Review Article

Pathophysiological mechanisms determining sex differences in circulating levels of cardiac natriuretic peptides and cardiac troponins

Aldo Clerico, Silvia Masotti, Veronica Musetti, Claudio Passino

Fondazione CNR Regione Toscana G. Monasterio and Scuola Superiore Sant'Anna, Pisa, Italy

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Correspondence to: Prof. Aldo Clerico, MD. Department of Laboratory Medicine, Fondazione G. Monasterio CNR - Regione Toscana, Via Giuseppe Moruzzi 1, 56124 Pisa, Italy. Email: clerico@ftgm.it.

Abstract: It is well known that adult fertile women have about two-fold higher circulating levels of cardiac natriuretic peptides (NPs) than men of the same age. Conversely, men have on average higher circulating levels of cardiac troponin I (cTnI) and T (cTnT) than women. Furthermore, the circulating levels of both cardiac NPs and troponins progressively increase after 65 years of age in both sexes. From a physiological point of view, these differences in circulating levels between cardiac NPs and troponins are closely related to production, secretion and peripheral degradation of these biomarkers; these complex physiological mechanisms are differently regulated in men and women. Some cardiac and extra-cardiac diseases with sex-related prevalence can also affect these physiological mechanisms, and in this way to allow more difficult the clinical interpretations of cardiac biomarker measurement with immunoassay methods. The aim of this review article is to discuss the available evidence of the possible different mechanisms related to production, secretion and degradation of cardiac NPs and cardiac troponins in men and women.

Keywords: Natriuretic peptides (NPs); brain natriuretic peptide (BNP); NT-proBNP; cardiac troponins; cTni; cTnT; specific cardiac biomarkers; heart failure (HF); acute myocardial infarction; precision medicine

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Background

It is well known that adult fertile women have higher circulating levels of cardiac natriuretic peptides (NPs) than men of the same age (1-5). On the contrary, men have on average higher circulating levels of cardiac troponin I (cTnI) and T (cTnT) than women (6). Furthermore, the circulating levels of both cardiac NPs and troponins progressively increase throughout the senescence in both sexes (1-6). These sex- dependent differences in circulating levels between NPs and cardiac troponins are closely related to complex physiological mechanisms, which are differently regulated in men and women (7-10). Some cardiac and extra-cardiac diseases with sex-related prevalence can also affect these physiological mechanisms, and in this way to allow more difficult the clinical interpretations of cardiac biomarker measurement with immunoassay methods (11-20).

The aim of this review article is to discuss the available evidence of the possible different mechanisms related to production, secretion and degradation of cardiac NPs and cardiac troponins in men and women.

NP system

Biochemical and physiological considerations

Human cardiomyocytes produce and secrete a family of related peptide hormones (i.e., cardiac natriuretic

Table 1 Distribution BNP values (ng/L), grouped according to 4 time periods from the birth to 12 years of life, measured in 253 healthy newborns and infants measured in the Authors laboratory with the BNP Triage Biosite for DxI platform (Beckman Coulter Diagnostics)

Groups (time periods from birth)	Number of individuals	Mean ± SD	Median	Range	97.5th percentile	P value
0–2 days	68	280.3±167.5	243.5	41–866	758.7	<0.0001 *
3–30 days	75	136.1±149.3	75	10–763	741.4	<0.0001**
1–12 months	46	20.3±10.7	19	5–45	43.9	–
1–12 years	64	15.7±8.9	13	4–46	39,8	–
All groups (0-12 years)	253	123.4±160.1	38	4–866	622.0	–

Range: minimum and maximum values; *, significantly higher than all the other next time period values; **, significantly higher than the values observed throughout the next time periods (i.e., 2–12 months and 2–12 years). The characteristics of the subjects studied are reported in detail elsewhere (8).

Table 2 Mean (± SD) plasma BNP concentrations (ng/L) of 292 healthy subjects, divided into four groups according to gender and age

Age groups	Men	Women	P value*
AGE 20–50 years [N]	5.9±6.0 [79]	10.0±8.3 [91]	<0.0001
AGE ≥50 years [N]	10.1±7.8 [53]	15.6±11.8 [68]	0.0033
P value*	0.0009	0.0020	

The subjects are the same reported in *Figure 1A* and *B*. The age cut-off of 50 years was chosen because corresponding to mean age of menopause in Western European countries. The number of subjects included in each subgroup is indicated within brackets. *, unpaired *t*-test using the logarithmic transformation of the original set of data. The characteristics of the studied population are reported in detail elsewhere (7). Plasma BNP was measured in the Authors' laboratory with a commercial two-site immunoradiometric assay (Shionoria BNP, Shionogi & Co., Japan). BNP, brain natriuretic peptide.

hormones) with similar biochemical structure and physiological activities (1,11). Cardiac NPs include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and their related peptides, while other NPs, such as C-type natriuretic peptide (CNP), Dendroaspis (or D-type) natriuretic peptide (DNP) and urodilatin, structurally related to the ANP/BNP peptide family, are not produced and secreted by cardiomyocytes, but by other tissues (1,11). All NPs share a similar peptide structure characterized by a similar peptide ring (1,11).

