Review

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Clinical relevance of biological variation of cardiac troponins

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Abstract: The high-sensitivity immunoassays for cardiac troponin I (hs-cTnI) and cardiac troponin T (hs-cTnT) are recommended by all the most recent international guidelines as gold standard laboratory methods for the detection of myocardial injury and diagnosis of acute myocardial infarction (AMI). In this review article, the Authors aimed at discussing the relevant biochemical, physiological, and clinical issues related to biological variability of cTnI and cTnT. Cardiac troponins, measured with hs-cTn methods, show a better clinical profile than the other cardio-specific biomarkers (such as the natriuretic peptides, BNP and NT-proBNP). In particular, the hs-cTn methods are characterized by a low intra-individual index of variation (<0.6) and reduced analytical imprecision (about 5% CV) at the clinical cut-off value (i.e., the 99th percentile URL value). Moreover, recent studies have reported that differences between two hs-cTn measured values (RCV) >30% can be considered statistically significant. These favourable biological characteristics and analytical performance of hs-cTn methods significantly improved the accuracy in the diagnostic process of acute coronary syndromes (ACS) in patients admitted to emergence department. In addition, several studies have demonstrated the clinical usefulness of cardiovascular risk evaluation with hs-cTn methods in some groups of patients with clinical conditions at high cardiovascular risk (such as systemic hypertension, severe obesity, diabetes mellitus, renal insufficiency, and chronic obstructive pulmonary disease). However, screening programs in the general population with hs-cTn methods for cardiovascular risk stratification require further investigation to define the optimal target populations, timing of measurement, and preventive interventions.

Keywords: biological variation; cardiac troponins; high-sensitivity methods; myocardial injury; reference change values.

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Introduction

The high-sensitivity immunoassays of cardiac troponin I (hs-cTnI) and cardiac troponin T (hs-cTnT) are recommended by all recent international guidelines as gold standard laboratory methods for the diagnosis of acute myocardial infarction (AMI) [1–4]. In particular, the AMI diagnosis requires a rise and/or fall of hs-cTn concentrations in patients with acute myocardial ischemia on serial testing, with at least one value above the 99th percentile of a reference population [1–4]. From a pathophysiological point of view, the fundamental statement of the Fourth Universal Definition of Myocardial Infarction concerns the definition of myocardial injury, which should be considered a distinct pathophysiological condition, with important clinical and also forensic relevance [3]. The most important corollary related to this statement is that myocardial injury, besides being a pre-requisite for the diagnosis of myocardial infarction, can be detected in many other cardiac or extra-cardiac conditions [1].

It is conceivable that a biomarker with a clinically favourable profile should have a within-subject biological variation (CV_i) lower than the between-subject biological variation (CV_g) [5, 6]. In particular, several recent studies reported that hs-cTn circulating levels in healthy adult subjects show significantly lower CV_i than CV_g values [6–12]. Considering both the statistical and clinical points of view, the very low index of "individual" variability should be considered when hs-cTn measured in a single patient is compared to the clinical cut-off value estimated

in a reference large population, such as the 99th percentile or cardiovascular risk values, which actually has a higher inter-individual variation [5, 6, 13–16].

The principal aims of this article are to evaluate the biochemical and physiological issues related to the low individuality index of cTn, and to discuss the relevant clinical implications of the biological variability of cTn compared to other cardiac biomarkers. In particular, the specific objectives of this review article are: (1) to consider the analytical challenges and pathophysiological interpretations related to hs-cTn methods, (2) to examine the basic concepts for determining the reference change values (RCV), (3) to review the results of previous studies on the biological variation of cTn, and (4) to discuss the potential benefits related to the utilization of hs-cTn methods in clinical practice in comparison with the analytical and biological characteristics of other biomarkers.

Analytical performances and pathophysiological interpretations related to hs-cTn methods

To date, all recent international guidelines strongly suggest that only high-sensitivity methods for cTn assay should be taken into consideration for the diagnosis of myocardial injury and AMI [1–4]. In particular, to meet the quality specifications required for a hs-cTn method two fundamental criteria need to be fulfilled [2]: (1) the error measurement (expressed as % CV) of the cTn concentration, corresponding to the 99th percentile value measured in the reference population (i.e., 99th percentile URL value) should be ≤10%; (2) assuming that women usually show significantly lower cTn levels than age-matched men [17, 18], measurable cTn concentrations should be obtainable at a value at, or above, the assay's LoD (Limit of Detection) in more than 50% of two populations including at least 300 women and men, respectively [2].

