Guidelines and Recommendations

Aldo Clerico*, Martina Zaninotto, Alberto Aimo, Daniela M. Cardinale, Ruggero Dittadi, Maria T. Sandri, Marco Alfonso Perrone, Lucia Belloni, Antonio Fortunato, Tommaso Trenti and Mario Plebani

Variability of cardiac troponin levels in normal subjects and in patients with cardiovascular diseases: analytical considerations and clinical relevance

A consensus document by the Study Group on Cardiac Biomarkers from Italian Society of Biochemical Chemistry (SIBioC) and European Ligand Assay Society (ELAS)

https://doi.org/10.1515/cclm-2022-1285
Received December 19, 2022; accepted January 9, 2023;
published online January 26, 2023

Abstract: In accordance with all the most recent international guidelines, the variation of circulating levels of cardiac troponins I and T, measured with high-sensitivity methods (hs-cTnI and hs-cTnT), should be used for the detection of acute myocardial injury. Recent experimental and clinical evidences have demonstrated that the evaluation of hs-cTnI and hs-cTnT variations is particularly relevant: a) for the differential diagnosis of Acute Coronary Syndromes (ACS) in patients admitted to the Emergency Department (ED); b) for the evaluation of cardiovascular risk in patients undergoing major cardiac or non-cardiac surgery, and in asymptomatic subjects of the general population aged >55 years and with co-morbidities; c) for the evaluation of cardiotoxicity caused by administration of some chemotherapy drugs in patients with malignant tumors. The aim of this document is to discuss the fundamental statistical and biological considerations on the intraindividual variability of hs-cTnI and hs-cTnT over time in the same individual. Firstly, it will be discussed in detail as the variations of circulating levels strictly depend not only on the analytical error of the method used but also on the intra-individual variability of the biomarker. Afterwards, the pathophysiological interpretation and the clinical relevance of the determination of the variability of the hs-cTnI and hs-cTnT values in patients with specific clinical conditions are discussed. Finally, the evaluation over time of the variation in circulating levels of hs-cTnI and hs-cTnT is proposed for a more accurate estimation of cardiovascular risk in asymptomatic subjects from the general population.

Keywords: acute myocardial infarction; biological variation; cardiac troponins; cardio-toxicity; cardiovascular risk; immunoassay methods; myocardial injury; natriuretic peptides.

This article has been co-published in *Clinical Chemistry and Laboratory Medicine* and *Biochimica Clinica* (DOI: https://doi.org/10.19186/BC_2023.005).

***Corresponding author: Aldo Clerico**, Scuola Superiore Sant'Anna e Fondazione CNR, Regione Toscana G. Monasterio, Pisa, Italy, E-mail: aldoclerico1948@gmail.com
Martina Zaninotto and Mario Plebani, Dipartimento di Medicina di Laboratorio, Università-Ospedale di Padova, Padova, Italy; and Azienda Ospedaliera Universitaria di Padova, e Facoltà di Medicina e Chirurgia, Università di Padova, Padova, Italy. https://orcid.org/0000-0002-0270-1711 (M. Plebani)
Alberto Aimo, Scuola Superiore Sant'Anna e Fondazione CNR, Regione Toscana G. Monasterio, Pisa, Italy
Daniela M. Cardinale, Unità di Cardioncologia, Istituto Europeo di Oncologia, IRCCS, Milan, Italy
Ruggero Dittadi, Unità di Medicina di Laboratorio, Ospedale dell'Angelo, e Centro Regionale dei Biomarcatori, Dipartimento di Patologia Clinica, Azienda ULSS 3, Mestre, Italy
Maria T. Sandri, Laboratorio Bianalisi, Carate Brianza, Monza e Brianza, Italy
Marco Alfonso Perrone, Dipartimento di Biochimica Clinica e Divisione di Cardiologia, Università e Ospedale di Tor Vergata, Rome, Italy
Lucia Belloni, Unità di Immunologia Clinica, Allergia e Biotecnologie Avanzate, Arcispedale Santa Maria Nuova-IRCCS, Reggio Emilia, Italy
Antonio Fortunato, Patologia Clinica, Area Vasta 5 ASUR Marche, Ascoli-Piceno, Italy
Tommaso Trenti, Dipartimento di Patologia Clinica e Laboratorio, Azienda USL of Modena, Modena, Italy

Introduction

In 2018, the Fourth Universal Definition of Myocardial Infarction (MI) introduced the term "myocardial injury" to define elevated cardiac troponin I or T, preferably measured with high-sensitivity assay methods (i.e., hs-cTnI and hs-cTnT) [1]. This document also established that the threshold value for myocardial injury is the 99th percentile of biomarker values estimated in a reference population (99th percentile upper reference limit [URL]) [1]. Furthermore, myocardial injury should be classified as "acute" when a significant increase or decrease in biomarker levels over a certain period of time is ascertained in patients admitted to the Emergency Department (ED) with chest pain. Otherwise, myocardial damage is defined as "chronic" when no significant variation in biomarker levels is found [1]. Importantly, this document proposed a nosographic entity (i.e., myocardial damage) that established a close connection between myocardial tissue damage and the results of a laboratory test [1].

Again in 2018, the document from the American Association for Clinical Chemistry (AACC) and International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine reported the quality specifications required for hs-cTnI and hs-cTnT methods [2]. The first criterion, which must be invariably present, is that these assays must be able to measure the 99th percentile URL with a coefficient of variation (CV) equal to or lower than 10%. The second criterion is that these assays must be able to measure biomarker levels above the limit of detection (LoD) in the majority of healthy adult subjects of both sexes. In detail, to obtain the 99th percentile URL with a confidence limit of 95%, this value must be calculated in a reference population including at least 300 men and 300 women considered in good health [2]. Indeed, women have usually lower circulating troponin levels than men of the same age; it is then essential that hs-cTn methods are able to measure circulating levels of the biomarker in the majority of healthy women in the reference population [2–4]. In order to satisfy these two criteria a considerable experimental effort from the manufacturers is required. In particular, some analytical considerations suggest that hs-cTnI and hs-cTnT methods should have a LoD≤3 ng/L [3–6]. Indeed, only in the last 10 years hs-cTnI and hs-cTnT methods meeting these quality specifications have become commercially available [2–6].

When using methods meeting such stringent quality specifications [1, 2], it appears evident that, besides the analytical error associated with the measurement of the 99th percentile URL, the significant intra-individual variations are crucially important. Some recent clinical studies have found that the evaluation of variations of hs-cTnI and hs-cTnT is particularly relevant: a) for the differential diagnosis of ACS in patients admitted to the ED [1, 7–9]; b) for the evaluation of cardiovascular risk in patients undergoing major cardiac or non-cardiac surgery [10, 11], as well as in asymptomatic subjects of the general population aged >55 years and with co-morbidities [12–14]; c) for the evaluation of cardiotoxicity caused by cancer therapies [15–17].

The principal aim of this document is to emphasize pathophysiological and clinical relevance of intra-individual variations of hs-cTnI and hs-cTnT. Firstly, we will discuss the close relationship between analytical sensitivity of immunometric methods and measurement error. In particular, the variability of hs-cTnI and hs-cTnT over time in the same individual depends both on the analytical error of the method used and on the intra-individual variability of the biomarker [12]. We will emphasize the notion that, although the hs-cTnI and hs-cTnT methods measure different biomarker concentrations in a same sample, the biomarker variations between two (or more) samples expressed as percentage are similar, especially for the values ≥ the 99th percentile URL. Afterwards, we will discuss the pathophysiological interpretation and the clinical relevance of variability of hs-cTnI and hs-cTnT circulating levels in normal subjects, as well as in patients with some specific clinical conditions. Finally, the importance of the evaluation over time of circulating levels of hs-cTnI and hs-cTnT in asymptomatic subjects from the general population will be also discussed [12, 13].

Analytical and pathophysiological characteristics of hs-cTn assay methods

Analytical sensitivity and measurement error

The analytical sensitivity parameters of the most common hs-cTnI methods are shown in Table 1. These data derive from the results of an Italian multicenter study that has compared the analytical performances of several hs-cTnI and hs-cTnT methods in a reference laboratory using standardized experimental protocols [6, 19–24]. It is well known that imprecision of each immunometric method, expressed as CV, has a curvilinear relationship with biomarker concentration [4]. For example, the results reported in Figure 1 show the imprecision profile of the Architect method (Abbott Diagnostics, Rome) for the measurement of hs-cTnI. The imprecision is very high for biomarker concentrations <3 ng/L, but it decreases progressively until reaching a plateau value corresponding to a CV≤5% for hs-cTnI concentrations hs-cTnI≥10 ng/L.

The results reported in Table 2 summarize the values relating to the imprecision profiles of the most common hs-

Table 1: Sensitivity of some assay methods for hs-cTnI.

Methods	LoB, ng/L	LoD, ng/L	LoQ 10% CV, ng/L	References
Architect	0.7	1.3	4.7	[6, 19, 21]
Access DxI	0.6	1.3	5.3	[6, 21]
ADVIA XPT	1.0	2.2	8.4	[6, 22]
Vitros	0.3	0.9	4.7	[24]

The values of the sensitivity parameters were evaluated in the reference laboratory during a study using plasma samples from an Italian population and standardized experimental protocols [6, 19, 21, 22, 24]. Architect: method Architect Highly Sensitive TnI for the Architect i1000SR platform (Abbott Diagnostics, Ref. B3P250) [6, 19, 21]. Access DxI: method Access hsTnI (IUO) for the DxI platform (REF B52699, Beckamn Coulter, Inc. Brea, CA 92821 USA) [6, 21]. ADVIA: method ADVIA Centaur High-Sensitivity Troponin I (TNIH) (Ref. 10994774-5) for the Centaur XPT platform (Siemens Healthineers, Milan, Italy) [6, 22]. Vitros: method VITROS High-Sensitivity Troponin I for the VITROS 3600 platform (Ortho Clinical Diagnostics, REF 6844436, Raritan, New Jersey, USA) [24].

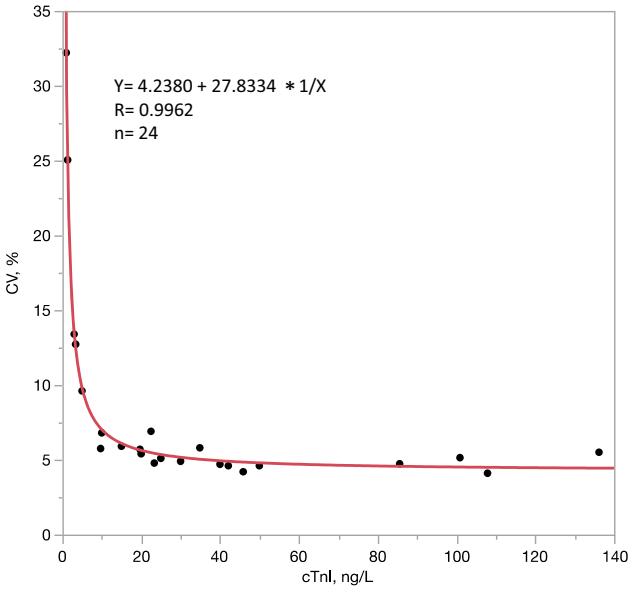

Figure 1: Inaccuracy profile of the Architect method (Abbott Diagnostics). The analytical imprecision values are reported as coefficient of variation (CV) on the y-axis while the concentration values of hs-cTnI, measured in 24 plasma samples, are reported on the x-axis. The results of the experimental measurements were interpolated by means of a reciprocal function (JMP 15.2.1, SAS Institute Inc.). Each sample was measured at least 13 times and in different working days. More details on the analytical procedure used for the calculation of imprecision profile of hs-cTnI or hs-cTnT methods were previously reported [4].

cTnI and the hs-cTnT method, as previously published [6, 18–24]. Figure 2 shows the average imprecision calculated (expressed as CV%) in 50 plasma samples with biomarker concentrations measured with the hs-cTnI and hs-cTnT, as reported in Table 2. These data demonstrate that the imprecision profiles of hs-cTnI and hs-cTnT methods are very similar if they are measured in the same reference laboratory and using standardized experimental protocols [4, 6, 18–24]. This result is no small thing given that there are systematic differences not only between values measured with the hs-cTnI and hs-cTnT methods in the same samples, but also between values measured with different hs-cTnI methods, which are significantly inhomogeneous [4, 6, 25].

Circulating levels of hs-cTnI and hs-cTnT in the reference population

Back in 2000, the consensus document of the European (ESC) and American Cardiology (ACC) Societies had established that the 99th percentile URL had clinical relevance [26]. At that time, however, there was no assay for cTnI or cTnT able to measure the 99th percentile URL with a CV≤10%, as requested by international guidelines [1–4]. For this reason, one might have thought that cTnI and cTnT could not be found in the circulation of healthy adults (i.e., without any heart damage). Over the last 15 years, thanks to the development of hs-cTnI and hs-cTnT methods (more than 20 times higher than the methods available in 2000) [4] and studies on the physiological renewal of the myocardium [3, 27–29], it was demonstrated that small amounts of the biomarker (on average 2–5 ng/L) are present in the circulation of healthy adult subjects [4–6]. These measurable concentrations of hs-cTnI and hs-cTnT are due to the sarcomere proteins that are released in the extra-cellular fluid after the death of cardiomyocytes that have completed their normal lifespan [3, 4, 27–32]. Marjot et al. calculated that the value of the 99th percentile URL of hs-cTnI and hs-cTnT corresponds to the amount of biomarker released by the death of approximately 40 mg of myocardial tissue [30]. The physiological renewal of the myocardium is greater in the infantile period and then progressively decreases to a value <1% per year, which remains stable in healthy adult subjects [30, 32]. Based on this experimental evidence [3, 4, 27–32], the median concentration of hs-TnI and hs-cTnT in healthy adult subjects corresponds to the renewal of ≤10 mg of cardiac tissue. This volume of cardiomyocytes is so small that it cannot be detected by even the most sensitive and sophisticated methods of non-invasive myocardial imaging [3, 4, 30]. It is also hypothesized that physiological renewal is proportional to cardiac mass, which could explain why biomarker values are on average higher in men (mean heart mass 300 g; range 280–340 g) than in women (average cardiac mass 250 g; range 230–280 g) [27–29].

