Guidelines and Recommendations

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Evidence on clinical relevance of cardiovascular risk evaluation in the general population using cardio-specific biomarkers

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Abstract: In recent years, the formulation of some immunoassays with high-sensitivity analytical performance allowed the accurate measurement of cardiac troponin I (cTnI) and T (cTnT) levels in reference subjects. Several studies have demonstrated the association between the risk of major cardiovascular events and cardiac troponin concentrations even for biomarker values within the reference intervals. High-sensitivity cTnI and cTnT methods (hs-cTn) enable to monitor myocardial renewal and remodelling, and to promptly identify patients at highest risk of heart failure. An early and effective treatment of individuals at higher cardiovascular risk may revert the initial myocardial remodelling and slow down heart failure progression. Specific clinical trials should be carried out to demonstrate the efficacy and efficiency of the general population screening by means of cost-benefit analysis, in order to better identify individuals at higher risk for heart failure (HF) progression with hs-cTn methods.

Keywords: cardiac natriuretic peptides; cardiac troponins; cardiovascular risk; high-sensitivity immunoassay; quality specification; reference population.

Introduction

Although there has been substantial improvement in clinical outcome in the recent decades, ischaemic heart disease remains the leading cause of morbidity and mortality in industrialized countries [1–3]. A large number of epidemiologic and clinical studies have confirmed the essential role of primary prevention in improving outcome of cardiovascular diseases [1–3]. The role of cardiovascular prevention in the general population is based on two fundamental clinical actions: accurate risk stratification

and appropriate interventions [1, 2]. Prevention strategies in the general population aim to slow the development of atherosclerotic cardiovascular disease by promoting a healthy lifestyle throughout all the lifespan [1–3]. In individuals at high score risk for development of atherosclerotic cardiovascular disease, an appropriate cardiovascular prevention should include clinical, pharmacological and multiple health behaviour change interventions [1–3].

In particular, specific pharmacological interventions are recommended for individuals with systemic arterial hypertension, elevated low-density lipoprotein cholesterol levels and diabetes mellitus (DM) [1–3]. Conversely, in people without established cardiovascular disease, the most recent meta-analyses have provided only equivocal evidence for reduction in incidence of cardiovascular diseases through multiple healthy lifestyle interventions [4–8]. However, the degree of effectiveness might be associated with the level of risk in the overall general population [2]. In 2017, a systematic review, including 31 studies (36,484 participants), evaluated the effectiveness of multiple lifestyle interventions on overall cardiovascular risk and traditional risk factors in people without established cardiovascular diseases [8]. This meta-analysis found modest, but statistically significant, effect on pooled net change in systolic blood pressure (16 trials), body mass index (BMI) (14 trials) and serum total cholesterol (14 trials) [8].

The early detection of individuals at higher cardiovascular risk should be the most important goal of the primary prevention in the general population. It is theoretically assumed that the cardiovascular risk in apparently healthy subjects is the result of actions of multiple, interacting genetic and environmental factors [1–3, 9–11]. According to the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) guidelines the natural history of heart failure (HF) can be divided in four progressive stages (from stage A to stage D) [12 ,13]. The first two stages include asymptomatic individuals who are at high risk (stage A), and those with structural heart disease but without signs or symptoms of HF (stage B). The last two stages include patients with signs and symptoms of HF: the patients responding to standard pharmacological treatment are included in stage C; while patients refractory to standard treatment and so requiring specialized interventions are included in the last stage D [12, 13]. The 5-years mortality rate increases progressively from the stage A to the stage D (i.e. up to about 50% for stage D patients) [12–14].

Many experimental and clinical studies have recently demonstrated that cardio-specific biomarkers (such as cardiac natriuretic peptides (cNP) and cardiac troponins) may help in the identification of apparently healthy subjects, who are at risk for accelerated progression towards symptomatic HF [9–11]. The use of cardiac-specific biomarkers for risk prediction in the general population was not even contemplated in international guidelines till 2010 [15], likely because only recently high-sensitivity immunoassays, able to measure the circulating levels of cardiac-specific biomarkers in the majority of apparent healthy individuals, have been finally introduced [16].

