Gender medicine in the evaluation of cardiovascular diseases: focus on cardiovascular biomarkers

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

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ABSTRACT

Many studies have demonstrated the crucial pathophysiological and clinical relevance to evaluate cardiac specific biomarkers (natriuretic peptides and cardiac troponins) for the diagnosis and risk prediction of cardiovascular diseases in the general population and in patients with cardiovascular diseases as well. However, some important pathophysiological aspects still need to be better clarified, such as the possible influence of the sex hormones on the circulating concentrations of cardiac biomarkers and therefore on the interpretation of changes in their circulating levels in healthy subjects and in patients with cardiovascular disease. The primary purpose of this document is to discuss the pathophysiological and clinical relevance of sex- and/or gender-related differences in circulating levels of cardiac biomarkers. In the first part of this document, the pathophysiological mechanisms related to age, sex, gonadal development and function (including pregnancy) will be discussed. In the second part, the pharmacological effects of sex hormone therapy will be discussed. Sex hormones can be prescribed to patients of both sexes as replacement therapy, for prevention or treatment. However, many transgender persons undergo treatment with gender-affirming hormone therapy in order to help aligning their physical traits and gender identity. Accordingly, the possible effect of sex steroid hormone administration on the circulating levels of cardiac-specific biomarkers will be discussed. The most recent studies confirm the pathophysiological hypothesis that the androgenic steroid hormones (particularly testosterone) are generally associated with higher levels of cardiac troponins. On the other hand, the administration of estrogenic steroid hormones (particularly estradiol) can stimulate the production and release of cardiac natriuretic hormones from cardiomyocytes.

Key words: *gender medicine, cardiovascular diseases, biomarkers*

This inter-society Document is jointly published in the Journals (or on the web sites) of the Societies that contributed to its drafting.

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Received: 17.07.2023 Revised: 18.07.2023 Accepted: 18.07.2023

Published on-line: 03.08.2023 DOI: 10.19186/BC_2023.058

INTRODUCTION

Many studies have now demonstrated the crucial pathophysiological and clinical relevance to evaluate cardiac specific biomarkers (natriuretic peptides and cardiac troponins) for the diagnosis and risk prediction of cardiovascular diseases. However, some important pathophysiological aspects still need to be better clarified, such as the possible influences of sex hormones on the circulating concentrations of cardiac biomarkers and therefore on the interpretation of changes in their circulating levels in healthy subjects and in patients with cardiovascular disease.

The terms "sex" and "gender" are not interchangeable. In agreement with the World Health Organization (WHO), the document of the Italian Ministry of Health dated May 6, 2019, entitled "Plan for the application and dissemination of Gender Medicine" (1), defines "gender" as the result of criteria built on social parameters about the behavior, actions and roles attributed to a gender and as a supporting element for health promotion. The term "sex" refers to the specific differences related to the functions of sex chromosomes, gonadal hormones, anatomy of the reproductive system, binary distinction between males and females, not including the condition of intersex (2,3). Conversely, the term "gender" is not binary, but refers to a broad spectrum of criteria built on social parameters about the behavior, actions and roles attributed to a gender and as a driving force for health promotion (1-3). Therefore, while sex is established by biological differences, the concept of gender varies according to culture, even when it is considered in the setting of the health status, leading to the creation of a new medicine field (1-3).

In agreement with international and national documents (1-3), Gender Medicine focuses on the influence of gender-related biological, socio-economic and cultural differences on the health and disease state. Many studies have confirmed that most diseases, including cardiovascular disorders, show different incidence, symptoms and severity related to sex or gender (1-9). Furthermore, both men and women can present a different response to drug therapy, as well as adverse reactions to many types of drugs (1-3,10-13). Finally, the access to the care suffers from significant inequalities linked to gender, even in Italy (1). The main purpose of Gender Medicine is to create prevention programs, define diagnostic and therapeutic approaches taking into account sex and gender-related differences. This goal is also in line with the recent trend of Laboratory Medicine to tailor the approach on the individual phenotype (14).

The circulating levels of cardiac natriuretic hormones (ANP and BNP) and cardiac troponin I (cTnI) and T (cTnT) are significantly influenced by age and sex (15-20). Furthermore, there is experimental evidence in animals and also in humans, obtained in apparently healthy individuals or patients with cardiovascular disease, that estrogens and androgens can differently modulate the production of natriuretic hormones and cardiac troponins by cardiomyocytes (6,7,15-20).

