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REVIEW

Sex-related differences in contemporary biomarkers for heart failure: a review

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The use of circulating biomarkers for heart failure (HF) is engrained in contemporary cardiovascular practice and provides objective information about various pathophysiological pathways associated with HF syndrome. However, biomarker profiles differ considerably among women and men. For instance, in the general population, markers of cardiac stretch (natriuretic peptides) and fibrosis (galectin-3) are higher in women, whereas markers of cardiac injury (cardiac troponins) and inflammation (sST2) are higher in men. Such differences may reflect sex-specific pathogenic processes associated with HF risk, but may also arise as a result of differences in sex hormone profiles and fat distribution. From a clinical perspective, sex-related differences in biomarker levels may affect the objectivity of biomarkers in HF management because what is considered to be 'normal' in one sex may not be so in the other. The objectives of this review are, therefore: (i) to examine the sex-specific dynamics of clinically relevant HF biomarkers in the general population, as well as in HF patients; (ii) to discuss the overlap between sex-related and obesity-related effects, and (iii) to identify knowledge gaps to stimulate research on sex-related differences in HF.

Keywords Heart failure • Biomarkers • Sex • Obesity • Prognostic value

Introduction

Heart failure (HF) is a multifactorial disorder characterized by impaired cardiac function, systemic inflammation and neurohormonal activation.[1,2] The most recent trends according to data from 4 million individuals indicate that the absolute number of incident HF cases was 9% higher in men than in women, but among older individuals (>80 years), the absolute number of HF cases was higher in women (*Figure 1*).[3] Whereas macrovascular coronary artery disease and myocardial infarction are leading causes of HF in men,[4–7] coronary microvascular dysfunction, hypertension and immuno-inflammatory mechanisms are thought to play a greater role in the development of HF in women.[4,8,9] Response of the myocardium to ischaemic injury and cardiovascular stress also differ between men and women. For instance, after an ischaemic insult to the heart, a ~10-fold higher apoptotic rate in the peri-infarct region has been observed in men compared with women.[10] When subjected to pressure overload, female hearts tend to remodel in a concentric pattern, whereas male hearts more often progress to an eccentric remodelling pattern.[10–12] However, the exact pathophysiological mechanisms that lead to these sex-related differences are yet to be elucidated.

Circulating HF biomarkers encompass a wide range of molecules (e.g. proteins, enzymes, hormones and gene products) present in blood and other body fluids, and furnish objective information about various biological or pathological processes associated with this syndrome.[13,14] Some are routinely used in clinical practice [e.g. natriuretic peptides (NPs)] to diagnose and estimate HF severity, and also to provide prognostic information beyond traditional cardiovascular risk factors. In addition to pre-analytical factors such as sample collection, storage and choice of assay, sex is a major factor influencing biomarker levels.[15] Biological sex-related differences in HF biomarkers may result from differences in genetic makeup, the direct effects of sex hormones, and also indirectly from differences in fat distribution among men and women.[16,17] However, information regarding the pathobiology of sex differences in HF biomarker concentrations is limited. The extent to which sex-related differences affect the utility of biomarkers in

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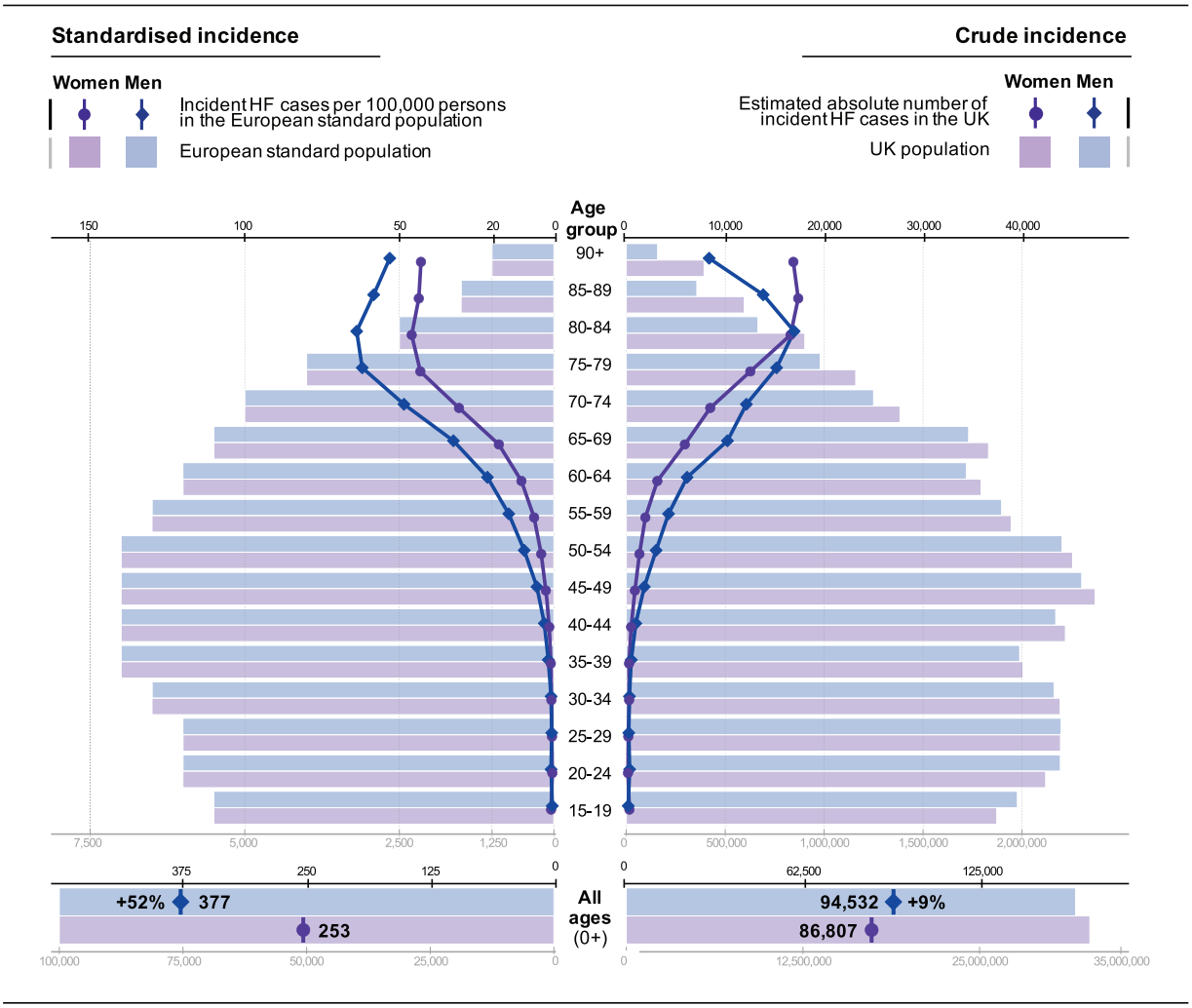