Aim

The aim of this document is to discuss the experimental and clinical evidences reported so far in the literature supporting the role of the measurement of cNP and cardiac troponin I (cTnI) and T (cTnT) in the detection of asymptomatic individuals, who are at higher risk for progression towards the symptomatic stages of HF.

Review of experimental and clinical results

Pathophysiological and clinical relevance of cardio-specific biomarkers in the prevention of cardiovascular risk

According to the international guidelines, the diagnosis of both acute and chronic HF relies on clinical judgement based on a combination of history, physical examination, appropriate investigations and laboratory tests [12–14]. Although more than 100 biomarkers have been suggested to be useful in the diagnosis, prognosis and/or risk stratification in HF patients [9–11, 16], only cardio-specific biomarkers are actually taken into consideration by the most recent international guidelines as the first-line biomarkers in risk stratification of HF [13, 14]. However, the 2019 position paper of the *Association of Preventive Cardiology of the European Society of Cardiology* states that it is necessary to definitively demonstrate the role of cardiac biomarkers (i.e. cNP and cTn) in risk stratification in the general population [2].

Cardiac natriuretic peptides

In 2004, Mueller et al. [17] evaluated 157 consecutive patients admitted for extensive cardiac evaluation and further 23 consecutive patients with symptomatic HF admitted for inpatient treatment. Receiver-operating characteristics (ROC) curves of brain natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in 43 patients with symptomatic stage HF vs. 137

asymptomatic subjects showed highly significant area under the curve (AUC) values. Furthermore, AUC values in 56 patients with asymptomatic structural heart disease and 81 subjects without structural disorder of the heart also showed highly significant AUC values. The results of this study suggest that both BNP and NT-proBNP assays are able to differentiate, in a population of asymptomatic individuals, those who have structural heart disease [17].

In the year 2007, Emdin et al. [18] reported that both BNP and NT-proBNP concentrations increased progressively in 820 individuals from stage A to stage D of HF. Moreover, there was a highly significant difference between the levels of both biomarkers found in HF patients from stage B to D compared to biomarker levels found in 182 apparently healthy subjects. Instead, no difference was found between BNP and NT-proBNP levels in apparently healthy subjects and 86 individuals in stage A of HF [18]. These data suggested for the first time that cNP can able to distinguish between a group of apparently healthy subjects and a group of individuals with structural alterations, but without HF symptoms and normal left ventricular ejection fraction (LVEF, mean 65%, Standard Error (SE) 4%) [18].

More recently, several studies confirmed that patients with type 2 DM have, on average, significantly higher BNP or NT-proBNP levels than a control group including apparently healthy subjects [19–24]. In particular, higher cNP values were found in DM patients with hypertension and coronary artery disease, while, on the contrary, significantly lower biomarker values were found in obese individuals with or without DM [19, 20, 23, 24].

Considering patients with systemic arterial hypertension (SH) [25], some studies suggest a close relationship that exists between cNP system and the origin and complications of chronic SH [26–28]. In particular, a very recent study reports that NT-proBNP significantly increased in 324 individuals (of a cohort of 2,309 individuals with normal blood pressure value at baseline) who developed a stable SH after a follow-up of 5 years [28]. The results of these studies [26–28], taken as a whole, strongly suggest that cNP assay is able to detect the individuals in the general populations, who are at risk to developing a stable SH in a short time.

Cardiac troponin I and T

Only after the year 2006, the set-up of a new generation of immunoassay methods with progressively better analytical performance allowed the detection of circulating cTnI and cTnT values not only in patients with cardiac or extracardiac diseases, but even in apparently healthy subjects [29–50]. Furthermore three meta-analyses [51–53], demonstrated that the cardiovascular risk tends to

increase also in some apparently healthy individuals of both sexes, who have cardiac troponin values below the 99th percentile upper reference limit (URL) (i.e. the cut-off value recommended by all the international guidelines for the diagnosis of MI) [54, 55]. However, only after the year 2015 some high-sensitivity methods for cardiac troponins (hs-cTn) allowed a reliable determination of cTn distribution parameters in the most part of healthy subjects [56–61], in accordance with quality specifications required by the most recent international guidelines [54, 55].