Considering these quality specifications, the accurate measurement of circulating cTn levels is a very hard challenge due to low biomarker concentrations observed in healthy adult subjects, especially women [17, 18]. Indeed, the 10% of cTnI values, measured with high-sensitivity methods in a large Italian population, are actually lower than 2 ng/L [19]. Considering the cTnT assay, about 20–25% of apparently healthy adult European and Chinese men and women [20–22] showed cTnT values ≤3 ng/L (i.e., the LoD of the method).

From a pathophysiological perspective, several authors have suggested that the circulating levels of hs-cTn measured in healthy adult subjects should be considered a reliable index of the physiological cardiomyocyte renewal [14–19, 23–25], which is defined as the ability to replace loss of cardiomyocytes by new ones [26]. In particular, some recent clinical studies report that the 99th percentile URL values of hs-cTn methods are on average ranging from 13 to 47 ng/L [13, 17–22], corresponding to about 30–40 mg of cardiomyocyte renewal [17, 23, 24]. Accordingly, the mean hs-cTn concentrations of about 3–5 ng/L, typical of adult healthy subjects, are related to a myocardial volume ≤10 mg. This amount of myocardial tissue is too low to be detected by non-invasive cardiac imaging, also including highly sensitive techniques, such as MRI or PET [14–19, 23, 24].

From a clinical point of view, several studies (including three meta-analyses) reported that hs-cTn values ≥99th percentile URL measured in general populations or elderly communities are significantly associated with an increased frequency of both cardiac mortality rate and major adverse cardiovascular events (MACE) [27–36]. In particular, one study reported that even small, but progressively increasing hs-cTnI values (e.g., about 5 ng/L) can significantly increase cardiovascular risk in asymptomatic individuals [29]. Due to the results of these studies [27–36], several Authors have recently proposed that hs-cTn assay may be a reliable laboratory test for early detection of patients with asymptomatic cardiac disease and at high risk for progression to heart failure [15–17, 37–39].

Biological variation and reference change value (RCV) of hs-cTn methods

Parameters of biological variation

Individual biological variation (CV_i) is usually defined as the random fluctuations of a biomarker around the individual homeostatic set point [5, 6]. This parameter is generally expressed as percent coefficient of variation (CV) of measured biomarker values, assuming that this index is relatively constant in apparently healthy individuals and also in individuals with stable disease [5, 6]. Moreover, the estimation of biological variation among several individuals, also expressed as CV, is termed between-subject biological variation (CV_g) [4–6]. Finally, the index of individuality is calculated according to Eq. (1) [5, 6], where CV_a is an estimation of analytical imprecision (expressed as CV) of a specific hs-cTn method [14, 19, 25, 40–43]:

$$\text{Index of Individuality} = \left(CV_a^2 + CV_i^2\right)^{1/2} \Big/ CV_g \quad (1)$$

From a clinical point of view, if the index of individuality is ≥1.4, then it may be more clinically useful to interpret the result of a test using population-based reference values. On the contrary, an individuality index ≤0.6 suggests a strong individuality of circulating levels of the biomarker, which, conversely, show large variations among different subjects. In this case, the population-based reference values become of little clinical use due to very large confidence intervals of cut-off values, such as 99th percentile URL or cardiovascular risk [5, 6].

The results of studies reporting data on biological variability, estimated using some hs-cTnI methods in adult healthy subjects, are summarized in Table 1. Four different hs-cTnI methods were used in studies on biological variation. These methods have different analytical performances, and evaluated populations with different demographic (especially sex ratio and age) and clinical characteristics [8, 11, 45–47]. Furthermore, different experimental protocols (i.e., time periods of study, temporal data analyse, and statistical analyses) were used. However, data reported in Table 1 demonstrated that the Index of Individuality (mean 0.32, SE 0.03) for hs-cTnI methods is on average significantly lower than 0.6 ($p<0.001$ by t test, n=8). Conversely, there is only one commercial method for hs-cTnT assay (i.e., the ECLIA Elecsys method by Roche Diagnostics), which, however, can run on different automated platforms. The results concerning the biological variation of hs-cTnT method are also reported in Table 1, Similarly to hs-cTnI, the Index of Individuality of the hs-cTnT method (on average 0.26) is significantly lower than 0.6 ($p<0.001$, n=4). Furthermore, the CV_i, CV_g and Index of Individuality are not significantly different between cTnI and cTnT methods; only the CV_a value tends to be lower for hs-cTnT than hs-cTnI methods ($p=0.0494$, non-paired t test).