All NPs share a direct diuretic, natriuretic and vasodilator effect and an inhibitory and protective action on inflammatory processes of myocardium, endothelium and smooth muscle cells, in this way modulating coagulation and fibrinolysis pathways, and inhibiting platelet activation (1,11). All the most important biological effects of NPs are mediated throughout two different guanylate cyclase-coupled receptors, NPR-A (more specific for ANP and BNP) and NPR-B (more specific for CNP) (12). A third specific receptor NPR-C, not coupled to a guanylate cyclase, has fundamentally a clearance function for all NPs (12).

Sex- and age-related physiological actions on cardiac NP system

Plasma BNP concentration is minimal in children with similar values in both sexes (*Table 1*), while sex-difference between hormone levels increase progressively throughout the adolescence, reaching values in normal cycling women about two-fold higher than men (*Table 2* and *Figure 1*) (1,2,8,10,21-24). These data suggest that sex steroid hormones may play a relevant physiological role in the regulation of production/secretion of cardiac NPs (1,2,7,9,11,21). According to this hypothesis, some Authors suggested that female steroid hormones, in particular estrogens, have a stimulating effect on the cardiac natriuretic hormone system (1,25-28). In particular, the stimulatory action of female steroid hormones on production/secretion of cardiac natriuretic hormones was reported in post-menopausal women, where the administration of estrogens induced a greater increase in plasma BNP than ANP (26,27). On the other hand, the male steroid hormone may have an inhibitory action on the production/secretion of ANP and BNP by myocardiocytes (9,16,25).

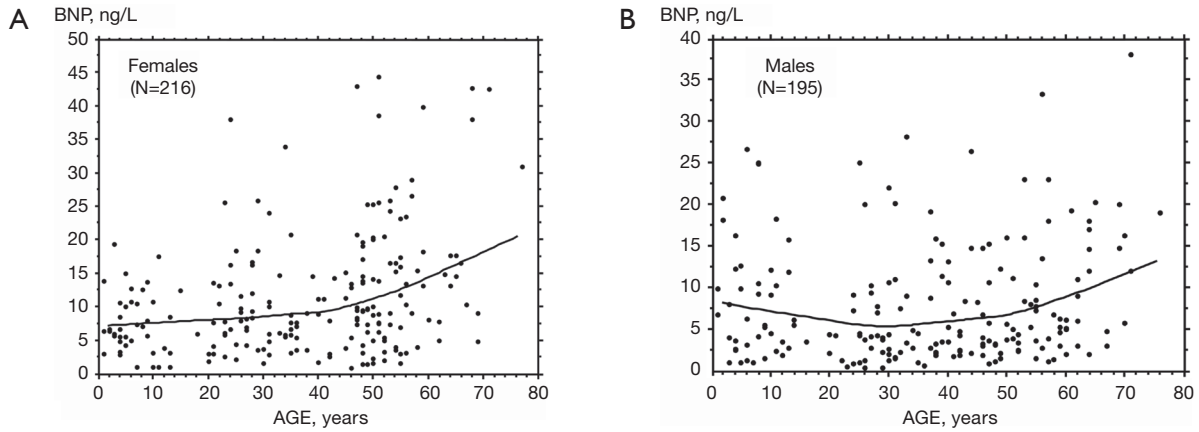

Figure 1 Variation of BNP levels in healthy females (A) and males (B). The age-dependent trend (continuous line) was assessed by the smoothing analysis using 66% of lowness (locally weighted scatterplot smoother). Plasma BNP was measured in the Authors' laboratory with a commercial two-site immunoradiometric assay (Shionoria BNP, Shionogi & Co., Japan). The characteristics of the studied population were reported in detail elsewhere (21). BNP, brain natriuretic peptide.

Indeed, transdermal testosterone administration to women with hypoandrogenemia due to hypopituitarism induced a significant fall in circulating NT-proBNP levels (29).