Using these hs-cTn methods [56–61], more accurate risk stratification was finally achieved in large cohorts from the general population [48–50, 62–70]. In particular, in 2017 Willeit et al. [66] published a meta-analysis including 28 studies, involving 154,052 individuals. These Authors reported that relative risks comparing the top vs. the bottom troponin tertiles were: 1.43 (1.31–1.56) for cardiovascular diseases (11,763 events), 1.67 (1.50–1.86) for fatal cardiovascular diseases (7,775 events), 1.59 (1.38–1.83) for cardiac disease (7,061 events) and 1.35 (1.23–1.48) for stroke (2,526 events) [66]. Moreover, the mortality risk related to cardiovascular diseases was more strongly associated to cTnT rather than to cTnI [66]. More recently, Welsh et al. [67] evaluate the association between cTnT and cTnI and other cardiovascular risk factors in a large general population cohort (19,501 individuals, age range 18–98 years). On average, higher cTn levels were found more frequently in older individuals with higher BMI, systolic blood pressure, and creatinine values, with a history of cardiovascular disease or diabetes, and use of cholesterol medications [67]. A composite 10 year cardiovascular disease risk score calculated in participants without prevalent cardiovascular disease and ≥35 years of age yielded not significantly different (p=0.34) positive associations with both cTnT and cTnI [67]. In the North-Trøndelag Health (HUNT) study [65], the tertile with the highest risk showed a cut-off value of 10 ng/L for women and 12 ng/L for men, while the 99th percentile URL values are 15.6 ng/L for women and 34.2 ng/L for men, respectively, as also suggested by the manufacturer (i.e. Architect hs-cTni method by Abbott Diagnostics). Therefore, the results of this study confirmed that the combined mortality and cardiovascular risk significantly increases even for cTnI values much below the 99th percentile URL values, divided for sex [65].

Take-home messages

–   A huge number of studies indicate that both cNP and cTn are able to detect the individuals at higher cardiovascular risk in the general population.
–   Measurement of cTnI and cTnT, using high-sensitivity methods, demonstrated that combined mortality and cardiovascular risk significantly increases even for

biomarker values below the 99th percentile URL values in the general population.

Pathophysiological characteristics and clinical interpretations of cardio-specific biomarkers

The cardio-specific biomarkers (i.e. cNP and cTn) actually show different, but complementary, pathophysiological characteristics.

The cardiac natriuretic hormone system (including ANP and BNP and their related peptides) is an essential component of the integrated systems of the mammalian body and, thus, plays a pivotal role in fluid, electrolyte and haemodynamic homoeostasis [71]. The close link between cNP system and counter-regulatory systems could explain the increase in circulating levels of BNP/NT-proBNP, not only in cardiac disease but also in several extra-cardiac clinical conditions (such as renal, pulmonary, hepatic, endocrinological, metabolic and inflammatory diseases) [71]. Indeed, several stressor situations or substances can activate the neuro-endocrine-immunological system in this way also inducing the activation of the cardiac natriuretic hormone system producing an increase in the circulation levels of ANP and BNP. According to these pathophysiological mechanisms, increased cNP levels indicate that the cardiac function is under stress.

The 2018 *Fourth Universal Definition of Myocardial Infarction* [55] states that: "the term myocardial injury should be used when there is evidence of elevated cardiac troponin values with at least one value above the 99th percentile URL". According to this definition, the detection in a patient of a cTn value upper this cut-off value, preferably measured with a high-sensitivity method, always indicates the presence of a myocardial injury, which should be accurately taken into consideration by clinicians. This document [55] also states that: "although elevated cTn values reflect injury to myocardial cells, they do not indicate the underlying pathophysiological mechanisms, and can arise following preload-induced mechanical stretch or physiological stresses in otherwise normal hearts". Several Authors suggested the working hypothesis that the release of cTn from cardiomyocytes may not always require myocardial cell death [34, 72–74]. Indeed, some experimental studies suggest different possibilities for the extrusion of proteins from reversibly injured cardiomyocytes, such as: transient increases in cell permeability due to cell wounds, formation and the release from membranous blebs or microparticles [34, 72–74]. In particular, a "reversible" injury has been taken into

