ESC HEART FAILURE
ESC Heart Failure 2022; **9**: 2084–2095
Published online 5 May 2022 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.13883

ORIGINAL ARTICLE

Circulating levels and prognostic cut-offs of sST2, hs-cTnT, and NT-proBNP in women vs. men with chronic heart failure

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Abstract

Aims To define plasma concentrations, determinants, and optimal prognostic cut-offs of soluble suppression of tumorigenesis-2 (sST2), high-sensitivity cardiac troponin T (hs-cTnT), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in women and men with chronic heart failure (HF).

Methods and results Individual data of patients from the Biomarkers In Heart Failure Outpatient Study (BIOS) Consortium with sST2, hs-cTnT, and NT-proBNP measured were analysed. The primary endpoint was a composite of 1 year cardiovascular death and HF hospitalization. The secondary endpoints were 5 year cardiovascular and all-cause death. The cohort included 4540 patients (age 67 ± 12 years, left ventricular ejection fraction 33 ± 13%, 1111 women, 25%). Women showed lower sST2 (24 vs. 27 ng/mL, $P < 0.001$) and hs-cTnT level (15 vs. 20 ng/L, $P < 0.001$), and similar concentrations of NT-proBNP (1540 vs. 1505 ng/L, $P = 0.408$). Although the three biomarkers were confirmed as independent predictors of outcome in both sexes, the optimal prognostic cut-off was lower in women for sST2 (28 vs. 31 ng/mL) and hs-cTnT (22 vs. 25 ng/L), while NT-proBNP cut-off was higher in women (2339 ng/L vs. 2145 ng/L). The use of sex-specific cut-offs improved risk prediction compared with the use of previously standardized prognostic cut-offs and allowed to reclassify the risk of many patients, to a greater extent in women than men, and for hs-cTnT than sST2 or NT-proBNP. Specifically, up to 18% men and up to 57% women were reclassified, by using the sex-specific cut-off of hs-cTnT for the endpoint of 5 year cardiovascular death.

Conclusions In patients with chronic HF, concentrations of sST2 and hs-cTnT, but not of NT-proBNP, are lower in women. Lower sST2 and hs-cTnT and higher NT-proBNP cut-offs for risk stratification could be used in women.

Keywords Chronic heart failure; Sex; Women; Prognosis; sST2; High-sensitivity troponin T; NT-proBNP

Received: 30 December 2021; Revised: 17 February 2022; Accepted: 2 March 2022
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Introduction

Chronic heart failure (HF) is a highly prevalent condition characterized by multiple clinical phenotypes.[1,2] Significant sex-related differences have been observed in HF patients. Although the lifetime risk of developing HF is similar for women and men, women more often show a preserved left ventricular ejection fraction (LVEF, HFpEF).[3] Distinct pathophysiological substrate may explain these differences, whereas ischaemic heart disease and neurohormonal activation prevail in men, mechanisms related to immune activation and inflammation may be prevalent in women.[3,4] Whether such different pathways may affect the prognostic role of HF biomarkers remains controversial.[5,6]

Soluble suppression of tumorigenesis-2 (sST2) and cardiac troponin T measured with high-sensitivity assay (hs-cTnT) are relevant biomarkers for risk stratification in HF.[7,8] Although male sex has been associated with higher concentrations of both sST2 and hs-cTnT in healthy individuals and in HF patients, the determinants of such discrepancy are unknown.[5,6] Furthermore, the possible influence of sex on the best cut-offs of sST2 and hs-cTnT for risk prediction in HF patients has never been investigated so far. Natriuretic peptides are essential tools for the diagnosis and risk prediction in HF.[9,10] In the general population, women show modestly higher concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) than men, possibly because of the effects of sex hormones and/or a different body-fat distribution.[11] The difference in natriuretic peptides' concentrations between sexes appears less prominent in HF patients,[12] likely because of a greater impact of disease-related factors, including more ischaemic heart disease in men and greater prevalence of HFpEF in women.[5,6] NT-proBNP is independently predictive of outcomes in both men and women, but the potential additional risk stratification of sex-specific marker thresholds is unknown.[13,14]

In the present study, we tested the impact of sex on sST2, hs-cTnT, and NT-proBNP concentrations, on their prognostic value and optimal cut-offs for risk prediction in a large international cohort of patients with chronic HF.

Methods

Study population

The Biomarkers In Heart Failure Outpatient Study (BIOS) consortium includes 13 cohorts of patients with stable chronic HF and available NT-proBNP. The dataset was built starting from a core population collected in 2018 (from 11 original cohorts) and used to perform an individual patient data meta-analysis on the prognostic value of hs-cTnT in chronic HF [n = 9289 (8)]. Then, patients from other trials with similar inclusion criteria were included (up to 15 681 individuals). For the present study, 4540 patients from six cohorts[15–20] with sST2, hs-cTnT, and NT-proBNP data were selected (Supporting Information, *Table S1*). Patients were clinically stable for at least 1 month before samples for markers were collected. Patients with acute coronary syndromes, cardiac surgery, or urgent hospitalization for acute HF in the previous 3 months, severe neurological conditions, active cancer, or liver failure were excluded.