Two very recent studies, using the Architect hs-cTnI [49] and hs-cTnT [12] methods, respectively, have further analysed the statistical, physiological and clinical limitations related to the evaluation of biological variation parameters assessed in apparently healthy subjects. The first limitation concerns the extreme difficulty to enrol a suitable number of "true" healthy adult volunteers, covering both sexes and all age groups [2, 6, 12–16, 50, 51]. Indeed, even the most sensitive hs-cTn methods are not able to measure cTn concentration ≥ limit of detection (LoD) in all the healthy subjects, especially women with age <40 years [13–19]. Another challenge is how to exclude the presence of asymptomatic cardiac disease in individual >65 years, without using expensive and also demanding clinical examinations [2, 13–19, 50, 51]. Accordingly, the estimated biological variation parameters may be strongly affected by the number and demographic or clinical characteristics of individuals enrolled in the study [6, 12, 49]. However, several recent studies reported that CV_i and Index of Individuality are similar in healthy subjects and patients with cardiac or renal diseases [6–10, 12, 47–50]. In particular, a recent systematic review [6] reported that the Index of Individuality is on average 0.14 (SE 0.02) in 15 studies including patients with cardiac or renal disease, i.e., even significantly lower than that observed in healthy subjects ($p<0.001$, nonpaired t test, analysis performed by Authors using data reported in the review).

From a physiological point of view, data reported in Table 1, considered as a whole, strongly support the hypothesis that the plasma hs-cTn concentration is a specific and stable index of an individual, strictly related to the physiological renewal of myocardial tissue [17, 23, 24]. The very low Index of Individuality should actually play an important clinical role, especially when compared with some other clinical cut-off values, estimated from a reference large population (such as the 99th percentile URL or a cardiovascular risk cut-off), which are characterized by a wider inter-individual variation [13–19, 51, 52].

The *Fourth Universal Definition of Myocardial Infarction* states that: "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 URL" [3]. In this clinical condition, a hs-cTn concentration value, characterized by a low Index of Individuality, is tested against a cut-off value with very large confidence intervals, resulting a significant reduction in diagnostic accuracy. For example, the 99th percentile URL values of hs-cTnI methods usually show confidence intervals ≥4 ng/L, even when measured in large population of healthy subjects (>1,000 people) with accurate statistical analysis, such as the bootstrap method (Table 2) [13–19, 51, 52]. Considering the hs-cTnT assay, recent studies have also reported confidence interval (CI 95%) for the 99th percentile URL value >4 ng/L [18]; in particular, the manufacturer suggests 13.9 ng/L (CI 95% 12.7–24.9 ng/L) for the 99th percentile value of the overall population [18].

On the contrary, the diagnosis for AMI is usually made using standard clinical algorithms based on the significant difference between two (or more) hs-cTn concentrations, collected during a fixed period of time in the same patient after admission to emergency department [1–4]. In particular, the most recent ESC 2020 guidelines recommend for a more rapid diagnosis of AMI to use the 0/1 h algorithm (best option, blood draw at 0 and 1 h after admission) or the 0/2 h algorithm (second best option, blood draw at 0 and

Table 1: Biological variation parameters measured in adult apparently healthy individuals with high-sensitivity cTnI and cTnT methods.