The 99th percentile URL of the hs-cTnI and hs-cTnT methods in the healthy reference population should be

Table 2: Imprecision profile of some methods for hs-cTnI and the method for hs-cTnT.

Sample	cTn interval, ng/L	cTnI Architect, CV%	cTnI Access, CV%	cTnI ADVIA, CV%	cTnI Vitros, CV %	cTnT ECLIA, CV%	Average, CV%
1	<3	32.2	34.6	65.5	33.0	58.5	44.8
2	≥3–<5	13.4	14.6	23.5	13.6	21.6	17.3
3	≥5–<10	9.6	9.8	15.1	9.7	14.2	11.7
4	≥10–<15	6.8	6.7	8.9	6.7	8.6	7.5
5	≥15–<20	5.9	5.7	6.8	5.7	6.8	6.2
6	≥20–<25	5.4	5.2	5.7	5.2	5.9	5.5
7	≥25–<30	5.1	4.8	5.1	4.9	5.3	5.0
8	≥30–<40	4.9	4.5	4.7	4.7	4.9	4.7
9	≥40–<50	4.7	4.4	4.1	4.5	4.5	4.4
10	≥50–<60	4.6	4.2	38	4.4	4.2	4.2

The last column provides the average values calculated through the different methods for each measured interval of the biomarker. The data reported in this table were partly taken from previously published studies performed in the reference laboratory of the SIBioC and ELAS Study Group for Cardiac Biomarkers [6, 19–25]. For each biomarker concentration range (column 2) a sample was chosen for each hs-cTnI or hs-cTnT method, which was then measured several times in successive days (n>13) to monitor the imprecision of the method. In total, 50 samples were chosen (10 for each method), prepared in the laboratory using heparin plasma from normal subjects or patients with cardiovascular disease to cover the entire concentration range of the marker from <3 ng/L to <60 ng/L. The biomarker values of these 50 samples were used to calculate the mean imprecision profile between hs-cTnI and hs-cTnT methods reported in Figure 2.

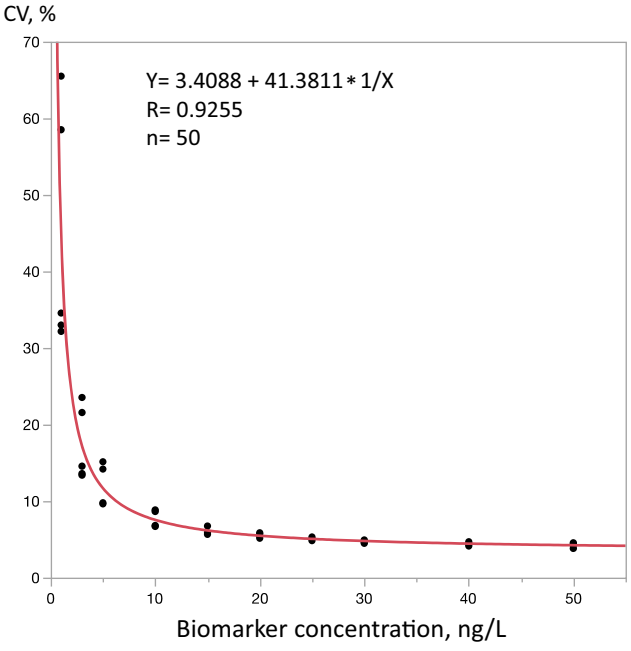

Figure 2: Average imprecision profile of 4 hs-cTnI methods and the hs-cTnT method. The values of the coefficient of variation (CV; y-axis) and biomarker concentration (x-axis) of the samples considered (10 for each method) are shown in Table 2. The results of the experimental measurements were interpolated by means of a reciprocal function (JMP 15.2.1, SAS Institute Inc.), as previously reported in detail [4]. Each sample was measured at least 13 times and in different days.

determined using standardized experimental protocols in agreement with the recommendations of guidelines and specific expert documents, which rely on pathophysiological and statistical considerations [2, 5, 33–41]. As reported in all the documents and guidelines [2, 5, 33–41], it is quite complex to establish the characteristics of the reference population required to calculate the distribution of hs-cTnI and hs-cTnT values. In principle, the reference population should consist of subjects aged >18 years of both sexes (with a sex ratio as close as possible to unity) comprising more than 600 surely "healthy" individuals, i.e., free from acute or chronic cardiovascular and non-cardiovascular disorders [2, 5, 33–40]. However, all studies on large populations including "apparently healthy" subjects, characterized on the basis of their clinical history, physical examination (including blood pressure control and body mass index), and some laboratory tests (including blood glucose, creatinine, blood count, electrolytes, lipid profile, C-reactive protein, BNP, plasma and urine protein analysis) as well as specific cardiac tests (such as the ECG and echocardiogram) have shown that hs-cTnI and hs-cTnT values progressively increase after the age of 55 in both sexes [5, 33–40]. This has been confirmed by many studies in different populations from Europe, North America, Australia and Asia, and using both hs-cTnI and hs-cTnT methods [5].

As for the statistical analysis, the calculation of the 99th percentile URL in the reference population may be challenging when outliers are present. The number of outliers that significantly influences the calculation of the 99th percentile tends to significantly and progressively decrease if increasingly restrictive inclusion criteria are used to enroll individuals in the reference population in order to exclude subjects with asymptomatic heart disease [2, 5, 34–38].

Another critical point in the estimation of the 99th percentile URL is the number of subjects enrolled in the

reference population. At least 300 subjects per group are required to estimate the 99th percentile URL with a 0.95 tolerance level, and at least 500 for a 0.99 tolerance level, respectively [39]. Designing a new study that aims to determine the 99th percentile URL in a reference population with adequate statistical confidence means to face two conflicting needs. On the one hand there is a need to increase as much as possible the number of enrolled subjects (to improve statistical confidence and increase the possibility of carrying out the analysis of sub-groups). On the other hand, only subjects who meet the criteria of "normality", in agreement with international guidelines, should be recruited [5, 34–40]. The complexity of enrollment and the overall costs become very high when trying to address both needs.

From a statistical perspective, the choice of the most appropriate statistical method to be used for the calculation of the 99th percentile is also critical [2, 5, 34–40]. The distribution of hs-cTnI and hs-cTnT values in the reference population is asymmetric and tends to be normally distributed only after log-transformation. Both parametric and non-parametric methods were used to calculate the 99th percentile URL value of hs-cTnI and hs-cTnT methods [2, 5, 33–40]. The EP28-A3c IFCC-CLSI guidelines [41] and the 2018 AAC and IFCC guidelines [2] recommend the use of non-parametric methods. This statistical approach has been most commonly used in population studies to calculate the 99th percentile URL [2, 5, 34], even if it is strongly influenced by the presence of outliers. More recently, the bootstrap method has been proposed [42]. It is more complicated from a mathematical point of view and generally requires the advice of expert statisticians and a high computing power [5, 37, 40]. The bootstrap method is considered less dependent on outliers and may be preferable, especially when a large amount of data is available [5, 37, 40, 42]. Regardless of the statistical approach to calculate the 99th percentile URL, a preliminary identification of the outliers is important [2, 4, 43–45]. The EP28-A3c IFCC-CLSI guidelines [41] recommend the Dixon method [43], but Tukey's method [44] has also been widely used [5, 39, 40, 45]. Both these tests assume a normal distribution of biomarker values in the reference population; therefore, the measured hs-cTnI and hs-cTnT concentrations should be log-transformed before the calculation of 99th percentile URL [5, 40]. In April 2022, the IFCC Committee on Clinical Application of Cardiac Bio-Markers published an important document that provides 7 specific recommendations covering all the main considerations on the statistical, analytical, pathophysiological and clinical aspects related to the estimation and interpretation of the 99th percentile URL values of the hs-cTnI and hs-cTnT methods [45].

Differences between cardiac troponins I and T

All guidelines [1, 2, 7, 8] basically agree that the hs-cTnI and hs-cTnT methods have similar diagnostic accuracy and can be used to diagnose an acute or chronic myocardial damage. However, cTnI and cTnT differ in terms of gene expression, biochemical features, physiological actions, and kinetics of release from cardiomyocytes (Table 3) [46]. Furthermore, the hs-cTnI and hs-cTnT methods have very different analytical characteristics [4, 47]. Based on these premises, it is important to check if hs-cTnI and hs-cTnT measurements give similar information in different clinical settings [4, 46].

Some studies have shown that the measurement of hs-cTnI and hs-cTnT in the same sample can sometimes provide discrepant results in some specific clinical conditions (particularly congenital neuro-muscular diseases and severe chronic inflammatory myopathies) or in some samples with substances that interfere with the immunometric systems used to measure the two troponins [4, 46, 47]. In addition, the two troponins have different kinetics, most likely because of a slower release of cTnT from necrotic cardiomyocytes and/or a longer circulating half-life than cTnI in patients with re-perfused MI [30, 31, 48–51].

After puberty, both hs-cTnI and hs-cTnT are higher in males than females. However, the difference between sexes is significantly higher for hs-cTnI than for hs-cTnT. In 2017 a meta-analysis [5] reported that the mean difference between the 99th percentile URL values of hs-cTnI (Architect method) measured in the two sexes in 10 studies of reference populations from different continents was 10.97 ng/L (95% confidence interval [CI] 7.10–14.85), while the difference for the 99th percentile URL value of hs-cTnT in 9 studies was 4.59 ng/L (95% CI 1.60–7.57). In agreement with these data, the manufacturers of all hs-cTnI methods recommend the use of the sex-specific 99th percentile URL, while the manufacturer of the hs-cTnT method recommends a single 99th percentile URL (13.9 ng/L; 95% CI 12.7–24.9 ng/L) [2, 5].

Take-home messages

- In the reference population of apparently healthy adult individuals (aged >18 years), hs-cTnI and hs-cTnT values are higher in men than in women of the same age and increase after 55 years in both sexes.
- Although the 99th percentile of the biomarker distribution in the reference population (99th percentile URL) is considered by all guidelines to be the decision value

Table 3: Biochemical and physiological characteristics of cTnI and cTnT.

Troponin	Molecular weight	Gene	Number of amino acids	Physiological function	Kinetics *in vivo*
cTnI	24 kDa	*TNN13* Chromosome 19 (19q13.4)	209	Binding to cTnC and actin	Quicker
cTnT	36 kDa	*TNNT2* Chromosome 1 (Iq32.3)	287	Binding to cTnC and tropomyosin	Slower and biphasic in some patients with myocardial infarction

Data reported in accordance with the reference [46].

for assessing the presence of heart damage (myocardial injury) and the differential diagnosis of ACS, the methods to calculate this reference limit are still controversial, in terms of the statistical approach and the composition of the reference population.

– For all hs-cTnI methods, the respective manufacturers recommend that the value of the 99th percentile URL be distinguished by sex, while the manufacturer of the hs-cTnT method recommends using a unique value for both sexes.

Temporal variations of cardiac troponins in healthy subject and in patients with cardiovascular diseases

hs-cTnI and hs-cTnT as individual indexes

Table 4 summarizes the main physiological characteristics of hs-cTnI and hs-cTnT and the analytical characteristics of the measurement methods according to the data reported in the literature [4, 6, 18, 22, 23, 46, 47, 52–56]. Overall, hs-cTnI and hs-cTnT values emerge as individual indexes, and then as possible tools for personalized medicine [57].

The individuality index (generally abbreviated as II) expresses the relationship between the intra-individual variability (CVi) and the general variability between individuals (CVg) for a biomarker [56, 58]. The cardiac-specific biomarkers natriuretic peptides and cardiac troponins have different II values [56, 59]. Natriuretic peptides (especially the peptide hormones ANP and BNP) have high CVi and CVg values, and their II is >0.6 [56, 59]. Conversely, the II of cardiac troponins is much lower (≤0.3) when these biomarkers are measured using hs-cTnI and hs-cTnT methods in healthy individuals (Table 5) [56, 60–65].

Some recent studies have demonstrated that the biological variation of cardiac troponins is similar in healthy subjects [56, 60–65] and patients with cardiac or non-cardiac diseases [66–71]. The results of clinical studies reported on PubMed (date of the latest search: 12 December 2022) concerning the evaluation of biological variability in healthy adult individuals using hs-cTnI and hs-cTnT methods are summarized in Table 5. Koerbin et al. [70] evaluated the intra-individual variability of hs-cTnI in patients admitted to the ED, in whom a MI was excluded. The CVi of hs-cTnI ranged from 10 to 20%, according to the specific setting, leading to values of II always <0.20 [70], in agreement with previous studies including only healthy subjects (Table 5) [56, 60–65]. Moreover, a recent meta-analysis [71] including 16 studies for cTnI (including 11 with hs-cTnI methods) and 15 hs-cTnT further confirmed that the value of II for both troponins, although evaluated over different periods of time

Table 4: Summary of the main physiological characteristics of hs-cTnI and hs-cTnT and of the analytical characteristics of the measurement methods.