Clinical data were collected at recruitment. LVEF was measured by 2D echocardiography through the modified Simpson's method.[21] HF was diagnosed following the recommendations of the European Society of Cardiology.[9] According to the universal definition of HF, patients were classified as having HFrEF (LVEF $\leq$ 40%), HF with mildly reduced LVEF (HFmrEF, LVEF 41–49%), or HFpEF (LVEF $\geq$ 50%).[9,22] The chronic kidney disease (CKD) epidemiology collaboration equation was used to calculate estimated glomerular filtration rate (eGFR).[23] The Presage® assay [limit of detection 1.3 ng/mL, measurement range up to 200 ng/mL, intra-assay CV $<$ 7%, inter-assay CV $<$ 9%] was used to measure sST2, the Roche Diagnostics® assay (limit of blank 3 ng/L, limit of detection 5 ng/L, 99th percentile value in apparently healthy individuals of 14 ng/L) for hs-cTnT, and the monoclonal electrochemiluminescence immunoassay method [Roche Diagnostics®; coefficient of variation $<$ 3% at cut-off value (150 ng/L)] for NT-proBNP. While sST2 was measured on EDTA plasma samples stored at $-$20°C, hs-cTnT and NT-proBNP were assayed at the time of recruitment.

Considering that biomarkers concentrations, which may oscillate over time, were assessed only at the time of enrolment in our patients, a composite of cardiovascular (CV) death and HF hospitalization at 1 year was considered as primary endpoint. Longer term hard endpoints, that is, 5 year CV and all-cause death, were instead reported as secondary endpoints. All patients provided informed consent for the study, which was approved by the Institutional Ethics Committee and conducted in accordance with the Declaration of Helsinki of the World Medical Association.

Statistical analysis

SPSS (IBM Statistics, Version 25.0, 2017) and R software (Version 3.2.3) and the related interface EZR (Saitama Medical

Center, Jichi Medical University, Saitama, Japan)[24] were used for statistical analysis. Normal distribution was assessed by plotting a histogram and running the Kolmogorov–Smirnov test. Normally distributed variables were reported as mean ± standard deviation, non-normally distributed variables as median and interquartile interval. Categorical data were reported as frequencies. The study population was distinguished into sex categories and quantitative variables were compared through the *t*-test for independent samples or the Mann–Whitney test, according to distribution, while χ^2 or Fisher test were used for qualitative variables. Further comparisons were performed across various patient subgroups [i.e. age, body mass index (BMI), and eGFR strata, LVEF classes, HF aetiology, history of atrial fibrillation (AF), hypertension, diabetes, or chronic obstructive pulmonary disease (COPD)]. Linear regression analysis was used to identify sex-specific predictors of sST2, hs-cTnT, and NT-proBNP, considering ln-transformed eGFR because non-normally distributed. As for sST2, hs-cTnT, and NT-proBNP concentrations, they were Log2-transformed before entering regression models, so that risk estimation should be considered for each doubling in their values.

At survival analysis, the independent prognostic value of sST2, hs-cTnT, and NT-proBNP was assessed through the Fine and Gray's proportional sub-hazards model (considering non-CV death as a competing risk for CV death, and all-cause death for HF hospitalization), adjusting the model for established outcome predictors[viz. age, LVEF, ischaemic aetiology, New York Heart Association (NYHA) class III–IV, history of AF, hypertension, diabetes mellitus, and CKD], and the incremental value of the three biomarkers when added to the model was evaluated through the difference in Harrell's *C*-statistic. The optimal biomarkers cut-offs for receiver-operating characteristics curves were assessed through the Youden's *J* statistics for each endpoint, and in men and women separately, whereas the DeLong's test was used to compare two receiver-operating characteristics curves. Cubic spline interpolation was carried out to represent the changes in risk according to biomarker values; five knots were considered and the biomarker value for which hazard ratio = 1 was chosen as the value corresponding to the inflection point of the curve, above which the slope of the curve becomes steeper. Patients were then stratified according to the number and the type of biomarkers over the calculated sex-specific cut-off, and the risk for the primary and secondary endpoints across these categories was expressed as relative risk (considering the patients with no increased biomarkers as the reference category). Kaplan–Meier method and log-rank statistics were used to estimate survival according to the number of increased biomarkers. The integrated discrimination improvement and continuous net reclassification improvement were calculated to assess reclassification of patients when considering the sex-specific cut-offs vs. single cut-offs applied to men and women alike, in a prognostic model further adjusted for patients' age and LVEF. Finally, to account for possible confounders unevenly influencing concentrations and prognostic cut-offs of sST2, hs-cTnT, and NT-proBNP in women and men, a propensity-score matching analysis was performed. To this purpose, a propensity score was calculated by a logistic regression model accounting for age, LVEF, aetiology of HF, NYHA class III–IV, and eGFR and the greedy nearest neighbour algorithm, with fixed calliper width of 0.2, was used to obtain a 1:1 matched-pairs cohort of women and men. Sex-specific differences in biomarkers concentrations and prognostic cut-offs for risk prediction were hence tested also within the matched cohort. Two-tailed P value < 0.05 were considered as significant.

Results

Study population

The cohort included 4540 patients (age 67 ± 12 years; 1111 women, 25%; LVEF 33 ± 13%; HFrEF 84%, HFmrEF 8%, HFpEF 8%) (*Table 1*). HFrEF was more prevalent in men (88% vs. 73%, $P < 0.001$), while HFmrEF and HFpEF in women (10% vs. 7% and 17% vs. 5%; both $P < 0.001$). Women less often had an ischaemic aetiology (55% vs. 70%, $P < 0.001$) and were more symptomatic (NYHA class III–IV in 52% vs. 44%, $P < 0.001$). Hypertension (71% vs. 62%, $P < 0.001$) and CKD (59% vs. 53%, $P < 0.001$) were more prevalent in women, while chronic obstructive pulmonary disease was slightly more common in men (COPD; 13% vs. 15%, $P = 0.006$).