Unfortunately, there are few experimental studies in animal models specifically designed to investigate the influence of sex steroid hormones on gene expression of cardiac natriuretic hormones, and most of these are focused on ANP rather than BNP (1,2,7,9). Furthermore, these studies have some important limitations: rodents rather than primates, and castrated rather than fertile animals have been studied. As a consequence, the results of these studied should be applied with extreme caution to healthy fertile men and women. In rats, ovariectomy and orchiectomy decreased atrial ANP mRNA transcripts *in vivo*, while the pretreatment for 7 days of Wistar female rats with estradiol and progesterone increased atrial ANP gene expression (30). On the other hand, only scarce and contradictory data have been reported on the action of male steroid hormones on the ANP/BNP systems (7,9). *In vivo* pre-treatment with testosterone for 7 days of gonadectomized Wistar male rats increases atrial ANP gene expression (30). Another study observed increased plasma ANP concentrations and atrial stores in the castrated male rats, while testosterone replacement decreased plasma ANP concentration in castrated male rats, but not atrial stores (31). Other studies reported that testosterone stimulates synthesis and secretion of both ventricular and atrial ANP in newborn rat atrial cultured myocytes (32,33).

As far as studies in humans are concerned, androgen receptor blockade and, to a lesser extent, androgen suppression cause an increase in NT-proBNP in men with prostate cancer (34). These data, taken as a whole, indicate that female sex steroid hormones (especially estrogens) have a stimulating action on cardiac endocrine function, while androgens an inhibitory effect.

Two large population-based studies evaluated the role of male sex steroid hormones on the regulation of cardiac endocrine function in humans. The Dallas Heart Study (25) reported no association between estrogen status and NT-proBNP levels, whereas a strong inverse association was found between free testosterone and NT-proBNP in young women (age range 35–49 years). Saenger *et al.* (35) reported an inverse association of NT-proBNP with serum testosterone, a direct association with sex hormone binding globulin (SHBG), and any significant association with circulating levels of estradiol in a large population of girls and boys (age range from 2 months up to 18 years).

Taking as whole the above mentioned data (1,2,5,7-11, 16,21-36), some authors assumed as working hypothesis that female and male sex steroid hormones together contribute to the regulation of production/secretion of ANP and BNP: estrogens may have a stimulatory, while androgens a inhibitory action (1,7,9,11,21). The inter-relationships between NPs and steroid hormones may be schematically represented by three distinct retroactive mechanisms (1,7-11,21,36). In normal cycling women NPs may share an inhibitory action on steroidogenesis, follicular development, granulosa cell maturation and

ovulation, while estrogens have a stimulatory effect on production/secretion of ANP and BNP production/secretion by cardiomyocytes (1,7-11,21,36). Conversely, in adult men NPs may share a stimulatory effect on testis steroidogenesis, while androgens may have an inhibitory effect on ANP and BNP production/secretion by cardiomyocytes (1,7-11,21,36). Adrenal corticosteroids (including glucocorticosteroids and mineral corticosteroids) show both direct and indirect stimulating actions on cardiac endocrine function, while NPs inhibit the production of corticosteroids by adrenal gland both directly (i.e., throughout the specific NPs receptors on zona fasciculata and glomerulosa cells of adrenal gland) and indirectly (i.e., by inhibiting the action of renin-angiotensin system) (1,7-11,21,36). This working hypothesis, however, requires further evidences: in particular, it should be demonstrated that sex steroids are actually able to affect the production/secretion of BNP in mammalian cardiomyocytes both in cell cultures and *in vivo*.

Cardiac NP system: pathophysiological and clinical considerations

NP system shares complex interactions with the neuro-hormonal system: several studies aimed at clarifying the possible relationships among cardiac endocrine function, sex and cardiovascular risk. Indeed, NPs exert several cardio-protective actions, including: (I) vasodilation; (II) increase in natriuresis and diuresis; (III) anti-hypertrophic and anti-remodeling effects on myocardial tissue; (IV) improvement in endothelial function and anti-atherosclerotic effects; (V) counter-regulatory action on sympathetic nervous system and several hormones, including aldosterone, angiotensin II, endothelins, renin, and vasopressin (1,11,37).

Cardio-protective effects of female steroids may explain why cardiovascular risk is significantly lower in healthy premenopausal women compared to men (16,26,38-40), while the sex-related risk difference is progressively abolished after menopause (38-40). These data suggest an important beneficial role of female sex steroids on pathophysiological mechanisms related to cardiovascular diseases (7,9,16,21,26-28). On the other hand, the complex inter-relationship between NP system and sex-related gonadal function could play a relevant role in determining the sex and age-related differences in cardiovascular pathophysiological mechanisms, clinical presentation, and disease course. Indeed, it is well known that sex and age-specific differences are actually observed in cardiovascular function, electrocardiogram, heart chamber volumes, arterial vessels sizes, microcirculation function and circulating biomarkers between males and females (15).