Figure 1 Overall and age-stratified incidence of heart failure (HF) in women and men. Standardized HF incidence (left panel) presents cases in 100 000 persons from the European standard population. Crude incidence (right panel) presents estimated absolute number of cases in the UK population (2014 census mid-year estimates). Age-standardized incidence of HF was 52% higher in men than in women. However, the total number of incident HF cases was only 9% higher in men. Reproduced with permission from Conrad *et al.*[3]

contemporary HF management is also unclear. The current review aims to address these issues.

Sex differences in heart failure biomarkers

In the following sections we will focus on the HF biomarkers with the greatest potential clinical relevance, based on the availability of robust biochemical assays and multiple publications demonstrating clinical utility beyond traditional HF risk factors.[13,14] These include NPs, as well as the more novel HF biomarkers,[18] which include cardiac troponins (cTns), galectin-3 and soluble interleukin-1 receptor-like 1 (sST2). We will also briefly discuss two potential HF biomarker candidates related to inflammation: growth differentiation factor-15 (GDF-15) and osteopontin. *Table 1* and *Figure 2* provide the reader with a synopsis of HF biomarkers and their chief sources, highlighting sex-specific aspects. *Figure 3B* illustrates sex-specific biomarker dynamics in healthy individuals and in HF patients. *Table 2* summarizes sex-specific data on the value of these biomarkers in HF prediction and prognosis.

Natriuretic peptides

Natriuretic peptides are a group of polypeptides secreted primarily by the heart, kidneys and the vascular endothelium. They regulate intravascular volume and arterial pressure, thereby maintaining fluid and cardiovascular homeostasis.[92,93] They are known to exert antifibrotic effects[94] and may also have a role in metabolic homeostasis.[95,96] The biological effects of NPs are usually mediated

Table 1 Heart failure biomarkers: major sources, impact of sex hormones and effects of obesity

Biomarkers (domains)	Major sources	Sex differences	
		Direct effect of sex hormones	Effects of adipose tissue
NPs[a] (myocardial stretch)	Heart (cardiomyocytes)[19]	**Present** • Testosterone suppresses NP levels[20–24] • Oestrogens may increase NP levels,[25] but more data needed	**Present** • Obesity is associated with lower levels of cardiac NPs[26–28] • In healthy individuals, male sex-related lowering of NPs is stronger than obesity-related effects,[26,27] which may explain lower NP levels in men despite lower fat mass
Cardiac troponins[b] (myocardial injury)	Heart (cardiomyocytes)[29]	**Unlikely**	**Present** • Obesity is associated with higher levels of cardiac troponins[30]
Galectin-3 (tissue fibrosis)	Adipose tissue,[31,32] lungs,[31] haematopoietic system Lesser extent: liver, heart (fibroblasts, resident macrophages)	**Unlikely**	**Strong** • Direct association with total body fat has been observed in both children and adults[33–36] • Higher percentage body fat may explain higher plasma levels in healthy women
sST2 (inflammation)	Lungs[37,38] Lesser extent: vascular endothelium, heart (cardiac endothelial cells, fibroblasts)[38,39]	**Unclear** • Weak correlation between sST2 and total testosterone/oestradiol in males[40] • Controversial evidence in women[40,41]	**Unlikely** • No significant association with body mass index in adults[41–43] • Weak association with waist circumference may exist[41]

NP, natriuretic peptide; sST2, soluble interleukin-1 receptor-like 1.
[a]NPs include N-terminal pro-B-type NP and B-type NP.
[b]Cardiac troponins include troponin T and I.

by binding to NP receptors (NPR-A and NPR-B), which are expressed in various tissues including the heart, vasculature, adipose tissue and kidneys.[97–99] Active clearance of NPs is facilitated via a third NP receptor (NPR-C), which is also widely distributed in many tissues including the adipose tissue and kidneys.[97,98] More general clearance mechanisms also exist, for instance, degradation of NPs by the enzyme neprilysin.[93,98,100]

Atrial NP (ANP) and B-type NP (BNP) are thought to be the most important NPs with regard to fluid regulation and blood pressure homeostasis, and are chiefly secreted by cardiomyocytes.[19] They bind to NPR-A, and elicit cardioprotective and antihypertensive effects by counter-regulating overactivity of the renin–angiotensin system, and also through natriuretic as well as vasodilatory effects.[93] They have an important role in contemporary HF management, with BNP and its amino-terminal-peptide fragment (NT-proBNP) being the most important molecules used to diagnose (or exclude) HF in patients presenting with acute dyspnoea (Class I, Level A evidence).[2,13,86,101]

