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Prognostic value of secretoneurin in chronic heart failure. Data from the GISSI-Heart Failure trial

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ABSTRACT

Background: Circulating secretoneurin (SN) concentrations have been found to provide prognostic information in patients with acute heart failure. We wanted to assess whether SN would improve prognostication also in patients with chronic heart failure (HF) in a large multicenter trial.

Methods: We measured plasma SN concentrations at randomization (n = 1224) and after 3 months (n = 1103) in patients with chronic, stable HF from the GISSI-HF study. The co-primary endpoints were *(1)* time to death or *(2)* admission to hospital for cardiovascular reasons.

Results: Mean age was 67 years and 80% were male. Median (quartile 1–3) SN concentrations were 42.6 (35.0–62.8) pmol/L on randomization and 42.0 (34.5–53.1) pmol/L after 3 months, which are higher than SN concentrations in healthy subjects. Higher SN concentrations at randomization were associated with lower body-mass index (BMI), lower systolic blood pressure, lower estimated glomerular filtration rate (eGFR), higher B-type natriuretic peptide (BNP) concentrations, and diagnosis of chronic obstructive pulmonary disease. During median follow-up of 3.9 years, 344 patients (27.0%) died. After adjusting for age, sex, left ventricular ejection fraction, BMI, functional class, ischemic etiology, heart rate, blood pressure, eGFR, bilirubin, comorbidities, and BNP concentrations, logarithmically transformed SN concentrations on randomization were associated with mortality (HR 2.60 (95% CI 1.01–6.70), p = 0.047). SN concentrations were also associated with admission to hospital for cardiovascular reasons, but the association was attenuated and no longer significant in multivariable analysis.

Conclusion: Plasma SN concentrations provided incremental prognostic information to established risk indices and biomarkers in a large cohort of chronic HF patients.

1. Introduction

Individual patients with chronic heart failure (HF) have very different prognosis, also within subgroups classified according to etiology and left ventricular ejection fraction (LVEF) [1]. Circulating protein biomarkers have the potential to identify vulnerable patients that could benefit from special follow-up and uptitration of guideline-directed medical therapy [2]. As B-type natriuretic peptide (BNP)-guided therapy has not demonstrated clear benefit over symptom-based management in major randomized-controlled trial [3], there is a need to identify additional prognostic biomarkers. Hence, biomarkers that provide incremental prognostic information to BNP, and preferably across the spectrum of LVEF, may prove important for future personalized management strategies in HF patients.

Secretoneurin (SN) is a 33-amino acid peptide belonging to the chromogranin-secretogranin protein family [4]. Myocardial SN production increases during HF development, and circulating SN concentrations are higher in HF patients compared to healthy subjects [5,6]. Circulating SN concentrations also increase after strenuous physical stress and in patients with impaired renal function [6–12]. Linking SN

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directly to HF pathophysiology, SN has been found to inhibit Ca^{2+}/calmodulin-dependent protein kinase IIδ (CaMKIIδ) activity in experimental models [6,13], with CaMKIIδ being an important kinase for HF development and outcome [14]. Myocardial and circulating SN concentrations were also higher in individuals with catecholaminergic polymorphic ventricular tachycardia (CPVT) compared to age- and gender-match healthy controls [13]. As patients with CPVT have diastolic Ca^{2+} leak in cardiomyocytes but no other myocardial structural dysfunction [15], this result supports a model of SN production and concentrations linked to dysfunctional cardiomyocyte Ca^{2+} regulation. Hence, circulating SN concentrations seem to integrate information on renal function, systemic stress, and myocardial dysfunction, which are all important factors for clinical outcome in HF patients. In line with this model, we and other groups have demonstrated additional prognostic information to established risk indices and BNP from circulating SN concentrations in a number of cohorts with myocardial dysfunction [6,8–12]. The correlation between circulating SN and BNP concentrations is also only moderate [6,8–10,12,13], which supports SN as a biomarker that provides information on pathophysiology not covered by BNP. Still, no information is currently available on the role of SN as a prognostic biomarker in patients with chronic HF. Accordingly, in this biomarker substudy of the large, multicenter GISSI-HF trial, we hypothesized that plasma SN concentrations would improve risk assessment over established risk indices in patients with stable, chronic HF.

