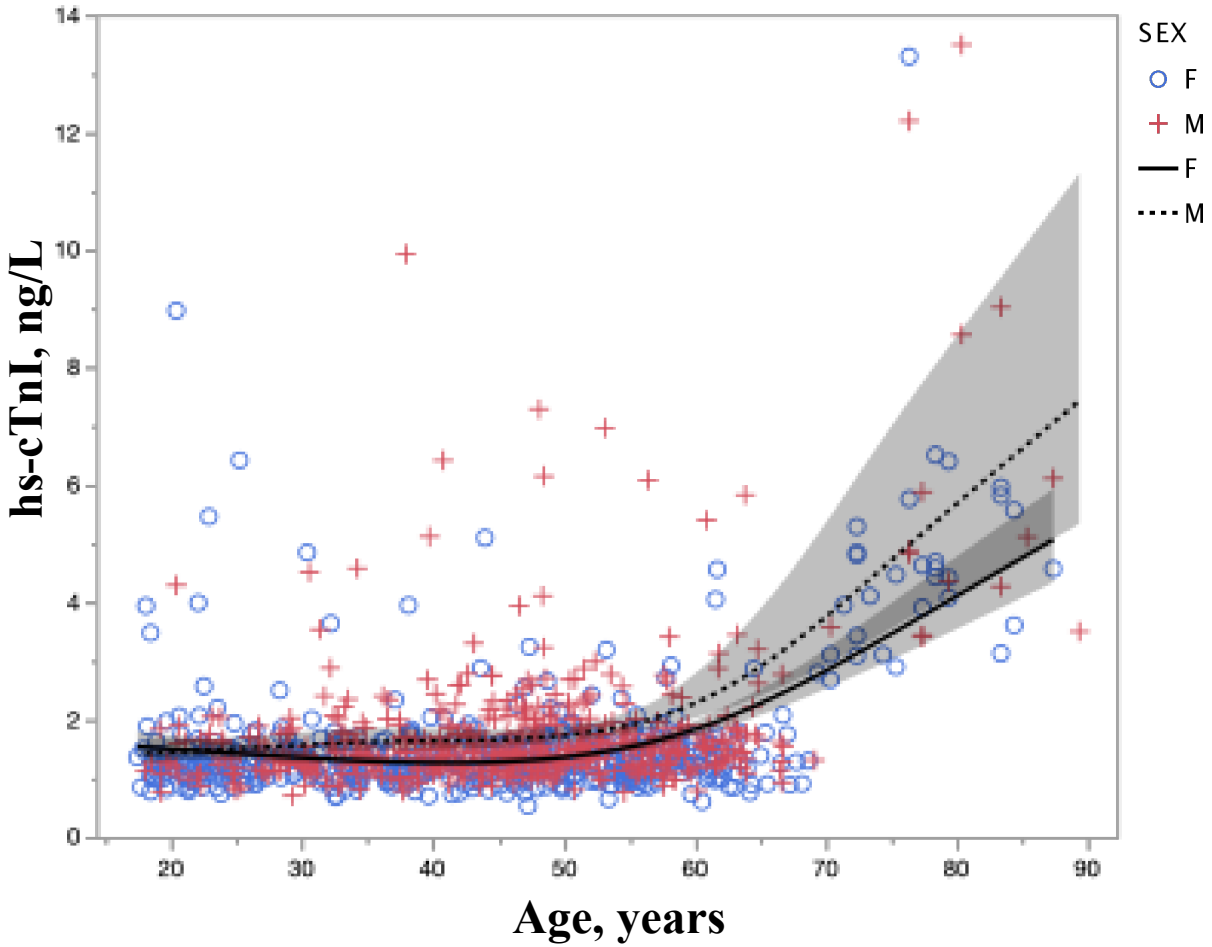

hs-cTnI, ng/L
Age, years
SEX
F
M
F
M
0
2
4
6
8
10
12
14
20
30
40
50
60
70
80
90

Figure 3

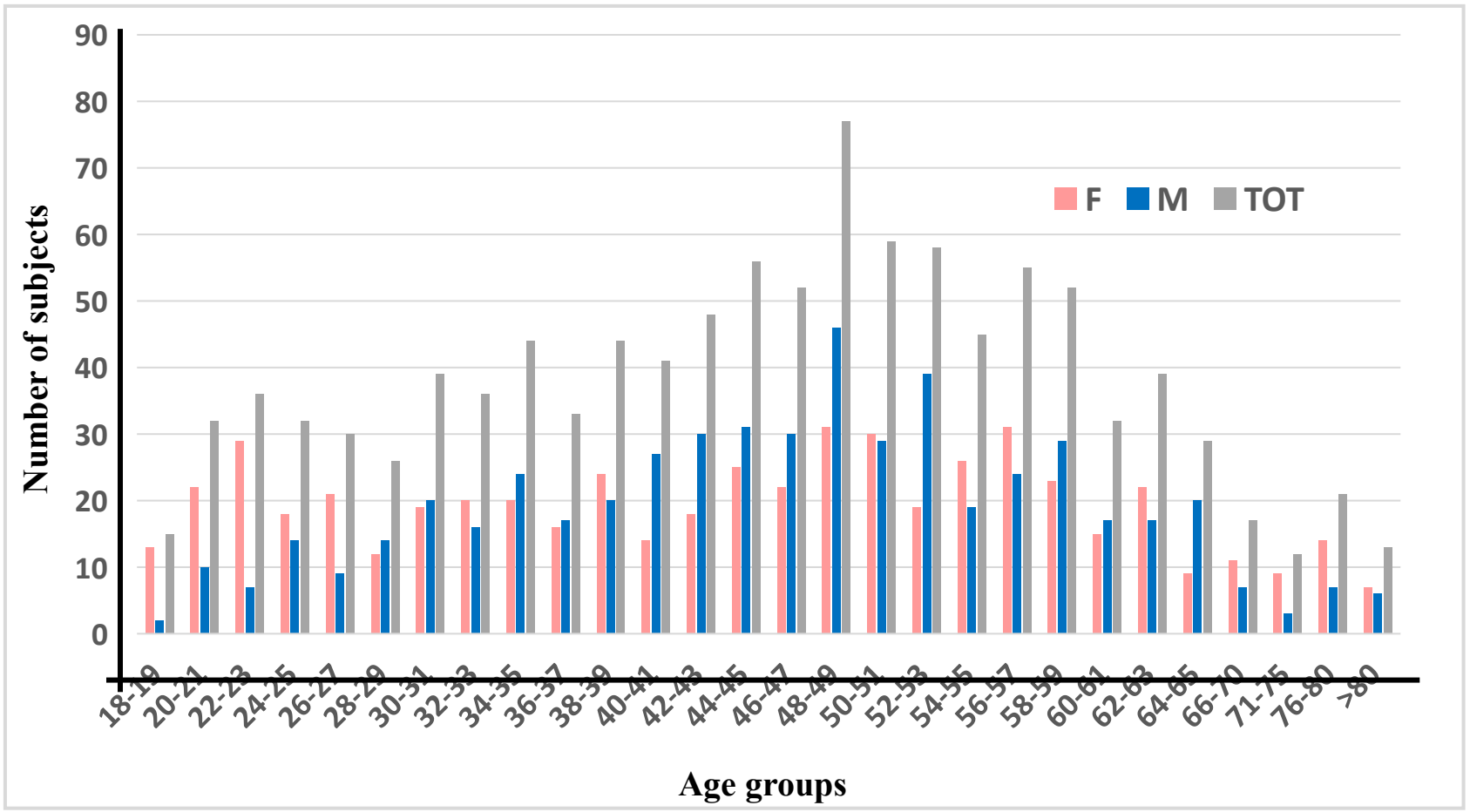

Distribution data of hs-cTnI levels measured in the studied population
Number of subjects
90
80
70
60
50
40
30
20
10
0
F
M
TOT
18-19
20-21
22-23
24-25
26-27
28-29
30-31
32-33
34-35
36-37
38-39
40-41
42-43
44-45
46-47
48-49
50-51
52-53
54-55
56-57
58-59
60-61
62-63
64-65
66-70
71-75
76-80
>80
Age groups