consideration in order to explain the release of troponin from cardiomyocytes after physical exercise in well-trained athletes [34, 72–74]. Although, at present time, the reasons of circulating cTn levels in healthy adult individuals at rest remain undetermined, some authors suggested the working hypothesis that the circulating levels of this biomarker, measured with high-sensitivity methods, are strictly related to the physiological renewal of cardiomyocytes [34, 72–74]. According to this hypothesis, the circulating levels of hs-cTn in healthy adult subjects should be considered as a reliable estimate of the physiological turnover of human myocardial tissue [72].

According to their different pathophysiological characteristics, circulating levels of cNP and cTn may be differently affected by pathophysiological mechanisms responsible of cardiac dysfunction and/or damage. An increment in circulating levels of both biomarkers suggests that some powerful stressor mechanisms have already caused relevant alterations on cardiac function (i.e. increased cNP levels), as well as a significant damage on cellular structure (i.e. increased hs-cTn levels). These finding are well in accordance with the results of a number of experimental and clinical studies reporting that individuals with both increased cardio-specific biomarkers have a more severe outcome than those with only one altered biomarker (usually cNP) [9–14, 75–78].

Take-home messages

– The measurement of cNP and cTn gives different, but complementary, pathophysiological and clinical information.
– A contemporaneous increase of the two cardio-specific biomarkers suggests that some powerful stressor mechanisms have already caused relevant alterations on both cardiac function and cellular structure.
– This finding explains why patients with both biomarkers increased show worse prognosis.

Comparison of analytical and biological characteristics of cardio-specific biomarkers

The two cardio-specific biomarkers have different analytical and biological characteristics. Due their specific biological action as peptide hormones, cNP are rapidly degraded both *in vivo* and *in vitro*. In particular, the active peptide BNP shows a plasma half-life of 15–20 min, because it is degraded by several plasma proteases; so only ethylene diamine teratacetic acid (EDTA) plasma sample should be used for measurement of BNP. Furthermore, the

production and release of cNP by cardiomyocytes is influenced by the rapid variations in activation of neuro-endocrine-immunological system, and so plasma BNP and NT-proBNP circulating levels show both large intra- and inter-individual variations (of about 30–50%) [16]. Due to counter-regulatory action of sex steroid hormones (i.e. female positive, male negative) on the production/release of cNP by cardiomyocytes, women show significantly higher level (up to 50%) of circulating BNP and NT-proBNP values during their fertile age up to age of menopause (about 55 years) than men of the same age [71, 79]. Furthermore, individuals of both sexes with higher BMI values (without cardiac disease and type 2 DM) show lower values than apparently healthy subjects with normal BMI values [80, 81]. However, overweight and obese patients with congestive heart failure show on average lower all-cause and cardiovascular mortality rates than patients with normal or lower BMI values [80, 81].

Cardiac troponins actually show a more favourable analytical and biological profile for a cardiovascular risk marker than cNP. Indeed, cardiac troponins are sarcomeric proteins with relatively high molecular weight (cTnI about 24 KDa, cTnT about 36 KDa), and they are also relatively stable both *in vivo* and *in vitro* [82]. From the analytical point of view, no specific recommendations related to sample matrix are currently reported by international guidelines for cTn assay, although in some clinical institutions heparinized blood samples may be preferred, especially for patients admitted to the emergency room [54, 83]. Considering the biological variation, several studies reported that hs-cTn circulating levels in healthy adult subjects show considerably lower intra-individual (from 4 to 12%) than inter-individual variations (about 50%) [11, 84–88]. These data suggest that 99th percentile URL of cTn concentration, if measured with high-sensitivity methods, may be considered as a reliable estimate of the physiological turnover of human myocardial tissue in healthy adult subjects [72, 89]. It is important to note that this very low intra-individual index of biological variation plays an important role when a hs-cTn value measured in a single subject/patient is compared to a clinical cut-off value estimated in a reference large population (such as the 99th percentile URL), which actually has a higher inter-individual variation [89]. This is the case when only one value above the 99th percentile URL is used for the evidence of myocardial injury in a patient [55]. Conversely, due the low biological individuality index of hs-cTn assay [11, 84–88], the use of algorithm based on serial change of the cardiac biomarker is recommended for early diagnosis of acute myocardial infarction (i.e. the sampling at 0–3 h after admission) [54, 55, 90]. Accordingly, the better is the