Reference	Method	Time frame	Clinical sample, n	CV_a, %	CV_i, %	CV_g, %	Index of Individuality
cTnI							
Wu et al. 2009 [45]	Singulex	4 h	12	8.3	9.7	57.0	0.21
Wu et al. 2009 [45]	Singulex	Eight weeks	17	15.0	14.0	63.0	0.39
Wu et al. 2012 [46]	Singulex	Nine months	17	15.2	27.9	70.9	0.45
Schinder et al. 2016 [47]	Abbott Architect	One week	20	4.8	14.5	44.0	0.3
Schinder et al. 2016 [47]	Abbott Architect	12 weeks	20	4.8	14.7	56.7	0.3
van der Linden et al. 2017 [8]	Abbott Architect	1 h	18	10.0	8.6	49.6	0.27
van der Linden et al. 2017 [8]	Abbott Architect	6 h	18	10.1	9.2	48.6	0.28
Zaninotto et al. 2020 [11]	Beckman Access UniCell DxI	0–7 h	35	7.1	4.2	23.4	0.35
Ceriotti et al. 2020 [48]	Singulex	10 weeks	89	11.6	16.6	44.3[a]	0.34[a]
Ceriotti et al. 2020 [48]	Siemens Ccntaur XPT	10 weeks	91	10.7	13.8	36.4[a]	0.40[a]
Mean cTnI (SE)				9.76 (1.15)	13.31 (2.01)	49.39 (4.29)	0.33 (0.02)
cTnT							
Aakre et al. 2014 [7]	ECLIA Modular	0–6 h	15	9.9	1.3	32.6	0.31
Corte et al. 2015 [48]	ECLIA Cobas e411	Five weeks	11	5.1	5.9	30.4	0.20
Fournier et al. 2017 [9]	ECLIA Cobas e602	24 h	17	4.2	9.7	47.2	0.22[b]
Mejers et al. 2017 [10]	ECLIA Modular	Four months	28	1.5	16.0	51.2	0.30
Mean cTnT (SE)				5.15 (1.73)	8.23 (3.11)	40.32 (5.18)	0.26 (0.03)
cTnI vs. cTnT				p=0.0475	p=0.2883	p=0.2579	p=0.1575

[a]The values for CV_g and Index of Individuality are calculated from values respectively reported in the original article divided for men and women [48]. [b]This Index value (not reported in the original article) was estimated by the authors according to formula 1 using the CV_a, CV_i, and CV_g values reported in the Table columns 5, 6 and 7, respectively [9]. SE, standard error; cTnI vs. cTnT, p-values concerning the statistical analysis (Wilcoxon test for non-parametric test for unequal two-samples) for difference between the values respectively reported for cTnI and cTnT.

2 h after admission) [4]. In this clinical condition, the better is the analytical performance of assay method and the lower is the biological intra-individual variation of cardio-specific biomarker, and the more accurate will be the estimation of variations between two (or more) serial measurements [5].

Reference change value (RCV)

The significant difference between serial measurements related to two (or more) samples collected from the same individual in different times is estimated with the Reference Change Value (RCV). The RCV is the percentage change which can be calculated by combining analytical and biological variation [5, 6]. The RCV is commonly used for the estimation of the significant difference between two (or more) values, collected at different times in the same individual (i.e. serial sampling). According to Callum G. Fraser [5], the bidirectional Z-score RCV between two results (expressed as confidence interval, CI 95%) can be calculated by considering both the analytical variability of the method (CV_a) and the intra-individual variability (CV_i), using Eq. (2):

$$RCV = 1.96\left[2\left(CV_a^2 + CV_1^2\right)\right]^{1/2} \quad (2)$$

As an example, we can assume that the analytical error of the most recent hs-cTnI and cTnT methods at the 99th percentile URL level is about 5% CV (i.e., the half of the value recommended by international guidelines), as estimated by means of imprecision profiles, as reported in some recent studies [19, 20, 40–43]. Moreover, the CV_i values are on average 13% and 8% for hs-cTnI and hs-cTnT, respectively, as summarized in Table 1. Considering a value of 10.5% (for the mean CV_i between hs-cTnI and hs-cTnT), and a value of 5% for CV_a, the RCV calculated according to Eq. (2) is on average approximately 32% at the 99th percentile level. Indeed, some recent studies estimated the RCV by means of imprecision profiles generated by repeatedly measuring several clinical samples and the results of several quality control samples circulated in annual External Quality Assessment (EQA) cycles [40, 53–55]. According to the results of these studies [40, 53–55], the estimated RCV (expressed as 95% CI) is on average 32.0% for the hs-cTnI and hs-cTnT values from 5 to 40 ng/L, as reported in Table 3. Taking into account the results reported in Table 3, it can be assumed that a difference ≥32% between the hs-cTn concentrations