Circulating levels of sex steroids and NPs show wide and differently related fluctuations throughout all the life span. Circulating levels of NPs are very high after birth and progressively fall throughout the first months of life (*Table 1*) (1,8,41-44), when, on the contrary, circulating sex steroids levels are lower than those of adult age. It is conceivable that high NP levels during neonatal period may help the adaptation of cardiovascular system of newborn to physiological conditions of extra-uterine life (8,43,44). In particular, NPs may alleviate the increased ventricular afterload of both ventricles after birth, and may also support heart function with a decreased preload in the first days of life (8,43-45).

During adolescence, NP levels of girls become progressively higher than boys, so that in the adult life fertile women (*Figure 1A* and *Table 2*) on average show BNP and NT-proBNP values about two-fold higher than men (*Figure 1B*) (1,8,16). It is conceivable that the NP action during the fertile period of women actually play a fundamental role in determining the sex-related differences in development, function, and disease susceptibility of cardiovascular system (16,46,47).

In particular during pregnancy, BNP and NT-proBNP levels show an early increase. NP levels are increased throughout all gestation until about 72 h after delivery with concentration peaks twice higher than in non-pregnant women (16,48-50). It is conceivable that NP system can significantly contribute to the regulation of fluids hemodynamics and cardiac function during pregnancy. Indeed, normal pregnant women show an increase in heart rate (from 10 to 20 bpm), cardiac output (up to 30–50%), but a fall in systemic vascular resistance (on average 20–30%) (50). In particular, the fall in systemic vascular resistance is observed only in the first period of pregnancy (up to 20 to 24 weeks of gestation), while in the last period of pregnancy there is a slow rise of systemic vascular resistance, however without reaching pre-pregnancy values (50). A similar trend is also found for mean arterial pressure with a fall (up to about 10%) in the first 20–24 weeks, and then a slow increase toward pre-pregnancy values approaching delivery (50). Adverse maternal cardiac events have been associated with high BNP concentrations (>100 ng/L), and its use as a negative predictive indicator appears to be of most value (50). In particular, NP assay was suggested to be useful in the diagnosis of pregnancy complications,

Table 3 99th URL values (ng/L) of some recent methods for cTnI assay divided according to sex

Populations	Architect method	Access hs cTnI method	ADVIA Centaur hs method
General population	26.2 ng/L	17.5 (12.6–20.7) ng/L	47.34 (36.39–64.27) ng/L
Women	15.6 ng/L	11.6 (8.4–18.3) ng/L	36.99 (30.22–72.63) ng/L
Men	34.2 ng/L	19.8 (14.0–42.9) ng/L	57.27 (38.58–90.15) ng/L
Number of subjects	1,531	1,098	2,010

The 99th URL values, reported in the table, are those suggested by the manufacturers. The 95% confidence limits are reported in brackets.

such as acute HF, preeclampsia or eclampsia (50-52). In preeclampsia, it was also suggested that NT-proBNP may reflect ventricular stress and subclinical cardiac dysfunction worsening, especially if fetal growth restriction is present (53).

After menopause, NP levels progressively increase in women, and sex-related differences in BNP and NT-proBNP concentrations progressively tend to reduce (1,8,16). High NP levels in some individuals over 65 years are likely related to the parallel increase in the incidence of heart failure (HF), especially women with asymptomatic or paucisymptomatic HF with preserved ejection fraction (HFpEF) (16,46,54-56). As a result, BNP and NT-proBNP assay is recommended by all the most recent international guidelines for early diagnosis and risk assessment in HF patients (47,57,58).

Cardiac troponin I and T

Analytical and pathophysiological considerations

All international guidelines and expert documents recommend that cTnI and cTnT should be the preferred biomarkers for differential diagnosis of acute coronary syndrome (ACS), and also that the decision cut-off value (i.e., 99th URL) should be measured with an imprecision of ≤10 CV% (59-62). However, measurement of the 99th URL of cTnI and cTnT levels is a challenging task, due to low biomarker concentrations in healthy subjects, especially women and children (6,21,36,59,60,63,64). For this reason, only after the year 2006, some manufacturers set-up the first new generation of cTnI and cTnT immunoassays with improved analytical sensitivity in accordance with the quality specifications indicated by international guidelines and consensus documents (6,21,36,64-67). According to 2018 IFCC Task Force on Clinical Applications of Cardiac Bio-Markers (Academy of AACC and Task Force of IFCC) (61), immunoassay methods should be able to measure cTnI and cTnT circulating levels even in the majority of healthy adult subjects of both sexes. In particular, high-sensitivity methods should also be able to measure troponin levels in the majority of healthy adult subjects (>50%) enrolled in large populations (more than 300 individuals) including both men and women.