Figure 4A

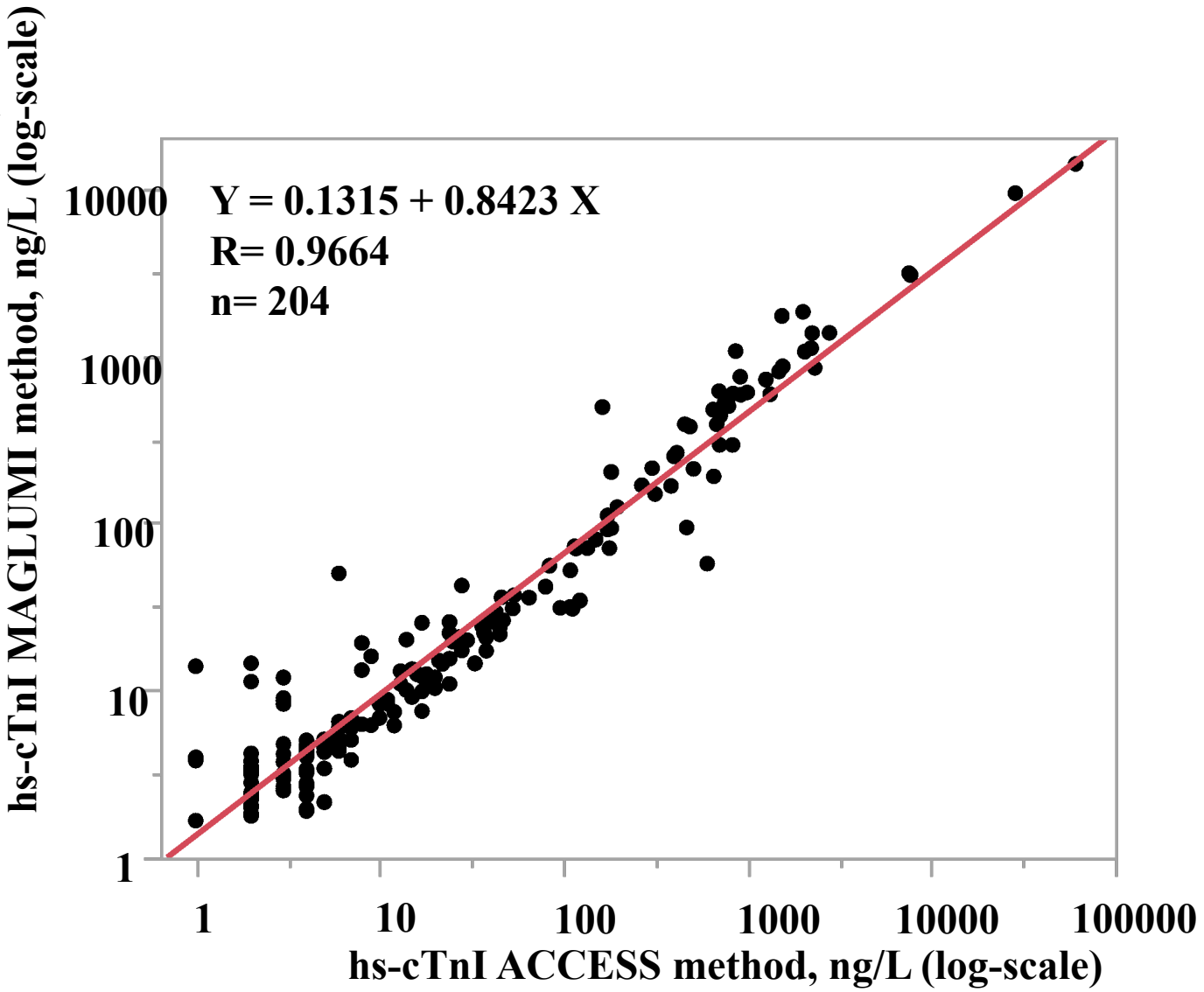

Y = 0.1315 + 0.8423 X
R= 0.9664
n= 204
hs-cTnI MAGLUMI method, ng/L (log-scale)
hs-cTnI ACCESS method, ng/L (log-scale)
10000
1000
100
10
1
1
10
100
1000
10000
100000