1 Cardiac troponins are cardiac-specific biomarkers [4, 46].
2 Concentrations of di hs-cTnI and hs-cTnT are stable at room temperatures for 6 h [52] and at least one year at −80 °C [53].
3 Cardiac troponins can be measured in both plasma and serum, although there may be a discrepancy between the values measured in the same blood sample transformed into plasma (with the addition of heparin or EDTA) or serum after centrifugation [4, 23].
4 The LoD value of the hs-cTnI and hs-cTnT methods is about 1–3 ng/L [4, 6, 18, 47].
5 The hs-cTnI and hs-cTnT methods are relatively inexpensive laboratory tests (around 5 euros for each sample).
6 The measurement of hs-cTnI or hs-cTnT can be obtained in less than 30 min with the most common automated platforms and recently also with some recent point of care testing (POCT) methods for the measurement of hs-cTnI [4, 54, 55].
7 Both cardiac troponins have an average intra-individual biological variability of about 10% CV and an average individuality index of 0.3 [56].
8 Although the values measured with the various methods of hs-cTnI and hs-cTnT are different and therefore have different reference limits, the reference change value (RCV), expressed as %, are similar for the biomarker values measured around the 99th percentile URL (mean RCV about 30%, minimum-maximum range, about 25–45%) [6, 23, 56].

Table 5: Summary of the results reported in the literature concerning the parameters of biological variability evaluated in healthy adult subjects using hs-cTnI and hs-cTnT methods.

Reference	Method	Period of time	Number of subjects studied	CVa, %	CVi, %	CVg, %	II
hs-cTnI							
Wu et al. 2009 [60]	Singulex	4 h	12	8.3	9.7	57.0	0.2
Wu et al. 2009 [60]	Singulex	8 weeks	17	15.0	14.0	63.0	0.4
Wu et al. 2012 [61]	Singulex	9 months	17	15.2	27.9	70.9	0.5
Schinder et al. 2016 [62]	Abbott Architect	1 week	20	4.8	14.5	44.0	0.3
Schinder et al. 2016 [62]	Abbott Architect	12 weeks	20	4.8	14.7	56.7	0.3
van der Linden et al. 2017 [63]	Abbott Architect	1 h	18	10.0	8.6	49.6	0.3
van der Linden et al. 2017 [63]	Abbott Architect	6 h	18	10.1	9.2	48.6	0.3
Zaninotto et al. 2020 [64]	Beckman Coulter Access UniCell DxI	7 h	35	7.1	4.2	23.4	0.4
Ceriotti et al. 2020 [65]	Singulex	10 weeks	89	11.6	16.6	–	–
Ceriotti et al. 2020 [65]	Siemens Centaur XPT	10 weeks	91	10.7	13.8	–	–
Calculated average of hs-cTnI methods				9.8	13.3	51.6	0.3
hs-cTnT							
Aakre et al. 2014 [66]	ECLIA Modular E	6 h	15	9.9	1.3	32.6	0.3
Corte et al. 2015 [67]	ECLIA Cobas e411	5 weeks	11	5.1	5.9	304	02
Fournier et al. 2017 [68]	ECLIA Cobas e602	24 h	17	4.2	9.7	47.2	–
Mejers et al. 2017 [69]	ECLIA Modular	4 months	28	1.5	16.0	51.2	0.3
Calculated average of hs-cTnT methods				5.2	8.2	40.3	0.3
Difference hs-cTnI vs hs-cTnT	Non-parametric Wilcoxon text			p=0.0475	p=0.2883	p=0.8651	p=0.2207

CVa indicates the analytical variability of the method (i.e., the analytical error expressed as a coefficient of variation, CV), CVi the intra-individual variability. The individuality index (generally abbreviated as II) expresses the relationship between the intra-individual variability (CVi) and the general variability between individuals (CVg) for a biomarker.

and in both healthy subjects and in patients with different clinical conditions, can range from a minimum 0.03 (in patients studied for ≤24 h) to a maximum of 0.44 (in healthy subjects studied for >24 h). Interestingly, hs-cTnI and hs-cTnT have similar II values than creatinine (about 0.3) [58], which is considered a biomarker closely related to skeletal muscle mass [3, 27–31].

A biomarker with an II value <0.6 is generally considered to have a good correlation with individual physiological characteristics (in particular sex, age and body mass) [58]. When we use a biomarker with an II value much lower than 0.6 (such as cTn and creatinine), even biologically significant variations over time could remain within the reference intervals estimated from the general population. To optimize diagnostic accuracy, it would therefore be appropriate not to refer to the classic reference intervals, but instead to use values measured on samples of the same individual collected at different times [58].

The 99th percentile URL value of the hs-cTnI and hs-cTnT methods has broad confidence limits

As shown in Table 6, the 95% CI values for the estimate of the 99th percentile URL concerning the three most popular hs-cTnI methods in European and North American countries show intervals >5 ng/L [6]. Similar results have been reported also for the hs-cTnT method [72, 73]. The large 95% CI for the estimate of the 99th percentile URL is due to the significant dispersion of hs-cTnI and hs-cTnT values in the reference population, reflecting sex-related differences (women have on average 30–40% lower values than males), age (ranging from 19 to more than 100 years), and body mass (which has a strictly relationship with myocardial mass) [2–6].

In agreement with the recommendations of the Fourth Universal Definition of MI [1], a single hs-cTnI or hs-cTnT

Table 6: Median and 99th percentile values (ng/L) measured with three hs-cTnI methods in an Italian reference population.

Method	Population	Median, ng/L	99th percentile (95% CI), ng/L
Architect	Women (n=699)	1.4	9.7 (6.8–12.4)
	Men (n=764)	2.1	17.2 (14.2–20.6)
Access	Women (n=703)	2.3	9.2 (7.2–14.2)
	Men (n=757)	3.2	14.0 (12.4–17.0)
ADVIA XPT	Women (n=679)	2.7	24.7 (16.3–37.8)
	Men (n=731)	3.9	41.8 (28.7–48.8)

The 99th percentile value was calculated with the bootstrap method, as previously reported in detail [6]. CI, confidence interval; n, number of subjects studied per group.

value greater than the 99th percentile URL is sufficient to diagnose a myocardial injury. Therefore, this diagnosis is influenced by the analytical performance of the assay method, which presents an imprecision (CVa) (on average 8.5% with hs-cTnI and hs-cTnT methods), and the intra-individual variability (CVi) of healthy adult subjects (on average 10.8% with hs-cTnI and hs-cTnT methods), as reported by the results of biological variability studies in healthy adult subjects (Table 5). From a clinical point of view, it is very important to take into consideration the wide variability of the threshold value (99th percentile URL). By analyzing the data shown in Table 6, the 95% CI of the 99th percentile for the three hs-cTnI methods has very large values, corresponding to about one half of the same 99th percentile URL. Furthermore, an increase in biomarker concentration of about 7–10 times must occur to go from the median value of hs-cTnI in healthy subjects (about 2–3 ng/L) to the 99th percentile value (Table 6). Similar considerations apply to the hs-cTnT method [18, 72, 73], even if there are significant differences between the two troponins in terms of analytical performance and pathophysiological characteristics [4, 5, 46, 50, 51].

Reference change value (RCV)

The Fourth Universal Definition of MI recommends that MI be diagnosed when myocardial injury is accompanied by signs and/or symptoms suggestive of acute myocardial ischemia [1]. In order to demonstrate an acute myocardial ischemia, recent guidelines and documents from international experts [7, 8, 74] have discussed the clinical importance of diagnostic algorithms based on the collection of serially collected blood samples taken immediately upon admission to the ED and after a few hours (1–3 or more hours) in patients with suspected non-ST-segment elevation MI (NSTEMI), using hs-cTnI and hs-cTnT methods.

The assessment of variations in hs-cTnI or hs-cTnT over a certain period of time should be more accurate and precise than the comparison between a single patient value and the 99th percentile URL, considering not only the mathematical/statistical point of view but also the intra-individual biological variability. In particular, the individuality index (generally abbreviated as II) expresses the relationship between the intra-individual variability (CVi) and the general variability between individuals (CVg) for a biomarker [56, 58].

The mathematical/statistical approach commonly recommended to evaluate the variation of a biomarker measured with the same method in two samples is the calculation of the reference change value (RCV) [58]:

$$RCV = 2^{½} \times Z \times [(CVa)2 + (CVi)2]^{½} \quad (1)$$

where CVa indicates the analytical variability of the method (i.e., the imprecision expressed as a coefficient of variation, CV), CVi the intra-individual variability of the subject, and Z the Zeta score, which for a bidirectional probability of 95% is 1.96 [58].

Regarding CVa, the data respectively reported in Figures 1 and 2 and Table 2, demonstrate that the analytical error of the hs-cTnI and hs-cTnT methods tends to be high for biomarker values <5 ng/L (with mean CV% values >44% for concentrations <3 ng). However, for all hs-cTnI and hs-cTnT methods, the analytical variability tends to decrease with increasing concentrations and then stabilizes on CV values around 5% for biomarker concentrations close to the 99th percentile (i.e., ≥12 ng/L).

As for CVi, in Table 5 are reported the results of studies evaluating the biological variability of cardiac troponins in healthy adult subjects using hs-cTnI (6 studies) and hs-cTnT methods (4 studies) [60–65]. Although these studies show substantial differences in terms of methods used and experimental protocols adopted (number of subjects studied and study period), the II is on average equal to 0.3 for both hs-cTnI and hs-cTnT methods [60–65]. In particular, the CVi value is on average 13.3% for the hs-cTnI methods and 8.2% for the hs-cTnT methods, but the difference is not significant (Table 5).

Koerbin et al. have recently confirmed that even in patients admitted to the ED without a definitive diagnosis of MI, the value of II is still <0.2, while the value of CVi depends on sex, age and time sampling, with CV values from 9.7 to 17.6 [70]. In a recent meta-analysis, CVi values ranging from 4.1 to 15.1 were found in clinical studies employing hs-cTnI (16 studies) and hs-cTnT (15 studies) methods [71]. In particular, II values for the hs-cTnI and hs-cTnT methods ranged from 0.03 to 0.15 over short sampling periods (a few hours), while they were higher, from 0.12 to 0.44, in studies with

longer sampling periods (weeks or months). Furthermore, populations including only healthy subjects showed higher variability and therefore also higher II values, compared to populations including non-healthy individuals [71].

The results of the most recent studies have clearly demonstrated that CVi values are relatively stable both in normal subjects (Table 5), and in patients with cardiovascular disease [70, 71], as well as those of CVa for all hs-cTnI and hs-cTnT methods (Table 2 and Figure 2). Accordingly, it is not surprising that RCV values (estimated with a probability of 95%) can vary on average by only 32.0% (with a minimum of 26.0% and a maximum of 45.4%) in the range of values between 5 ng/L and 40 ng/L, considering 3 different hs-cTnI methods and the hs-cTnT method, as reported in Table 7 [20, 23, 56, 75–77].

Take-home messages

- The II value is much lower for cardiac troponins than the ther cardiac biomarkers (also including natriuretic peptides), being on average 0.3 for the hs-cTnI methods and the hs-cTnT method [56, 60–71].
- The 95% CI for the 99th percentile URL, which represents the threshold limit for the diagnosis of myocardial injury and MI, shows a large variability in the reference population as it depends not only on the analytical performances of hs-cTnI or hs-cTnT methods, but also on age, sex and body mass characteristics of the reference population [2, 3, 5, 12, 30, 33–36].
- Although there are systematic differences between the measured biomarker concentrations, the imprecision profiles of the hs-cTnI and hs-cTnT methods are very similar [9, 20, 23, 56, 70, 71, 75–77].
- RCV values (estimated with a 95% probability) for a series of two samples have been reported to vary on average by 32% (with a minimum of approximately 25% and a maximum of approximately 45%), considering many both experimental and clinical studies [9, 20, 23, 56, 70, 71, 75–77].

Table 7: RCV values for hs-cTnI and hs-cTnT methods for range of values from 5 ng/L to 40 ng/L.

Method	RCV, % (mean ± SE)	RCV, % (minimum–maximum)	References
hs-cTnI			
Architect	31.3 (1.5)	28.4–36.9	[23, 56]
Access	31.0 (1.9)	27.5–38.1	[56, 76]
ADVIA XPT	33.4 (3.0)	27.4–43.8	[56, 75]
hs-cTnT			
ECLIA	32.4 (3.5)	26.0–45.4	[20, 56]
Global mean (SE)	32.0 (0.5)	26.0–45.4	

RCV% values were calculated according to Eq. (1) in a reference laboratory using standardized protocols, as detailed previously [20, 23, 56, 75, 76].

Clinical relevance of biological variability

Myocardial infarction (MI)

According to 2021 AHA/ACC clinical practical guideline, of all patients admitted to Emergence Department (ED) with chest pain in United States, only 5.1% will have an acute coronary syndrome (ACS) and more than half will ultimately be found to have a noncardiac cause [78] Nonetheless, chest pain is the most common symptom of CAD in both men and women. Considering all ED patients with chest pain, only about 5% of these patients have an acute coronary syndrome (ACS), and more than half will ultimately be found to have no cardiac cause related to chest pain [78]. Nonetheless, chest pain is the most common symptom of coronary artery syndrome (CAD) in both men and women [78, 79]. The definition of MI, according to the *Fourth Universal Definition of Myocardial Infarction* [1], has been previously discussed in detail in the Introduction section of this document. It is important to emphasize that chronic myocardial damage is much more frequent than acute myocardial injury in patients (especially the elderly and with co-morbidities) in which the measurement of hs-cTnI and hs-cTnT is usually performed [79, 80]. Thus, the importance of the distinction between acute injury, characterized by ≥20% changes in hs-cTnI levels [1], and chronic heart damage with stable biomarker levels (<20% change) is clinically very important [79, 80].