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

Population groups	Number of subjects	99th percentile, ng/L	Confidence interval 95%, ng/L	Confidence interval 99%, ng/L
Architect method				
Whole population	1463	14.4	12.0–17.5	11.4–19.2
Women	699	9.7	6.8–12.4	6.7–12.5
Men	764	17.2	14.2–20.6	13.4–23.9
Access method				
Whole population	1460	13.1	11.8–15.2	11.7–16.8
Women	703	9.2	7.2–14.2	6.6–16.8
Men	757	14.0	12.4–17	12.1–19.5
Advia XPT method				
Whole population	1411	33.5	26.2–42.8	25.2–47.1
Women	680	24.7	16.3–37.8	15.8–40.2
Men	731	41.8	28.7–48.8	26.6–52.2

measured in two samples, collected at different times in the same individual, is significant with a confidence interval of 95% (CI 95%). Of course, the percentage RCV can be also expressed as absolute reference change values by multiplying the percentage RVC by the target hs-cTn concentration (expressed in ng/L), as recently reported for some hs-cTn methods [40, 53–55].

Clinical relevance of reference change values

Acute coronary syndrome (ACS)

Both RCV and delta changes values have been for long time used to demonstrate a significant difference between the hs-cTn concentrations measured in two (or more) clinical samples collected from patients with ACS admitted to the emergency department [56–63]. There are, however, some mathematical and analytical differences between these two parameters [60, 64]. The RCV is an individual-specific parameter, which can be directly calculated from biological variation of the cardiac troponin (I or T) and analytical imprecision of hs-cTn assay, according to Eq. (2) [5]. The accurate estimation of RCV is a very difficult task requiring a standardized experimental protocol and often the use of sophisticated statistical analyses for the estimation of biological variation of a biomarker in healthy subjects [5, 6, 8, 9, 11, 13, 60, 64]. As an example, Figure 1 summarizes the mean imprecision profile calculated by measuring 10 quality control samples (hs-cTn concentrations from about 1 to 50 ng/L) with three hs-cTnI and the hs-cTnT methods, as previously reported [40, 53–55] (Supplementary Material). These data indicate that the imprecision profile of these hs-cTnI and hs-cTnT methods are very similar with CV at the 99th percentile URL value ≤5%. According to Eq. (2), the imprecision profile can be used for the estimation of the CV_a for the calculation of RCV, as reported in Table 3 for the hs-cTnI and hs-cTnT methods [40, 53–55].

On the contrary, the estimation of delta change value is based on the simple arithmetic difference equation between the cTn concentrations measured in two (or more) samples collected from the same subject or patient at different times. Unfortunately, the magnitude of change in hs-cTn concentrations related to a significant rise and/or fall was not specified by international guidelines [1, 3, 4, 6]. To estimate the statistical significance of the difference between two measurement values, Eq. (2) can be modified in order to allow the estimation of the Z-score, according to Eq. (3).

$$\text{Z} - \text{score} = \Delta \Big/ \left[2\left(\text{CV}_a^2 + \text{CV}_i^2\right)\right]^{1/2} \quad (3)$$

Table 3: RCV (expressed as 95% CI) of some hs-cTnI and hs-cTnT methods for biomarker values within the normal range and around the 99th percentile URL values, according to references [40, 53–55].

hs-cTn concentration, ng/L	RCV – hs-cTnI Architect (95% CI %)	RCV – hs-cTnI Access (95% CI %)	RCV – hs-ADVIA XPT (95% CI %)	RCV – hs-cTnT ECLIA[a] (95% CI %)
5	36.9	38.1	43.8	45.4
10	31.6	31.3	35.1	33.0
15	30.1	29.4	31.3	29.6
20	29.4	28.7	29.5	28.0
40	28.4	27.5	27.4	26.0
Mean value (SE)	31.28 (1.50)	31.00 (1.88)	33.40 (2.89)	32.44 (3.48)
Reference	Musetti et al. 2019 [53]	Clerico et al. 2019 [55]	Clerico et al. 2019 [54]	Ndreu et al. 2019 [40]

[a]The RCV values for the hs-cTnT methods were calculated using the CV_a values, estimated by the imprecision profile, as previously reported [40], and the mean CV_i value 8.23%, as reported in Table 1. SE, Standard Error.