2. Methods

2.1. Study design

The GISSI-HF trial included 6975 patients with clinical evidence of chronic and stable HF (New York Heart Association functional class II-IV), of any etiology and level of LVEF, in a randomized, double-blind, placebo-controlled, multicenter study [16–18]. The main research questions for the GISSI-HF trial were whether rosuvastatin or n-3 polyunsaturated fatty acids (PUFA) would provide benefit on top of conventional HF treatment in patients with moderate to severe HF. The GISSI-HF trial used two randomizations in sequence: first randomization to n-3 PUFA/placebo, and thereafter randomization to rosuvastatin/placebo. Patients already taking a statin or having contraindications to statin therapy were not randomized to rosuvastatin/placebo. The co-primary outcome measures of GISSI-HF were *(1)* time to death or *(2)* admission to hospital for cardiovascular reasons during follow-up. We have previously reported the protocol and the main results of GISSI-HF where the addition of n-3 PUFA to conventional HF therapy provided a small beneficial effect on both primary outcome measures, while we found no significant effects for rosuvastatin [17,18].

The GISSI-HF trial had a prospectively planned biomarker substudy. In the biomarker substudy, we collected blood samples at randomization and after three months in a subset of 1233 patients from 51 centers of the main GISSI-HF trial. For the study on SN, we had blood samples available from 1224 patients at randomization and 1103 patients after 3 months. The endpoints of the biomarker substudy are the same as the endpoints of the main GISSI-HF trial.

The Local Ethics Committee approved the GISSI-HF trial and we obtained informed and signed consent from all participants prior to study commencement. The data are stored at the CREACTIVE Coordinating Center (Mario Negri Institute, Milano, Italy). Data are available upon justified request to the CREACTIVE study Steering Committee.

2.2. Blood sampling procedures

We collected blood samples by venipuncture of an antecubital vein after patients had rested for minimum 15 min in a supine position. We used tubes containing EDTA as anticoagulant and all blood samples were centrifuged at 4 °C within 10 min. All participating centers shipped plasma aliquots on dry ice to a central laboratory where samples were stored at –70 °C until used for biomarker studies.

2.3. Determination of biomarker concentrations

Plasma SN concentrations were measured by a commercially available CE-marked ELISA assay (CardiNor AS, Oslo, Norway) as previously described [19,20]. The SN ELISA assay has range 10–250 pmol/L and intra-assay and inter-assay coefficients of variation of <5% and <7%, respectively. We used an immunometric method to measure BNP (IRMA, Shionogi, Osaka, Japan) [21]. All plasma biomarkers were assayed in blinded fashion and as a single batch. We calculated estimated glomerular filtration rate (eGFR) with the simplified MDRD equation [22]. We report methodology for studies on long-term storage and SN concentrations in the Supplementary material.

2.4. Statistical methods

We present categorical variables as proportions and continuous variables as mean (±standard deviation [SD]) or median (quartile [Q] 1–3). The associations between SN tertiles and baseline variables were investigated with the Chi-square test for categorical variables, by analysis of variance for normally distributed continuous variables, and by the non-parametric Kruskal-Wallis test for non-normally distributed continuous data.

We used the Spearman rank method to assess correlations between SN concentrations and continuous variables. To assess the independent determinants of elevated baseline concentration of SN (continuous variable), we used a multivariable linear regression model with baseline patient characteristics. For all variables, we first created a univariate model, and only variables significant at the univariate level were included in the multivariate model. Due to a right-skewed distributions of SN (Fig. 1) and BNP concentrations, we log-transformed SN and BNP prior to regression analyses.