Concentrations and predictors of biomarkers in women and men

In the whole cohort, sST2 was lower in women than men [24 ng/mL (17–36) vs. 27 ng/mL (20–40), $P < 0.001$] (*Figure 1*) and within most subgroup except for patients older than 75 years, underweight, with LVEF > 40%, or history of AF (*Table S2*). Some sST2-independent predictors were common to both sexes (LVEF, AF, diabetes, and haemoglobin), while BMI, ischaemic aetiology, and eGFR among men only (*Tables S3* and *S4*).

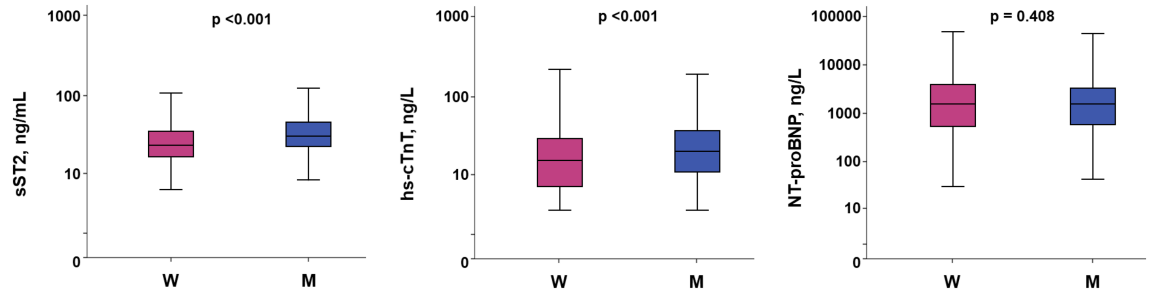
Similar to sST2, hs-cTnT was lower in women [15 ng/L (7–29) vs. 20 ng/L (11–36), $P < 0.001$] in the whole population (*Figure 1*). This difference was observed in all subgroups except for patients with HFmrEF, underweight, and in those with eGFR < 30 mL/min/1.73 m^2 (*Table S2*). Increasing age, BMI, and presence of AF, hypertension, diabetes, haemoglobin, and reduced eGFR independently predicted

Table 1 General features of the study population and comparisons between women (W) and men (M)

	All patients (n = 4540)	W (n = 1111, 25%)	M (n = 3429, 75%)	P
Clinical features				
Age (years)	67 ± 12	69 ± 12	66 ± 11	<**0.001**
BMI (kg/m2)	27 ± 5	27 ± 6	27 ± 5	0.067
LVEF (%)	31 ± 11	35 ± 13	29 ± 10	<**0.001**
HFrEF, *n* (%)	2824 (84)	804 (73)	3020 (88)	<**0.001**
HFmrEF, *n* (%)	339 (8)	114 (10)	225 (7)	<**0.001**
HFpEF, *n* (%)	341 (8)	187 (17)	154 (5)	<**0.001**
Ischaemic aetiology, *n* (%)	3003 (66)	607 (55)	2396 (70)	<**0.001**
NYHA class III–IV, *n* (%)	1091 (46)	576 (52)	1516 (44)	<**0.001**
Comorbidities				
Atrial fibrillation, *n* (%)	907 (20)	224 (20)	683 (20)	0.468
Hypertension, *n* (%)	2896 (64)	786 (71)	2110 (62)	<**0.001**
Diabetes mellitus, *n* (%)	1816 (40)	467 (42)	1349 (39)	0.095
Hb (g/dL)	13.2 ± 1.7	12.3 ± 1.5	13.5 ± 1.7	<**0.001**
Creatinine (mg/dL)	1.2 (1.0–1.5)	1.1 (0.9–1.3)	1.3 (1.1–1.5)	<**0.001**
eGFR (mL/min/1.73 m2)	57 (45–69)	54 (41–68)	58 (46–70)	<**0.001**
CKD stage 3–5, *n* (%)	2461 (54)	654 (59)	1807 (53)	**0.001**
COPD, *n* (%)	635 (14)	140 (13)	495 (15)	**0.006**
Biomarkers				
sST2 (ng/mL)	26 (19–39)	24 (17–36)	27 (20–40)	<**0.001**
hs-cTnT (ng/L)	19 (10–35)	15 (7–29)	20 (11–36)	<**0.001**
NT-proBNP (ng/L)	1525 (579–3457)	1540 (554–3982)	1505 (586–3320)	0.408
Therapies				
β-Blockers, *n* (%)	2910 (64)	716 (64)	2194 (64)	0.404
ACEi/ARB, *n* (%)	3824 (84)	918 (83)	2906 (85)	**0.042**
MRA, *n* (%)	1178 (26)	246 (22)	932 (27)	<**0.001**

ACEi, angiotensin converter enzyme inhibitors; ARB, angiotensin II receptor blockers; BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; hs-cTnT, high-sensitivity cardiac Troponin T; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonists; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; sST2, soluble suppression of tumorigenesis-2.
Values are presented as *n*, %; mean ± standard deviation, or median (interquartile interval).

Figure 1 Concentrations of sST2, hs-cTnT, and NT-proBNP in women and men with chronic heart failure. In the study population, both sST2 and hs-cTnT concentrations were significantly higher in men than in women (both $P < 0.001$), while those of NT-proBNP did not differ significantly between women (W) and men (M) ($P = 0.408$). hs-cTnT, high-sensitivity cardiac Troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.



hs-cTnT in both sexes, while LVEF and COPD were predictive only in men (*Tables S3* and *S4*).

N-terminal pro-B-type natriuretic peptide did not differ between women and men in the whole study population [1540 ng/L (554–3982) vs. 1505 ng/L (586–3320), $P = 0.408$] (*Figure 1*). Women displayed higher NT-proBNP in the overweight, HFmrEF, and non-diabetic subgroups, while NT-proBNP was lower in women aged < 45 years and in those without COPD (*Table S2*). Age, BMI, LVEF, AF, haemoglobin, and eGFR independently predicted NT-proBNP concentrations in both sexes, whereas hypertension and diabetes predicted NT-proBNP only in men (*Tables S3* and *S4*).