In the general population, circulating levels of cardiac NPs are approximately two-fold higher in women compared with men (*Figure 3B*),[26,27,44,45] although such differences are not observed before puberty.[102] Currently, there is strong clinical evidence demonstrating that testosterone lowers cardiac NP levels,[20–24,103,104] which may partly explain the relative cardiac NP deficiency in men. The exact mechanism through which testosterone reduces cardiac NP levels remains poorly understood, although up-regulation of neprilysin activity by testosterone may be one possible explanation.[105,106]

The role of female sex hormones in modulating plasma concentrations of cardiac NPs appears to be complicated: although oestrogen may increase cardiac NP levels by directly increasing cardiac NP gene expression and release,[107,108] or by increasing the NPR-A to NPR-C ratio,[109–111] there are also reports suggesting that oestrogen increases neprilysin activity.[112,113] In the clinical setting, evidence regarding the association of endogenous female sex hormones with higher cardiac NP levels is limited; some studies, however, indicate that exogenous female hormone therapy may contribute to higher cardiac NP levels.[25,114]

In HF patients, sex differences in cardiac NP levels are inconsistent,[46–49] and on an average, their levels appear to be slightly higher in men (*Figure 3B*). This suggests that in diseased states associated with massive cardiac NP production, such as HF, more 'subtle' effects of sex hormones are overridden, and plasma levels may no longer reflect sex-specific changes. Nevertheless, HF is a complex phenotype, and differences in NP levels between men and women with HF should be interpreted with caution because

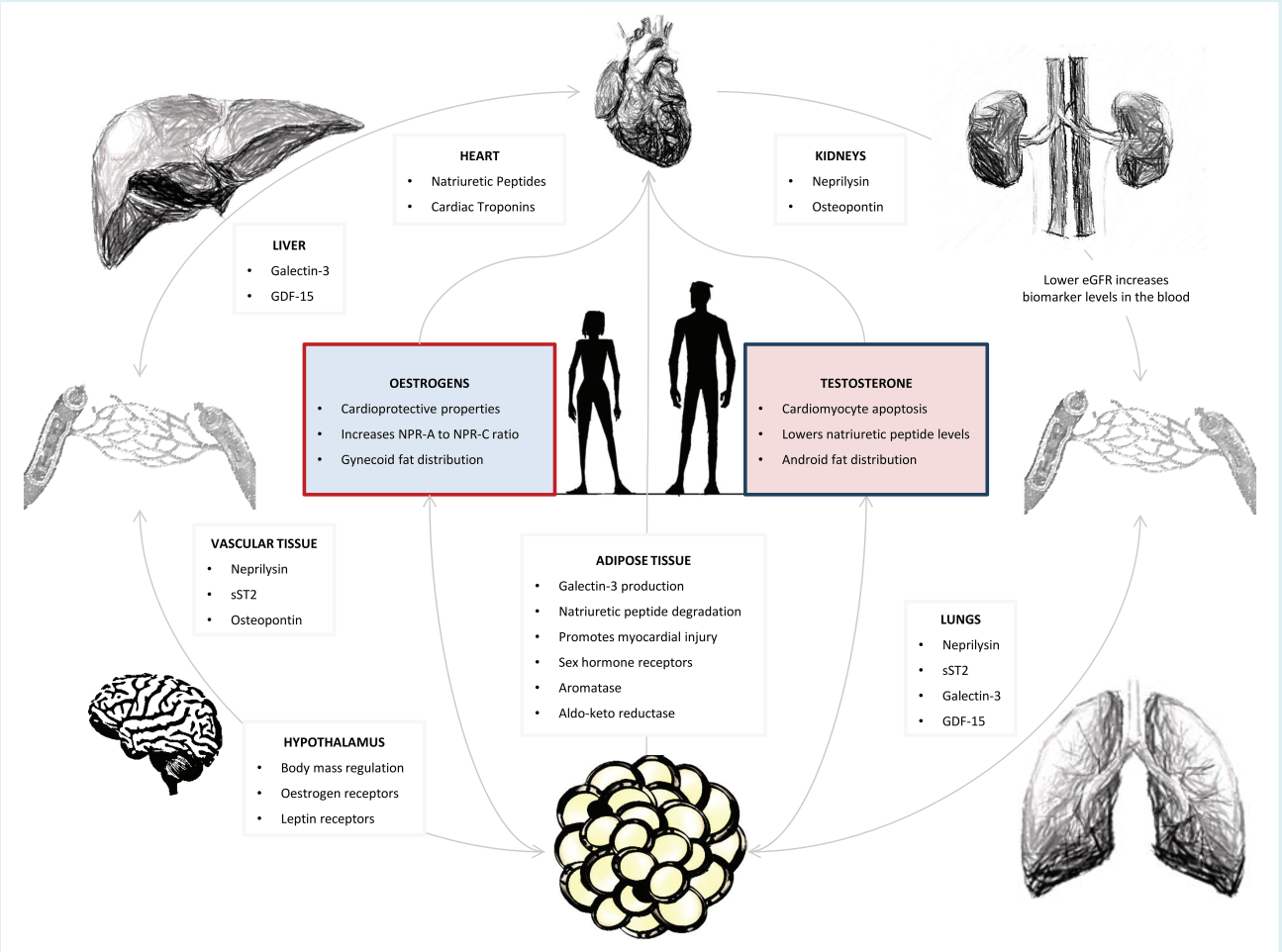

Figure 2 Heart failure biomarkers include cardiac-specific as well as non-cardiac biomarkers. This figure highlights the impact of sex hormones and adiposity on plasma concentrations of heart failure biomarkers. eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; NPR, natriuretic peptide receptor; sST2, soluble interleukin-like receptor-like 1.

such differences may relate to differential prevalence of HF with reduced ejection fraction (HFrEF) vs. HF with preserved ejection fraction (HFpEF) among men and women.[5,87,115,116]

Lower cardiac natriuretic peptide levels in heavier individuals: is this sex-related or obesity-related?