The prognostic value of SN was examined by stratifying SN into tertiles and by using the log-rank test. We also calculated univariate and multivariable Cox proportional hazard regression models with SN concentrations at randomization. We provide 95% confidence intervals for all hazard ratios (HR). In multivariable analysis, we first adjusted for age and sex (model #1). In a second, comprehensive model, we adjusted for age, sex, LVEF, body-mass index (BMI), New York Heart Association (NYHA) functional class, ischemic etiology for HF, heart rate, systolic blood pressure, bilirubin, eGFR, diabetes mellitus, atrial fibrillation, and diagnosis of chronic obstructive pulmonary disease (COPD). Finally, in model #3 we adjusted for all of the variables included in model #2 plus BNP concentrations.

We tested the effect of study drugs (n-3 PUFA vs. placebo or rosuvastatin vs. placebo) on 3-month changes of SN concentrations with ANCOVA, after adjusting for baseline SN concentrations.

We performed statistics with SPSS software version 27.0 (IBM Corp., Armonk, N.Y. USA). All P-values are two-tailed and considered significant if <0.05. HR and TO drafted the statistical analysis plan and all statistical analyses were performed by JM under the supervision of RL at the Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.

3. Results

3.1. Baseline characteristics

Median SN concentration at randomization in the 1224 patients enrolled in this biomarker substudy was 42.6 (35.0–62.8) pmol/L and median BNP concentration was 141 (61–292) ng/L. We present SN distribution at randomization in Fig. 1 and patient characteristics stratified by SN concentrations in Table 1. Patients with high SN concentrations were older, a higher proportion were female, they had lower BMI and higher NYHA functional class, and more patients with high SN concentrations had ischemic etiology for HF compared to patients with