Biomarkers, outcome, and sex

Over a median 24 month follow-up duration,[17–31] the primary endpoint of 1 year CV death or HF hospitalization occurred in

DOI: 10.1002/ehf2.13883

868 patients (19%), with no significant difference between men and women (P = 0.689). On a 5 year follow-up, 1041 (23%) patients died and 777 (75%) for CV causes. Women showed a better 5 year survival than men (P = 0.010 for all-cause death, P = 0.018 for CV death) (*Table S5*). At multivariable regression analyses, sST2, hs-cTnT, and NT-proBNP independently predicted the primary and secondary endpoints in both sexes in a model adjusted for age, LVEF, ischaemic aetiology, NYHA class III–IV, history of AF, hypertension, diabetes mellitus, and CKD (*Table 2*), with no significant difference for the primary endpoint and for 5 year CV death (all P for interaction > 0.05). hs-cTnT and NT-proBNP were stronger predictors of 5 year all-cause death in men than in women (P for interaction 0.031 and 0.024, respectively). Moreover, the three biomarkers remained independent predictors of the primary endpoint also when forced into the same model (all $P < 0.001$), whereas progressively adding NT-proBNP, hs-cTnT, and sST2 to clinical covariates significantly improved the accuracy of risk prediction (assessed as the Δ C-statistics) in both women and men (*Table 3*).

Sex-specific cut-offs

The optimal cut-off in predicting CV death or hospitalization for HF was lower in women than in men for both sST2 (28 ng/mL vs. 31 ng/mL) and hs-cTnT (22 ng/L vs. 25 ng/L), while it was slightly higher among women (2339 ng/L vs. 2145 ng/L) for NT-proBNP. Similar results were found for CV death or all-cause mortality (*Table 4*). The differences among cut-offs were confirmed when searching for the inflection points of the spline curves for either the primary (*Figure 2*) or secondary endpoints (*Figures* S1 and S2).

The risk of primary and secondary endpoints increased in parallel with the number of biomarkers higher than or equal to sex-specific and endpoint-specific cut-offs (*Figure 3*). When considering the different combinations of elevated biomarkers, women with elevated hs-cTnT and NT-proBNP, but normal sST2, and those with elevated sST2 and hs-cTnT, but normal NT-proBNP, had a 10-fold higher risk for the primary endpoint than the reference category (patients with all biomarkers below cut-offs), compared with a five-fold higher risk in men. Furthermore, both women and men with all three biomarkers elevated had the greatest risk for the greatest risk for the primary endpoint, up to 15-fold higher in men, and to 22-fold higher in women, as further shown in the Kaplan–Meier curves reported in *Figure* S3. The use of sex-specific cut-offs of sST2 and NT-proBNP, compared with the use of non-sex-specific prognostic cut-offs (1 year CV death or HF hospitalization: 31 ng/mL for sST2, 23 ng/L for hs-cTnT, and 2198 ng/L for NT-proBNP; 5 year CV death: 28 ng/mL for sST2, 23 ng/L for hs-cTnT,

Table 2 Biomarkers and outcome in women (W) and men (M)

		W						M					
		Univariable analysis			Multivariable analysis[a]			Univariable analysis			Multivariable analysis[a]		
Endpoint	Biomarker	SHR	95%CI	P	SHR	95%CI	P	SHR	95%CI	P	SHR	95%CI	P	P for interaction
1 year CV death or HF hospitalization	sST2	1.91	1.68–2.17	<0.001	1.64	1.35–1.99	**<0.001**	1.82	1.66–1.99	<0.001	1.76	1.59–1.95	**<0.001**	0.711
	hs-cTnT	1.43	1.33–1.55	<0.001	1.29	1.18–1.43	**<0.001**	1.57	1.49–1.66	<0.001	1.53	1.43–1.63	**<0.001**	0.100
	NT-proBNP	1.40	1.30–1.50	<0.001	1.30	1.19–1.43	**<0.001**	1.47	1.39–1.54	<0.001	1.41	1.32–1.51	**<0.001**	0.144
5 year CV death	sST2	1.77	1.49–2.10	<0.001	1.39	1.13–1.71	**0.002**	1.56	1.41–1.74	<0.001	1.42	1.26–1.60	**<0.001**	0.518
	hs-cTnT	1.50	1.41–1.60	<0.001	1.22	1.09–1.34	**<0.001**	1.56	1.48–1.65	<0.001	1.40	1.29–1.51	**<0.001**	0.176
	NT-proBNP	1.46	1.34–1.59	<0.001	1.12	1.00–1.26	**0.050**	1.55	1.47–1.62	<0.001	1.30	1.22–1.49	**<0.001**	0.158
5 year all-cause death	sST2	1.62	1.44–1.83	<0.001	1.41	1.20–1.65	**<0.001**	1.63	1.50–1.76	<0.001	1.56	1.41–1.73	**<0.001**	0.944
	hs-cTnT	1.45	1.37–1.55	<0.001	1.24	1.13–1.37	**<0.001**	1.53	1.46–1-60	<0.001	1.46	1.37–1.56	**<0.001**	**0.031**
	NT-proBNP	1.39	1.30–1.50	<0.001	1.14	1.04–1.26	**0.007**	1.48	1.42–1.53	<0.001	1.38	1.30–1.47	**<0.001**	**0.024**

CV, cardiovascular; HF, heart failure; hs-cTnT, high-sensitivity cardiac Troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SHR, sub-distribution hazard ratio; sST2, soluble suppression of tumorigenesis-2.