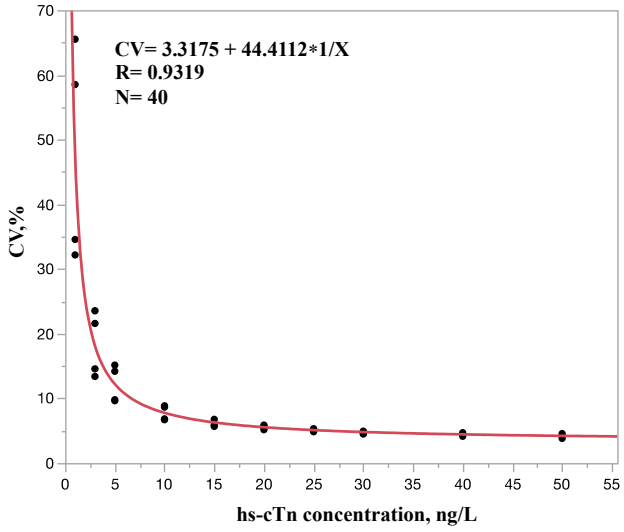

Figure 1: Mean imprecision profile calculated among three hs-cTnI methods (i.e. Architect, Access and ADVIA Centaur XPT) and the ECLIA hs-cTnT method.
For the calculation of the mean imprecision profile, 10 target cTn concentrations (from 1 to 50 ng/L) were used, estimated from the original imprecision profiles previously described in the references [18–20, 40–43, 53–55], and reported in the Supplementary Material.

In this equation, Δ is the difference in hs-cTn concentrations, and the terms CV_a and CV_i indicate the analytical and biological variation, respectively. According to Eq. (3), assuming a p-value of 0.05 to define statistical significance, a Z-score value >1.96 would mean that an observed difference in hs-cTn concentrations is unlikely to have occurred by chance [6, 58, 60]. In other words, according Eq. (3), there is only a 5% chance that a healthy subject may have variations in hs-cTn concentration that exceed the RCV [6, 58, 60].

The Fourth Universal Definition of Myocardial Infarction [3] states that the diagnosis of AMI should be made when a significant rise and/or fall in hs-cTn concentrations is detected in a patient with at least one biomarker value above the 99th percentile URL in the setting of acute myocardial ischaemia. Consequently, it is theoretically conceivable that significant variations at the level of the 99th percentile URL value should assume critical importance for the diagnosis of myocardial injury and infarction. In particular, some recent studies have estimated the RCV for some cTnI and cTnT concentrations around the 99th percentile values (i.e. from 5 to 40 ng/L) [40–43]. These data (summarized in Table 3) have indicated that on average variations in hs-cTn concentrations (expressed as percentage RCV) more than 32% are statistically significant with a CI of 95% [40–43]. However, even if a variation is statistically significant, there is no guarantee that it is also clinically relevant. The clinical relevance of RCV or delta changes of hs-cTn methods in emergency patients with chest pain are usually evaluated using appropriate statistical methods (such as C-statistics or/and logistic regression analyses) [6, 52, 56, 57, 60–62, 65–67].

However, even the use of sophisticated statistical analyses does not protect clinicians from making errors concerning interpretation of clinical results obtained with hs-cTn assay. Clinical judgement remains of paramount importance: results of hs-cTn assay should be interpreted according to the clinical setting, taking into account the results of history, physical examination, electrocardiogram and other investigation results [1–4, 6, 52, 67, 68]. Furthermore, there may be a potential role for the clinical laboratory to provide further interpretative comments to improve clinician interpretation of hs-cTn results [2, 52, 68, 69]. In particular, the time since onset of symptoms is crucial when interpreting a change in hs-cTn concentrations [1–4, 6, 52, 67–69]. There may be very little change in cTn concentrations near peak of cTn kinetic or late after an acute myocardial injury [1, 6, 52, 69]. Furthermore, the increase in hs-cTn levels is frequently be more rapid than their decline, especially in patients with ACS-STEMI (acute coronary syndrome with ST-segment elevation myocardial infarction) [1, 6, 52, 69].

In last 20 years, the most impressive clinical issue related to the progressive improvement in the analytical sensitivity of cTn immunoassay method is a continuous downward trend in diagnosis number of unstable angina associated with a reciprocal increment in diagnosis of ACS-NSTEMI (acute coronary syndrome with Non-ST-segment elevation myocardial infarction) [1, 70, 71]. According to the Fourth Universal Definition of Myocardial Infarction [3], the patients with unstable angina have a clinical setting compatible with myocardial ischaemia, but do not actually show any hs-cTn value above the 99th percentile URL. Therefore, some patients with unstable angina may have a significant increment in hs-cTn levels but falling within the normal interval (i.e., without any value above the 99th percentile URL). This clinical situation has become possible only after introduction in clinical laboratory practice of hs-cTn immunoassay methods with LoD values ≤5 ng/L (i.e., values 3–5-fold lower than 99th percentile URL) [14–18]. Some Authors have suggested that unstable angina should be considered as an intermediate syndrome between stable angina and AMI [71]. This hypothesis is in accordance with the evidences indicating that patients with unstable angina have a substantially lower risk of death and appear to derive less benefit from intensified antiplatelet therapy as well as early invasive

strategy then patients with ACS-NSTEMI or STEMI [1, 70–72].