analytical performance of assay method and the lower is the biological intra-individual variation of cardio-specific biomarker, and the more accurate will be the estimation of variations between two (or more) serial measurements [91]. In particular, the error measurement of the most recent hs-cTnI and cTnT methods actually show measurement errors at the 99th percentile value of about 5% coefficient of variation (CV) (i.e. the half of the value recommended by international guidelines) [61, 92].

Take-home messages

– cTnI and cTnT actually show a more favourable analytical and biological profile for a cardiovascular risk marker than cNP.
– cTnI and cTnT are more stable *in vivo* and *in vitro* and have a lower intra-individual biological variation than cNP.

Design and result interpretation of experimental protocols for cardiovascular risk evaluation in the general population

Clinical studies for cardiovascular risk evaluation in the general population generally use an experimental protocol including one or more biomarkers measured at the time of individual enrolment in the study cohort (basal sample) and then the evaluation of the association between these biomarker basal values with cardiovascular outcome [93–96]. Considering that the risk is usually evaluated by means of regression analysis models, the variability of distribution values of biomarkers in the general population may be critical in the statistical analysis. In Table 1, a summary of 11 studies using hs-cTnI methods for risk stratification in the general population is reported; in the major part of these studies (10/11) the hs-cTn Architect method (Abbott Diagnostics) was used. These studies markedly differ for characteristics of the studied populations (sex, age, number of individuals), follow-up times (from 2 to 20 years), cut-off values for risk evaluation, and outcomes. In particular, there are no data on evaluation of cardiovascular risk for the Asia Pacific general populations [95]. Considering the studies using the cTnT assay for risk stratification in the general population, in 2016 a meta-analysis [62], including 22 studies involving 64,855 participants, reported that elevated cTnT values in asymptomatic individuals in the community are associated with a three-fold increased risk of all-cause and cardiovascular mortality.

A very important issue to take into consideration for risk evaluation in general population studies is that both cardio-specific biomarkers (i.e. cNPs and cardiac troponins) show a non-normal distribution of circulating levels [18, 56, 57, 61, 79]. In particular, the distributions of circulating cTnI values, measured with high-sensitivity methods in a large population of healthy adult subjects, show highly asymmetric distributions [56, 61]. Data reported in Figure 1, taken as a whole, represent a snapshot of hs-cTnI distribution values in a large Italian population including 1,463 apparently healthy adult subjects of both sexes (F/M ratio 0.95) with age range from 18 to 86 years, enrolled in a multicentre study endorsed by the *Italian Societies of Laboratory Medicine ELAS and SIBioC* [61]. According to the hypothesis that the plasma hs-cTn concentration is a reliable index of physiological renewal of cardiomyocytes, data reported in Figure 1 indicate that an increment of about 15–20 folds the median cardiomyocyte renewal of healthy adult subjects is required in order to exceed the clinical cut-off value recommended for the diagnosis of myocardial injury (i.e. the 99th percentile URL value). Furthermore, the variability of hs-cTn circulating levels in large population of adult healthy subjects is increased by the combined effects of sex and age. Women have, on average, significantly lower cTn concentrations than men of the same age, while in both sexes the

biomarker values progressively increase after the age of 55 years, as suggested by data reported in Figure 1.