To perform a correct estimate of changes in hs-cTnI and hs-cTnT values, the first sample should be collected immediately upon admission to the ED and the following one within a few hours (commonly from 1 to 3 h, but in some cases even 6 or 12 h), as established by guideline-recommended diagnostic algorithms [7, 8, 74]. However, even the most recent guidelines are not in agreement on which is the most accurate algorithm and more importantly the one with the best cost/benefit ratio [7, 8, 74, 77–79].

The faster algorithms, with a sampling on admission to the ED and the second after 1 h or after 2 h, have the advantage of obtaining a faster rule-out of patients compared to the classic 0–3 h algorithm [8]. In particular, the 2020 ESC guidelines [8] recommend rapid algorithms (0–1 and 0–2 h) for rule-

in and rule-out, suggesting significant changes (delta change, expressed in ng/L) specific to each hs-cTnI and hs-cTnT method, including two hs-cTnI point-of-care tests (POCT). However, reliable evidence for the delta values for the fastest algorithms is still lacking for some hs-cTnI methods [7–9, 74]. The 2020 ESC guidelines also recommend specific levels for rule-in and rule-out at time 0 (i.e., using only one admission value). Generally, the cut-off level indicated for the rule-out at time 0 is lower than or equal to the LoD value of the method, and has a negative predictive value (NPV) equal to 99% [8, 9, 77, 79].

In particular, the HIGH-US study evaluated the accuracy of the 0/1-h algorithm using the hs-cTnI method (Siemens Atellica Immunoassay) in 2,113 adult individuals presenting to the ED of 29 US medical centers with suspected AMI [81]. In this study, 1,065 patients (50.4%) were ruled-out, with a negative predictive value of 99.7% and sensitivity of 98.7% (95% CI from 99.2 to 99.9% and from 96.3 to 99.6%, respectively), whereas 265 patients (12.6%) were ruled-in, having a positive predictive value of 69.4% and specificity of 95.7% (95% CI 63.6%–74.7% and 94.7–96.5%, respectively). The remaining 783 patients (37.1%) were classified as having continued evaluations, with an acute myocardial infarction incidence of 5.6% (95% confidence interval 4.2–7.5%) [81]. Furthermore, in this study equivalent results were observed using the most rapid the 0/1-h algorithm or the 0/2-to 3-h algorithms.

Nevertheless, some recent expert documents and guidelines suggest that it is always necessary to ensure that the symptoms began at least 3 h before the collection, because hs-cTnI and hs-cTnT levels increase more slowly in the first hours and therefore it is more difficult to detect a significant variation in the biomarker levels [8, 9, 77, 79]. The levels recommended by the ESC 2020 guidelines for rule-in at time 0 are approximately 5 times or more the value of the 99th percentile URL [8, 78, 80]. However, the positive predictive value (PPV) of rapid rule-in algorithms is on average 70–75%, so these patients always require further non-invasive and invasive investigations to confirm the diagnosis of MI (such as coronary angiography) [7].

Overall, the table of hs-TnI and hs-cTnT values reported in the ESC 2020 guidelines is not easy to understand and interpret. The multiplicity of data reported requires a careful reading and almost certainly repeated re-considerations [7]. Even after a careful examination there are still some points open to discussion [7–9, 73, 77–79].

The values of delta recommended by the 2020 ESC guidelines were derived from multicenter studies where the optimal accuracy was calculated for both sensitivity (NPV=99%) and specificity (PPV=70%) [7]. However, while the optimal delta values for the hs-cTnT method and the Architect hs-cTnI method have been validated in multiple studies, few studies are available for the other hs-cTnI methods (including the POCT methods) [7, 8, 54, 55, 74, 81–87]. It is conceivable that the optimal values found in a single study strictly depend on the demographic characteristics (especially age and sex distribution) and clinical conditions of the enrolled patients [2, 5, 7, 34–40, 45, 72–74].

Another point concerns the sex specificity of cut-off values or delta, since it is well known that the values of the 99th percentile URL differ significantly between men and women [1, 5, 7, 9, 45, 74, 77, 79]. The 2020 ESC guidelines recommend equal cut-off values (for a single measurement) or delta (for two measurements) for men and women [8], while other guidelines support the use of different cut-off values among men and women, because this distinction of reference values by sex seems to improve diagnostic accuracy, especially in women [1, 7, 9, 45, 74, 79, 88, 89]. The 2020 ESC guidelines seem to take into consideration for the choice of cut-off values and delta recommended only a few studies, performed in a few institutions, evidently selected by the members of the ESC Task Force for the high quality of the experimental design and the characteristics and clinical demographics of the patient populations enrolled in the studies [8, 81–87]. Indeed, the delta values suggested by 2020 ESC guidelines [8] for the management of NSTEMI patients can vary according to methods, to rule-in/rule-out algorithms, and probably also to sex, at least for the hs-cTnI methods [2, 7, 9, 38, 54, 74, 79].

On the contrary, the RCV values (expressed as %) are less subject to variations among hs-cTnI and hs-cTnT methods [9, 20, 23, 56, 70, 71, 75–77]. In particular, RCV values for a series of two samples were reported to vary on average by 32% for concentrations values of hs-cTnI and hs-cTnT methods for concentrations values from 5 ng/L to 40 ng/L (Table 7), in agreement with many experimental and clinical studies, including both healthy adult subjects and patients admitted to the ED in whom the presence of acute heart damage was excluded [9, 20, 23, 45, 56, 70, 71, 75–77]. The percent change value (RCV%) for evaluating the kinetics of hs-cTnI and hs-cTnT in patients admitted to the ED with suspected MI is recommended by many guidelines or expert papers [1, 7, 45, 74, 78, 79]. Generally speaking, as a thumb rule, it seems much easier to use the RCV values expressed as a percentage rather than absolute changes (expressed as ng/L) to evaluate the biomarker kinetics using hs-cTnI methods for the diagnosis of acute myocardial injury, according to the Fourth Universal Definition of Myocardial Infarction [1].

Considering the different recommendations among the most recent international guidelines concerning the use of delta change or RCV values for the rule-in/rule-out of patients admitted to the ED with suspected MI, several expert documents and international guidelines strongly recommend a very close collaboration between clinicians and laboratorians in order to better understand issues related to the analytical characteristics and clinical performances of all hs-cTnI and hs-cTnT methods, especially concerning LoD, 99th percentile URL, and cut-off (like as delta changes) values [2, 4, 7, 9, 38, 54, 74, 79]. The ultimate goal of this collaboration should be choice of the rule-in/rule-out algorithm more appropriated for patients admitted to the ED with suspected MI in their clinical institution.

The evaluation of the kinetics of hs-cTnI and hs-cTnT values as RCV% has also the advantage of providing a more accurate estimate of the risk of cardiac disease even in patients presenting with chronic or acute non-ischemic myocardial damage [78–80].

It is now commonly ascertained that the use of hs-cTnI and hs-cTnT methods has led to a progressive increase in the incidence of NSTEMI compared unstable angina [8, 9, 78, 80]. In 2020, Chapman et al. [80] confirmed that patients with myocardial injury or NSTEMI are at a higher risk of death from non-cardiac events, occurring within 30 days in approximately one third of these patients due to complications from lung disease or sepsis [80]. However, patients with cardiac injury or NSTEMI have a lower mortality rate from major adverse cardiovascular events (MACE) than patients with STEMI [9, 78, 80]. However, according to this study [80], 1/6 of patients with heart damage or NSTEMI are at risk of death from MACE within 1 year; this risk is 3 times higher than in patients with no evidence of heart damage.

Take-home messages

- The algorithms for the NSTEMI diagnosis with a sampling on admission to the ED and the second one after 1 or 2 h have the advantage of a faster rule-out compared to the classic 0–3 h algorithm [7, 8].
- The 2020 ESC guidelines [8] recommend cut-off values (for a single measurement) or delta (for two measurements in series) equal for men and women, while other recent guidelines or international documents support the use of different threshold values between men and women, especially using the hs-cTnI methods [7, 9, 74, 79].
- To evaluate the variations of hs-cTnI and hs-cTnT values in patients admitted to the ED with suspected NSTEMI, it seems much easier to use RCV values expressed as a percentage (using a value ≥30% as a threshold) rather than as delta (i.e., expressed in concentration difference in ng/L), as recommended by the 2020 ESC guidelines.
- Considering the different recommendations among the most recent international guidelines, clinicians and laboratorians should collaborate together in order to choose the rule-in/rule-out algorithms more appropriated for patients admitted to the ED with suspected MI in their clinical institution [2, 7, 9, 38, 74, 79].

Cardiovascular risk in the general population

As a consequence of the recent introduction of hs-cTnI and hs-cTnT methods in the clinical practice [2–6, 12–14], several meta-analyses have demonstrated that some “apparently healthy” individuals with hs-cTnI and hs-cTnT concentrations in the upper tertile have a significantly worse cardiovascular outcome [14, 90–93]. Furthermore, hs-cTnI and hs-cTnT values increase progressively after 55 years in both sexes in the general population [3, 5, 12, 13, 33–40], and cardiovascular disease becomes more prevalent after the fifth or sixth decade of life [94, 95].

The close association between senescence and increased circulating levels of hs-cTnI and hs-cTnT might be usefully explained through the notion of “inflammaging” (or “inflammageing”), introduced by Franceschi et al. in 2000 [96]. Inflammaging defines a set of pathophysiological mechanisms in older individuals who have a very high susceptibility to disease manifestations, disability, frailty and premature death [97–99]. This condition is typically associated with high circulating levels of inflammatory biomarkers [96–99]. Recent evidence has shown that inflammaging is a hallmark of cardiovascular diseases typical of the elderly, such as atherosclerosis, systemic arterial hypertension and rapid progression to heart failure (HF) [98–101]. For this reason, inflammageing, cardiovascular disease and life expectancy are closely related [94, 98, 100–103].

The Senescence-Associated Secretory Phenotype (SASP) has been attributed to senescent cells secreting high levels of pro-inflammatory cytokines, immuno-modulators, angiogenic growth factors, metalloproteases, especially in tissues with a low rate of cell renewal such as the myocardium [99, 102, 103] (Figure 3). Chronic activation of the pathogenetic mechanisms linked to SASP stimulates not only the secretion of natriuretic peptides [101, 104, 105], but also may produce some cytotoxic effects on cardiomyocytes inducing a myocardial damage [100]. Consequently, the mechanisms

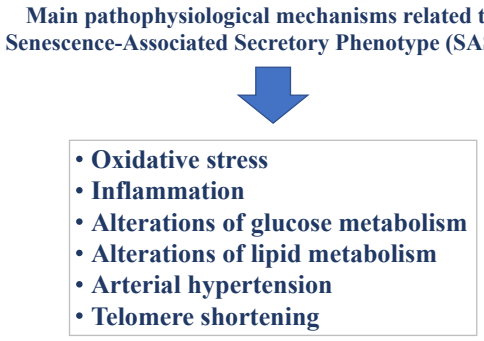

Figure 3: Main mechanisms related of the secretory phenotype associated with senescence (SASP).

related to SASP is strictly related to a progressive reduction of cardiomyocytes in the myocardial tissue of the elderly, which are gradually replaced by fibrotic tissue [102, 103, 106–109]. This progressive ventricular remodeling is pathological, because it induces a progressive decline in myocardial function and beyond a certain point is considered irreversible [110].

According to the ACC/AHA 2005 guidelines on the diagnosis and management of chronic HF in the adult [111], the natural history of HF can be divided into four periods in which the first two (stages A and B), which are still asymptomatic and without the typical signs of the disease, are considered still reversible. If an adequate therapy is instituted early, the patient can regain a normal cardiac function [110, 111]. Conversely, in the last two stages (C and D) symptoms are present and there are alterations of the structure and function of the myocardium that are deemed irreversible. A therapeutic intervention is just able to slow down disease progression [110, 111].

In 2017, the results of the MORGAM/BiomarCaRE study were published, supporting the hypothesis that repeated measures of hs-cTnI or hs-cTnT are able to highlight individuals in the general population at higher risk for cardiovascular events in subsequent years [112]. This study collected data from a Danish population (3,975 participants, with an age at baseline of 30–60 years, 51% female, apparently healthy) followed with a total of 26 years (from 1982 to 2009). The hs-cTnI values (Architect i2000SR method, Abbott Diagnostics) were measured in this population in samples collected in 3 series every 5 years [112]. The median concentration of hs-cTnI in the population ranged from 2.6 ng/L at baseline to 3.4 ng/L after a 10-year period. The change process of hs-cTnI values, modelled using a joint (longitudinal and survival) model, was able to predict the 10-year cardiovascular risk (with 581 events) with an HR of 1.31 (95% CI 1.15–1.48) after adjustment for cardiovascular risk factors on an individual basis [112].

In agreement with the literature [14, 90–93, 112], a progressive increase of even a few ng/L (variation ≥30%) of hs-cTnI and hs-cTnT, even lower than the 99th percentile URL, may suggest a progressive increase in cardiac remodeling in subjects who are still asymptomatic [12, 13]. The hs-cTnI and hs-cTnT thresholds have been identified for a low, intermediate and high risk, as reported in Table 8 [12, 13, 113–116]. For example, a progressive increase >30% over a few months in a subject at risk could indicate a progressive remodeling with rapid evolution towards symptomatic HF. According to the recommendations of two very recent documents [12, 13], these data should stimulate the clinician to investigate the causes of the remodeling. In particular, these two recent documents suggest the use of hs-cTnI and hs-cTnT measurement in the general population to detect early asymptomatic individuals at higher risk for progression to symptomatic HF or for developing MACE over the medium to long term (≥6 months), such as patients aged >55 years and/or with comorbidities (obesity, diabetes, hypercholesterolemia, hypertension, atherosclerosis, chronic kidney or lung disease) [12, 13]. Despite the experimental evidence and the favorable results of numerous clinical studies [12–14, 90–93, 112–116], no international guidelines still recommend the use in the general population of the hs-cTnI and hs-TnT measurement for cardiovascular risk assessment due to the lack of studies on the risk/benefit assessment of this screening.