From a clinical point of view, it is important to stress that high-sensitivity cTnI and cTnT immunoassay methods should be able to detect even minimal (microscopic) amount of myocardial damage (36,59,60). In particular, some recent immunoassay methods should measure 99th URL values, corresponding to about 10–40 ng/L for cTnI (*Table 3*) and to 14 ng/L for cTnT, with an error ≤10% CV (*Table 4*) (64,68-72). Using an experimental protocol, including rat, ovine and human myocardial tissues, Marjot *et al.* (59) recently demonstrated that circulating cTnI and cTnT levels, corresponding to 99th URL value, are related to necrosis of about 40 mg of myocardium. In *Table 4*, the column named "Ratio" reports the calculation between the 99th URL values (suggested by the manufacturer) (*Table 3*) and the cTnI value measured with a CV% equal to 10% (i.e., LoQ 10%). These results indicate that the cTnI methods with ratio values ≥4 measure the 99th URL values with an imprecision (expressed as CV) of about 4–6% (63,68,69), which is significantly lower than the CV value required by international guidelines (61,62).

Therefore, according to the data reported in *Tables 3* and 4, only the cTnI and cTnT immunoassay methods with a LoD value of about 1–2 ng/L and a ratio values ≥4 should be considered high-sensitivity methods. Of course, the high-sensitivity cTnI methods should be also able to measure cTnI levels in the majority of healthy adult men and women (61). However, at the moment of preparation of this review article, only for the Architect method several independent studies were available in literature

Table 4 Analytical parameters of the some immunoassay methods for cTnI and cTn

Methods	LoB (ng/L)	LoD (ng/L)	LoQ 20% CV (ng/L)	LoQ 10% CV (ng/L)	Ratio	References
cTnI						
Architect	0.7	1.3	1.8	4.7	5	(64)
Access DxI	0.6	1.3	2.1	5.3	4	(68)
ADVIA	1.0	2.2	3.5	8.4	5.6	(69)
AIA	1.1	2.1	15.0	30.9	1	(70)
cTnT						
ECLIA	3	3-5	6	13	1.3	(71)

Data reported in this Table were obtained in the laboratory of Fondazione CNR Regione Toscana G. Monasterio (Pisa, Italy) according to the references (64,68-71). The ratio value was calculated by dividing the 99th URL value, suggested by the manufacturer, and the LoQ 10% CV evaluated in the reference laboratory. Architect, STAT Architect highly Sensitive TnI for Architect i1000SR platform (Abbott Diagnostics, Ref. B3P250); Access DxI, Access hsTnI (IUO) for DxI platform (REF B52699, Beckman Coulter, Inc. Brea, CA 92821 USA); ADVIA, ADVIA Centaur High-Sensitivity Troponin I (TNIH) (Ref. 10994774-5) for Centaur XPT platform (Siemens Healthineers Diagnostics, Erlangen, Germany); AIA, CLEIA method (CL AIA-PACK cTnI TEST) for automated AIA-CL2400 platform (TOSOH BIOSCIENCE, Tessenderlo, Belgium); ECLIA, ECLIA hs-cTnT method (Ref. 05092744) for Cobas e411 platform (Roche Diagnostics, Mannheim, Germany).

demonstrating that this method is able to measure cTnI levels in the majority of healthy adult women and men (63).

It is important to note that the analytical sensitivity of the last generation of cTn immunoassays (*Table 4*) is much better than the spatial resolution of the most recent NMR cardiac imaging techniques, which are not able to detect necrosis of 40 mg of myocardial tissue (36,59,60). Experimental data indicate that the adult mammalian heart (including human) is capable of limited renewal of cardiomyocytes by means of mitosis and/or cellular replacement originating from stem cells (73-75). Consequently, circulating cTnI and cTnT levels, measured with high-sensitivity methods in healthy subjects, could be envisaged as a reliable index of the physiological renewal of human cardiomyocytes (36).

However, two important clinical points should be taken into-account. First, it is important to underline that increased levels of cardiac biomarker demonstrate the presence of myocardial damage, but cTnI or cTnT assay is not able to indicate the specific mechanism of myocardial injury (36,59,60,62). Thus, increased cTn values, in the absence of clinical evidence of ischemia, should prompt a search for other causes of cardiac damage (*Table 5*). Moreover, currently available high-sensitivity methods demonstrate no threshold below which cTn variations are harmless and without negative implications for prognosis (36,59,60,63,76-87). Therefore, the high-sensitivity cTnI and cTnT immunoassay methods are essential for risk assessment, clinical stratification and prognosis of patients with cardiovascular diseases even in general population (36,59,60,63,76-87).