Figure 4B

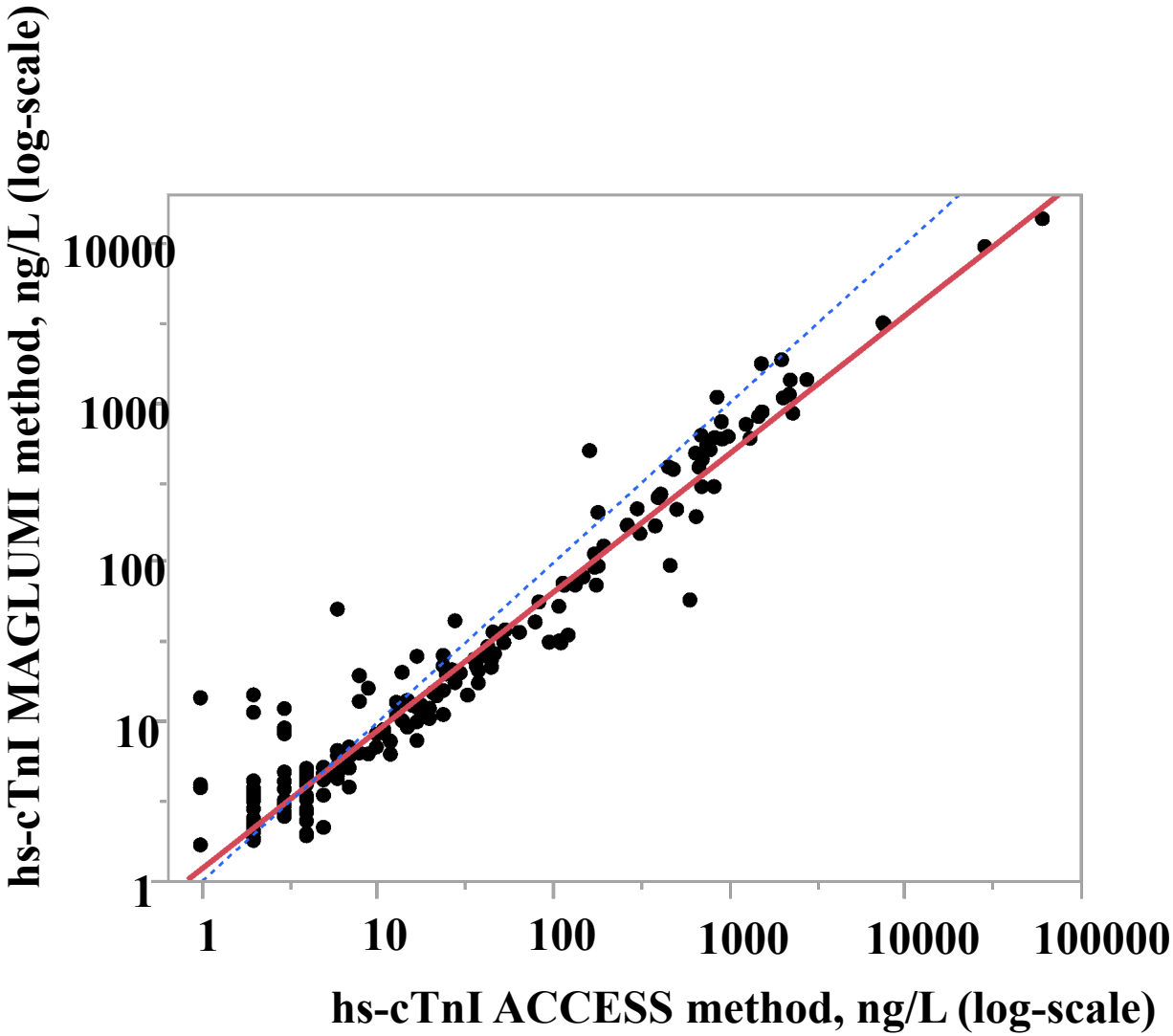

hs-cTnI MAGLUMI method, ng/L (log-scale)
10000
1000
100
10
1
1
10
100
1000
10000
100000
hs-cTnI ACCESS method, ng/L (log-scale)