The primary purpose of this document is to discuss the pathophysiological and clinical relevance of sex- and/or gender-related differences in circulating levels of cardiac biomarkers. In the first part of this document, the mechanisms related to age, sex, gonadal development and function (including pregnancy) and the presence of cardiovascular diseases will be discussed, all of which can influence the circulating levels of cardiovascular biomarkers. In the second part, the pharmacological effects of sex hormone therapy will be discussed. Sex hormones can be prescribed to patients of both sexes as replacement therapy, for prevention or treatment. Furthermore, many transgender persons undergo treatment with gender-affirming hormone therapy in order to help aligning their physical traits and gender identity. Accordingly, the possible effect of sex steroid hormone administration on the circulating levels of cardiac-specific biomarkers will be discussed. The most recent studies confirm the pathophysiological hypothesis that the androgenic steroid hormones (particularly testosterone) are generally associated with higher levels of cardiac troponins. On the other hand, the administration of estrogenic steroid hormones (particularly estradiol) can stimulate the production and release of cardiac natriuretic hormones from cardiomyocytes.

PATHOPHYSIOLOGICAL MECHANISMS

Impact of sex/gender on cardiovascular mortality in the general population

Globally, cardiovascular disease is the first cause of death in Western countries and contributes significantly to the reduction of life expectancy. A drastic reduction in the mortality from cardiovascular diseases could lead to an improvement in longevity of about 6 years (21). A close association exists between sex/gender, age and the development of cardiovascular diseases. The most recent epidemiological data about the Italian population in 2020 confirmed this correlation between age, sex and mortality (22). In Italy, the life expectancy at birth is 79.7 years in men and 84.4 years in women. At 65 years, life expectancy is 19.9 years (18.2 for men, 21.6 for women) (22). Men develop cardiovascular disease earlier (i.e., after 55 years), while women are affected more often by specific conditions such as Takotsubo cardiomyopathy or heart failure with preserved ejection fraction (HFpEF) (5,6,8).

To explain the association between senescence and increased mortality from cardiovascular disease, Franceschi et al. introduced in 2000 the term "inflammaging" (or inflammageing) (23). Inflammaging defines a set of mechanisms active in older individuals who present a very high susceptibility to chronic disorders of various organs and systems, disability, frailty and premature death (24-27). The clinical condition of inflammaging is typically associated with elevated circulating levels of pro-inflammatory biomarkers (24-27)

Furthermore, the mechanisms related to inflammaging are hallmarks of the cardiovascular diseases, typical of the elderly, such as atherosclerosis, systemic arterial hypertension and rapid progression towards heart failure (HF) (27-31). For this reason, the activation of inflammatory mechanisms, the development of cardiovascular disease in old age and the consequent decrease in life expectancy are closely intertwined (27-31).

Influence of age, aging and sex on circulating levels of cardiac biomarkers

The activation of the Senescence-Associated Secretory Phenotype (SASP) is closely associated with the typical phenotype of senescent cells that secrete high levels of pro-inflammatory cytokines, immuno-modulators, angiogenic growth factors, metalloproteases, particularly in tissues with a low rate of cell renewal such as the myocardium (27,32,33). The chronic activation of the mechanisms related to SASP stimulates not only the secretion of cNPs (34,35), but also cytotoxic effects on cardiomyocytes that induce myocardial damage and therefore an increase in cardiac biomarkers circulating concentrations (27, 32, 33). Importantly, the circulating levels of cNPs and cardiac troponins (cTs) differ in the two sexes during the various ages of life (15-20,27). The underlying mechanisms could be usefully investigated to refine the clinical use of cardiac biomarkers (27,36,37).

Natriuretic peptides

Experimental and clinical studies have demonstrated that cardiac natriuretic hormones (ANP and BNP) are secreted and released specifically by atrial (ANP) and ventricular (BNP) cardiomyocytes in response to stressful stimuli that activate the neuro-endocrine and immunological systems (15,16,38-40). Moreover, the circulating levels of cardiac natriuretic peptides (cNPs) are about two-fold higher in fertile women than in men, with a further increase during pregnancy, or hormonal therapy with estrogen (41-45). In males, cNPs progressively increase after 50 years and this increase in circulating levels is closely connected with an increased incidence of cardiomyopathies. After menopause, the levels of cNPs increase also in women, and then the difference between the levels of the biomarkers between the two sexes tend to decrease during senescence (43,44).

The production and release of cNPs are regulated in cardiomyocytes by means of the membrane sensors specific for mechanical (stretching of the atrial or ventricular wall) or physical stimuli (such as hypoxia), or by specific receptors stimulated by hormones (estrogens), pro-inflammatory cytokines, and growth or tumor factors (15,16,38-42). Most of the intra-cytoplasmic pathways regulating the production of cNPs at the nuclear level, appear to be mediated by means of Nuclear Factor kappa B (NF-kB), which is a nuclear transcription factor activated in cardiomyocytes by cytokines, growth factors, chemokines, adhesion molecules by means of specific membrane receptors (16,46,47).