Obesity is known to promote a state of relative cardiac NP deficiency.[27,117,118] We recently showed that, in the general population, lower NT-proBNP levels in heavier individuals are better explained by sex than by obesity.[26] In other words, (male) sex-related lowering of NT-proBNP was more prominent than obesity-associated reduction in NT-proBNP levels (*Figure 4*). These observations may have clinical consequences with regard to the choice of optimal cut-off value to rule out HF. For instance, current guidelines recommend a universal NT-proBNP cut-off (125 ng/L in the non-acute setting) to exclude HF with confidence, and a reduced cut-off (~50% lower) in obese individuals.[88] However, median NT-proBNP levels are usually in the range of 45–70 ng/L in women, and 25–40 ng/L in men.[26,27] Given that, in the general population, sex strongly impacts cardiac NP levels (more so than even obesity), we argue that sex-specific cutpoints to rule out HF[119] (e.g. lower NT-proBNP cutpoints in men) should be embraced.

By contrast, in HF patients, sex-related effects appear to be subtle (*Figure 3B*), and obesity may play a greater role.[28,120–122] In fact, NT-proBNP levels are up to 60% lower in obese HF patients compared with their lean counterparts.[123] This suggests that in HF patients, a lower cutpoint should potentially be considered in obese individuals to estimate disease severity, and sex-specific cutpoints may be redundant. Future studies should examine this hypothesis in HF patients and also among individual HF subtypes.

Heart failure prediction and prognosis

In addition to their utility in HF diagnosis, NPs serve as valuable tools in preventive cardiovascular medicine, and strongly predict incident HF in the general population.[2,18,27,88,101] In a meta-analysis

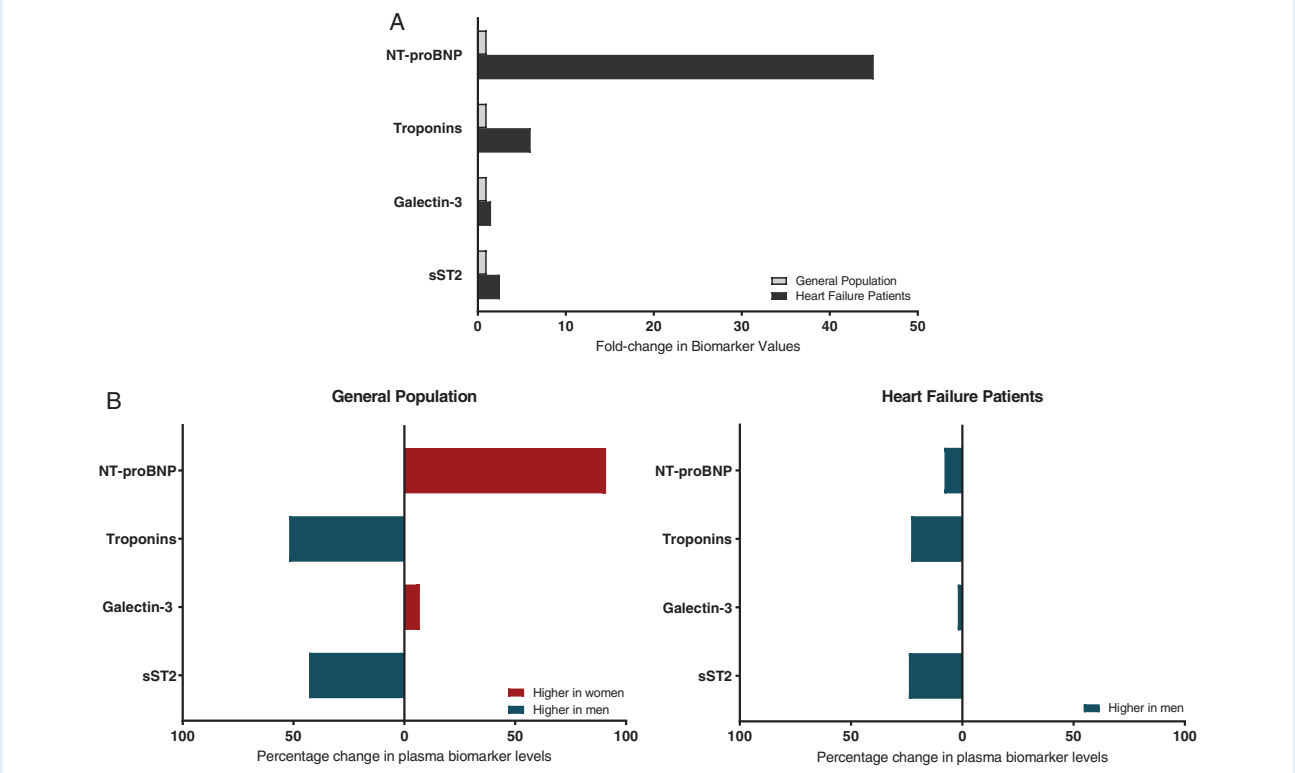

Figure 3 (*A*) An overview of relative proportions (i.e. fold change) of biomarker levels in heart failure (HF) patients (black) compared with community-dwelling individuals (grey) using pooled data from multiple studies.[24–27,30,33,40–42,44–85] On average, N-terminal pro-B-type natriuretic peptide (NT-proBNP) is ∼45-fold higher in HF patients compared with healthy individuals, followed by troponins (∼6-fold), soluble interleukin-1 receptor-like 1 (sST2, ∼2.5-fold), and galectin-3 (∼1.5-fold). (*B*) Impact of sex on circulating biomarker levels in the general population and in HF patients. The x-axis represents percentage increase in biomarker concentrations in women compared with men (red), and in men compared with women (blue). In community-dwelling individuals, NT-proBNP levels are ∼90% higher in women compared with men. Galectin-3 is also slightly higher in women, whereas cardiac troponins and sST2 are higher in men. In HF patients, sex-related differences in biomarker levels are attenuated, and on an average, all biomarkers are higher in men. The reader is advised to consider assay-related differences for more exact representation. Troponins include cardiac troponins T and I.