Assessment of cardiovascular risk in the general population

Due to their excellent analytical performance (LoD value ≤3 ng/L), hs-cTn immunoassays allow the accurate evaluation of cardiovascular risk in the general population because these laboratory methods are able to effectively measure the biomarker circulating levels in the major part of healthy adult subjects [14–19]. Indeed, a huge number of studies [28–36, 73–83], including also three meta-analyses [27, 28, 73], have recently demonstrated that the cardiovascular risk tends to increase even in some apparently healthy individuals of both sexes, who currently show circulating levels of hs-cTn below the 99th percentile URL value (Table 4). In particular, the North-Trøndelag Health (HUNT) study [31] evaluated the cardiovascular risk with a hs-cTnI method in a cohort of a general population, including 9,005 participants free from known cardiovascular disease at baseline with a median follow-up period of 13.9 years. The addition of hs-cTnI to multivariate regression models for risk prediction led to a net reclassification improvement of 0.35 (95% CI from 0.27 to 0.42), superior to that previously obtained with classical cardiovascular risk factors [31]. It is important to note that the tertile with the highest risk showed a cut-off value of 10 ng/L for women and 12 ng/L for men [15, 16, 31] (Table 4). Therefore, the results of this study confirmed that the combined mortality and cardiovascular risk significantly increases even for cTnI values much below the 99th percentile URL values, divided for sex, as suggested by the manufacturer (i.e., 15.6 ng/L for women and 34.2 ng/L for men) [31].

Moreover, the *MORGAM/BiomarCaRe* study reported that serial measurements (n≥3) of biomarkers, collected throughout five years, in the general population [29] improves the 10-year prediction of cardiovascular risk in 3875 participants, aged 30–60 years at enrollment (51% female, disease free at baseline). Although during the 10-year follow-up the median hs-cTnI concentration changed less than 1 ng/L (i.e. from 2.6 to 3.4 ng/L), however, the change in biomarker concentrations more accurately predicted the cardiovascular risk in the general population than the most recent measurement [29]. A more recent study confirmed these results, suggesting also that for refinement of risk prediction models, the most recent measurement of hs-cTnI may be preferred in clinical practice in order to reduce the cost of screening [82].

From a clinical point of view, some important issues should be taken into consideration about the evaluation of cardiovascular risk in the general population [6, 15–17, 52, 69, 83]. Due to the very low intra-individual biological variation of cardiac troponins, serial measurements of hs-cTn significantly improve prognostic accuracy [29, 46–50]. However, practically, a single measurement of cTn using high-sensitivity methods should be adequate for the prediction of cardiovascular risk [29, 82]. Finally, it is important to note that the values for risk prediction are strictly method- and population-dependent and far below the current cut-off values of hs-cTn methods (i.e. the 99th percentile URL values suggested by the manufacturers) [15, 16, 83].

Table 4: Suggested cut-off values for risk stratification in the general population using cTnI and cTnT assays, measured with high-sensitivity methods.

cTnI[a]	Women	Men
Low	<4 ng/L	<6 ng/L
Moderate	4–10 ng/L	6–12 ng/L
High	>10 ng/L	>12 ng/L
cTn[b]		**Total population**
Low		≤3 ng/L
Moderate		3.0–5.7 ng/L
High		≥5.8 ng/L

[a]Data obtained with the hs-cTnI Architect method (Abbott Diagnostics), according to the references [15, 16, 31, 83, 85]. [b]Data obtained with the ECLIA hs-cTnT Elecsys method (Roche Diagnostics) according to the references [15, 16, 33, 84, 85].

Comparison of analytical and biological profiles among the cardio-specific biomarkers

Cardiac troponins, measured with high-sensitivity methods, actually show a significant different analytical and biological profile compared to other cardiovascular risk markers, especially the other cardio-specific biomarkers, namely the natriuretic peptides (in particular, BNP and NT-proBNP) [15, 16, 44, 84–89]. Indeed, the BNP and NT-proBNP have intra-individual and inter-individual variations (on average about 40–60%) and also Index of Individuality (about 0.64–1.0) higher than that of cTn [63, 84, 85, 87–91].