An identical progression of hs-cTnI values in both sexes after the age of 55 years was previously reported by the *MORGAM/BiomarCaRe* study by using serial measurements of biomarkers in the general population [63]. This study investigated whether the change in three repeated measures of hs-cTnI collected 5 years apart improves 10-year prediction of cardiovascular risk in 3,875 participants, aged 30–60 years at enrolment (51% female, disease free at baseline) [63]. This study found that median hs-cTnI concentrations changed from 2.6 ng/L to 3.4 ng/L over 10 years. Furthermore, the change in hs-cTnI values throughout 10 year follow-up more accurately predicted the cardiovascular risk in the general population than the most recent measurement [63]. However, in order to simplify the experimental protocol for primary prevention using hs-cTnI assay, these Authors suggested that a single measurement of the cardio-specific biomarker might be sufficient for the 10 year prediction of cardiovascular risk [63]. A more recent study confirmed these results, suggesting that for refinement of risk prediction models, the most recent measurement of hs-cTnI may be preferred in clinical practice [69]. Considering the large systematic differences among hs-cTnI methods [61, 83, 89], the cut-off values for cardiovascular risk should be strictly method-

Table 1: Summary of studies using hs-cTnI methods for risk stratification in the general population.

Study	Method	Number of subjects	SEX, (%♀)	Mean age, years	Follow-up years	Cut-off value, ng/L	Outcome
Minnesota heart Study (Apple 2012) [37]	Erenna System (Singulex)	464	39.2	67.9	8–15	>10.19 ng/L	Cardiovascular death
PIVUS Study (Eggers 2013) [38]	hs-cTnI Architect (Abbott Diagnostics)	826	50.0	70	5	Not reported	Overall death
MORGAM Study (Zeller 2014) [44]	hs-cTnI Architect (Abbott Diagnostics)	15340	50.5	49	29	>4.7 ng/L (♀) >7.0 ng/L (♂)	MACE, cardiovascular death
HUNT Study (Omland 2015) [43]	hs-cTnI Architect (Abbott Diagnostics)	9712	54.3	50.0	13.9	>3.85 ng/L (♀) >5.65 ng/L (♂)	Cardiovascular death
JUPITER Trial (Everett 2015) [76]	hs-cTnI Architect (Abbott Diagnostics)	12956	36.2	65	2.0	≥3.9 ng/L (♀) ≥4.6 ng/L (♂)	MACE, overall death
AGES Study (Thorsteinsdottir 2016) [48]	hs-cTnI Architect (Abbott Diagnostics)	5691	57.5	77 (range 66–98)	10	>10.6 ng/L	MACE, overall death
BiomarCaRE Consortium (Blankenberg 2016) [50]	hs-cTnI Architect (Abbott Diagnostics)	74738	47.8	52.2	13.8	≥6 ng/l	MACE, overall death
MORGAM /BiomarCaRE (Hughes 2017) [63]	hs-cTnI Architect (Abbott Diagnostics)	3785	48.7	45.5 (range 30–60)	10	Not reported	MACE
Busselton Health Study (Zhu 2018) [68]	hs-cTnI Architect (Abbott Diagnostics)	3939	57.1	52.4	20	≥4 ng/L (♀) ≥6 ng/L (♂)	MACE
HUNT Study (Sigurdardottir 2018) [65]	hs-cTnI Architect (Abbott Diagnostics)	9005	55.5	48.5	13.9	>10 ng/L (♀) >12 ng/L (♂)	Hospitalization, MACE, overall death
ARIC Study (Jia 2019) [70]	hs-cTnI Architect (Abbott Diagnostics)	8121	57.7	62.7 (range 54–74)	15	≥4 ng/L (♀) ≥6 ng/L (♂)	Hospitalization, MACE, overall death