of 40 prospective studies (95 617 participants, 2212 HF events), the risk ratio for HF (comparing the top and bottom thirds of NT-proBNP concentrations after sex stratification and adjustment for clinical risk factors) was higher in men than in women [4.25 vs. 2.44; $P < 0.001$].[50] Another recently conducted prospective study including participants from four cohorts ($n = 78\,657$) also reported a similar trend: NT-proBNP (measured in 30 443 individuals) was more strongly associated with incident HF in men than in women [hazard ratio (HR) 1.89 vs. 1.54; $P = 0.006$].[51] NPs also strongly predict outcomes in HF[46–48,52–59,87] with some evidence that NT-proBNP may be a superior predictor of mortality and HF readmission in men.[49]

Cardiac troponins

The troponin complex consists of three subunits regulating actin–myosin interaction: troponin C (TnC; the calcium-binding subunit), troponin T (TnT; the tropomyosin-binding subunit), and troponin I (TnI; the inhibitory subunit).[124] Troponins relevant to cardiology practice include cardiac-specific isoforms of TnT and TnI (i.e. cTns).[125] Even minor elevations in circulating cTns raise the suspicion of ongoing cardiac damage[29,30,126] although such findings do not provide any information about the cause of myocardial injury.

In healthy individuals, circulating cTn levels are higher in men than women.[127,128] For instance, median values were ∼53% higher in men using the Roche Diagnostics cTnT assay [pooled median values ± standard deviation (SD): 5.5 ± 2.2 ng/L in men vs. 3.6 ± 1.3 ng/L in women],[60–64] and ∼44% higher in men with the Abbott cTnI assay (2.6 ± 1.1 ng/L in men vs. 1.8 ± 1.0 ng/L in women).[60,62,65] An illustrative overview of sex-related differences in the 99th percentile values for cTnT assay (Roche Diagnostics) and cTnI assays (Abbott Diagnostics, Beckman Coulter, Singulex and Siemens) using data from over 30 population-based studies was recently provided by Romiti and colleagues.[128]

In HF patients, plasma cTn levels rise several fold (*Figure 3A*),[66,129,130] and on average, men have higher cTn levels compared with women (*Figure 3B*).[67–69] For example, in a study

Table 2 Sex-specific predictive and prognostic value of heart failure biomarkers

Biomarkers	Predicting incident heart failure		Predicting outcomes in heart failure	
	Total population	Sex-specific data	Total population	Sex-specific data
Natriuretic peptides[a]	Strong evidence[50,51,53]	• RR in men > women: 4.25 vs. 2.44 ($P<0.001$). Type of study: meta-analysis of prospective cohort studies[c]; $n=95\,617$[50] • HR in men > women: 1.89 (95% CI 1.75–2.05) vs. 1.54 (95% CI 1.37–1.74) ($P=0.006$). Type of study: prospective cohort study[d]; $n=30\,443$[51] • Sex-specific cutpoints for HF diagnosis/prediction not routinely used in clinical practice[86]	Strong evidence[2,18,87,88]	• HR for composite events in men > women: 1.74 (95% CI 1.25–2.43) vs. 1.17 (95% CI 0.84–1.56). Type of study: prospective cohort study enrolling patients with acute HF; $n=2280$[49]
Cardiac troponins[b]	Strong evidence[53,60,70,89]	• HR comparable in men and women: 2.29 (95% CI 1.64–3.21) vs. 2.18 (95% CI 1.68–2.81). Type of study: meta-analysis of prospective cohort studies[e]; $n=67\,073$[70]	Strong emerging evidence[71,73]	• HR for all-cause mortality comparable in men and women using a universal cTnT cutpoint of 18 ng/L [1.48 (95% CI 1.41–1.57) vs. 1.48 (95% CI 1.34–1.62)]. Type of study: meta-analysis of cohort studies enrolling patients with chronic HF; $n=9289$.[73] • HR for composite events in men > women using cTnI assay [3.33 (95% CI 1.82–6.09) vs. 1.35 (95% CI 0.94–1.93)]. Type of study: prospective cohort study enrolling patients with HF with preserved ejection fraction; $n=1096$.[74]
Galectin-3	May predict incident HF[80] Serial measurements preferable[90,91]	• Limited	Moderate evidence[14,80] Universal cutpoint: 17.8 μg/L	• Limited
sST2	May predict incident HF[53,82]	• Limited	Strong emerging evidence[83–85] Universal cutpoint: 35 μg/L	• Limited

CI, confidence interval; cTnI, cardiac troponin I; cTnT, cardiac troponin-T; RR, risk ratio; HR, hazard ratio; HF, heart failure; sST2, soluble interleukin-1 receptor-like 1.
[a]Natriuretic peptides include N-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide.
[b]Cardiac troponins include cTnT and cTnI.
[c]Community-dwelling individuals without baseline cardiovascular disease were included for analyses. Sex-specific secondary analysis was performed in a subset.
[d]Community-dwelling individuals without baseline HF were included for analyses. N-terminal pro-B-type natriuretic peptide was measured in 30 443 individuals.
[e]Community-dwelling individuals without baseline HF were included for analyses. Sex-specific secondary analysis was performed in a subset.

including stable HF patients, median cTnT levels were 23 ng/L in men and 18 ng/L in women.[67] Several mechanisms have been proposed to explain raised cTns in HF,[131,132] but the exact pathophysiology of sex-related differences remains to be elucidated. We postulate that a greater prevalence of cardiac comorbidities[133–135] (e.g. atrial fibrillation, ventricular arrhythmias, coronary artery disease, cardiomyopathies, myocarditis) and male-specific hormonal mechanisms[136] (e.g. testosterone-induced hypertrophy and apoptosis of cardiomyocytes) contribute to higher cTn levels in men with HF. By contrast, more subtle mechanisms of myocardial injury[137,138] (e.g. coronary microvascular disease), along with the cardioprotective effects of oestrogen[139–142] (e.g. suppression of cardiomyocyte apoptosis), may translate into relatively lower cTn levels in women presenting with HF.