From a clinical perspective, cNPs (especially BNP and NT-proBNP) are considered the biomarkers of first choice by all the most recent national and international guidelines for early diagnosis, therapy monitoring and risk prediction of the patients with HF (37,48-50). In particular, the 2021 European Society of Cardiology (ESC) guidelines consider the measurement of cNPs (BNP and NT-proBNP) essential to diagnose HFpEF, being EF within normal limits (49). cNPs measurement is recommended in outpatients (diagnostic criteria: ≥35 ng/L for BNP; ≥125 ng/L for NT-proBNP) and in hospitalized patients with clinical symptoms of decompensation (≥100 ng/L for BNP; ≥300 ng/L for NT-proBNP) (49). The same cut-off values are proposed for all assay methods, age categories, and sex. Nonetheless, different methods show significant differences between the measured values of the biomarker (even up to 50%). Furthermore, the cut-off values depend on sex (higher in women) and increase progressively with age (>55 years) with an intra-individual variability of about 50% for BNP (less for NT-proBNP) (37,48,51-53).

Cardiac troponins

From a pathophysiological perspective, the measurement of cardiac troponins I and T with high sensitivity methods (hs-cTnI and hs-cTnT) provides an estimate of the physiological or pathological turnover of the myocardial cell (cardiomyocyte renewal) (17,18, 54,55). In fact, the hs-cTnI and hs-cTnT methods have shown that, on average, healthy adult subjects show values of the 99th percentile upper reference limit (99th percentile URL; 15-45 ng/L) that are compatible with a daily cardiac turnover of about 30-40 mg (17,18,54,55). Since myocardial cell turnover is closely correlated with the total myocardial mass (higher in men), this explains the difference between the two sexes in the circulating values of hs-cTnI and hs-cTnT, with usually higher values in the men in comparison to women of the same age (17, 18,54-57).

Figure 1 shows the temporal trend of the circulating levels of cTnI, measured with an hs method, from the neonatal period to senescence in 1594 apparently healthy subjects from an Italian reference population (mean age 44.6 years; interquartile range, IQR, 30-59 years; ratio F/M:793/801=0.99), enrolled in a multicenter study organized by the Italian Cardiac Biomarkers Study Group between 2016 and 2019 (18,20,60-65). Soon after birth, hs-cTnI values are elevated in both sexes and that the biomarker levels tend to decrease until puberty, which occurs earlier in females. At puberty, hs-cTn values increase more in males than in females, therefore the biomarker values in adult males are significantly higher than those observed in fertile women at the same age. After 55 years, a progressive increase in hs-cTn values is observed in both sexes, more significant in women after menopause, so in the old age the difference between the values found in the two sexes tends to decrease (18,20,60-65).

The higher hs-cTnI and hs-cTnT levels in age-

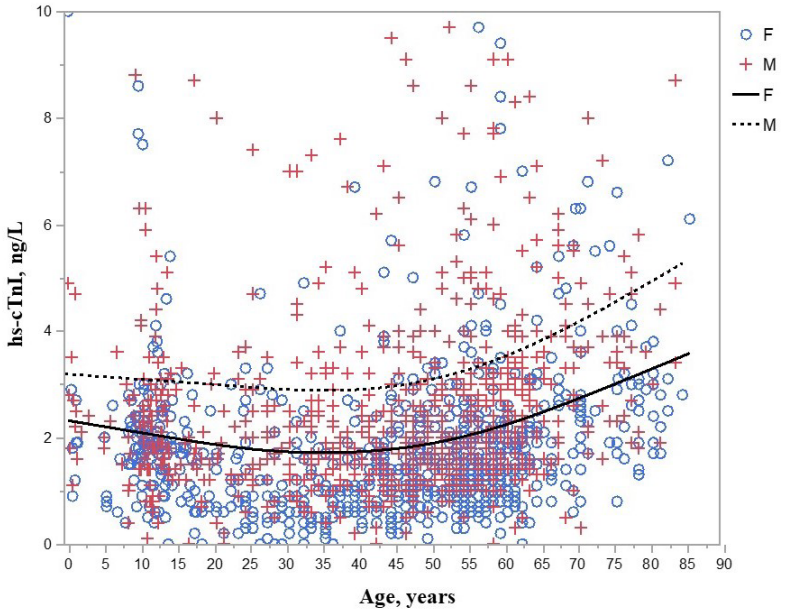

Figure 1

The figure illustrates the two sex-specific curvilinear relationships between the age (in the X-axis, age interval 18-90 years, ratio F/M:793/801=0.99) and hs-cTni concentration values (Y-axis), measured with the Architect method (Abbott Diagnostics), in 1594 adult subjects, enrolled in a reference Italian population from 2016 to 2019, as previously reported in detail (18,20,60-64). The data were analyzed using a spline polynomial model using the JMP 17 statistical software (version 17.0, Copyright © 2022 JMP Statistical Discovery LLC).

matched males compared to females during all ages of life, are related to the myocardial mass and cardiac renewal. Indeed, *in vivo* ultrasound and magnetic resonance studies, as well as studies in Anatomy and Forensic Medicine, which have evaluated myocardial mass at various ages in apparently normal subjects, have always demonstrated a greater myocardial mass in males compared to females (66,67). In fact, the weight of the heart is on average 300 g (range 280-340 g) in men, and 250 g (range 230-280 g) in women (68).