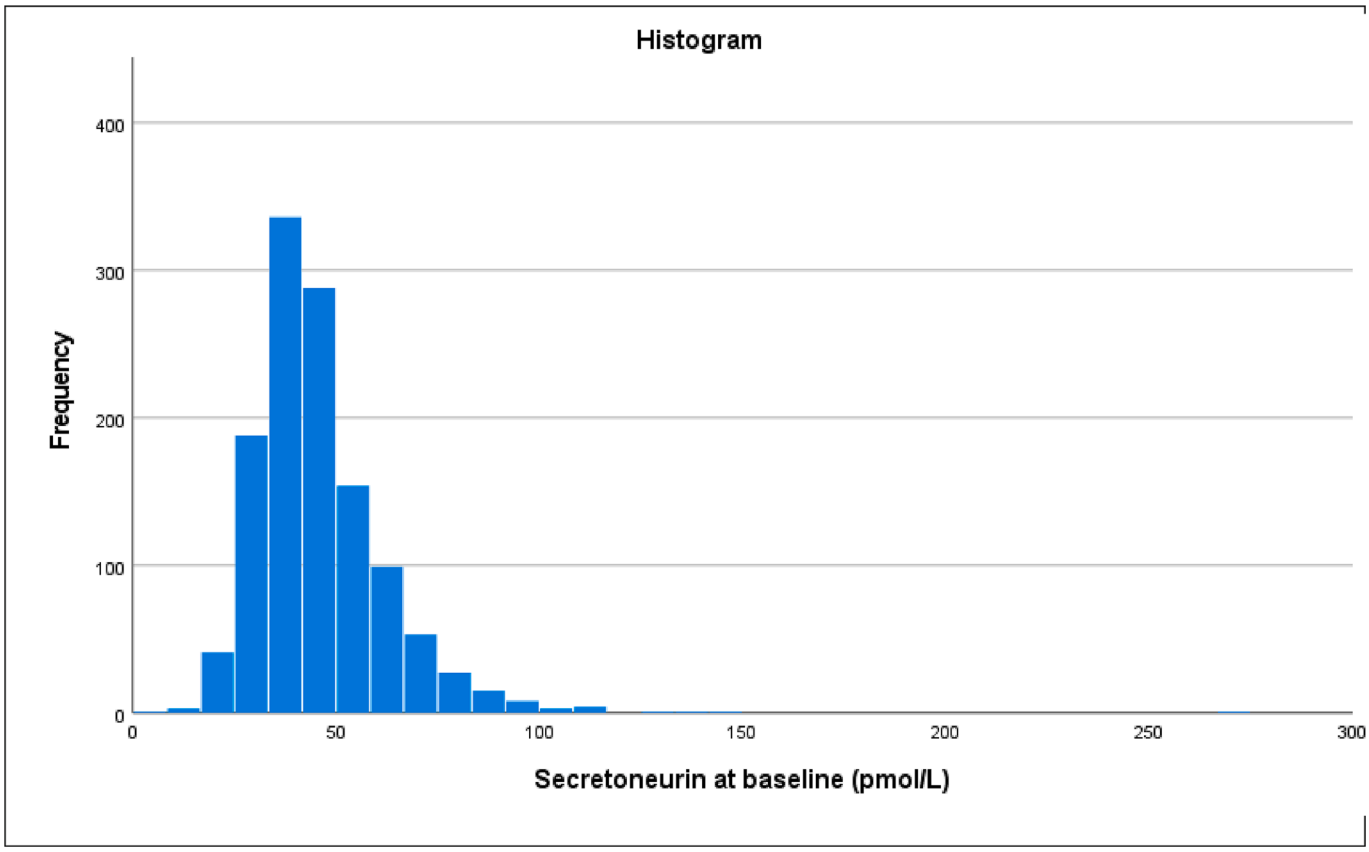

Fig. 1. Distribution of SN concentrations measured at randomization in the GISSI-HF trial (n = 1224).

Table 1
Characteristics of the population stratified by baseline SN concentrations (pmol/L).

N		Tertile 1 N = 415 SN: 5.0–37.4	Tertile 2 N = 407 SN: 37.5–48.6	Tertile 3 N = 402 SN: 48.7–273.8	P
Age	mean ± SD	63.1 ± 11.0	67.0 ± 10.4	70.6 ± 9.6	<0.001
Males	(%)	362 (87.2%)	306 (75.2%)	315 (78.4%)	<0.001
LVEF	mean ± SD	33.9 ± 9.4	33.2 ± 9.9	32.6 ± 9.5	0.152
BMI	mean ± SD	27.7 ± 4.9	26.5 ± 4.0	26.3 ± 4.4	<0.001
Ischemic etiology	(%)	189 (45.5%)	199 (48.9%)	232 (57.7%)	0.002
NYHA class III-IV	(%)	83 (20.0%)	109 (26.8%)	129 (32.1%)	0.002
Heart rate	mean ± SD	71.0 ± 12.6	69.8 ± 13.3	73.5 ± 14.5	<0.001
Systolic blood pressure	mean ± SD	129 ± 20	125 ± 18	122 ± 17	<0.001
Diastolic blood pressure	mean ± SD	79 ± 11	77 ± 10	74 ± 11	<0.001
Serum sodium	mean ± SD	139 ± 12	139 ± 10	139 ± 8	0.766
eGFR (mL/min/1.73 m^2)	MDRD	81.0 ± 20.6	70.4 ± 20.8	54.5 ± 20.2	<0.001
Serum bilirubin	mean ± SD	0.82 ± 0.53	0.79 ± 0.52	0.79 ± 0.62	0.660
Diabetes mellitus	(%)	97 (23.4%)	97 (23.8%)	125 (31.1%)	0.019
Atrial fibrillation	(%)	77 (18.6%)	65 (16.0%)	85 (21.1%)	0.167
COPD	(%)	59 (14.2%)	73 (17.9%)	95 (23.6%)	0.002
ACEi or ARBs	(%)	402 (96.9%)	392 (96.3%)	384 (95.5%)	0.597
Beta-blockers	(%)	296 (71.3%)	281 (69.0%)	255 (63.4%)	0.046
Diuretics	(%)	357 (86.0%)	369 (90.7%)	384 (95.5%)	<0.001
Digoxin	(%)	133 (32.0%)	142 (34.9%)	140 (34.8%)	0.617
Aldosterone antagonists	(%)	153 (36.9%)	182 (44.7%)	195 (48.5%)	0.003
BNP, ng/L	median [Q1-Q3]	108 [43–195]	149 [68–286]	196 [74–357]	<0.001
HFrEF (LVEF < 40%)	(%)	326 (78.6%)	335 (82.3%)	331 (82.3%)	0.447
HFmrEF (LVEF 40–49%)	(%)	65 (15.7%)	47 (11.5%)	49 (12.2%)	
HFpEF (LVEF ≥ 50%)	(%)	24 (5.8%)	25 (6.1%)	22 (5.5%)	
All-cause mortality	(%)	76 (18.3%)	96 (23.6%)	158 (39.3%)	<0.001
All-cause mortality or CV hospitalizations	(%)	241 (58.1%)	251 (61.7%)	291 (72.4%)	<0.001