[a]Model adjusted for age, left ventricular ejection fraction, ischaemic aetiology, New York Heart Association class III–IV, atrial fibrillation, hypertension, diabetes mellitus, chronic kidney disease stage III–V. sST2, hs-cTnT, and NT-proBNP were Log2-transformed before entering into regressions so that risk estimation should be considered for each doubling in their concentrations.

Table 3 Improvement in risk prediction for the primary endpoint by progressively adding NT-proBNP, hs-cTnT, and sST2 to clinical covariates

Sex	Adjusted model[a]	*C*-statistics (95% CI)	Δ *C*-statistics	*P*
W	+NT-proBNP	0.72 (0.68–0.76)	0.03 (0.01–0.05)	**0.005**
	+NT-proBNP + hs-cTnT	0.74 (0.70–0.77)	0.05 (0.02–0.07)	**<0.001**
	+NT-proBNP + hs-cTnT + sST2	0.75 (0.71–0.78)	0.06 (0.03–0.09)	**<0.001**
M	+NT-proBNP	0.69 (0.67–0.72)	0.07 (0.05–0.09)	**<0.001**
	+NT-proBNP + hs-cTnT	0.73 (0.70–0.75)	0.09 (0.08–0.12)	**<0.001**
	+NT-proBNP + hs-cTnT + sST2	0.74 (0.71–0.76)	0.11 (0.09–0.14)	**<0.001**

hs-cTnT, high-sensitivity cardiac Troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.

[a]Model adjusted for age, left ventricular ejection fraction, ischaemic aetiology, New York Heart Association class III–IV, atrial fibrillation, hypertension, diabetes mellitus, and chronic kidney disease stage III–V. sST2, hs-cTnT, and NT-proBNP were Log2-transformed before entering into regressions.

Table 4 Best cut-offs of sST2, hs-cTnT, and NT-proBNP for predicting outcomes in women (W) and men (M)

Biomarker	Endpoints	Sex	Best cut-off	AUC (95% CI)	Sens	Spec
sST2	1 year CV death or HF hospitalization	W	28 ng/mL	0.687 (0.631–0.701)	0.647 (0.559–0.694)	0.651 (0.610–0.674)
		M	31 ng/mL	0.653 (0.642–0.672)	0.612 (0.560–0.639)	0.634 (0.610–0.646)
	5 year CV death	W	26 ng/mL	0.602 (0.564–0.645)	0.593 (0.512–0.668)	0.564 (0.532–0.597)
		M	28 ng/mL	0.574 (0.549–0.602)	0.597 (0.557–0.636)	0.532 (0.512–0.549)
	5 year all-cause death	W	26 ng/mL	0.623 (0.594–0.642)	0.624 (0.557–0.687)	0.583 (0.550–0.616)
		M	29 ng/mL	0.600 (0.574–0.632)	0.585 (0.550–0.619)	0.574 (0.554–0.592)
hs-cTnT	1 year CV death or HF hospitalization	W	22 ng/L	0.745 (0.719–0.832)	0.661 (0.584–0.717)	0.721 (0.675–0.735)
		M	25 ng/L	0.713 (0.687–0.734)	0.669 (0.631–0.707)	0.659 (0.625–0.690)
	5 year CV death	W	18 ng/L	0.708 (0.689–0.736)	0.695(0.625–0.769)	0.626 (0.588–0.651)
		M	24 ng/L	0.655 (0.631–0.672)	0.616 (0.560–0.639)	0.610 (0.586–0.644)
	5 year all-cause death	W	18 ng/L	0.715 (0.695–0.738)	0.695 (0.635–0.758)	0.647 (0.608–0.672)
		M	23 ng/L	0.668 (0.642–0.684)	0.636 (0.613–0.680)	0.622 (0.591–0.629)
NT-proBNP	1 year CV death or HF hospitalization	W	2339 ng/L	0.712 (0.688–0.732)	0.643 (0.550–0.685)	0.682 (0.641–0.703)
		M	2145 ng/L	0.694 (0.672–0.723)	0.615 (0.577–0.656)	0.675 (0.654–0.869)
	5 year CV death	W	2304 ng/L	0.693 (0.669–0.734)	0.683 (0.606–0.752)	0.665 (0.634–0.695)
		M	1971 ng/L	0.682 (0.648–0.704)	0.636 (0.612–0.664)	0.637 (0.609–0.656)
	5 year all-cause death	W	2303 ng/L	0.693 (0.671–0.723)	0.650 (0.584–0.712)	0.681 (0.649–0.712)
		M	1848 ng/L	0.691 (0.668–0.712)	0.645 (0.612–0.679)	0.638 (0.619–0.656)

CV, cardiovascular; HF, heart failure; hs-cTnT, high-sensitivity cardiac Troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.

and 1975 ng/L for NT-proBNP; 5 year all-cause death: 28 ng/mL for sST2, 22 ng/L for hs-cTnT, and 2136 ng/L for NT-proBNP), improved risk reclassification in women for each endpoint, while the improvement was less apparent for hs-cTnT (*Table S6*). As reported in *Table S7*, the use of sex-specific cut-offs for risk prediction allowed to reclassify the risk of a substantial amount of patients, more in women than men, and for hs-cTnT than sST2 or NT-proBNP. Specifically, up to 18% men and up to 57% women were reclassified, by using the sex-specific cut-off of hs-cTnT for the endpoint of 5 year CV death.