Take-home messages

– Cardiovascular risk is significantly higher in apparently healthy subjects in the reference population with concentrations of hs-cTnI and hs-cTnT in the upper tertile of the biomarker distribution [12–14, 90–93, 112–116].

Table 8: Threshold values for the evaluation of cardiovascular risk in the general population from the literature.

cTnI[a]	Women	Men
Low risk	<4 ng/L	<6 ng/L
Intermediate risk	4–10 ng/L	6–12 ng/L
High risk	>10 ng/L	>12 ng/L
cTnT[b]		**Whole population**
Low risk		≤3 ng/L
Intermediate risk		3.0–5.7 ng/L
High risk		≥5.8 ng/L

[a]Only the data relating to the hs-cTnI Architect Method (Abbott Diagnostics) are reported because this is the method most often evaluated in published studies [12, 13, 112–116]. [b]Method ECLIA hs-cTnT Elecsys (Roche Diagnostics) [12, 13, 116].

– hs-cTnI and hs-cTnT can be measured in the general population to detect early asymptomatic individuals at higher risk of progressing to symptomatic HF or developing MACE over ≥6 months, such as patients aged >55 years and with comorbidities [12–14].
– There is still a lack of accurate studies that demonstrate the favorable cost/benefit profile of a cardiovascular risk screening with serial measurements of hs-cTn in the general population [12–14].

Cardiovascular risk assessment in patients undergoing major non-cardiac surgery

About half of peri-operative cardiac deaths occur in patients, undergoing major non-cardiac surgery, who do not have a history of heart disease [117]. Cardiac complications after non-cardiac surgery depend on the type of surgery, the specific clinical condition, the individual responses to surgical stress, and the effects of anesthesia [117]. This stress response begins with the onset of tissue damage, which releases neuro-endocrine and pro-inflammatory factors into the circulation, which in turn can induce alterations in the action of the peripheral nervous system (vagal and sympathetic), circulating volume and distribution of extracellular fluid. These pathogenetic mechanisms induce an increase in oxygen demand by tissues (including myocardial tissue) and an imbalance between thrombotic and fibrinolytic activity (with an increased risk of coronary thrombosis) [117].

The 2014 ESC/European Society of Anesthesiology (ESA) guidelines report that approximately 167,000 cardiac complications occur each year in European countries from major non-cardiac surgery, of which 19,000 are life-threatening [117]. Non-cardiac surgery has a rate of complications between 7 and 11% and a mortality rate of 0.8–1.5% worldwide, with 42% of deaths due to cardiac complications [117–119]. More recently, the risk of complications has progressively decreased due to the improvement of surgical and anesthesia procedures, but in 2018 Sellers et al. [120] have reported that the 30-day mortality rate for patients undergoing major non-cardiac surgery still varied between 0.5 and 2%, mostly because of cardiovascular complications.

The use of the hs-cTnI and hs-cTnT methods has shown that these biomarkers can increase in some patients undergoing surgery, and this increase has been associated with a higher risk of complications and death [121, 122]. The notion of Myocardial Injury after Non-cardiac Surgery (MINS) was introduced to denote the specific clinical condition of ischemic myocardial damage detected during major non-cardiac surgery [121–123]; conversely, the Fourth Universal Definition of Myocardial Infarction [1] specifically refers to patients admitted to ED with ACS.

MINS is due to a supply-demand mismatch of the myocardium or to coronary thrombosis, and this clinical condition is associated with an increased risk of mortality or MACE 30 days to 2 years after major non-cardiac surgery [122]. MINS is defined by hs-cTnI or hs-cTnT values ≥99th percentile URL of the method up to 30 days during or after non-cardiac surgery, which are not attributable to other well-known and frequent non-ischemic causes of biomarker increase (such as arrhythmias, HF, myocarditis) [11, 123–125].

To assess more accurately the peri-operative risk in patients who must undergo major surgery for non-cardiac causes, the 2017 Canadian Cardiovascular Society guidelines [125] recommend the determination of natriuretic peptides (BNP or N-terminal pro-BNP [NT-proBNP]) before surgery. In particular, the measurement of natriuretic peptides is recommended to improve the estimate of the risk of peri-operative MACE, especially in patients over 65 years of age, or aged 45–64 years but with cardiovascular disease or a high cardiovascular risk [125].

Some recent papers [11, 124–126] strongly recommend that cardiac-specific biomarkers (BNP/NT-proBNP and hs-cTnI/hs-cTnT) should be always measured before major non-cardiac surgery, especially in patients with a high cardiovascular risk. Indeed, the measurement of cardiac-specific biomarkers before surgery allows to more accurately identify patients with a higher cardiovascular risk [11–13, 111, 124–127]. Moreover, a more complete profile of the clinical status and the cardiovascular risk before surgery can allow a better choice of the type of surgery, a preventive pharmacological therapy with regard to possible peri-operative complications and also a tailored monitoring during and after surgery [11, 125]. Finally, it is conceivable that a measurement of hs-cTnI or hs-cTnT before the intervention makes it easier to diagnose the presence of MINS, using samples taken during the peri- and post-operative period and thus detecting any significant increase in biomarker levels from baseline and above the 99th percentile URL [124].

Take-home messages

– Non-cardiac surgeries have a complication rate between 7 and 11% and a mortality rate of 0.5–2%, with up to 42% due to cardiac complications [117–120].
– The term Myocardial Injury after Non-cardiac Surgery (MINS) was introduced to denote the occurrence of acute

ischemic myocardial damage during major non-cardiac surgery [11, 122, 125].
- MINS is identified by signs and symptoms of myocardial ischemia, hs-cTnI or hs-cTnT ≥99th percentile URL of the method during the intra-and post-operative period, up to 30 days after non-cardiac surgery [11, 122, 123, 125].
- It is conceivable that the pre-intervention measurement of hs-cTnI or hs-cTnT makes it easier to diagnose the presence of MINS, comparing the values obtained pre-operatively with those carried out subsequently during the peri-and post-operative period and thus ascertaining any significant increase biomarker levels from baseline and above the 99th percentile URL [124].

Evaluation of cardiac damage associated with the use of cardiotoxic drugs

Several chemotherapy drugs have a cardiotoxic effect in patients being treated for tumors, including not only the more utilized drugs, like as cyclophosphamide, taxanes or 5-fluoropirimidines, but also the more recent monoclonal antibodies, the tyrosin-kinase inhibitors, and the D-1 immune checkpoint blockers [128]. All these chemotherapy drugs can induce several cardiovascular complications ranging from myocardial dysfunction and HF to acute coronary syndromes, morphological and functional abnormalities of heart valves, arrhythmias, systemic arterial hypertension, pulmonary hypertension, pericardial complications, thrombo-embolism, and stroke [128–130].

As several definitions have been proposed for cardiotoxicity [131], some discrepancies are available in the literature concerning both diagnosis and management of cardiovascular complications induced by chemo-therapy [129–131]. In order to harmonize the different definitions of cardiotoxicities and the management of patients with tumors treated with chemotherapy, two consensus documents, including practical clinical guidelines, have been recently proposed by the International Cardio-Oncology Society (IC-OS), the European Hematology Association (EHA), and the European Society for Therapeutic Radiology and Oncology (ESTRO) [132, 133]. These documents provide consensus definitions for the most commonly reported cardiovascular complications related to chemotherapy administration, including: cardiomyopathy, heart failure, myocarditis, vascular toxicity, hypertension, arrhythmias and QTc prolongation [132, 133].

The detection of cardiac damage, not related to symptoms or signs of coronary ischemia, may indicate a high risk of a progressive worsening of cardiac function up to overt HF [15, 16, 129–131, 134–138]. The 2022 ESC guidelines on Cardio-Oncology [133] recommend (class 1, level C) the measurement of NPs and/or cardiac troponins in all patients with tumors at risk of developing cardiac dysfunction following cancer chemotherapy.

It is important to note that the measurement of natriuretic peptides and cardiac troponins provide complementary pathophysiological and clinical information even in patients treated with cardio-toxic drugs [16, 127, 131]. In fact, high levels of BNP or NT-proBNP indicate that chemotherapy therapy has produced an activation of the neuro-endocrine system and of the pro-inflammatory cytokine system capable of stressing cardiac function [127, 128, 139]. On the other hand, the detection of hs-cTnI and hs-cTnT values above the 99th percentile URL indicates the presence of a myocardial damage related to a biomarker release from cardiomyocytes for some weeks or even months, suggesting a progressive development of cardiac symptomatic dysfunction [140, 141].

Progressively higher levels of natriuretic peptides (BNP and NT-proBNP), but especially of hs-cTnI and hs-cTnT, after two or more courses of chemotherapy therapy, seem to identify patients with a pathological rate of cardiomyocyte death and a higher risk of progressive ventricular remodeling [130–135]. As also recommended by the 2022 ESC guidelines [133], there is a need to perform a basal measurement (i.e., before treatment) of BNP/NT-proBNP and hs-cTnI/hs-cTnT in every patient receiving some cycles of potentially cardiotoxic drugs, to evaluate any changes in these markers following therapy. Importantly, considering the systematic differences both between the measurement methods of BNP/NT-proBNP [142, 143], and hs-cTnI/hs-cTnT [4–6, 38, 45], it is necessary to monitor the patient during the various cycles of therapy with serial measurements performed with the same method and possibly in the same laboratory.

Take-home messages

- Many studies indicate that in patients with tumors treated with chemotherapy, the measurement of hs-cTnI and hs-cTnT can allow to detect a heart damage, which is generally associated with a high risk of progressive worsening of heart function up to overt HF [130–138].
- The 2022 ESC Guidelines on Cardio-Oncology recommend (class 1, level C) the measurement of BNP/NT-proBNP and/or hs-cTnI/hs-TnT in all patients with tumors at risk of developing cardiac dysfunction following cancer therapy [133].

- Every patient who will be subjected to courses of potentially cardiotoxic chemotherapy drugs, must carry out a basal (pre-treatment) measurement of BNP/NT-proBNP and hs-cTnI/hs-cTnT for the assessment of cardiovascular risk [132–134].
- As there are systematic differences among immunoassay methods, it is necessary to follow the patient during the various cycles of therapy with serial cardio-specific biomarker measurements performed with the same method and possibly in the same laboratory [134].

Summary and future perspectives

In these first 20 years of the 21st century, the assay methods to measure cardiac troponins have acquired an increasingly greater clinical relevance [3, 4]. This is due not only to the biochemical and biological characteristics of these biomarkers, but also to the development of extremely sensitive immunometric assay for hs-cTnI and hs-cTnT with LoD value ranging from 1.5 to 3 ng/L (Table 1) [3, 4]. Furthermore, the biochemical and biological characteristics of biomarkers guarantee an absolute cardiac specificity (especially for hs-cTnI methods) [4], and a very low intra-individual variability, with an average Individuality Index of 0.3 (Table 5) [56]. From a clinical perspective, it is particularly important that, in apparently healthy (and so still asymptomatic) subjects, the hs-cTnI and hs-cTnT values in the upper tertile of distribution range are associated with an increase in mortality and of MACE compared to subjects with concentrations within the first tertile [12–14, 90–93].

The biological characteristics of the biomarkers and an analytical error <10% for concentrations around the 99th percentile URL (Table 2 and Figure 2) allow to define as clinically significant changes of about 30% (with an interval ranging from 25 to 45%) between two serial measurements in the same individual with the same hs-cTnI or hs-cTnT method [3, 4, 20, 23, 24, 46, 47, 56]. The circulating levels of hs-cTnI e hs-cTnT can rapidly increase in a few hours like as in patients with AMI [1, 2, 7–9, 30, 31, 74, 79, 80], or more slowly, but progressively, over the course of some months as in patients with HF (stages C and D) [30, 31, 144]. In HF patients, multiple mechanisms may lead to myocyte necrosis, apoptosis, mitochondrial autophagy, or reversible injury with increased myocyte membrane permeability, all resulting in cardiac troponin release into the circulation [30, 31, 144–147].

The evaluation of the kinetics of hs-cTnI and hs-cTnT values is relevant also for the detection of cardiac damage during major non-cardiac surgery [11, 117–125] or therapy with cardiotoxic cancer drugs [128–138]. Accordingly, a blood sampling to measure cardiac-specific biomarkers, especially hs-cTnI and hs-cTnT, should become routine practice before non-cardiac surgery or cancer therapy. The baseline value will be useful to evaluate the cardiovascular risk before surgery or drug administration, because some patients still asymptomatic,but with a high cardiovascular risk, may experience a myocardial damage during surgery [11, 119–125]. The same pathophysiological reasoning must be taken into consideration in patients with tumors who have to undergo repeated administration of potentially cardiotoxic drugs [128–138]. Without a baseline value of hs-cTnI or hs-cTnT it is not possible to perform an accurate assessment of biomarker kinetics, because some patients may already have hs-cTnI and hs-cTnT values higher than the 99th percentile URL before surgery or cancer therapy [134, 135]. Moreover, some patients with hs-cTnI and hs-cTnT values in the first or second tertile (therefore within normal limits and at low or medium risk) may exhibit after surgery or after the first cycle of therapy with chemotherapy an increase in circulating levels up to the upper tertile or even above the 99th percentile URL (Table 8) [134, 135]. Clinicians should consider these patients at higher risk for further medium or short-term cardiovascular events, such as progression to symptomatic HF, and therefore provide more careful monitoring and cardio-protective therapy [11–13, 17, 123–125, 132–135].