low SN concentrations. Patients with high SN concentrations also had higher heart rate, lower blood pressure, worse renal function, higher BNP concentrations, and the prevalence of diabetes mellitus and COPD was higher. The use of aldosterone antagonist and diuretics was higher and the use of β-blockers lower in patients with high SN concentrations. Patients with high SN concentrations on study randomization also had

worse clinical outcome compared to patients with low SN concentrations. Information on SN concentrations in selected subgroups is presented in Table 2. We found no significant difference in SN concentrations between patients with LVEF ≥50% compared to patients with LVEF <50%.

SN concentrations correlated with a large number of continuous variables (Supplementary Table 1). We found especially strong correlations between SN concentrations measured at randomization and after 3 months (rho = 0.66, p < 0.001) and between SN concentrations and eGFR (rho = -0.53, p < 0.001). In contrast, the correlation between SN and BNP was modest (rho = 0.22, p < 0.001) and the correlation coefficient between SN and LVEF was rho = -0.08 (p = 0.008).

By univariate and multivariable linear regression analysis, we found low BMI, low systolic blood pressure, low eGFR, high BNP concentrations, and a prior diagnosis of COPD to be associated with increasing SN concentrations at randomization (Supplementary Table 2 and Table 3).

SN concentrations in frozen plasma samples did not change during 24-month storage at −80 °C (Supplementary Table 3).

3.2. Baseline SN concentrations and clinical outcomes

During a median follow-up of 3.9 years (interquartile range 3.1–4.6 years), 330 patients (27%) died. SN plasma concentrations at randomization were strongly associated with time to death, as presented in Fig. 2 (log-rank test: p < 0.001). SN concentrations were also associated with mortality in Cox hazard regression analysis, including in a comprehensive multivariable model that adjusted for clinical risk factors and BNP concentrations (Table 4).

During follow-up, 783 patients (64%) were admitted to hospital for cardiovascular reasons. SN concentrations at randomization were associated with hospitalization for cardiovascular reasons (Supplementary Table 4). This association was significant also after adjustment for age and sex, but was attenuated and no longer significant in comprehensive multivariable models.

Table 2
SN concentrations (pmol/L) in selected subgroups of patients.

		Age		p
		<70 years, N = 707	≥70 years, N = 517	
SN	Baseline	39.6 [33.6–48.6]	46.5 [38.0–57.5]	<0.001
—	3 months	39.7 [32.6–49.4]	46.7 [38.5–57.3]	<0.001

		NYHA		p
		II, N=903	III or IV, N=321	
SN	Baseline	41.7 [34.4–51.5]	45.4 [37.1–55.8]	<0.001
—	3 months	41.4 [34.1–50.9]	47.8 [36.6–60.8]	<0.001

		eGFR			p
		<60, N=450	60–89, N=561	≥90, N=206	
SN	Baseline	51.8 [44.0–62.2]	39.2 [33.4–47.3]	36.7 [30.5–41.3]	<0.001
—	3 months	51.0 [42.2–61.3]	39.6 [33.0–47.6]	35.7 [29.9–42.3]	<0.001

		BMI			p
		<25, N=434	25–29.9, N=533	≥30, N=257	
SN	Baseline	44.8 [37.2–56.2]	42.6 [35.0–51.7]	38.9 [32.3–49.0]	<0.001
—	3 months	45.3 [36.1–55.6]	41.7 [34.6–51.3]	39.5 [31.5–52.2]	<0.001

		LVEF		p
		<50%, N=1164	≥50%, N=60	
SN	Baseline	42.5 [35.0–52.9]	45.0 [34.5–51.8]	0.888
—	3 months	42.1 [34.5–53.2]	39.9 [33.6–51.0]	0.286

Table 3
Variables associated with increasing SN concentrations (continuous variable) in multivariable linear regression analysis.