Sex and age-related difference in circulating levels of cTnI and cTnT

There are actually some fundamental issues to discuss about the estimation of 99th URL values: (I) there are sex-related differences in circulating cTnI and cTnT levels due to lower 99th URL values in women than men; (II) there are also age-related differences in circulating cTn levels; (III) there are still large variations among the 99th URL values suggested by manufacturers even among the most recent cTnI assays (*Table 3*). These issues can lead to misclassification in the differential diagnosis of ACS, and these may be particularly problematic for women, resulting in underdiagnosis of acute myocardial infarction (AMI) in women (88).

Using high-sensitivity methods, sex-related difference in circulating levels of both cTnI and cTnT has been confirmed in healthy adult subjects (16,36,60,63,65-67,71,72). In particular, considering the most recent studies including large populations of different ethnic origins, a meta-analysis reported that there is a significant mean difference of 10.97 ng/L (95% CI: 7.10–14.85 ng/L) between the cTnI concentrations (measured by Architect method) of adult men and women, while the sex-related

Table 5 Elevation of cardiac troponin values because of myocardial injury, according to references (36,60,62,67)

Injury related to primary myocardial ischemia
Plaque rupture
Intraluminal coronary artery thrombus formation
Injury related to supply/demand imbalance of myocardial ischemia
Tachy-/brady-arrhythmias
Aortic dissection or severe aortic valve disease
Hypertrophic cardiomyopathy
Cardiogenic, hypovolemic, or septic shock
Severe respiratory failure
Severe anemia
Hypertension with or without LVH
Coronary spasm
Coronary embolism or vasculitis
Coronary endothelial dysfunction without significant cardiac disease
Injury not related to myocardial ischemia
Cardiac contusion, surgery, ablation, pacing, or defibrillator shocks
Rhabdomyolysis with cardiac involvement
Myocarditis
Cardiotoxic agents (such as anthracyclines), Herceptin, drugs of abuse (such as cocaine)
Multifactorial or indeterminate myocardial injury
Heart failure (chronic and acute)
Stress cardiomyopathy (such as Takotsubo disease)
Severe pulmonary embolism or pulmonary hypertension
Sepsis, critically ill patients
Renal failure (chronic and acute)
Severe acute neurological diseases (such as stroke and subarachnoid hemorrhage)
Infiltrative diseases (such as amyloidosis and sarcoidosis)
Strenuous exercise

difference for cTnT (measured by ECLIA method) is on average 4.59 ng/L (95% CI: 1.60–7.57 ng/L) (63). It is usually assumed that this sex difference in cTn circulating levels is due to different cardiac mass (15) (and so also cardiomyocyte renewal) between adult healthy men and women (36,59,60).

From a clinical point of view, a fundamental question is whether the use of sex-specific cutoff values for high-sensitivity cTnI and/or cTnT assays actually allows a more accurate detection of patients with non-ST-segment elevation acute coronary syndromes (NSTEMI-ACS), who are at higher risk of presenting major cardiovascular events in the short term. Considering high-sensitivity cTnI assays, several studies confirmed that the use of sex-specific decision values significantly improve both diagnostic accuracy of ACS and risk stratification in women (83,88-91). However, a very recent study (92) reported some conflicting results regarding the cost-benefit ratio of hs-cTnI assay concerning sex-related cut-off values in patients with suspected ACS. Indeed, Shah *et al.* (92) confirmed that the hs-cTnI method prompted reclassification of 1,771 (17%) of 10,360 patients with myocardial injury or infarction, but these reclassified patients did not show a lower subsequent incidence of myocardial infarction or cardiovascular death after a follow-up of 12 months. These results question whether the diagnostic threshold for AMI should be based on the 99th centile derived from a normal reference population. A very recent study demonstrated that intra-individual (within-subject) biological variation of cTnI in healthy adult subjects and patients with chronic kidney disease is about 8–10% (expressed as CV), while the between-subject variation is about 3-fold higher (on average about 61–62%), if evaluated by means of a high-sensitivity immunoassay method (93). These data open the question how to clinically interpreter the cTnI variations significantly greater than the LoD value, measured with high-sensitivity immunoassays, which still are within the 99th URL value in patients in the setting of ACS (60,67,76).

Considering the cTnT assay, several evidences indicate that sex-specific clinical decision values are not particularly useful for cTnT assay (94-96). Sex-related differences between the results of cTnI and cTnT in ACS patients are somewhat expected considering the lower sex-dependence of cTnT compared to cTnI (63).