As observed in the most recent guidelines [7, 11, 74, 78, 79, 125, 132, 133], further studies are needed to define the best way to evaluate the kinetics of hs-cTnI and hs-cTnT in specific clinical conditions. In particular, it is important to evaluate whether the rapid algorithms (0–1 h or 0–2 h) are more effective and convenient than the classic 0–3 h algorithm in patients admitted to the ED with suspicion of NSTEMI even outside of the clinical and research institutions where the clinical studies considered by the guidelines are typically performed [7–9, 74, 78, 79]. Furthermore, there are insufficient studies that evaluate whether the screening for cardiovascular risk assessment in the general population through the measurement of hs-cTnI and hs-cTnT has a favorable cost/benefit profile, at least in individuals more at risk due to advanced age and/or the presence of comorbidities (Table 8) [12–14]. Finally, further randomized and multicenter studies are needed to evaluate the efficacy and convenience of measuring cardiac-specific biomarkers (BNP/NT-proBNP and hs-cTnI/hs-cTnT) in patients with tumors undergoing potentially cardiotoxic therapies [128–138].

The development of hs-cTnI and hs-cTnT methods with increasingly better analytical performance, easy to use and with short turnaround times has not yet come to an end, and

further important developments are expected [47, 54, 55, 148–156]. Recently, some POCT methods for the measurement of hs-cTnI have received analytical and clinical validation and have become commercially available [151–154]. These methods are able to guarantee a fast turnaround time and excellent sensitivity and reproducibility, without losing specificity [151–154]. These POCT methods for hs-cTnI could allow an efficient screening of patients with suspected NSTEMI at home, in decentralized clinics or in ambulance with a significant reduction in the time of diagnosis and hospitalization [47, 54, 55, 148, 149]. Furthermore, sone of these methods can also use drops of blood for the measurement of hs-cTnI, thus avoiding the need for a blood sample from the vein, which can be very useful especially in newborns and infants [47, 54, 55, 149, 157].

Finally, the recent development of new and more sensitive biosensors could allow the preparation of wearable devices capable of measuring cardiac troponins with transdermal methods, allowing a quasi-continuous monitoring of circulating levels of hs-cTnI, which could be very useful in adult or pediatric patients admitted to the ED or intensive care units [55, 155, 156].

Final remarks and suggestions

The following proposals on the use of the hs-cTnI and hs-cTn methods derive from a critical reappraisal of the recommendations supported by the most recent international guidelines.

- All the most recent guidelines recommend the use of hs-cTnI and hs-cTnT methods for prognosis, diagnosis and management of cardiovascular disease [1, 2, 7–9, 11, 78, 79].
- For all hs-cTnI methods, the manufacturers recommend that the reference value (i.e., the 99th percentile URL) be distinct by sex, while the manufacturer of the hs-cTnT method recommends using a single value for both sexes [2, 5].
- In agreement with the recommendations of the Fourth Universal Definition of Myocardial Infarction [1], the finding in a patient of a single hs-cTnI or hs-cTnT value greater than the 99th percentile URL is sufficient to diagnose the presence of cardiac damage (i.e., myocardial injury).
- All international guidelines [1, 2, 7, 8] agree that the hs-cTnI and hs-cTnT methods have on average a similar diagnostic accuracy and therefore allow to detect an acute or chronic damage to the myocardium.
- The algorithms for the NSTEMI diagnosis with a sampling on admission to the ED and the second after 1 h or 2 h have the advantage of obtaining a faster rule-out of patients compared to the classic 0–3 h algorithm in patients with suspected NSTEMI [7–9, 78, 79], but are probably not applicable in all hospitals at present time.
- The 2020 ESC guidelines for rule-in and rule-out of patients suspected of NSTEMI recommend cut-off values (for a single measurement) or delta (for two measurements in series) equal for men and women [8], while other international guidelines support the use of different threshold values between men and women, especially for the hs-cTnI methods [1, 7, 45, 74, 79, 88, 89].
- To evaluate the kinetics of hs-cTnI and hs-cTnT values in patients admitted to the ED with suspected NSTEMI, it seems much easier to use the RCV values expressed as a percentage (using a threshold value ≥30%) rather than delta (i.e., as an absolute difference), as recommended by the 2020 ESC guidelines [8].
- Although two recent papers have suggested the measurement of hs-cTnI and hs-cTnT in the general population to detect early symptomatic individuals at higher risk [12, 13], there is still a lack of accurate studies demonstrating a favorable cost/benefit ratio for this screening.
- The latest guidelines recommend both pre-and post-operative measurement of hs-cTnI or hs-cTnT in patients undergoing major non-cardiac surgery, because it allows for a better pre-operative risk assessment cardiovascular, and to diagnose the presence of MINS and/or other cardiac complications during or immediately after surgery [11, 122–125].
- The 2022 ESC Guidelines on Cardio-Oncology [133] recommend (class 1, level C) the measurement of cardiac natriuretic peptides (BNP/NT-proBNP) and/or hs-cTnI/hs-cTnT in all patients with tumors at risk of developing cardiac dysfunction following cancer therapy.
- Each patient who will have to undergo cycles of potentially cardiotoxic chemotherapy drugs should perform a basal (pre-therapy) measurement of BNP/NT-proBNP and hs-cTnI/hs-cTnT to assess changes following the start of treatment. It is recommended to carry out biomarker measurements with the same method and possibly in the same laboratory.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.
Informed consent: Not applicable.
Ethical approval: Not applicable.

References

1. Thygesen K, Alpert JS, Jaffe AS, Chaitman B, Bax JJ, Morrow DA, et al. Fourth Universal definition of myocardial infarction (2018). Eur Heart J 2019;40:237–69.
2. Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, et al. 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 2018;64:645–55.
3. Clerico A, Giannoni A, Prontera C, Giovannini S. High-sensitivity troponin: a new tool for pathophysiological investigation and clinical practice. Adv Clin Chem 2009;49:1–30.
4. Clerico A, Zaninotto M, Padoan A, Masotti S, Musetti V, Prontera C, et al. Evaluation of analytical performance of immunoassay methods for cTnI and cTnT: from theory to practice. Adv Clin Chem 2019;93: 239–62.
5. Clerico A, Zaninotto M, Ripoli M, Masotti S, Prontera C, Passino C, et al. The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology, and clinical implications. Clin Chem Lab Med 2017;55:1634–51.
6. Clerico A, Ripoli A, Zaninotto M, Masotti S, Musetti V, Ciaccio M, et al. 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. Clin Chim Acta 2019;496:25–34.
7. NICE. High-sensitivity troponin tests for the early rule out of NSTEMI. Diagnostics guidance; 2020. Available from: www.nice.org.uk/guidance/dg40.
8. Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. The task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). 2020 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2021;42: 1289–387.
9. Clerico A, Zaninotto M, Aimo A, Dittadi R, Cosseddu D, Perrone M, et al. Use of high-sensitivity cardiac troponins in the emergency department for the early rule-in and rule-out of acute myocardial infarction without persistent ST-segment elevation (NSTEMI) in Italy. Clin Chem Lab Med 2021;60:169–82.
10. Clerico A, Zaninotto M, Aimo A, Musetti V, Perrone M, Padoan A, et al. Evaluation of the cardiovascular risk in patients undergoing major non-cardiac surgery: role of cardiac-specific biomarkers. A consensus document by the Inter-society study group on cardiac biomarkers of the Italian societies of clinical biochemistry: European Ligand Assay Society (ELAS), Italian section; Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC); Società Italiana di Patologia Clinica e Medicina di Laboratorio (SIPMel). Clin Chem Lab Med 2022; 60:1525–42.
11. Ruetzler K, Smilowitz NR, Berger JS, Devereaux PJ, Maron BA, Newby LK, et al. Diagnosis and management of patients with myocardial injury after noncardiac surgery: a scientific statement from the American heart association. Circulation 2021;144:e287–305.
12. Clerico A, Zaninotto M, Passino C, Aspromonte N, Piepoli MF, Migliardi M, et al. Evidence on clinical relevance of cardiovascular risk evaluation in the general population using cardio-specific biomarkers. Clin Chem Lab Med 2021;59:79–90.
13. Farmakis D, Mueller C, Apple FS. High-sensitivity cardiac troponin assays for cardiovascular risk stratification in the general population. Eur Heart J 2020;41:4050–6.
14. Aimo A, Georgiopoulos G, Panichella G, Vergaro G, Passino C, Emdin M, et al. High-sensitivity troponins for outcome prediction in the general population: a systematic review and meta-analysis. Eur J Intern Med 2022;98:61–8.
15. Cardinale D, Biasillo G, Salvatici M, Sandri MT, Cipolla CM. Using biomarkers to predict and to prevent cardiotoxicity of cancer therapy. Expert Rev Mol Diagn 2017;17:245–56.
16. Pudil R, Mueller C, Čelutkienė J, Henriksen PA, Lenihan D, Dent S, et al. Role of serum biomarkers in cancer patients receiving cardiotoxic cancer therapies: a position statement from the cardio-oncology study group of the heart failure association and the cardio-oncology council of the European society of cardiology. Eur J Heart Fail 2020;22: 1966–83.
17. Cardinale D, Milss NL, Mueller C. Study group on biomarkers of the ESC association for acute cardiovascular care. cardiac biomarkers in the field of cardio-oncology. Eur Heart J Acute Cardiovasc Care 2022; 11:e1–2.
18. Franzini M, Lorenzoni V, Masotti S, Prontera C, Chiappino D, Della Latta D, et al. 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 2015; 438:376–81.
19. Caselli C, Cangemi G, Masotti S, Ragusa R, Gennai I, Del Ry S, et al. 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 2016;458:68–71.
20. Ndreu R, Musetti V, Masotti S, Zaninotto M, Prontera C, Zucchelli GC, et al. Evaluation of the cTnT immunoassay using quality control samples. Clin Chim Acta 2019;495:269–70.
21. Masotti S, Prontera C, Musetti V, Storti S, Ndreu R, Zucchelli GC, et al. Evaluation of analytical performance of a new high-sensitivity immunoassay for cardiac troponin I. Clin Chem Lab Med 2018;56: 492–501.
22. Musetti V, Masotti S, Prontera C, Storti S, Ndreu R, Zucchelli GC, et al. 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 2018;56: e229–31.
23. Musetti V, Masotti S, Prontera C, Ndreu R, Zucchelli GC, Passino C, et al. 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;57: e241–3.
24. Masotti S, Musetti V, Prontera C, Storti S, Passino C, Clerico A. Evaluation and comparison with other high-sensitivity methods of analytical performance and measured values of a new laboratory test for cardiac troponin I assay. J Appl Lab Med 2021;6:1237–50.