Variable	ß (SE)	t	p
BMI (+1 kg/m^2)	−0.003 (0.001)	−3.482	0.001
Systolic blood pressure (+1 mmHg)	−0.001 (0.0003)	−2.174	0.030
eGFR (+1 mL/min/1.73 m^2)	−0.003 (0.0002)	−15.628	<0.001
COPD	0.020 (0.010)	2.061	0.039
BNP (logtransformed)	0.014(0.007)	2.127	0.034
	0.14		

Linear regression for log-transformed SN was adjusted for the following variables, which were all significantly associated with baseline SN concentrations in univariate analyses: age, sex, BMI, ischemic etiology for HF, heart rate, systolic and diastolic blood pressure, eGFR, COPD, Beta-blockers, diuretics, aldosterone antagonists, and log-transformed BNP.

3.3. Effect of study drugs on SN concentrations at 3-month follow-up

Median SN concentrations were 42.0 (34.5–53.1) pmol/L after 3 months. The majority of patients had stable SN concentrations over time, as reflected by rho = 0.66 (p < 0.001) for correlation between baseline and 3 months SN concentrations (Supplementary Table 1). Three-month changes in SN concentrations were not significantly different in patients randomized to 1 g/day n-3 PUFA or 10 mg/d rosuvastatin compared to patients on placebo (Supplementary Table 5).

4. Discussion

In this substudy from a large, randomized-controlled clinical trial, we report for the first time that SN measurements provide incremental prognostic information to established risk indices and biomarkers in patients with chronic HF. We found no differences in SN concentrations between HF patients with LVEF above or below 50%, which supports SN as a biomarker across HF subgroups.

Cardiologists have used biomarkers in clinical practice for many years, but primarily to establish the correct diagnosis [2]. As B-type natriuretic peptides are cardiac-specific, these peptides provide sensitive and specific information related to cardiac function [2]. Hence, guidelines recommend B-type natriuretic peptide measurement in patients with dyspnea and other symptoms suggestive of HF [1]. Studies have also indicated a potential of B-type natriuretic peptides to guide therapy. However, as a large multicenter randomized-controlled trial failed to verify that B-type natriuretic peptide-guided therapy is superior to conventional symptom-based management [3], there is a need for additional studies related to biomarker-guided therapy in HF patients. Pertinent to this point, large groups of HF patients do not receive guideline-directed medical or device therapy [1]. Accordingly, B-type natriuretic peptide-guided therapy should be further tested, and possibly in combination with novel biomarkers that complement the B-type natriuretic peptides. Ideally, such biomarkers should reflect additional pathophysiology to the B-type natriuretic peptides, have limited physiological variability, and demonstrate utility across HF subtypes.

SN is a candidate biomarker in patients with myocardial dysfunction that could prove useful as a complement to the B-type natriuretic peptides [23]. In contrast to B-type natriuretic peptides, SN production in the myocardium is not primarily linked to volume overload and LV remodeling [6,11], but rather could be linked to dysfunctional cardiomyocyte Ca^{2+} handling [13]. Accordingly, the pathophysiology of SN seems to differ from the mechanism that primary regulate B-type natriuretic peptide production. We now strengthen a model of SN as complementary to the B-type natriuretic peptides as we find SN measurements to provide prognostic information on top of established risk indices and BNP measurements. Of note, comparing SN concentrations in our study with prior data from healthy subjects and low-risk patients with chest pain [19,20], SN concentrations are not increased in all chronic HF patients, but rather in a subgroup with worse prognosis.