As far as the age-related differences are concerned, several studies published after the year 2005 demonstrated that cTn levels progressively increase in apparently healthy adult subjects aged over 65 years (60,63,71,72). Higher circulating levels of cTnI and cTnT in elderly healthy subjects may be caused by an increased release of these proteins from cardiomyocytes as well as by a decreased turnover, or by the combined action of these

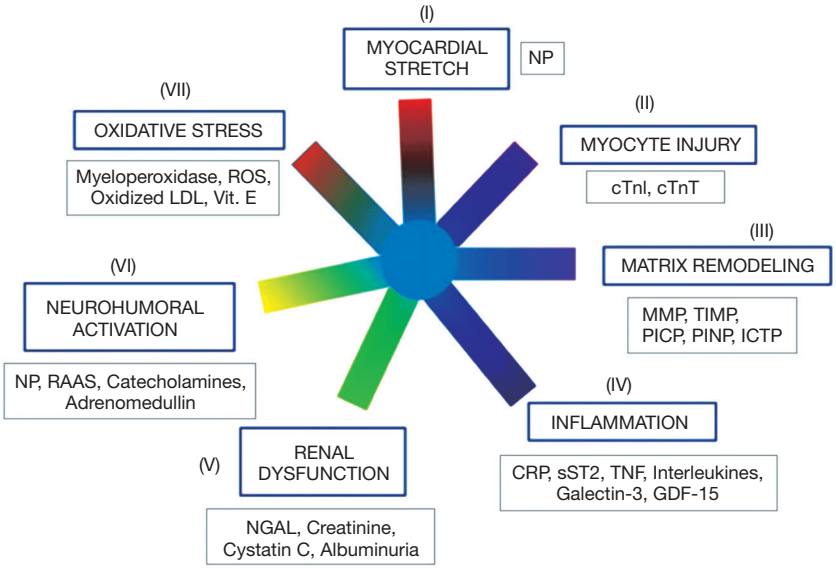

Figure 2 According to Braunwald (101), the figure reports the seven major classes of biomarkers related to main pathophysiological mechanisms strictly related to HF. As also demonstrated by several studies (56,97,99,102-104), these biomarkers are also closely linked to progression and clinical severity of HF. HF, heart failure.

two mechanisms (36,59,60). Indeed, several age-associated disorders can cause the death of cardiomyocytes, with the consequent release of sarcolemma proteins, including cTnI and cTnT (63,97,98).

It is conceivable that the improvement in analytical performance of immunoassay methods will continue in the next future until reaching the exceptional objective to measure the circulating levels of biomarker in more than 99% of individuals with cTnI and cTnT values above the LoD, including women and pediatric subjects. Indeed, comparing the results of cTnI immunoassay methods reported in 2004 (99) to those found in more recent studies (68-70,100), the bias among methods is greatly reduced (from about 20 folds to about two folds) and analytical performances are also significantly better. These results suggest that may be also possible to reach in a short period of time the ambitious objective to measure cTnI and cTnT concentrations at LoD level with an imprecision ≤20%.

Incremental diagnostic and prognostic risk using different cardiac specific biomarkers

In clinical routine, assessment of cardiovascular risk is usually based on the assay of several biomarkers, including NPs, cTn and, often, other biomarkers (in particular: inflammatory and fibrosis biomarkers, growth factors, neuro-hormones, cytokines, biomarkerts of oxidative stress, and biomarkers of renal function and injury) (*Figure 2*) (101-103,105,106). This approach is defined as Multi-Markers (MM) or global risk model (101-108). It is conceivable that only some of biomarkers included in MM models show sex-related variations. Novel risk biomarkers should be evaluated in several phases, including initial proof of concept, prospective validation in independent populations, documentation of incremental information in MM-models, assessment of effects on patient management and outcomes, and ultimately, cost-effectiveness (104,107-110).

Biomarkers that do not change the management of a disease unlikely will significantly affect patient outcome and therefore will not be cost-effective (101,103-107,109,110). According to Braunwald (101), seven major classes of pathophysiological mechanisms are strictly related to pathogenesis of HF (*Figure 2*). Therefore, the biomarkers related to these seven classes of pathophysiological mechanisms are likely linked to progression and clinical severity of HF (*Figure 2*). As a result, a good strategy could be to include in MM models only one biomarker for each of these seven classes.