According to data from the study conducted by Ndumele and colleagues ($n=9507$), obesity was strongly associated with elevated cTns.[30] It is hypothesized that adipokines released from the fat tissue may potentiate cardio-deleterious signals or even directly damage the cardiac tissue,[143] resulting in adverse cardiac remodelling[144,145] and in cardiac steatosis.[146] Given the differences

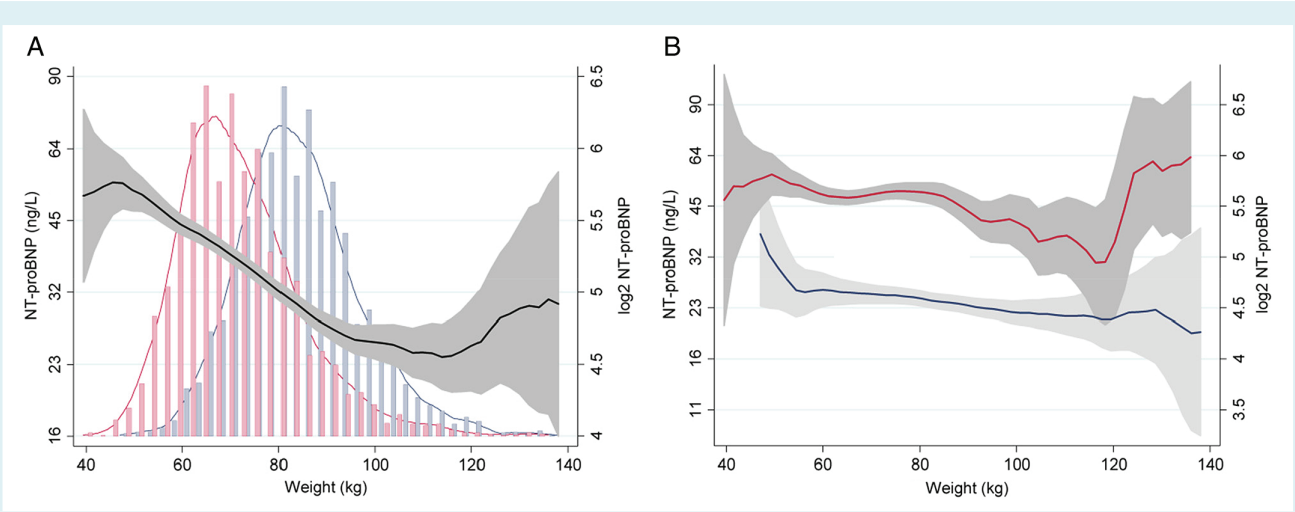

Figure 4 Impact of sex and obesity on N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels in the general population. In the general population, lower NT-proBNP levels in heavier individuals can be better explained by (male) sex than by obesity. (*A*) Black lines represent median NT-proBNP levels in the overall population; grey bands represent prediction intervals of median NT-proBNP; histograms represent distribution of bodyweight in men (blue) and women (red). (*B*) Sex-specific associations of body weight and NT-proBNP. Blue lines represent median NT-proBNP levels in men; red lines represent median NT-proBNP levels in women; grey bands represent prediction intervals of median NT-proBNP. Reproduced with permission from Suthahar *et al.*[26]

in fat distribution among men and women,[147] and the higher global prevalence of obesity in women,[148] examining sex differences in obesity cardiomyopathy may potentially be an exciting avenue of research.

Heart failure prediction and prognosis

The value of cTns in HF diagnosis is limited. However, cTns strongly predict incident HF in the general population[53,60,89,126], and in a meta-analysis of 16 studies (67 063 individuals and 4165 HF events), the predictive value of cTns for incident HF was comparable in men and women (*Table 2*).[70] cTns can also potentially be used to risk-stratify HF patients, although the level of evidence for this is currently lower than for NPs.[2,13,101] Nevertheless, evidence offered by the current body of literature is gaining momentum, emphasizing the strong and independent performance of cTns in prognosticating outcomes in both acute[71,72] and chronic[73] HF patients. In a meta-analysis of 11 cohort studies including chronic HF patients ($n = 9289$), cTnT was a robust predictor of outcomes, and the prognostic value of cTnT for all-cause death was similar in men and women[73] (*Table 2*). Recently Gohar and colleagues reported that both cTnT and cTnI strongly predicted outcome (all-cause mortality or HF rehospitalization) in patients with HFpEF. Interestingly, cTnT was similarly associated with adverse events in both sexes, whereas cTnI (measured using a more sensitive assay) was more strongly associated with adverse events in men with HFpEF (HR 3.33, $P < 0.001$) than in women with HFpEF (HR 1.35, $P = 0.100$).[74] Nevertheless, limited data on sex-related differences in the prognostic value of cTns in HF patients preclude the drawing of any definitive conclusions.