Biomarker's concentration and prognostic cut-offs in propensity matched women and men

After propensity-score matching, 1566 patients (n = 783 women, 50%) were selected. As reported in *Table S8*, patients' age (68 ± 12 years vs. 68 ± 11 years, P = 0.957), LVEF (31 ± 12 vs. 32 ± 12%, P = 0.542), eGFR [54 (41–64) vs. 53 mL/min/1.73 m^2 (41–66)], as well as prevalence of LVEF classes (84% HFrEF, 8% HFmrEF, and 8% HFpEF for both sexes, P = 1.000), and of ischaemic aetiology (64% for both sexes, P = 1.000), and of NYHA class III–IV (51% vs. 55%, P = 0.116) were similar between sexes.

Both sST2 and hs-cTnT concentrations were lower in women [22 ng/mL (16–33) and 14 ng/L (7–28)] than in men [39 ng/mL (26–57) and 33 ng/L (21–53)] (both $P < 0.001$). In the matched population, also NT-proBNP was lower in women than in men [2764 ng/L (1462–6286) vs. 1531 ng/L (553–4121), $P < 0.001$]. The analyses of the matched population confirmed that the optimal cut-offs of sST2 and hs-cTnT for the prediction of the primary and secondary endpoints were lower in women than in men. Differently from the whole cohort, NT-proBNP prognostic cut-offs were also slightly lower in women than in men (*Table S9*).

Figure 2 P-spine curves for the best cut-offs of sST2, hs-cTnT, and NT-proBNP in predicting the risk of cardiovascular death or hospitalization for heart failure in women and men. The spline curves show how the event-risk changes with the increase of sST2, hs-cTnT, and NT-proBNP in either women (W) or men (M). The dashed lines represent the upper and lower limits of 95% confidence interval for each curve. hs-cTnT, high-sensitivity cardiac Troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.

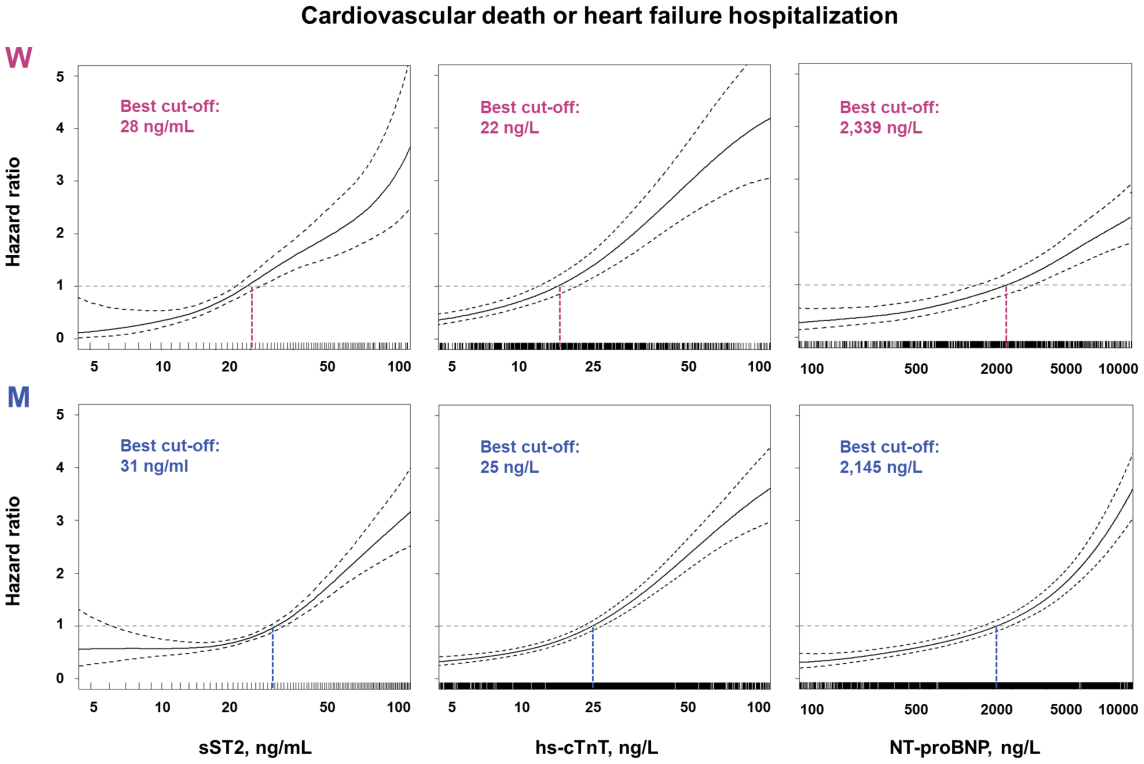
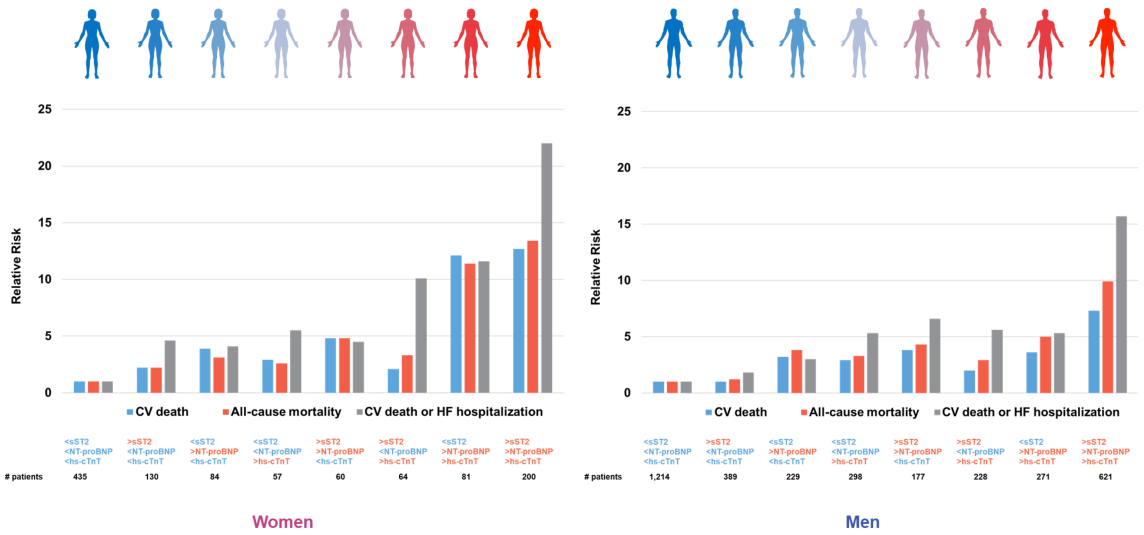