The higher hs-cTnI and hs-cTnT levels in the newborn, which has an average heart weight of about 21 g (67), suggest a greatly increase in cardiac remodeling due to the adaptive mechanisms activated soon after the birth to make the cardiovascular and pulmonary systems of the newborn most suitable for the extra-uterine life (69).

Finally, the progressive increase in circulating levels of cNPs and hs-cTns in both sexes after 55 years suggests that the increase in cardiac-specific biomarkers depends on the activation of the SASP phenotype and therefore to inflammaging, which is more prominent in the most fragile people, who suffers from comorbidities such as obesity, diabetes mellitus, and chronic pulmonary or renal diseases (27,32-35,70,71).

Pregnancy

Cardiac disease is the leading cause of maternal mortality in the European and North American countries, so accurate cardiovascular diagnoses in pregnancy are essential. (72-74). In 2023, the document "Consensus Bundle on Cardiac Conditions in Obstetric Care" published in US, describes that women who died for cardiovascular complications during pregnancy or postpartum were not diagnosed with a cardiac disease before death and that more than 80% of all pregnancy-related deaths, regardless of cause, were preventable (73). In addition, this document indicates that some common obstetric complications, like as pre-eclampsia and gestational diabetes, strongly increase the cardiovascular risk (73).

Considering these data, it is conceivable that an accurate evaluation of cardiovascular risk should be carried out in all women at the beginning of pregnancy (72,73). Accordingly, it may be clinically interesting to discuss the clinical relevance of the cardiac specific biomarkers measurement in order to further improve the cardiovascular risk evaluation in pregnant women.

Natriuretic peptides

All the clinical studies, including women in apparent good health and without any complications during pregnancy, agree that the levels of BNP and NT-proBNP are higher in pregnant than in non-pregnant women (74, 75). In particular, BNP and NT-proBNP values are already elevated in the first trimester of pregnancy and then tend to decrease slightly during the course of the second and third trimesters until they reach a plateau value, slightly higher than that in non-pregnant women (74,75). NT-proBNP values are always higher than those of BNP in both pregnant and non-pregnant women (74,76).

Umazume et al. (76) evaluated the variation of BNP and NT-proBNP levels in 51 pregnant women mean age (SD) 34.4 (5,1) years, by measuring the biomarker levels during pregnancy (one sample per trimester) and in the post-partum period (one blood sample taken in the first

postpartum week and another one about 1 month after delivery) (76). During pregnancy, cNPs levels are higher in the first trimester with a slow but progressive decrease of the levels in the second and third trimesters. In the first days postpartum, there is a marked increase in the cNPs levels with a progressive back to normal values after the first week after delivery (76).

BNP values measured in pregnant women should be considered method-dependent, even if no studies have specifically evaluated the systematic difference between methods in a large populations of pregnant women. Furthermore, studies related to the possible differences in circulating levels of NPs in populations with different ethnicities are also lacking.

Physiologically, changes in BNP and NT-proBNP levels in women with normal pregnancies are probably due to the profound changes in cardiovascular and renal function, as well as to the increase in circulating plasma volume, partially related to the activation of the renin-angiotensin-aldosterone system (74-78). In particular, cardiac output increases by 20% in the first 8 weeks, mainly due to peripheral vasodilation, while blood pressure remains stable even if peripheral resistance tends to decrease after the first trimester (74,78). Childbirth further activates the neurohormonal system producing a marked increase in NPs and cardiovascular indices (74,79).

Two recent meta-analyses suggest that persistent and elevated levels of BNP or NT-proBNP during the second half of pregnancy, should be considered an indication for the presence of complications (such as HF and pre-eclampsia) (80,81). Furthermore, the presence of complications is significantly associated to dystocia and neonatal suffering (80,81). However, further studies are needed to confirm these results, because the studies so far available in the literature are heterogeneous in terms of number of women enrolled, sampling time during pregnancy, and methods used to measure BNP and NT-proBNP (80,81).