25. Clerico A, Zaninotto M, Padoan A, Ndreu R, Musetti V, Masotti S, et al. Harmonization of two hs-cTnI methods based on recalibration of measured quality control and clinical samples. Clin Chim Acta 2020; 510:150–6.
26. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined: a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36: 959–69.
27. Buja LM, Vela D. Cardiomyocyte death and renewal in the normal and diseased heart. Cardiovasc Pathol 2008;17:349–74.
28. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, et al. Evidence for cardiomyocyte renewal in humans. Science 2009;324:98–102.
29. Bergmann O, Zdunek S, Felker A, Salhpoor M, Alkass K, Bernard S, et al. Dynamics of cell generation and turnover in the human heart. Cell 2015;161:1566–75.
30. Marjot J, Kaier TE, Martin ED, Reji SS, Copeland O, Iqbal M, et al. Quantifying the release of biomarkers of myocardial necrosis from cardiac myocytes and intact myocardium. Clin Chem 2017;63:990–6.
31. Mair J, Lindahl B, Hammarsten O, Müller C, Giannitsis E, Huber K, et al. How is cardiac troponin released from injured myocardium? Eur Heart J Acute Cardiovasc Care 2018;6:553–60.
32. Eschenhagen T, Bolli R, Braun T, Field LJ, Fleischmann BK, Frisén J, et al. Cardiomyocyte regeneration: a consensus statement. Circulation 2017;136:680–6.
33. Collinson PO, Heung YM, Gaze D, Boa F, Senior R, Christenson R, et al. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. Clin Chem 2012;58:219–25.
34. Sandoval Y, Apple FS. The global need to define normality: the 99th percentile value of cardiac troponin. Clin Chem 2014;60:455–62.
35. Hickman PE, Lindahl B, Cullen L, Koerbin G, Tate J, Potter JM. Decision limits and the reporting of cardiac troponin: meeting the needs of both the cardiologist and the ED physician. Crit Rev Clin Lab Sci 2015; 52:28–44.
36. Krintus M, Kozinski M, Boudry P, Lackner K, Lefèvre G, Lennartz L, et al. Defining normality in a European multinational cohort: critical factors influencing the 99th percentile upper reference limit for high sensitivity cardiac troponin I. Int J Cardiol 2015;187:256–63.
37. Eggers KM, Apple FS, Lind L, Lindahl B. The applied statistical approach highly influences the 99th percentile of cardiac troponin I. Clin Biochem 2016;49:1109–12.
38. Apple FS, Sandoval Y, Jaffe AS, Ordonez-Llanos J, IFCC Task Force on Clinical Applications of Cardiac Bio-Markers. Cardiac troponin assays: guide to understanding analytical characteristics and their impact on clinical care. Clin Chem 2017;63:73–81.
39. Hickman PE, Badrick T, Wilson SR, McGill D. Reporting of cardiac troponin problems with the 99th population percentile. Clin Chim Acta 2007;38:182–3.
40. Hickman PE, Koerbin G, Potter JM, Abhayaratna WP. Statistical considerations for determining high-sensitivity cardiac troponin reference intervals. Clin Biochem 2017;50:502–5.
41. Clinical Laboratory Standards Institute. Defining, establishing and verifying reference intervals in the clinical laboratory; approved guideline, 3rd ed. Wayne (PA): CLSI Document EP28-A3c, CLSI; 2008.
42. Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. Stat Med 2000;19: 1141–64.
43. Dixon WJ. Processing data for outliers. Biometrics 1983;9:74–89.
44. Tukey W. Exploratory data analysis. Reading (PA): Addison-Wesley; 1977.
45. Aakre KM, Saenger AK, Body R, Collinson P, Hammarsten O, Jaffe AS, et al. Analytical considerations in deriving 99th percentile upper reference limits for high-sensitivity cardiac troponin assays: educational recommendations from the IFCC Committee on clinical application of cardiac bio-markers. Clin Chem 2022;68:1022–30.
46. Perrone MA, Storti S, Salvadori S, Pecori A, Bernardini S, Romeo F, et al. Cardiac troponins: are there any differences between T and I? J Cardiovasc Med 2021;22:797–805.
47. Clerico A, Zaninotto M, Passino C, Padoan A, Migliardi M, Plebani M. High-sensitivity methods for cardiac troponins: the mission is not over yet. Adv Clin Chem 2021;103:215–52.
48. Laugaudin G, Kuster N, Petition A. Kinetics of high sensitivity cardiac troponin T and I differ in patients with ST-segment elevation myocardial infarction treated by primary coronary intervention. Eur Heart J Acute Cardiovasc Care 2015;5:354–63.
49. Solecki K, Dupuy AM, Kuster N, Leclercq F, Gervasoni R, Macia JC, et al. Kinetics of high sensitivity cardiac troponin T or troponin I compared to creatine kinase in patients with revascularized acute myocardial infarction. Clin Chem Lab Med 2015;53:707–14.
50. Pickering JW, Young JM, George PM, Pemberton CJ, Watson A, Aldous SJ, et al. Early kinetic profiles of troponin I and T measured by high-sensitivity assays in patients with myocardial infarction. Clin Chim Acta 2020;505:15–25.
51. Starnberg K, Fridén V, Muslimovic A, Ricksten SE, Nyström S, Forsgard N, et al. A possible mechanism behind faster clearance and higher peak concentrations of cardiac troponin I compared with troponin T in acute myocardial infarction. Clin Chem 2020;66:333–41.
52. Wu AH, Shea E, Lu QT, Minyard J, Bui K, Hsu JC, et al. Short-and long-term cardiac troponin I analyte stability in plasma and serum from healthy volunteers by use of an ultrasensitive, single-molecule counting assay. Clin Chem 2009;55:2057–9.
53. Egger M, Dieplinger B, Mueller T. One-year in vitro stability of cardiac troponins and galectin-3 in different sample types. Clin Chim Acta 2018;476:117–22.
54. Clerico A, Zaninotto M, Passino C, Padoan A, Migliardi M, Plebani M. High-sensitivity assay for cardiac troponins with POCT methods. The future is soon. Clin Chem Lab Med 2021;59:1477–8.
55. Clerico A, Aimo A, Zaninotto M, Pebani M. Transdermal measurement of cardiac troponins: the future is now. Clin Chem Lab Med 2022;60: 1133–5.
56. Clerico A, Padoan A, Zaninotto M, Passino C, Plebani M. Clinical relevance of biological variation of cardiac troponins. Clin Chem Lab Med 2021;59:641–52.
57. Prodan Žitnik I, Černe D, Mancini I, Simi L, Pazzagli M, Di Chiara R, et al. Personalized laboratory medicine: a patient-centered future approach. Clin Chem Lab Med 2018;56:1981–91.
58. Fraser CG. Biological variation: from principles to practice. Washington, DC: AACC Press; 2001.
59. Clerico A, Zucchelli GC, Pilo A, Passino C, Emdin M. Clinical relevance of biological variation: the lesson of brain natriuretic peptide (BNP) and NT-proBNP assay. Clin Chem Lab Med 2006;44:366–78.
60. Wu AH, Lu QA, Todd J, Moecks J, Wians F. Short-and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. Clin Chem 2009;55: 52–8.
61. Wu AH, Akhigbe P, Wians F. Long-term biological variation in cardiac troponin I. Clin Biochem 2012;60:638–47.

62. Schindler EI, Szymanski JJ, Hock KG, Geltman EM, Scott MG. Short-and long-term biologic variability of Galectin-3 and other cardiac biomarkers in patients with stable heart failure and healthy adults. Clin Chem 2016;62:360–6.
63. van der Linden N, Hilderink JM, Cornelis T, Kimenai DM, Klinkenberg LJJ, van Doorn WP, et al. Twenty-four-hour biological variation profiles of cardiac troponin I in individuals with or without chronic kidney disease. Clin Chem 2017;63:1655–6.
64. Zaninotto M, Padoan A, Mion MM, Marinova M, Plebani M. Short-term biological variation and diurnal rhythm of cardiac troponin I (Access hs-TnI) in healthy subjects. Clin Chim Acta 2020;504:163–7.
65. Ceriotti F, Díaz-Garzón Marco J, Fernández-Calle P, Maregnani A, Aarsand AK, Coskun A, et al. The European Biological Variation Study (EuBIVAS): weekly biological variation of cardiac troponin I estimated by the use of two different high-sensitivity cardiac troponin I assays. Clin Chem Lab Med 2020;58:1741–7.
66. Aakre KM, Røraas T, Petersen PH, Svarstad E, Sellevoli H, Skadberg Ø, et al. Weekly and 90-minute biological variations in cardiac troponin T and cardiac troponin I in hemodialysis patients and healthy controls. Clin Chem 2014;60:838–47.
67. Corte Z, García C, Venta R. Biological variation of cardiac troponin T in patients with end-stage renal disease and in healthy individuals. Ann Clin Biochem 2015;52:53–60.
68. Fournier S, Iten L, Marques-Vidal P, Boulat O, Bardy D, Beggah A, et al. Circadian rhythm of blood cardiac troponin T concentration. Clin Res Cardiol 2017;106:1026–32.
69. Meijers WC, van der Velde AR, Muller Kobold AC, Dijck-Brouwer J, Wu AH, Jaffe A, et al. Variability of biomarkers in patients with chronic heart failure and healthy controls. Eur J Heart Fail 2017;19: 357–65.
70. Koerbin G, Potter JM, Pinto do Nascimento M, Cullen L, Scanlan SL, Woods C, et al. The intra-individual variation of cardiac troponin I: the effects of sex, age, climatic season, and time between samples. Clin Chem Lab Med 2022;60:1001–9.
71. Diaz-Garzon J, Fernandez-Calle P, Sandberg S, Özcürümez M, Bartlett WA, Coskun A, et al. Biological variation of cardiac troponins in health and disease: a systematic review and meta-analysis. Clin Chem 2021;67:256–64.
72. Giannitsis E, Mueller-Hennessen M, Zeller T, Schuebler A, Auric M, Biener M, et al. Gender-specific reference values for high-sensitivity cardiac troponin T and I in well-phenotyped healthy individuals and validity of high-sensitivity assay designation. Clin Biochem 2020;78: 18–24.
73. Gärtner C, Langhammer R, Schmidt M, Federbusch M, Wirkner K, Löffler M, et al. Revisited upper reference limits for highly sensitive cardiac troponin T in relation to age, sex, and renal function. J Clin Med 2021;10:5508.
74. Apple FS, Collinson PO, Kavsak PA, Body R, Ordóñez-Llanos J, Saenger AK, et al. Getting cardiac troponin right: Appraisal of the 2020 European society of cardiology guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation by the international federation of clinical chemistry and laboratory medicine committee on clinical Applications of cardiac bio-markers. Clin Chem 2021;67:730–5.
75. Clerico A, Masotti S, Musetti V, Ripoli A, Aloe R, Di Pietro M, et al. Evaluation of 99th percentile and reference change values of the hs-cTnI method Using ADVIA Centaur XPT platform: a multicenter study. Clin Chim Acta 2019;495:161–6.
76. Clerico A, Ripoli A, Masotti S, Musetti V, Aloe R, Dipalo M, et al. Evaluation of 99th percentile and reference change values of a high sensitivity cTnI method: a multicentre study. Clin Chim Acta 2019;493: 156–61.
77. Clerico A, Aimo A, Zaninotto M, Plebani M. Diagnostic algorithms for non-ST-segment elevation myocardial infarction: open issues. Clin Chem Lab Med 2021;59:1761–71.
78. Gulati M, Levy PD, Mukherjee D, Amsterdam E, Bhatt DL, Birtcher KK, et al. 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR guideline for the evaluation and diagnosis of chest pain: a report of the American College of Cardiology/American Heart Association joint committee on clinical practice guidelines. Circulation 2021;144:e368–454.
79. Sandoval Y, Apple FS, Mahler SA, Body R, Collinson PO, Jaffe AS. High-sensitivity cardiac troponin and the 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR guidelines for the evaluation and diagnosis of acute chest pain. Circulation 2022;146:569–81.
80. Chapman AR, Adamson PD, Shah ASV, Anand A, Strachan FE, Ferry AV, et al. High-sensitivity cardiac troponin and the Universal definition of myocardial infarction. Circulation 2020;141:161–71.
81. Nowak RM, Christenson RH, Jacobsen G, McCord J, Apple FS, Singer AJ, et al. Performance of novel high-sensitivity cardiac troponin I assays for 0/1-hour and 0/2-to 3-hour evaluations for acute myocardial infarction: results from the HIGH-US study. Ann Emerg Med 2020;76:1–13.
82. Twerenbold R, Badertscher P, Boeddinghaus J, Nestelberger T, Wildi K, Puelacher C, et al. 0/1-hour triage algorithm for myocardial infarction in patients with renal dysfunction. Circulation 2018;137:436–51.
83. Boeddinghaus J, Nestelberger T, Twerenbold R, Neumann JT, Lindahl B, Giannitsis E, APACE, BACC, and TRAPID-AMI Investigators, et al. Impact of age on the performance of the ESC 0/1halgorithms for early diagnosis of myocardial infarction. Eur Heart J 2018;39:3780–94.
84. Boeddinghaus J, Twerenbold R, Nestelberger T, Badertscher P, Wildi K, Puelacher C, et al. APACE Investigators. Clinical validation of a novel high-sensitivity cardiac troponin i assay for early diagnosis of acute myocardial infarction. Clin Chem 2018;64:1347–60.
85. Mueller C, Giannitsis E, Christ M, Ordóñez-Llanos J, deFilippi C, McCord J, et al. Investigators TRAPID-AMI. Multicenter evaluation of a 0-hour/1-hour algorithm in the diagnosis of myocardial infarction with high-sensitivity cardiac troponin T. Ann Emerg Med 2016;68:76–87.e4.
86. Neumann JT, Sorensen NA, Schwemer T, Ojeda F, Bourry R, Sciacca V, et al. Diagnosis of myocardial infarction using a high-sensitivity troponin I 1-hour algorithm. JAMA Cardiol 2016;1:397–404.
87. Twerenbold R, Neumann JT, Sörensen NA, Ojeda F, Karakas M, Boeddinghaus J, et al. Prospective validation of the 0/1-h algorithm for early diagnosis of myocardial infarction. J Am Coll Cardiol 2018;72: 620–32.
88. Ola O, Akula A, De Michieli L, Dworak M, Crockford E, Lobo R, et al. Clinical impact of high-sensitivity cardiac troponin T implementation in the community. J Am Coll Cardiol 2021;77:3160–70.
89. Shah AS, Griffiths M, Lee KK, McAllister DA, Hunter AL, Ferry AV, et al. High sensitivity cardiac troponin and the under-diagnosis of myocardial infarction in women: prospective cohort study. Br Med J 2015;350:7873.
90. Sze J, Mooney J, Barzi F, Hillis GS, Chow CK. Cardiac troponin and its relationship to cardiovascular outcomes in community populations – a systematic review and meta-analysis. Heart Lung Circ 2016;25: 217–28.
91. Van der Lindel N, Klinkenberg LJJ, Bekers O, Loon LJCV, Dieijen-Visser MPV, Zeegers MP, et al. Prognostic value of basal high-sensitive cardiac troponin levels on mortality in the general population: a meta-analysis. Medicine 2016;95:e5703.
92. Willeit P, Welsh P, Evans JDW, Tschiderer L, Boachie C, Jukema JW, et al. High-sensitivity cardiac troponin concentration and risk of first-ever