Figure 3 Relative risk of adverse events across biomarkers-based subgroups of women and men with chronic heart failure. Patients were classified according to the number of biomarkers over the sex-specific prognostic cut-offs calculated for each endpoint (as reported in *Table 4*). The subgroup with no elevated biomarkers was considered as reference category. CV, cardiovascular; HF, heart failure; hs-cTnT, high-sensitivity cardiac Troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.



Discussion

In a large international cohort of patients with chronic HF, sST2 and hs-cTnT concentrations were lower in women, while NT-proBNP did not differ between sexes. Nevertheless, no sex difference in sST2 concentrations was observed in the elderly, in underweight patients, in those with HFmrEF or HFpEF, or with history of AF, while underweight patients, those with HFmrEF, or with advanced CKD did not show sex difference in hs-cTnT concentrations. Conversely, NT-proBNP concentrations were higher in women in the overweight subset, in patients with HFmrEF, and in those without diabetes, while they were higher in men, in younger patients, and in those without COPD. The three biomarkers were independent predictors of adverse outcomes in both sexes, whereas the optimal cut-offs for risk prediction were lower in women for sST2 and hs-cTnT. While patients with all the three biomarkers over the cut-offs showed the greatest risk of adverse events in both sexes, high hs-cTnT combined with high sST2, NT-proBNP, or both was associated with a greater risk of CV death or HF hospitalization in women than in men. Finally, the use of sex-specific cut-offs improved event prediction compared with the use of standardized prognostic cut-offs.

Sex-related differences in concentrations and predictors of heart failure biomarkers

Male sex has been associated to higher sST2 concentrations in both healthy individuals[25] and in adults with HF.[26,27] In a community-based population (n = 3450 individuals, 55% women), age was associated to higher sST2 and increasing BMI to lower sST2 concentration in women but not in men.[25] In the present study, sST2 concentrations did not differ between men and women among patients older than 75 years, confirming a possible sex-specific relation with ageing. Conversely, sST2 concentrations decreased with increasing BMI in men but not in women, suggesting a potential interplay between sex, disease severity, and body fat.[28] Differences in sST2 across LVEF categories is controversial. Although in a sub-analysis of the Trial of Intensified vs. standard Medical therapy in Elderly patients with Congestive Heart Failure (TIME-CHF, n = 622 patients) sST2 concentrations did not significantly differ across the LVEF spectrum,[29] they were slightly higher in HFpEF when accounting for possible confounders (e.g. age, sex, and BMI).[18] In our study including 4540 HF patients, sST2 concentrations increased in parallel to LVEF in men and did so even more markedly in women. Whereas the possible determinants of such findings remain to be specifically investigated, the differences in the pathophysiological substrates behind HFrEF, HFmrEF, and HFpEF syndromes (e.g. neurohormonal activation, profibrotic and proinflammatory pathways, and cardiac and extracardiac comorbidities) may be a plausible explanation.[3] Finally, although higher sST2 concentrations have been identified as a marker of renal dysfunction as well,[7] in our study, eGFR was an independent (negative) predictor of sST2 in men but not in women. To the best of our knowledge, this sex-related difference in the association between sST2 and renal function had never been reported before and could be the object of future studies.

Higher hs-cTnT in men has been observed in the general population and among HF patients.[17,30–32] A greater burden of coronary heart disease and AF,[33,34] and testosterone-mediated cardiac damage pathways[35] have been proposed as possible mechanisms, as has the greater LV mass in men. In our cohort, hs-cTnT was higher in men across most subgroups (regardless of age, HF aetiology, and comorbidities), except for underweight patients and those with an eGFR < 30 mL/min/1.73 m^2, in whom greater HF severity (i.e. cardiac cachexia) and/or influence of advanced kidney disease, respectively, could overcome sex-related differences.[36,37]

In this population of individuals with mostly HFrEF, we did not observe significant differences in NT-proBNP concentrations between men and women, in line with previous findings in patients with HF.[12,38,39] In agreement with a recent study from our group,[14] including a different and larger cohort (n = 12,763), women had higher NT-proBNP than men in the overweight subset, supporting the existence of a subtle sex-specific interaction between body-fat and natriuretic peptides in chronic HF.[5,6,11] In the present study, NT-proBNP concentrations were lower in women than in men in patients younger than 45 years. Although the complex interplay between sex hormones oscillations and the metabolism of natriuretic peptide could partially explain such sex difference in NT-proBNP concentrations across age categories, clear pathophysiological evidence is missing in the context of HF.[40–44] Finally, sex-specific interactions between comorbidities and natriuretic peptides concentrations have been poorly investigated so far. While diabetes had been correlated to higher concentrations of NT-proBNP,[45] in our population, such relation was present only in men.