cTnI, ng/L
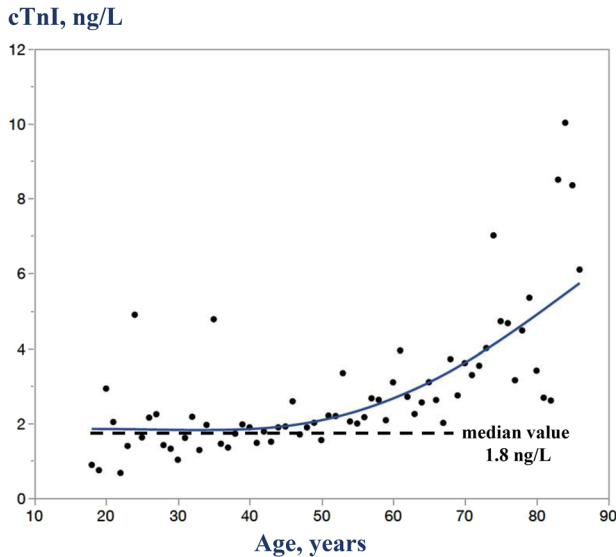
Figure 1: Relationship between plasma cTnI concentration values (Y axis) and age (X axis) in 1,463 apparently healthy subjects of both sexes (range 18–86 years, F/M = 0.95) measured with the Architect hs-cTnI method [61].
The non-linear trend between age and hs-cTnI was evaluated by means of a regression spline analysis. For spline analysis, the mean values of hs-cTnI concentrations, included in several small intervals of age, were calculated and then reported in the graph. Finally, a non-linear regression trend was interpolated considering these mean hs-cTnI values. The median value of the distribution of hs-cTnI values was also indicated with a dashed line.

dependent. Furthermore, the cost/benefit of the singular cut-off values, compared to serial measurements, for the assessment of cardiovascular risk should be evaluated by appropriately designed clinical studies.

Finally, an important question concerns the possible differences between cTnI and cTnT in the stratification of cardiovascular risk. Two recent studies [67, 97] reported that there are some differences between cTnI and cTnT in terms of their association with composite cardiovascular diseases and with specific cardiovascular outcomes, even if these two biomarkers have similar strong associations with risk of cardiovascular death and HF. In particular, the cTnI assay may be more specific for cardiovascular risk, whereas the cTnT assay may be more strongly associated with non-cardiovascular mortality [97]. Accordingly, cTnI and cTnT assays may indicate distinct and complementary pathophysiological and predictive information in the general population [67, 97].

A stratification strategy for cardiovascular risk in the general population by means of some classical or cardiac-specific biomarkers was suggested by some international guidelines or authoritative documents [1, 2, 96]. Very recently, Farmakis et al. [96] suggest a putative cardiovascular strategy for the general population based on established risk factor, especially the calculation of European Society of Cardiology (ESC) score [1, 2], and hs-cTn assay. This document takes in consideration only one cut-off value for hs-cTnI, while it is conceivable that the cut-off values for cardiovascular risk stratification should be method-dependent, as the 99th percentile URL values actually are [11, 36, 54, 61, 89]. In Table 2, the putative cut-off values for risk stratification in general population for cTnT and hs-cTnI are reported according to the most recent studies [56, 61, 64, 67, 95–97]. In Table 2, for cTnI assay is reported the cut-off value for risk stratification related to only one cTnI assay (i.e. the hs-cTnI Architect method), because at present time there are no data available in the literature for other commercial hs-cTnI methods.

Take-home messages

– Due to the low intra-individual biological variation of cTnI and cTnT serial measurement of the biomarker should significantly improve prognostic accuracy.
– However, practically, a single measurement of cTn using high-sensitivity methods should be adequate for the prediction of cardiovascular risk [63, 69].
– The values for risk prediction are strictly method-dependent and probably far below the current cut-off values of hs-cTn methods (i.e. the 99th percentile URL values suggested by the manufacturers) (Table 2).

Conclusive remarks

Although the introduction of high-sensitivity methods allowing an accurate detection of cTn levels in healthy adults is very recent insight [34, 54, 61, 72–74], a large number of studies has indicated that the cardiovascular risk progressively increases in the general population even for cTn values below the 99th percentile URL (i.e. the recommended cut-off for the detection of myocardial injury and diagnosis of myocardial infarction) [48–50, 62–70].

From a clinical perspective, an increase in hs-cTnI levels, even of only 5–10 ng/L over some months in a patient with a suspect of cardiomyopathy, should suggest an initial myocardial remodelling, ultimately culminating in symptomatic heart failure. Indeed, cTnI distribution in the reference population indicate that an individual with a cTnI concentration equal to the median value (about 2 ng/L) should increase his/her myocardial renewal of about 14-fold in order to reach the 99th percentile URL value (about 28 ng/L for the reference population including both sexes) (Figure 1).