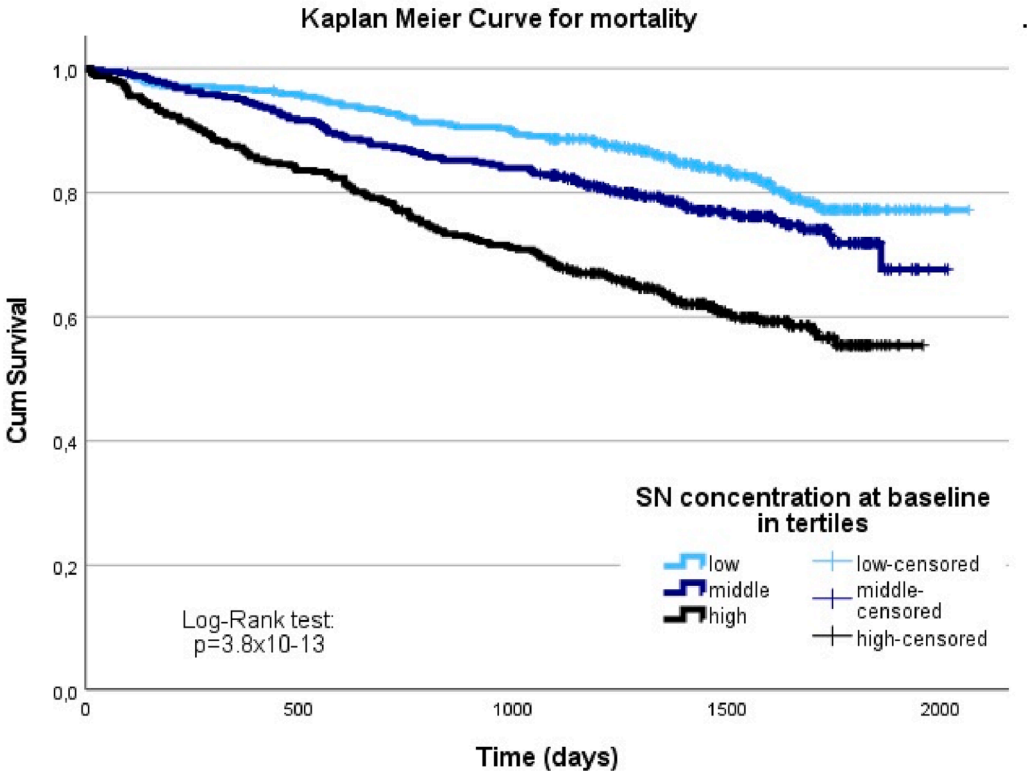

Fig. 2. Association between baseline secretoneurin (SN) concentrations by tertiles and all-cause mortality in patients with chronic heart failure.

Table 4
Univariate and multivariable Cox proportional hazard models for time to death during follow-up.

	Univariate		
	HR	**95 %CI**	**P**
SN, randomization	15.45	7.84–30.42	<0.001
	Multivariable		
	HR	**95 %CI**	**P**
Model #1: SN, randomization	10.16	4.78–21.60	<0.001
Model #2: SN, randomization	3.43	1.37–8.64	0.009
Model #3: SN, randomization	2.60	1.011–6.70	0.047

Model #1: adjusted for age and sex.
Model #2: adjusted for age, sex, LVEF, BMI, NYHA functional class, ischemic etiology for HF, heart rate, systolic blood pressure, bilirubin, eGFR, diabetes mellitus, atrial fibrillation, and diagnosis of COPD.
Model #3: adjusted for all variables in model #2 plus BNP concentrations.

Accordingly, although prior data found SN concentrations to stay high during hospitalization for acute HF patients [6], our new data indicates that only a subgroup of chronic HF patients have long-term elevated SN concentrations. Additional studies are needed to establish the dynamics of SN over time in HF patients, including whether single measurement or serial measurements are most informative. Still, for a novel biomarker to have clinical potential a number of criteria should been met [24]. First, there is a need for a robust method to measure the biomarker. We recently reported the development of a CE-marked SN ELISA test and demonstrated favorable results for the SN ELISA compared to the established, in-house SN RIA [19]. Using the SN ELISA, we have also found low day-to-day variability in SN concentrations among healthy subjects [20], which supports that SN has low noise-to-signal ratio as a biomarker. Hence, the SN ELISA provides a valuable platform to further validate and test SN, which seems to have potential as a new cardiac biomarker [2,23].