Galectin-3

Galectin-3 is a pro-fibrotic protein secreted by several cell types including macrophages, and is involved in pathways leading to fibrosis of various organs including the heart, lungs, liver and kidneys.[31] Unlike NPs and cTns, plasma levels of galectin-3 are chiefly maintained by contributions from non-cardiac sources (e.g. adipose tissue, lungs, haematopoietic tissue, liver).[31,32] According to data from four large population-based studies (using BG Medicine,[33,75] Alere,[76] or ARCHITECHT[77] assays), women consistently exhibited slightly higher levels of galectin-3 than men (pooled median value ± SD: 13.2 ± 1.2 μg/L in women and 12.3 ± 1.4 μg/L in men) (*Figure 3B*). The reason for this sex-specific effect is unknown although differences in fat mass may be a likely explanation. Indeed, strong associations between adiposity and galectin-3 levels have been observed in both population-based studies[33–35] and animal studies.[32,149] Recently, a comprehensive analysis was performed in children ($n = 170$) using more accurate estimates of body fat mass and distribution [i.e. with dual energy X-ray absorptiometry (DEXA)].[36] A strong association between total body fat and galectin-3 levels was observed, indicating that adipose tissue mass, and not the direct effect of sex hormones, would better explain the galectin-3 'excess' in women. Galectin-3 levels are generally higher in HF patients than in healthy individuals[78] (*Figure 3A*). For instance, the pooled median galectin-3 value ± SD in HF patients from multiple studies[78] (using BG Medicine, Alere or ARCHITECHT assays) was 18.8 ± 2.8 μg/L. Interestingly, in HF patients, sex differences in plasma concentrations of galectin-3 are inconsistent, and on an average, men tend to have slightly higher galectin-3 levels than women[52,79] (*Figure 3B*). This suggests that in HF, the production and clearance of galectin-3 change so that the dynamics

and biology governing homeostasis under normal circumstances no longer operate in disease.

Heart failure prediction and prognosis

Galectin-3 was significantly associated with incident HF in community-dwelling individuals from the FHS ($n = 3353$)[75] and FINRISK ($n = 8444$)[77] studies, but not in the PREVEND cohort ($n = 8569$).[150,151] In a recent meta-analysis of 18 studies ($n = 32\,350$),[80] as well as in a pooled analysis of four community-based cohorts ($n = 22\,756$),[53] galectin-3 remained associated with incident HF. However, none of these studies evaluated sex-specific associations of galectin-3 with incident HF as the primary outcome. In the FINRISK cohort, sex-stratified subanalyses were conducted and galectin-3 levels appeared to be similarly associated with HF in both sexes.[77]

As galectin-3 is a relatively stable biomarker, serial measurements would provide more precise information about an ongoing disease process (e.g. cardiac fibrosis) compared with a random one-time measurement. Indeed, longitudinal changes in galectin-3 levels predicted incident HF in both the FHS ($n = 2477$) and PREVEND ($n = 5958$) cohorts, also after extensive adjustment for cardiovascular risk factors.[90,91] To date, no study has examined whether longitudinal changes in galectin-3 predict new-onset HF differentially in men and women.

Galectin-3 measurements can be used for risk stratification and prognostication in acute and chronic HF patients [Class IIb recommendation; American College of Cardiology (ACC)/American Heart Association (AHA) HF guidelines],[13,14,101,152] and low discharge galectin-3 values (<10th percentile) identify a relatively stable and low-risk subpopulation of HF patients.[153] We lack data on the sex-specific prognostic value of galectin-3 in HF patients.

Soluble interleukin-1 receptor-like 1

The soluble form of ST2 (sST2) is speculated to indirectly promote myocardial damage by acting as a 'decoy' receptor of interleukin-33 (IL-33); that is, circulating sST2 binds to IL-33 and blocks the cardioprotective effects generated by the interaction between IL-33 and the transmembrane ST2 ligand (i.e. IL-33/ST2L interaction).[154] Non-cardiac sources, particularly pulmonary tissue,[37,38] may be more important in maintaining plasma sST2 levels, although production from vasculature and cardiac endothelial cells has also been recognized.[39]

Sex differences in sST2 levels are not observed in children aged <15 years.[155] However, sex differences become apparent in older children (≥15 years), with males demonstrating higher levels of sST2 compared with females.[155] These sex-related differences persist in both healthy individuals[41,43,156,157] (average median values ± SD: 24.0 ± 0.78 μg/L in men and 17.2 ± 1.18 μg/L in women), as well as in HF patients[52,81,158] (*Figure 3B*). Although male sex appears to be consistently associated with higher sST2 levels, the direct effect of sex hormones may only partly explain this phenomenon. For instance, in men, both testosterone levels as well as estradiol were significantly (but weakly) associated with sST2 levels.[40] In women, exogenous oestrogen therapy was associated with lower sST2 levels,[41] whereas in another study sex hormones did not correlate with sST2 levels.[40] Therefore, other potential mechanisms that would better explain this difference (also in HF) need to be elucidated. Finally, a significant association between obesity and sST2 levels has not been reported in population-based studies,[40,42,156] although some animal studies indicate that sST2 expression is decreased in adipose tissue, heart and liver of obese mice compared with non-obese controls.[159]

Heart failure prediction and prognosis

Elevated sST2 levels predict incident HF to some extent,[53,82] but sex-specific data are limited. Currently, sST2 has only a Class IIb recommendation for risk stratification in acute and chronic HF patients (ACC/AHA HF guidelines),[13,101] and a universal prognostic cutpoint of 35 μg/L has been proposed.[13,82] However, current data indicate that sST2 measurements predict outcomes in both acute[83] and chronic[84] HF patients. Recently, Emdin and colleagues demonstrated that in chronic HF patients ($n = 4268$), sST2 was significantly associated with HF hospitalization and mortality and also provided prognostic information beyond NT-proBNP and cTnT.[85] Whether sST2 measurements predict HF outcomes differentially in men and women, and whether choosing sex-specific cutpoints would further refine risk prediction in HF patients is not currently known, and should be investigated in future studies.

Potential heart failure biomarkers: growth differentiation factor-15 and osteopontin

Growth differentiation factor-15 is a member of the transforming growth factor-β (TGF-β) cytokine superfamily with anti-apoptotic, anti-hypertrophic and anti-inflammatory properties. It is abundantly expressed in extracardiac tissues (e.g. lungs, liver and kidneys),[32,160,161] whereas the heart has only moderate GDF-15 expression.[32] Sex differences in plasma levels are not clearly observed,[162] although women may have slightly lower GDF-15 levels than men.[163,164] GDF-15 is strongly associated with incident HF[165,166] and can potentially be used in conjunction with other HF biomarkers to optimize HF prediction.[165] GDF-15 also strongly predicts outcomes in HF patients.[164,167–169] However, sex-specific data are lacking.