In conclusions, the results of most recent clinical studies support the hypothesis that hs-cTn methods are able to monitor myocardial renewal and remodelling mechanisms, thus promptly identify individuals at highest risk to develop symptomatic heart failure, possibly resulting in early diagnosis and improved prognosis [34, 48–50, 62–70, 72–74]. Indeed, an early and effective treatment is required in high risk individuals in order to revert the initial myocardial remodelling and slow down progression toward to heart failure [9, 11, 98]. Therefore, these results should promote some clinical studies specifically evaluating the cost-benefit of a screening in the general population in order to identify individuals at high cardiovascular risk, and in particular those at high-risk for progression toward symptomatic heart failure, by using the hs-Tn methods. Futhermore, the screening programs of cardiovascular risk stratification and prevention strategies incorporating hs-cTn requires further investigation to define the optimal target populations, timing of measurement, and preventive interventions [96].

Future perspectives

Gaps in the knowledge

– Cost-benefit analysis of serial measurements of cardio-specific biomarkers in the general population is needed.
– The role of cardiac biomarkers (i.e. natriuretic peptide and cardiac troponin blood concentration) in risk stratification in comparison with other biomarkers should be better evaluated.
– The role of cardiac biomarkers (i.e. natriuretic peptide and cardiac troponin blood concentration) in risk stratification related to pharmacological treatment should be better evaluated.

Works in progress

Taken in considerations all the evidences so far available, the future studies on risk stratification in the general population should be designed considering the following issues:

– The enrolment of individuals in these studies should follow the indications reported in 2016 and 2019 guidelines of the *European Association for Cardiovascular Prevention and Rehabilitation* [1, 2].
– Individuals of both sexes with age >55 years and the stage B of the heart failure should be evaluated with hs-cTn assay (cTnI or cTnT).
– If the hs-cTn value is below the upper limit of the interquartile range of the method, additional hs-cTn measurements may be performed after 2–3 years, if the clinical conditions of the subject are stable.
– If the hs-cTn value is in the third tertile of the method, BNP/NT-proBNP should be measured as well. The subjects should be re-evaluated after 6–12 months in order to evaluate a progressive increase in ventricular myocardial remodelling.
– In accordance with 2018 Fourth Universal Definition of Myocardial Infarction, subjects with only one hs-cTn value >99th percentile URL should be considered as having myocardial injury [55]. However, to distinguish between analytical interference and reversible or persistent myocardial injury the hs-cTn measurement should be repeated together with BNP/NT-proBNP. As subjects with confirmed higher hs-cTn and cNP values are at high cardiovascular risk, they should be accurately evaluated for the presence of asymptomatic cardiac alterations or extra-cardiac diseases able to cause myocardial injury [34, 55, 72–74].
– There is a plethora of candidate cardiovascular risk biomarkers, including cytokines, peptides, proteins, metabolites and circulating nucleic acids [10–14, 45, 98, 99]. However, at present time, these putative new markers seem to be clinically useful for a more accurate stratification of risk only in patients with elevated cNP and hs-cTn values, who are at high risk for an accelerated progression to symptomatic HF [10–14, 45, 98, 99]. It is important to note that any aspirant new

Table 2: Suggested cut-off values for risk stratification in the general population using cTnI and cTnT assays, measured with high-sensitivity methods.

cTnI[a]	Women	Men
Low	<4 ng/L	<6 ng/L
Moderate	4–10 ng/L	6–12 ng/L
High	>10 ng/L	>10 ng/L

cTnT[b]	Total Population	
Low	≤3 ng/L	
Moderate	3.0–5.7 ng/L	
High	≥5.8 ng/L	

[a] hs-cTnI Architect method (Abbott Diagnostics) [56, 61, 65, 67, 95–97]. [b] ElectroChemiLuminescenceImmunoAssay (ECLIA) hs-cTnT Elecsys method (Roche Diagnostics) [97].

favourite will have to satisfy rigorous assessment of their ability to facilitate improved clinical outcomes before they enter routine clinical practice [10, 11, 16, 36, 45, 93, 94, 98, 99].

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