Cardiac troponins

There are few studies reporting the variations of cTns levels during pregnancy using hs-cTnI or hs-cTnT methods (79,82-85). According to the results of a systematic review published in 2021 (82), 10 studies only (9 studies for cTnI and one study for cTnT) have measured the circulating troponin levels in women with normal or complicated pregnancy, but only two of these used hs-cTnI methods (83,84). The results of this systematic review suggest that the levels of cTnI and cTnT in normally pregnant women are not significantly different from healthy, non-pregnant women (82).

A more recent study by Minhas et al. (85) analyzed data on 622 pregnant and 2358 non-pregnant women (aged 18 to 40 years) from the US Centers for Disease Control and Prevention (National Health and Nutrition Examination Survey) (86,87). Less than 5% of women had diabetes and less than 10% were hypertensive. The values of hs-cTnT and hs-cTnI were measured with 3 different methods (Architect Abbott Laboratories, Siemens Healthcare Diagnostics, VITROS Ortho Clinical Diagnostics) on samples collected and stored from 1999 to 2004 (85). Women with uncomplicated pregnancies had hs-cTnI and hs-cTnT values similar to those of non-pregnant women, both before and after statistical adjustment, considering all demographics parameters and cardiovascular risk factors (i.e., age, race, ethnicity, diabetes, blood pressure value, glomerular filtration rate value) (85).

Evaluating all studies now available (79,82-85), in women with pregnancies who proceed towards childbirth without any type of complication, the levels of hs-cTnI and hs-cTnT are substantially stable and similar to those of the pre-partum period.

Only recently, some hs-cTnI or hs-cTnT methods have been used to evaluate the pathophysiological and clinical relevance of biomarkers in women with pregnancies complicated by diabetes, hypertension, eclampsia or cardiomyopathy (82-84,89-93). Overall, elevated levels of hs-cTnI and hs-cTnT in pregnancy are significantly associated with a higher risk of cardiovascular complications, dystocia and fetal distress (82-84,89-93).

Women with complicated pregnancies show frequently an increased of hs-cTnI and hs-cTnT circulating levels as well as an activation of the NPs system (82,90,93). In particular, Furenäs et al. (90) suggest as cut-off values, NT-proBNP <300 ng/L to exclude the diagnosis of HF, and hs-cTnT <14 ng/L to exclude the presence of myocardial damage in pregnant or peripartum women.

Moreover, several studies have reported that both cardiac-specific biomarkers predict delivery outcome, and also fetus and newborn health (80-85,87-93). However, the only few studies available are heterogeneous in terms of the number of women enrolled, the presence of a control group with normal pregnancy, the type of complications (e.g., hypertension, eclampsia, diabetes, cardiomyopathies), the biomarkers evaluated and the analytical performance of the methods used.

Despite these limitations, some pathophysiological and clinical considerations can be taking into account to direct clinicians towards a more appropriate use of cardiac biomarkers in the assessment of cardiovascular risk in pregnant women. Although consolidated data on cut-off values are not currently available, it seems important to use the variation of hs-cTnI and hs-cTnT values in the evaluation of cardiovascular risk, as suggested by a recent document of the Italian Study Group on Cardiac Biomarkers for other conditions (i.e., differential diagnosis of acute coronary syndrome, assessment of the presence of myocardial damage during major surgery or treatment with cardiotoxic drugs, such as chemotherapy or drugs) (94).

When cardiac involvement or a worsening of pre-existing cardiovascular conditions is suspected in a pregnant woman, clinicians should evaluate the percentage changes in hs-cTnI and hs-cTnT using as threshold a ≥30% change from the pre-pregnancy value of the biomarker (if available) or using multiple blood samples during the same pregnancy (94). Furthermore, since all studies indicate that the values of hs-cTnI and hs-cTnT in pregnant women without complications are

similar to those in non-pregnant women, finding that a value above the 99th URL percentile, specific for female reference population, should be sufficient to diagnose myocardial injury, as recommended by the guidelines (94,95). As suggested by Dockree et al. (82), there is a need to define reference intervals for the hs-cTnI and hs-cTnT methods in pregnant women, which should be calculated using samples collected throughout pregnancy and taking into account gestational age, body mass index, and other factors or clinical conditions that can modify the normal course of pregnancy.

We may suggest to measure hs-cTnI or hs-cTnT during the first trimester of pregnancy to estimate the cardiovascular risk, at least in pregnant women of advanced age or who present comorbidities before pregnancy (hypertension, obesity, diabetes) or when cardiomyopathy is suspected. Since the values of hs-cTnI and hs-cTnT are method-dependent, the measurement should be performed in the same laboratory using the same method to best estimate the biomarker variability (94).