Table 1 - Analytical performance of the MAGLUMI® CLIA hs-cTnI method

	LoB (ng/L)	LoD (ng/L)	LoQ 20% CV (ng/L)	LoQ 10% CV (ng/L)
Present Study	1.22	1.46	2.59	5.38
Manufacturer	0.50	1.00	2.00	-

LoB: Limit of Blank; LoD: Limit of Detection; LoQ: Limit of Quantitation [14].

Table 2- Imprecision profiles of the MAGLUMI CLIA hs-cTnI and also of the most popular hs-cTnI methods used in the Italian clinical laboratories, as previously reported in some experimental and clinical studies supported by the Italian Study Group of Cardiac Biomarkers [14,15,22,26-32].

hs-cTnI (ng/L)	ARCHITECT (CV%)	ACCESS (CV%)	ADVIA (CV%)	VITROS (CV%)	MAGLUMI (CV%)
1	32.2	34.6	65.5	33.0	50.5
3	13.4	14.6	23.5	13.6	17.4
5	9.6	9.8	15.1	9.7	10.7
10	6.8	6.7	8.9	6.7	5.7
15	5.9	5.7	6.8	5.7	4.1
20	5.4	5.2	5.7	5.2	3.2
25	5.1	4.8	5.1	4.9	2.7
30	4.9	4.5	4.7	4.7	2.4
40	4.7	4.4	4.1	4.5	2.0

- ARCHITECT: ARCHITECT hscTnI STAT method, Abbott Diagnostics.
- ACCESS: ACCESS High Sensitivity Troponin I Assay, Beckman Coulter.
- ADVIA: ADVIA Centaur High Sensitivity Troponin I Assay, Siemens Halthinners.
- VITROS: VITROS® High Sensitivity Troponin I Assay, OrthoClinical Diagnostics.
- MAGLUMI: MAGLUMI® CLIA hs-cTnI, SNIBE Diagnostics.

Table 3- Age and sex 99th URL values for hs-cTnI calculated in two Reference Italian populations using two different statistical programs.

Statistical Program	Females 99th URL [CI 95%], ng/L	Males 99th URL [CI 95%], ng/L	Total Population 99th URL [CI 95%], ng/L
Age 18-70 years			
JMP-17	4.82 [3.91 - 6.43], N= 490	6.70 [5.17 - 14.10], N= 500	6.06 [4.58 - 7.29], N= 990
Package boot in R	4.59 [3.67 - 6.42]	6.42 [5.12 - 14.10]	5.86 [4.58 - 7.01]
Age ≥ 71 years			
JMP-17	13.50 [6.67 - 13.81], N= 31	35.32 [14.63 - 36.11], N= 16	33.93 [13.31 - 34.34], N= 47
Boot Package in R	11.33 [6.23 – 13.30]	30.84 [11.72 – 33.90]	24.72 [12.21 – 33.90]
Age > 18 years			
JMP-17	5.98 [5.33 - 8.97], N= 521	9.96 [6.45 - 18.00], N= 516	7.30 [6.12 - 13.28], N= 1037
Package boot in R	5.93 [5.29 – 8.48]	9.79 [6.37 – 17.41]	7.18 [6.08 – 12.20]

Table 4- Results of the multiple linear correlation analysis considering hs-cTnI (log10 transformed), as independent variable, and NT-proBNP (log10 transformed), sex (dichotomous variable) and BMI as dependent variables.

	Estimation	Standard Error	P
Intercept	0.0080595	0.03632	0.8244
log NT-proBNP (ng/L)	0.0763198	0.013185	<0.0001
Sex (F)	-0.048755	0.005569	<0.0001
BMI	0.0027133	0.001335	<0.0424

The multiple linear correlation analysis was made with the program JMP-17 (JMP Statistical Discovery LLC, 920 SAS Camp Drive Cary, NC 27513).