cardiovascular outcomes in 154,052 participants. J Am Coll Cardiol 2017;70:558–68.
93. Lippi G, Cervellin G, Sanchis-Gomar F. Predicting mortality with cardiac troponins: recent insights from meta-analyses. Diagnosis 2019;8:37–49.
94. North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. Circ Res 2012;110:1097–108.
95. United Nation Department of Economic and Social Affairs Population World Population Prospects 2019. Highlights. New York: United Nations; 2019:1–46 pp.
96. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000;908:244–54.
97. Calder PC, Bosco N, Bourdet-Sicard R, Capuron L, Delzenne N, Doré J, et al. Health relevance of the modification of low grade infiammation in ageing (infiammageing) and the role of nutrition. Ageing Res Rev 2017;40:95–119.
98. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol 2018;15:505–22.
99. Liu D, Richardson G, Benli FM, Park C, de Souza JV, Bronowska AK, et al. Infiammageing in the cardiovascular system: mechanisms, emerging targets, and novel therapeutic strategies. Clin Sci 2020;134:2243–62.
100. Yan M, Sun S, Xu K, Huang X, Dou L, Pang J, et al. Cardiac aging: from basic research to therapeutics. Oxid Med Cell Longev 2021;2021: 9570325.
101. Pietri P, Stefanidis C. Cardiovascular aging and longevity: JACC state-of-the-art review. J Am Coll Cardiol 2021;77:189–204.
102. Cianflone E, Torella M, Biamonte F, De Angelis A, Urbanek K, Costanzo FS, et al. Targeting cardiac stem cell senescence to treat cardiac aging and disease. Cells 2020;9:1558.
103. Kirkland JL, Tchkonia T. Cellular senescence: a translational perspective. EBioMedicine 2017;21:21–8.
104. Itagaki T, Motoki H, Otagiri K, Machida K, Takeuchi T, Kanai M, et al. Inflammation-based assessment for the risk stratification of mortality in patients with heart failure. Sci Rep 2021;11:14989.
105. McKechnie DG, Papacosta AO, Lennon LT, Welsh P, Whincup PH, Wannamethee SG. Inflammatory markers and incident heart failure in older men: the role of NT-proBNP. Biomarkers Med 2021;15:413–25.
106. Coppé JP, Kauser K, Campisi J, Beauséjour CM. Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem 2006;281:29568–74.
107. Kuilman T, Peeper DS. Senescence-messaging secretome: SMS-ing cellular stress. Nat Rev Cancer 2009;9:81–94.
108. de Boer RA, De Keulenaer G, Bauersachs J, Brutsaert D, Cleland JG, Diez J, et al. Towards better definition, quantification and treatment of fibrosis in heart failure. A scientific roadmap by the committee of translational research of the Heart Failure Association (HFA) of the European Society of Cardiology. Eur J Heart Fail 2019;21:272–85.
109. Li H, Hastings MH, Rhee J, Trager LE, Roh JD, Rosenzweig A. Targeting age-related pathways in heart failure. Circ Res 2020;126:533–51.
110. Braunwald E. Heart failure. J Am Coll Cardiol 2013;1:1–20.
111. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American college of cardiology/American heart association Task force on practice guidelines (writing committee to update the 2001 guidelines for the evaluation and management of heart failure): developed in collaboration with the American college of chest physicians and the international society for heart and lung transplantation: endorsed by the heart rhythm society. Circulation 2005;112:e154–235.
112. Hughes MF, Ojeda F, Saarela O, Jørgensen T, Zeller T, Palosaari T, et al. Association of repeatedly measured high-sensitivity-assayed troponin I with cardiovascular disease events in a general population from the MORGAM/BiomarCaRE Study. Clin Chem 2017;63:334–42.
113. Sigurdardottir FD, Lynbakken MN, Holmen OL, Dalen H, Hveem K, Røsjø H, et al. 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 2018;121:949–55.
114. Welsh P, Preiss D, Shah ASV, McAllister D, Briggs A, Boachie C, et al. Comparison between high-sensitivity cardiac troponin T and cardiac troponin I in a large general population cohort. Clin Chem 2018;64: 1607–16.
115. Lam CSP, Castillo R, Ho DT, Kasliwal RR, Khurana R, Naik S, et al. High-sensitivity troponin I for cardiovascular risk stratification in the general asymptomatic population: perspectives from Asia-Pacific. Int J Cardiol 2019;282:93–8.
116. Welsh P, Preiss D, Hayward C, Shah ASV, McAllister D, Briggs A, et al. Cardiac troponin T and troponin I in the general population. Comparing and contrasting their genetic determinants and associations with outcomes. Circulation 2019;139:2754–64.
117. Kristensen SD, Knuuti J, Saraste A, Anker S, Bøtker HE, Hert SD, et al. 2014 ESC/ESA guidelines on non-cardiac surgery: cardiovascular assessment and management: the joint task force on non-cardiac surgery: cardiovascular assessment and management of the European Society of Cardiology (ESC) and the European Society of Anaesthesiology (ESA). Eur Heart J 2014;35:2383–431.
118. Haynes AB, Weiser TG, Berry WR, Lipsitz SR, Breizat AH, Dellinger EP, et al. A surgical safety check list to reduce morbidity and mortality in a global population. N Engl J Med 2009;360:491–9.
119. Devereaux PJ, Chan MT, Alonso-Coello P, Walsh M, Berwanger O, Villar JC, et al. Association between post-operative troponin levels and 30-day mortality among patients undergoing noncardiac surgery. JAMA 2012;307:2295–304.
120. Sellers D, Srinivas C, Djaiani G. Cardiovascular complications after non-cardiac surgery. Anaesthesia 2018;73(1 Suppl):34–42.
121. Botto F, Alonso Coello P, Chan MTV, Villar JC, Xavier D, Srinathan S, et al. Myocardial injury after noncardiac surgery: a large, international, prospective cohort study establishing diagnostic criteria, characteristics, predictors, and 30-day outcomes. Anesthesiology 2014;120:564–78.
122. Devereaux PJ, Biccard BM, Sigamani A, Xavier D, Chan MTV, Srinathan SK, et al. Writing committee for the VISION Study Investigators. Association of postoperative high-sensitivity troponin levels with myocardial injury and 30-day mortality among patients undergoing noncardiac surgery. JAMA 2017;317:1642–51.
123. Devereaux PJ, Szczeklik W. Myocardial injury after non-cardiac surgery: diagnosis and management. Eur Heart J 2020;41:3083–91.
124. Clerico A, Zaninotto M, Aimo A, Musetti V, Perrone M, Padoan A, et al. Evaluation of the cardiovascular risk in patients Undergoing major non-cardiac surgery: role of cardiac-specific biomarkers. Clin Chem Lab Med 2022;60:1525–42.
125. Duceppe E, Parlow J, MacDonald P, Lyons K, McMullen M, Srinathan S, et al. Canadian cardiovascular society guidelines on perioperative cardiac risk assessment and management for patients who undergo noncardiac surgery. Can J Cardiol 2017;33:17–32.
126. Emdin M, Passino C, Prontera C, Fontana M, Poletti R, Gabutti A, et al. Comparison of Brain Natriuretic Peptide (BNP) and amino-terminal

ProBNP for early diagnosis of heart failure. Clin Chem 2007;53: 1289–97.
127. Perrone AM, Zaninotto M, Masotti S, Musetti V, Padoan A, Prontera C, et al. The combined measurement of high-sensitivity cardiac troponins and natriuretic peptides: a useful tool for clinicians? J Cardiovasc Med 2020;21:953–63.
128. Curigliano G, Cardinale D, Dent S, Criscitiello C, Aseyev O, Lenihan D, et al. Cardiotoxicity of anticancer treatments: epidemiology, detection, and management. CA A Cancer J Clin 2016;66:309–25.
129. Alexandre J, Cautela J, Edrhy S, Damaj GL, Salem JE, Barlesi F, et al. Cardiovascular toxicity related to cancer treatment: a pragmatic approach to the American and European Cardio-Oncology Guidelines. J Am Heart Assoc 2020;9:e018403.
130. Cardinale DM, Fabiani I, Cipolla CM. Cardiotoxicity of anthracyclines. Font Cardiovasc Med 2020;7:26.
131. Cardinale DM, Zaninotto M, Cipolla CM, Passino C, Plebani M, Clerico A. Cardiotoxic effects and myocardial injury: the search for a more precise definition of drug cardiotoxicity. Clin Chem Lab Med 2020;59:51–7.
132. Herrmann J, Lenihan D, Armenian S, Barac A, Blaes A, Cardinale D, et al. Defining cardiovascular toxicities of cancer therapies: an International Cardio-Oncology Society (IC-OS) consensus statement. Eur Heart J 2022;43:280–99.
133. Lyon AR, López-Fernández T, Couch LS, Asteggiano R, Aznar MC, Bergler-Klein J, et al. 2022 ESC guidelines on cardio-oncology developed in collaboration with the European Hematology association (EHA), the European society for therapeutic Radiology and oncology (ESTRO) and the international cardio-oncology society (IC-OS). Eur Heart J 2022;43:4229–361.
134. Clerico A, Cardinale DM, Zaninotto M, Aspromonte N, Sandri MT, Passino C, et al. High-sensitivity cardiac troponin I and T methods for the early detection of myocardial injury in patients on chemotherapy. Clin Chem Lab Med 2021;59:513–21.
135. Semeraro GC, Cipolla CM, Cardinale DM. Role of cardiac biomarkers in cancer patients. Cancers 2021;13:5426.
136. Mueller C, McDonald K, de Boer RA, Maisel A, Cleland JGF, Kozhuharov N, et al. Heart Failure Association of the European Society of Cardiology practical guidance on the use of natriuretic peptide concentrations. Eur J Heart Fail 2019;21:715–31.
137. Feola M, Garrone O, Occelli M, Francini A, Biggi A, Visconti G, et al. Cardiotoxicity after anthracycline chemotherapy in breast carcinoma: effects on left ventricular ejection fraction, troponin I and brain natriuretic peptide. Int J Cardiol 2011;148:194–8.
138. Demissei BG, Hubbard RA, Zhang L, Smith AM, Sheline K, McDonald C, et al. Changes in cardiovascular biomarkers with breast cancer therapy and associations with cardiac dysfunction. J Am Heart Assoc 2020;9:e014708.
139. Vittorini S, Clerico A. Cardiovascular biomarkers: increasing impact of laboratory medicine in cardiology practice. Clin Chem Lab Med 2008; 46:748–63.
140. Cardinale D, Colombo A, Sandri MT, Lamantia G, Colombo N, Civelli M, et al. Prevention of high-dose chemotherapy-induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. Circulation 2006;114:2474–81.
141. Cardinale D, Colombo A, Torrisi R, Sandri MT, Civelli M, Salvatici M, et al. Trastuzumab-induced cardiotoxicity: clinical and prognostic implications of troponin I evaluation. J Clin Oncol 2010;28:3910–3.
142. Clerico A, Zaninotto M, Prontera C, Giovannini S, Ndreu R, Franzini M, et al. State of the art of BNP and NT-proBNP immunoassays: the CardioOrmoCheck study. Clin Chim Acta 2012;414:112–9.
143. Clerico A, Passino C, Franzini M, Emdin M. Cardiac biomarker testing in the clinical laboratory: where do we stand? General overview of the methodology with special emphasis on natriuretic peptides. Clin Chim Acta 2015;443:17–24.
144. Kociol RD, Pang PS, Gheorghiade M, Fonarow GC, O'Connor CM, Felker GM. Troponin elevation in heart failure prevalence, mechanisms, and clinical implications. J Am Coll Cardiol 2010;56: 1071–8.
145. Kitmitto A, Baudoin F, Cartwrigh EJ. Cardiomyocyte damage control in heart failure and the role of the sarcolemma. J Muscle Res Cell Motil 2019;40:319–33.
146. Forte M, Schirone L, Ameri P, Basso C, Catalucci D, Modica J, et al. The role of mitochondrial dynamics in cardiovascular diseases. Br J Pharmacol 2020;178:2060–76.
147. Li A, Gao M, Liu B, Fang Z, Sun Y, Li S, et al. Mitochondrial autophagy: molecular mechanisms and implications for cardiovascular disease. Cell Death Dis 2022;13:444.
148. Regan B, O'Kennedy R, Collins D. Point-of-Care compatibility of Ultra-sensitive detection techniques for the cardiac biomarker troponin I – challenges and potential value. Biosensors 2018;8:114.
149. Collinson PO, Saenger AK, Apple FS, IFCC C-CB. High sensitivity, contemporary and point-of-care cardiac troponin assays: educational aids developed by the IFCC Committee on Clinical Application of Cardiac Bio-Markers. Clin Chem Lab Med 2019;57:623–32.
150. Schols AMR, Stakenborg JPG, Dinant GJ, Willemsen RTA, Cals JWL. Point-of-care testing in primary care patients with acute cardiopulmonary symptoms: a systematic review. Fam Pract 2018;35: 4–12.
151. Sörensen NA, Neumann JT, Ojeda F, Giannitsis E, Spanuth E, Blankenberg S, et al. Diagnostic evaluation of a high-sensitivity troponin I point-of-care assay. Clin Chem 2019;65:1592–601.
152. Boeddinghaus J, Nestelberger T, Koechlin L, Wussler D, Lopez-Ayala P, Walter JE, et al. Early diagnosis of myocardial infarction with point-of-care high-sensitivity cardiac troponin I. J Am Coll Cardiol 2020;75: 1111–24.
153. Apple FS, Schulz K, Schmidt CW, Van Domburg TSY, Fonville JM, de Theije FK. Determination of sex-specific 99th percentile upper reference limits for a point of care high sensitivity cardiac troponin I assay. Clin Chem Lab Med 2021;59:1574–8.
154. Gopi V, Milles B, Spanuth E, Müller-Hennessen M, Biener M, Stoyanov K, et al. Comparison of the analytical performance of the PATHFAST high sensitivity cardiac troponin I using fresh whole blood versus fresh plasma samples. Clin Chem Lab Med 2021;59: 1579–84.
155. Titus J, Wu AHB, Biswal S, Burman A, Sengupta SP, Sengupta PP. Development and preliminary validation of infrared spectroscopic device for transdermal assessment of elevated cardiac troponin. Commun Med 2022;2:42.
156. Zhan T, Su Y, Lai W, Chen Z, Zhang C. A dry chemistry-based ultrasensitive electrochemiluminescence immunosensor for sample-to-answer detection of cardiac troponin I. Biosens Bioelectron 2022; 214:114494.
157. Clerico A, Aimo A, Cantinotti M. High-sensitivity cardiac troponins in pediatric population. Clin Chem Lab Med 2022;60:18–23.