PHARMACOLOGICAL EFFECTS OF SEX HORMONE THERAPY

Sex hormones can be prescribed to patients of both sexes as replacement therapy, for prevention or treatment (96-105). Transgender people frequently embark on a gender-affirming journey through hormone therapy (96,97). It seems useful to discuss the pathophysiological effects on the cardiovascular system of sex steroid hormones in both sexes. In particular, the possible effect of sex steroid hormone administration on the circulating levels of cardiac-specific biomarkers will be discussed.

Hormone replacement therapy and cardiovascular risk

Replacement therapy with sex steroid hormones (HRT) is indicated in women (with estrogen hormones) and in men (with testosterone) in case of primary or secondary hypogonadism (98-105). The purpose of HRT is to provide the hormones that are necessary for an adequate and physiological development in puberty, or to integrate the reduced functionality of the genital glands (ovary and testicles) in adulthood (98,99,102,105). More specifically, the ideal HRT should guarantee circulating levels of steroid hormones comparable to those expected by gender and age. If the HRT achieves this primary objective, there should be no alterations in cardiovascular function and therefore also the values of NPs and cTns should be in line with those expected for age and gender (36,70).

In recent years there has been much debate on the cost/benefit of HRT to treat the symptoms of hypo-estrogenism and to reduce long-term complications in women suffering from ovarian insufficiency and/or premature menopause (100-102,106-112). Premature Ovarian Insufficiency (POI) is a syndrome defined as insufficiency of ovarian activity before 40 years, affecting about 1% of women (113). Over the last few years, many definitions have been proposed, such as: primary insufficiency of the ovary, premature menopause, premature ovarian insufficiency; the latest guidelines of the European Society of Human Reproduction and Embryology (ESHRE) recommend the term POI to define this syndrome (113).

A meta-analysis published in 2022 (112) indicated that the replacement therapy including estrogen and progesterone (contraceptives enclosed) may be beneficial in women with POI, not only in countering the symptoms of hypo-estrogenism, but also in preserving bone metabolism and preventing atrophy of the uterus. Considering cardiovascular risk, HRT improves the lipid profile using transdermal estradiol, whereas transdermal or vaginal use of estrogen hormone administration can reduce blood pressure (112-116).

The original studies on HRT (112-116) are relatively few, not recent and carried out on women with POIs, who are only a small fraction of the much larger population of women who are still fertile, taking contraceptives, or women in peri- or post-menopause starting HTR therapy to reduce menopausal symptoms (99,101,106-108,111). In 2019, Palacios et al. (100) stated that there was a general agreement that hormonal therapy, based on guideline recommendations (100,117,118), may present a favorable risk/benefit ratio in women initiating it between the ages of 50 and 59 or within 10 years of the onset of menopause. HRT is able to reduce vasomotor and urogenital symptoms, prevent the loss of bone matrix and therefore the possibility of fractures, improve sexual function and therefore the general quality of life of menopausal women.

In 2019, a meta-analysis (119) reported data from 31 randomized trials comparing women (n=40521) on HRT *versus* those who were not. Women on HRT were divided into two subgroups according to the date of treatment initiation: young HTR (starting HRT <60 years) and elderly HTR (>60 years). In fact, the age of onset seems to play a significant role in determining the risk/benefit ratio in terms of all-cause or cardiac death and cerebrovascular events (119). The young HTR group appears to benefit from hormonal treatment in terms of a reduction of all-cause and cardiac mortality, and a decrease in the number of cardiac events. However, all HTR women may be at higher risk of stroke, transient ischemic attacks and systemic embolism events, which progressively increase with age (119). Important limitations of these studies are the lack of accurate information on the starting date of HRT and the heterogeneities in composition, formulation and administration route of HRT (119).

A more recent meta-analysis sought to evaluate the effects of HTR treatment on the lipid profile in menopausal women by selecting 73 studies (120). HTR treatment showed a positive effect on the lipid profile of menopausal women: in particular, the transdermal treatment with tibolone seemed particularly effective in decreasing high triglyceride levels (120).

Mills et al. (121) recently reported that HRT has a role in modulating the risk of dementia, but the overall outcome depends on the age of the start of therapy, dosage and formulation of the treatment, as well as the presence of

other comorbidities and risk factors. Furthermore, some women may suffer from cardiovascular disease or high cardiovascular risk before menopause (122), therefore careful evaluation to select female candidates who can benefit from HRT seems to be necessary.

Role of cardiac-specific biomarkers in risk assessment of women taking contraceptives or on hormone replacment therapy

Unlike the studies on the general population (36,37,70,71), the specific studies assessing cardiovascular risk in women on contraceptive or HRT treatment did not consider the measurement of cardiac biomarkers for risk estimation, but only the traditional risk factors such as age, gender, lipid profile and comorbidities (such as obesity, diabetes and hypertension) (111,112,114,116,119-122). Instead, the measurement of cardiac-specific biomarkers can lead to a more accurate estimate of the cardiovascular risk even in women on contraceptive or HRT treatment.