All the most recent guidelines (47,57,58,103) state that NPs and cTn assay should be considered as the first line biomarkers for risk evaluation in general

population and HF patients. These recommendations are based on a huge number of experimental evidences, which have demonstrated that measurement of NPs and cTn significantly and independently improve both risk stratification and clinical outcome of HF patients (47,57, 58,103). According to *Figure 2*, NPs and cTn independently contribute to cardiovascular risk assessment because are involved in different pathophysiological mechanisms related to cardiac dysfunction and progression of heart failure (i.e., detection of myocardial tissue injury and cardiac stress, respectively). Several studies (111-116) reported that these two biomarkers are increased even in the first two asymptomatic stages (A and B) of natural history of HF (47). However, the 2010 ACCF/AHA guidelines reported that NP measurement is not recommended for cardiovascular risk assessment in asymptomatic adults (Class III: no benefit; level of evidence: B) (117). Indeed, at present time, we need some specific clinical trials, based on cohorts of asymptomatic individuals, which are designed for early assessment of individuals at high risk of HF by means of NPs and cTn using high-sensitivity method. In particular, these clinical studies should demonstrate that early treatment is able to improve clinical outcomes, such as: reversion of adverse remodeling, slowing down in HF progression and/or reduction in mortality rate and major adverse cardiovascular events in individuals at high risk for HF with increased NP and cTn levels.

From a statistical viewpoint, when using decision values (such as 99th URL) it may be inadvisable to dichotomize continuous variables for multiple regression analyses, because this approach can significantly reduce information (118). Several recent studies, using high-sensitivity methods, demonstrated that a progressive increment in biomarker levels, even within the reference interval values, can significantly increase cardiovascular risk scores (76-79). Consequently, sex-related decision values have no clinical relevance in risk assessment.

Conflicting results have been reported when several biomarkers were added to regression models, already including NPs and cTn for risk stratification in general population or HF patients (47,57,58,109). In particular, although some inflammatory biomarkers (such as sST2, galectin-3 and GDF-15) are significantly related to clinical outcome in univariate analyses, correlations often vanished when these variables are tested in MM models including NPs and/or cTn measured with high-sensitivity methods (103). This effect is in part expected because these biomarkers (such as sST2, galectin-3 and GDF-15) have similar pathophysiological mechanisms, related to activation/ regulation of cytokines system. As a result, these variables can introduce some collinearity when tested together in MM models (103). However, some recent results indicate that in MM models, also including NPs and cTn, some inflammatory biomarkers are also able to independently contribute to regression in patients with acute HF even when tested together in the same MM model (119).

Future perspectives

Due to the progressive improvement in analytical performance of immunoassays (6,36,60,63,66), LoQ values at 20% CV have progressively approximated those at LoD level of cTnI methods (*Table 4*). These results suggest that may be also possible to reach in a short time the objective to measure cTnI and cTnT concentrations at the LoD level with an imprecision ≤20%: a goal inconceivable only 5 years ago.

As far as the BNP immunoassay methods are concerned, several studies demonstrated that the IRMA method (by Shionogi Diagnostic Division, Japan), the ADVIA method for the Centaur platform (by Siemens Health Care Diagnostics) and ST AIA-PACK method for the AIA platform (by TOSOH Corporation, Tokyo, Japan) measured greatly lower (up to the half) BNP values in comparison with other immunoassays, such as the POCT Triage method (by Alere Diagnostics), the BNP Triage Biosite for Access and UniCell DxI platforms (by Beckman Coulter Diagnostics), the MEIA method for the AxSYM platform and the chemiluminescent microparticle immunoassay for ARCHITECT platform (both by Abbotts Diagnostics) (3). However, more recent results (120) indicate that the bias between BNP ADVIA and Access methods is significantly decreased (from approximately 50% to roughly 20%) compared to that reported with the previous generation of BNP ADVIA method (121-123). The results of this recent study also confirm that differences between NT-proBNP methods (i.e., <20%) are lower than that observed between BNP methods (120).

Further studies, including some based on results of external quality control programs, are needed to confirm that there is a trend to a progressive harmonization among the results of the most popular immunoassay methods for NPs and cTn. Some recent results (3,63,97,120,122,123), indicating both a progressive harmonization of results and better analytical performances, are good news for clinicians. If this trend to better harmonization for NP and cTn immunoassays will be confirmed also in the next

future, it will be possible to adopt similar clinical decision values for the diagnosis of heart failure independently of the immunoassay method used. According to the Precision Medicine principles, this improvement in harmonization of clinical results and in analytical performance of NP and cTn methods will allow an early and more accurate diagnosis of cardiac disease, as well as a better stratification of cardiovascular risk within the reference interval values of biomarkers. These improvements will also allow to better distinguish features of specific groups of individuals/patients, and also to better individualize and personalize therapies (124).

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Footnote

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