Furthermore, the natriuretic peptides are rapidly degraded both *in vivo* than *in vitro*, especially the active hormone BNP (plasma half-life about 20–40 min),

while the cTn are relatively stable more *in vivo* than *in vitro* [15, 16, 44, 84, 85]. According to larger and more asymmetrical intra- and inter-individual distributions of natriuretic peptides, the more accurate (but also more complex) method using the log-normal approach described by Fokkema et al. [88] should be preferred for evaluation of biological variation of BNP/NT-proBNP in healthy subjects. Furthermore, hourly, diurnal and monthly variations of hs-cTn levels measured in healthy volunteers are greatly more stable than those of natriuretic peptides [44–50, 64, 84, 85, 87–91]. Therefore, taking all these differences between analytical and biological characteristics of cardiac-specific biomarkers as a whole, cardiac troponins actually show a more favourable biomarker profile than natriuretic peptides [15, 16, 44, 84, 85].

Considering the pathophysiological and clinical characteristics, circulating levels of natriuretic peptides and cardiac troponins are differently affected by pathophysiological mechanisms related to cardiac dysfunction and/or damage [15, 16, 44, 84–86]. An increase in circulating levels of both cardio-specific biomarkers suggests that some powerful stressor mechanisms have already caused relevant alterations on cardiac function (i.e. increased BNP/NT-proBNP levels), as well as a significant damage on cellular structure (i.e. increased hs-cTn levels) [15, 16, 44, 84–86]. Finally, these finding are well in accordance with many experimental and clinical studies reporting that individuals with both increased cardio-specific biomarkers have a more severe clinical outcome than those with only one altered biomarker [84–85, 92–94]. These data, taking as a whole, actually explain why the hs-cTn methods add almost always incremental and independent pathophysiological and clinical information compared to BNP/NT-proBNP assay [15, 16, 44, 84–86].

Conclusive and prospective remarks

To make the diagnosis of myocardial injury, clinicians should compare a single value of hs-cTn, which has a very low intra-individual (CV_i) (Table 1), with the 99th percentile URL value, which, on the contrary, has both inter-individual variability (CV_g) and confidence interval very large (Table 2). Furthermore, other confounding variables may affect the 99th percentile URL value, such as the analytical performance of immunoassay methods and the demographic characteristics related to the reference population, such as sex, age, genetic determinants and ethnicity [18, 21, 50, 51, 95, 96]. Unfortunately, a lot of cardiac and even extracardiac clinical conditions may cause an increase in hs-cTn levels above the 99th percentile URL value [1, 3, 18, 21, 38, 50, 51, 95, 96]. Accordingly, only a minority of patients admitted to the emergency department with a hs-cTn value above the 99th percentile URL values actually has an AMI [1, 3, 51, 95, 96]. Clinical algorithms using serial sample testing with hs-cTn methods significantly increase the diagnostic accuracy of AMI in patient admitted to emergency department, due to the favourable profile of the cardiac biomarker based on the very low intra-individual variability (Table 1) and the excellent analytical performance at the 99th percentile level (CV about 5%) (Figure 1) [1–4, 6]. Indeed, recent studies reported that RCV >30% between two measurements with hs-cTn methods can be considered statistically significant [40, 53–55], suggesting the diagnosis of AMI in patients admitted to the emergence department with clinical evidence of acute myocardial ischaemia [1, 3].

Although recent guidelines do not yet recommend the use of hs-cTn methods for screening of cardiovascular risk in the general population [97], several experimental evidences actually suggest the clinical usefulness of cardiovascular risk evaluation with hs-cTn methods in some groups of patients at high risk of progressive myocardial damage (such as systemic hypertension, severe obesity, diabetes mellitus, chronic kidney disease, and chronic obstructive pulmonary disease) [16, 83]. Indeed, in these patients even a small (5–10 ng/L) increase in hs-cTn level over time (months) may suggest a progressive myocardial remodelling, ultimately culminating in symptomatic heart failure [16]. Therefore, these results should promote clinical studies specifically designed at assessing the cost-benefit of screening programs based on hs-cTn assays and at defining the optimal target populations, timing of measurement, and preventive interventions [16, 83].

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