An analysis by the United Nations (Department of Economic and Social Affair) published in 2019, reports that, on average, from 20% to 50% of women in reproductive age use contraceptive drugs in European and North American countries (123). Therefore, a significant percentage of women on contraceptive treatment has been likely enrolled in the studies recently carried out in Western countries for the evaluation of cardiovascular risk in the general population (70,71). In fact, some studies concerning cardiovascular risk evaluation in the reference adult healthy populations do not consider the use of contraceptives or HRT, or at least do not specifically declare that this hormone treatment is taken into account in the multivariable statistical analyses (124-129).

Recent studies have also highlighted the relevance to measure the variation over time of cardio-specific biomarkers, especially hs-cTnI and hs-cTnT, for a more accurate and early detection in the general population of asymptomatic subjects, who are at high cardiovascular risk or already suffer from cardiac damage (36,37,70, 129). Several studies have demonstrated that variations of hs-cTnI and hs-cTnT values greater than 30% in asymptomatic subjects or patients with cardiovascular disease, should be considered clinically significant because associated with an increased cardiovascular risk (36,37,70). Indeed, a variation between two consecutive measurements greater than 30% indicates that the increase in hs-cTnI and hs-cTnT levels cannot be explained by the combined effect of analytical error and biological variance. On the opposite, this biomarker variation may be attributable to a myocardial injury if the hs-cTnI and hs-cTnT levels are greater than the cut-off (i.e., the 99th percentile URL) value (36,37,70,95). The early detection of a high cardiovascular risk score or a cardiac damage in an asymptomatic adult individual can suggest a clinical intervention designed to delay or even to stop the evolution towards a symptomatic HF (36,37, 70,130).

Future studies on the cardiovascular risk assessment in women from the general population need to be designed considering the use of the contraceptive treatment (or HRT) together with evaluation of NPs and cTns to verify whether hormonal treatment is associated with significant changes in biomarkers and consequently also in the outcome, such as death and Major Adverse Cardiovascular Events (MACE).

Transgender people and cardiovascular risk

Some transgender people choose to embark on a gender affirmation path using hormonal steroid-based treatment (96,97,131-133). A systematic review published in 2019 (132) reports that transgender people represent about 0.6-1.1% of the general population in some European countries (such as Belgium and the Netherlands), in particular 1.1% of people classified as male at birth and about 0.6%-0.8% of people classified as female at birth.

In transgenders who choose to use feminizing hormone therapy, the long-term administration of estrogens or drugs that decrease the activity of androgen hormones are associated with an increased risk of thromboembolic events (132,133). However, the cardiovascular risk is drug-dependent and therefore can be decreased by optimizing hormonal therapy (132).

There are a few studies (including some systematic reviews and meta-analyses) on cardiovascular risk that specifically address transgender people who have undertaken therapy with testosterone or other hormones with androgenic activity (134-140). Overall, testosterone treatment may be quite safe in the short or medium term with regard to cardiovascular risk (including the development of hypertension) and the risk of tumors, while the long-term effects need to be further investigated (132,139,140).

Some recent articles report data about circulating levels of cardiac biomarkers in transgender people and their relevance in respect to the cardiovascular risk (141-147). Kulprachakarn et al. (142) conducted a study including a treated population [mean (SD) treatment time 6.65 (0.52) years) and a control group, enrolled from a total population of 200 Thai transgender women (100 treated with estrogens and 100 controls, mean age of 24 (5) years). Testosterone levels were significantly higher in the untreated group than in the estrogen-treated group [6.37(0.23) *versus* 4.30 (0.23) ng/mL, p<0.001], while no significant difference was found between groups for 17-beta estradiol levels (142). The Authors assessed cardiovascular risk by measuring the lipid profile, glucose, C-reactive protein, NT-proBNP (ELISA ABCAM method, ab263877; sensitivity 11.5 ng/L; linearity from 21.9 ng/L to 1400 ng/L), and cTnI using a non-high sensitive method (ELISA method ABCAM, ab200016; sensitivity 4.4 ng/L, linearity between 31.3 ng/L to 4000 ng/L). In the treated group, significantly higher levels were found for cTnI [mean 29 (51) *versus* 14 (14) ng/L, p=0.040], and HDL cholesterol [50.14 (1.28) *versus* 56.43 (1.28) mg/dL, p=0.001], while a decrease for C-reactive protein levels [3.44 (6.82) *versus* 3.28 (5.80), p=0.031], and no changes for NT-proBNP and the other parameters (142).