Osteopontin is a secreted matricellular glycoprotein expressed primarily in extracardiac tissues (e.g. the kidneys and luminal epithelial surfaces of various organs).[170] Osteopontin expression is up-regulated in HF, hypertension and various inflammatory conditions including obesity.[171–175] High cardiac osteopontin expression promotes myocardial fibrosis and increases left ventricular stiffness by facilitating the formation of insoluble collagen.[174,176] Interestingly, osteopontin deficiency ameliorates myocardial fibrosis and improves cardiac function,[177] indicating that osteopontin may emerge as an attractive biotarget in the treatment of cardiovascular disease.[178] In humans, plasma osteopontin levels appear to be lower in women,[179,180] and it is suggested that oestrogen suppresses osteopontin expression in the vascular

tissue.[181] Currently, there is strong evidence highlighting the prognostic value of osteopontin in HF patients,[182–184] although sex-specific data are lacking.

State-of-the-art: the relevance of sex-specific dynamics in heart failure biomarkers

Heart failure biomarkers are indispensable tools in contemporary cardiovascular medicine, and may play an even greater role in the future. Overall, it appears that sex-specific dynamics in biomarker levels operate primarily in healthy individuals and to a lesser extent in HF patients. Interestingly, biomarkers displaying lower levels in healthy women (cTns and sST2) also display lower levels in women with HF. By contrast, biomarkers displaying higher levels in healthy women (NPs and galectin-3) do not consistently exhibit higher levels in women with HF. Although these observations may be intriguing from a biological point of view, their clinical relevance is likely to be limited.

Two potential exceptions could be NPs and cTns, in which sex-specific differences have been repeatedly observed, but these

Table 3 Future directions: potential research questions

HF biomarkers	Knowledge gaps
Natriuretic peptides (NPs)	• What are the mechanisms through which testosterone lowers plasma cardiac NP levels? • What is the role of female sex hormones in modulating plasma NP levels? • How do sex hormones affect neprilysin levels/activity? • When NPs are used to rule out HF, are sex-specific cutpoints relevant? • In HF patients, are baseline sex-related differences in NP levels absent (or present) when HF subtypes are separately considered? • Does obesity-associated lowering of NP levels in HF patients have a significant sex-related component?
Cardiac troponins (cTns)	• Are sex-specific cTn cutpoints relevant in predicting incident HF, and in predicting outcomes in HF? • Do obesity-related myocardial injury mechanisms differ between men and women?
Galectin-3	• Do longitudinal changes in galectin-3 predict incident HF and outcomes related to HF differentially in men and in women? Is the predictive value of galectin-3 different in lean vs. overweight individuals?
sST2	• Why are sST2 levels consistently higher in men than in women? What is the role of sex hormone levels in determining sST2 levels? • Will sex-specific sST2 cutpoints improve HF risk prediction?

HF, heart failure; sST2, soluble interleukin-1 receptor-like 1.

Table 4 Reporting template for sex-specific biomarker analysis

	Recommendations
1. Sex-specific plasma concentrations	• Sex-specific plasma biomarker concentrations should be provided, even if significant baseline differences are not observed • Age-adjusted biomarker concentrations should be provided where necessary
2. Sex-specific cutpoints	• In biomarkers displaying (clinically relevant) baseline sex differences, optimal sex-specific cutpoints to predict heart failure, diagnose (rule in/rule out) heart failure, or prognosticate outcomes in heart failure should be identified • If no sex-specific cutpoint was identified, this should also be mentioned
3. Sex-specific risk ratios	• Crude and age-standardized event rates in men and women should be mentioned • When comparing risk ratios, studies should not only provide *P*-values for sex*biomarker interaction on a multiplicative scale, but also hazard ratios or odds ratios of the interaction term along with the corresponding 95% confidence intervals • Sex-stratified coefficients should be provided (at least in the supplementary information) for future meta-analysis of results[185]
4. Sex-specific prediction models using biomarkers	• Sole reliance on improvement in C-statistic (discrimination) to identify sex-specific predictive utility of biomarkers (beyond an established clinical model) is not advised due to its limited sensitivity[186–188] • Other often ignored measures such as the Wald statistic, likelihood ratio test, chi-squared statistic and Akaike/Bayesian information criteria are more powerful in assessing model improvement,[188] and should also be considered in sex-specific biomarker selection

differences have not (yet) been used in sex-specific diagnostic or prediction models. In this context, we would like to reiterate that in the general population, male sex explains lower cardiac NP levels to a greater extent than obesity. Therefore, using sex-specific cutpoints (i.e. lower cutpoints in men) may (theoretically) rule out HF more accurately in men and this deserves further study. In contrast to NPs, circulating cTn levels are lower in women than in men. Although the clinical relevance of sex-specific cTn cutpoints in HF prevention is currently under-recognized, the development of ultra-sensitive cTn assays may unmask subtle sex-related differences. This, together with the generation of high-quality data, could potentially lead to the clinical application of sex-specific cutpoints (i.e. lower cutpoints in women), which may help to identify future HF risk, as well as risk associated with HF more effectively in women.

In summary, we have reviewed sex-specific aspects of key HF biomarkers, and highlighted the fact that our current understanding of factors contributing to sex-related differences in HF biomarkers, and the clinical relevance of these findings, is insufficient. We have identified several knowledge gaps that could potentially serve as "focus points" for future research on sex-related differences in HF biomarkers (*Table 3*). We also provide key recommendations for sex-specific biomarker analyses in *Table 4*,[185–188] and strongly advocate that future studies should examine the clinical value of HF biomarkers in men and women separately. Such an approach may uncover important sex-related differences,[185] and may ultimately improve HF management and patient care.

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