Sex differences in prognostic significance and cut-offs for risk prediction of heart failure biomarkers

As previously reported,[7,8,24] sST2, hs-cTnT, and NT-proBNP hold independent prognostic significance in men and women with chronic HF.

Circulating sST2 has been shown to predict prognosis in patients with either acute[46] or chronic HF,[7] also beyond NT-proBNP and hs-cTnT,[47] and regardless of possible confounders including sex.[7,46,47] To our knowledge, while the use of sex-specific sST2 cut-offs has been shown to have

incremental value for risk prediction in the general population,[48] there is currently no data in HF settings. In our population, the optimal sST2 cut-offs for each endpoint were ~10% lower in women than men. Of note, this difference was less pronounced than for hs-cTnT, possibly secondary to the lower interindividual variability of sST2.[49]

The prognostic significance of cardiac troponins in chronic HF had been more extensively investigated than sST2. In a study by Gohar *et al*. including patients with either HFrEF (n = 853) or HFpEF (n = 243), both hs-cTnT and hs-cTnI were associated with the endpoint of all-cause mortality or first hospitalization for HF.[49] While no sex-related difference was observed for hs-cTnT, hs-cTnI predicted poor outcome in men ($P < 0.001$) but not in women with HFpEF (P = 0.10), but the possible mechanisms behind such difference remained unknown.[50] In an individual patient data meta-analysis, hs-cTnT was a strong predictor of all-cause mortality, CV mortality, and hospitalizations in a prognostic model including sex.[8] Beyond confirming the prognostic value of hs-cTnT across LVEF strata and independent of other covariates, we identified for the first-time sex-specific optimal cut-offs for risk prediction that were up to 25% higher in men than women. Furthermore, our analysis of the prognostic impact of the combined elevation of different biomarkers pointed out a major impact of hs-cTnT elevation, as an index of ongoing cardiac damage, in women than in men, as high hs-cTnT concentrations (combined with high sST2, NT-proBNP, or both) were associated to a larger increase in relative risk of primary and secondary endpoints in women than in men. This suggests that chronic hs-cTnT release should be regarded as a negative prognostic sign, particularly in the female population.

The possible sex difference in NT-proBNP cut-offs for risk stratification in patients with chronic HF is unknown, and no specific adjustment is currently advised.[13] However, considering the spreading use of NT-proBNP also as entry criteria or surrogate survival endpoint in observational and clinical studies, the definition of patient-tailored reference values may be necessary.[51,52] In the present study, the optimal prognostic cut-offs of NT-proBNP were higher in women than in men. Although such results were confirmed also when stratifying patients into BMI categories,[14] after propensity-score matching, NT-proBNP concentrations and cut-offs for risk prediction were higher in men than in women, possibly reflecting the larger prevalence of AF in men than in women in the matched population.

Study limitations

First, clinical and laboratory variables were only assessed at the time of recruitment; therefore, any possible variation during follow-up could not be taken into account. Second, women accounted for only a minority (25%) of the study population, and HFrEF was more prevalent in the female individuals of this study than in other cohorts. Moreover, the proportion of patients with HFpEF and HFmrEF were in general low. To overcome such disparities between sexes, we further performed a propensity-score matching analysis, although its results should be viewed with caution, as the sample size is smaller and possibly not representative of a real population. The relatively large study population (n = 4540 patients, with 1111 women) allows reliable comparisons, regressions, and survival analyses between sex categories in the whole population, while the differences observed between smaller subgroups, including LVEF strata, should be regarded with greater caution and considered as exploratory; further regression or survival analysis were not performed to avoid model overfitting. Third, BMI was used to estimate body composition; although it does not discriminate lean from fat mass or body-fat distribution, BMI is correlated with total body fat content and is commonly evaluated in large cohort studies.[14] Finally, the study population, although composed of patients with chronic HF, was assembled from different cohorts with slightly different characteristics.[7,14]

Conclusions

In a large population of patients with chronic HF, both sST2 and hs-cTnT concentrations were lower in women than in men, while those of NT-proBNP were similar between sexes, albeit some exception emerged in specific subsets of patients. Whereas sST2, hs-cTnT, and NT-proBNP independently predicted adverse events in both sexes, risk prediction should take into account differences in sex-specific prognostic cut-offs.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Original study cohorts composing the study population.
Table S2. Subgroup analysis for biomarkers concentrations in women vs. men.
Table S3. Predictors of sST2, hs-cTnT, and NT-proBNP concentrations in women.
Table S4. Predictors of sST2, hs-cTnT, and NT-proBNP concentrations in men.
Table S5. Number of events for the primary and secondary endpoints in women and men of the study population.
Table S6. Improvement in risk prediction by using sex-specific prognostic cut-offs of sST2, hs-cTnT, and NT-proBNP for each endpoint in women and men with chronic heart failure.

Table S7. Percentage of patients reclassified by using sex-specific prognostic cut-offs of sST2, hs-cTnT, and NT-proBNP for each endpoint in women and men with chronic heart failure.

Table S8. Sex-related differences in baseline characteristics and biomarkers concentrations after propensity-score matching.

Table S9. Best cut-offs of sST2, hs-cTnT, and NT-proBNP for predicting outcomes in women (W) and men (M) after propensity-score matching.

Figure S1. P-spine curves for the best cut-offs of sST2, hs-cTnT, and NT-proBNP in predicting the risk of cardiovascular death in women and men.

Figure S2. P-spine curves for the best cut-offs of sST2, hs-cTnT, and NT-proBNP in predicting the risk of all-cause mortality in women and men.

Figure S3. Kaplan–Meier curves for the composite endpoint of cardiovascular death and heart failure hospitalization according to the number of biomarkers above the cut-off values in women and men.

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