More recently, Boone et al. (147) using two different hs-cTnI methods (Architect Abbott Diagnostics and Access Beckman Coulter) and the hs-cTnT method (Elecsys Roche Diagnostics) evaluated the differences in biomarker values between a group of 15 transgender men (median age 37 years, IQR 17-56 years) and 48 transgender women (median age 63 years, IQR 17-78 years). All subjects received hormone therapy with a median treatment of 6 years (3.3-17 years) in transgender women (commonly treated with estradiol combined with an anti-androgen) and 3.6 years (2.1-5.2 years) in transgender men (treated with testosterone) (147). No difference was found between hs-cTnI or hs-cTnT values in the two groups of transgender women and men. The results of this study are strongly limited by the number of subjects studied, especially transgender men (only 23.8% of the total), and the different age between the two groups (26 years between the median age values) (147).

Greene et al. (145) evaluated the distribution of hs-cTnI (Architect method, Abbott Diagnostics) and NT-proBNP (Elecsys method, Roche Diagnostics) values in apparently healthy transgender people taking testosterone [n=79; mean (SD) age 28.8 (7.8) years] or estradiol [n=93; mean age 35.1 (11.7) years] for 12 months or more. In this study, the hs-cTnI values measured in men were significantly higher than in transgender women ($p<0.001$). Conversely, NT-proBNP values were significantly higher ($p=0.001$) in women than in transgender men (145). The results of this study (145) are in line with the hypothesis that elevated levels of androgens (particularly testosterone) are generally associated with higher hs-cTnI values (18), while elevated levels of female sex steroid hormones (particularly estradiol) stimulate the production and release of NPs from cardiomyocytes (16,17). Furthermore, sex steroid hormone levels rather than gender assigned at birth influence the levels of cardiac-specific biomarkers (145).

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

During the last 10 years, the progressive adoption of the new hs-cTnI and hs-cTnT methods in the clinical laboratory practice allowed a more accurate evaluation of the circulating levels of the biomarkers in healthy subjects and in patients with cardiovascular diseases (17,152). All the most recent studies confirm that NPs and cTns show a different behavior not only in the two sexes during the various ages of life (15-20,27), but also in transgender people treated with hormone therapy (96,97,131-135).

Recent data suggest that it could be useful to investigate why there are changes in cardiac-specific biomarkers in transgender people, in order to improve their use both for an earlier assessment of cardiovascular risk and for a more accurate diagnosis, prognosis and therapy (97,141-151). The most recent studies (97,141-151) confirm the pathophysiological hypothesis that androgenic steroid hormones (particularly testosterone) are generally associated with higher hs-cTnI levels (18). On the other hand, estrogenic steroid hormones (particularly estradiol) stimulate the production and release of cardiac natriuretic hormones from cardiomyocytes (16,17).

In 2023, a document from the Centers for Disease Control and Prevention (CDC) reported that the maternal mortality rate for 2021 was 32.9 deaths per 100 000 live births, compared with a rate of 23.8 in 2020 and 20.1 in 2019 (152). Moreover, the mortality rates for black women were significantly higher than rates for white and hispanic women, being the observed increase from 2020 to 2021 for all race and for hispanic-origin groups statistically significant (153, 154). This increase in mortality rate was probably caused by COVID-19 pandemic (154-156). However, it is well known that the cardiovascular complications were the first cause of death in patients affect by COVID-19 pandemic (154-156). Indeed, increased levels of hs-cTnI and hs-cTnT upper the 99th percentile URL are usually observed in patients with previous cardiovascular diseases, suggesting the presence of an acute myocardial injury or infarction (154, 155). Accordingly, these results suggest the clinical relevance of the accurate evaluation of the cardiovascular risk by measuring cardio-specific biomarkers during pregnancy and post-partum, especially in women with an history of cardiovascular disease before pregnancy (154).

New studies are needed to evaluate whether the physiological levels of the sex steroid hormones in cis-gender people could play a different pathophysiological role on the development of cardiovascular diseases compared to sex steroid hormones assumed for gender affirmation. In fact, there are few studies performed on transgender people that have used hs-cTnI and hs-cTnT methods for cardiovascular risk assessment (97,148-151). Furthermore, even the few studies available up to now present major limitations due to the small number of transgender people enrolled, the experimental design (few studies present a comparative evaluation between cis-gender/trans-gender control groups and trans-gender treated groups), the different compositions and routes of administration of the HRT drugs, the age of initiation and duration of HRT.

Finally, it is necessary to design studies evaluating the cardiovascular risk in women from the general population taking into account the use of contraceptive treatment (or HRT) as a possible variable that can influence the circulating levels of NPs and cTns to verify if hormonal treatment is associated with significant changes in biomarkers and consequently also in the outcome, such as all-cause and cardiovascular death and MACE.

CONFLICT OF INTEREST

None

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