A second criterion for novel biomarkers is that the biomarker provides consistent information across cohorts [24]. For prognostic biomarkers, this would mean strong and incremental risk information to established clinical risk indices and cardiac biomarkers. Pertinent to this point; SN has been found to provide strong and consistent prognostic information across several cohorts and studies, including patients with acute HF, patients with ventricular arrhythmia-induced cardiac arrest, patients with cardiovascular related-acute respiratory failure, patients with aortic stenosis, and patients with sepsis-induced myocardial dysfunction [6,8–12]. In these studies, circulating SN concentrations added prognostic information to a number of clinical risk factors and established biomarkers like the B-type natriuretic peptides. The present study supports and extends prior studies by demonstrating incremental prognostic information from SN in patients with chronic HF from a large randomized-controlled multicenter trial. Of note, we have previously reported that chromogranin A, which is another novel biomarker candidate from the chromogranin-secretogranin protein family, did not improve prognostication in the GISSI-HF trial [25].

The final criterion for novel biomarkers is whether information from the biomarker will help physicians treat patients better [24]. Whether SN can improve patient management needs to be tested in prospective clinical trials. However, this and prior studies indicate that high SN concentrations should alert the physician that the patient is high-risk for adverse events in the near future. Accordingly, it is imperative that such high-risk patients receive guideline-directed medical or device therapy, and that pharmacological treatment is uptitrated to the maximum tolerated dose [1]. Established HF therapy will also target pathophysiology associated with high SN concentrations. SN is released from systemic and peripheral nerve endings and linked to dysfunctional cardiomyocyte Ca^{2+} handling [4,6,13], which support the need for β-blocker inhibition in HF patients with high SN concentrations. In contrast, we found low β-blocker use and poor clinical outcome in HF

patients with high SN concentrations in the GISSI-HF trial. Whether SN-guided strategies can improve guideline adherence and clinical outcomes in HF patients should be explored in new trials.

This study has some strengths and limitations. One strength is the large population of patients with chronic HF that were enrolled into a multicenter, randomized-controlled clinical trial. This ensures close follow-up of the patients for the clinical endpoints. We also processed samples uniformly and biomarkers were measured as a single batch and in a single laboratory with personnel unaware of patient characteristics or clinical events when performing the analysis. Limitations include lack of detailed echocardiography, which precludes analysis into correlations between SN concentrations and systolic and diastolic indices in this cohort. The GISSI-HF trial was also conducted prior to the introduction of the most recent HF medication, such as sacubitril-angiotensin and sodium-glucose cotransport-2 inhibitors. Whether such novel HF therapies affect SN concentrations and the prognostic utility of SN measurements in HF patients should be examined in other cohorts. Samples in the GISSI HF trial had been stored for several years at −70 °C and we cannot exclude influence by storage on SN concentrations. Still, as we demonstrate excellent stability for SN concentrations in frozen samples stored up to 24 months, and as degradation is expected to affect our results in a negative way, we believe degradation has not had major impact on our results. Recruitment of patients from a large number of centers should neither influence SN concentrations.

In conclusion, we find that SN concentrations measured at randomization in the large GISSI-HF trial provided incremental prognostic information to established risk indices and biomarkers in patients with chronic HF. The role of SN as a possible novel clinical biomarker in HF patients requires further studies, including more detailed information on therapeutic implications of high SN concentrations.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: HR and TO have intellectual property rights for the use of SN as a biomarker in CVD, HR and TO have stocks in CardiNor AS, which holds the patent for SN as a CV biomarker, and HR, AHO, and TO have received consultant fees from CardiNor AS. HR has also received consultant fee from SpinChip Diagnostics.

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The sponsors had no role in any of the following: design and conduct of the study, collection, management, analysis and interpretation of the data, or preparation, review and approval of the manuscript.

Appendix. Participating centers and investigators

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Appendix A. Supplementary data

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