Circulating levels and prognostic value of soluble ST2 in heart failure are less influenced by age than N-terminal pro-B-type natriuretic peptide and high-sensitivity troponin T

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Aims N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity troponin T (hs-TnT) and soluble suppression of tumorigenesis-2 (sST2) predict outcome in chronic heart failure (HF). We assessed the influence of age on circulating levels and prognostic significance of these biomarkers.

Methods and results Individual data from 5301 patients with chronic HF and NT-proBNP, hs-TnT, and sST2 data were evaluated. Patients were stratified according to age: <60 years (n = 1332, 25%), 60–69 years (n = 1628, 31%), 70–79 years (n = 1662, 31%), and ≥ 80 years (n = 679, 13%). Patients (median age 66 years, 75% men, median left ventricular ejection fraction 28%, 64% with ischaemic HF) had median NT-proBNP 1564 ng/L, hs-TnT 21 ng/L, and sST2 29 ng/mL. Age independently predicted NT-proBNP and hs-TnT, but not sST2. The best NT-proBNP and hs-TnT cut-offs for 1-year and 5-year all-cause and cardiovascular mortality and 1- to 12-month HF hospitalization increased with age, while the best sST2 cut-offs did not. When stratifying patients according to age- and outcome-specific cut-offs, this stratification yielded independent prognostic significance over NT-proBNP levels only, or the composite of NT-proBNP and hs-TnT, and improved risk prediction for most endpoints. Finally, absolute NT-proBNP, hs-TnT, and sST2 levels predicted outcomes independent of age, sex, left ventricular ejection fraction category, ethnic group, and other variables.

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Conclusions Soluble ST2 is less influenced by age than NT-proBNP or hs-TnT; all these biomarkers predict outcome regardless of age. The use of age- and outcome-specific cut-offs of NT-proBNP, hs-TnT and sST2 allows more accurate risk stratification than NT-proBNP alone or the combination of NT-proBNP and hs-TnT.

Keywords sST2 • Biomarkers • Age • Prognosis • Heart failure

Introduction

The burden of chronic heart failure (HF) is expected to increase because of population ageing.[1] Measurement of circulating levels of HF biomarkers may provide insight on disease severity, thus allowing clinicians to tailor the therapeutic strategy on the individual patient. Nonetheless, the optimal use of biomarkers for risk stratification is controversial, also because several aspects have not been specifically analysed, including the influence of age on some HF biomarkers, and the need for age-specific cut-offs for risk prediction.[2,3]

In both healthy subjects and HF patients, circulating levels of B-type natriuretic peptides are significantly influenced by age, as well as by cardiovascular (e.g. atrial fibrillation) and non-cardiovascular conditions [such as anaemia and chronic kidney disease (CKD)], whose prevalence increases with age.[3] For this reason, higher N-terminal pro-B-type natriuretic peptide (NT-proBNP) cut-offs have been proposed for risk stratification among elderly patients.[4] Similarly, circulating troponin levels [namely, high-sensitivity troponin T (hs-TnT)] tend to rise with age, at least partially because of declining renal function.[5] With respect to the relationship between soluble suppression of tumorigenesis-2 (sST2) and age, several small, single-centre studies found no correlation between sST2 levels and age,[6,7] and age was not an independent predictor of sST2 even in a sub-analysis of the Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial.[8] On the other hand, all these studies included only patients with systolic HF [left ventricular ejection fraction (LVEF) <40% or <45%], and, to our knowledge, the impact of age on the prognostic value of sST2 has never been specifically analysed so far.[9]

In the present study we performed a dedicated analysis of the relationship between age, circulating levels of HF biomarkers, and patient outcome in an international cohort of chronic HF patients with data available for NT-proBNP, hs-TnT, and sST2.

Methods

Patient population

The Biomarkers Of heart failure Study (BIOS) consortium includes 13 cohorts of patients with stable chronic HF and NT-proBNP and hs-TnT data. This consortium was constituted by merging the dataset created for a previous individual patient data meta-analysis (n = 9289)[10] with the Prospective Evaluation of Outcome in Patients with heart failure with preserved Left ventricular Ejection fraction (PEOPLE) and Singapore Heart failure Outcomes and Phenotypes (SHOP) cohorts (n = 941 and n = 1099, respectively).[11] Total patient number was 11 329. For the present study, we selected patients with available data on age, LVEF, hs-TnT, NT-proBNP, and sST2. This study then included 5301 patients.

Biohumoral evaluation

In all studies NT-proBNP was measured through the monoclonal electrochemiluminescence immunoassay method [Roche Diagnostics®; coefficient of variation (CV) <3% at cut-off value (150 ng/L)],[12] troponin T through the only hs-TnT assay available [Roche Diagnostics®, Basel, Switzerland; limit of blank 3 ng/L, limit of detection (LOD) 5 ng/L, 99th percentile value in apparently healthy individuals of 14 ng/L],[13] and sST2 with the Presage® assay (LOD 1.3 ng/mL, measurement range up to 200 ng/mL, intra-assay CV <7%, inter-assay CV <9%).[14] These biomarkers were assayed in a core laboratory for each study; NT-proBNP and hs-TnT were assayed during each of the original studies, while sST2 was measured on EDTA plasma samples stored at −20°C. Samples were collected during an outpatient visit; patients were in a condition of clinical stability, with no need for changes in therapy from at least 1 month.

The estimated glomerular filtration rate (eGFR) was calculated through the CKD Epidemiology Collaboration equation[15]; patients on dialysis were excluded.

Statistical analysis

IBM SPSS Statistics (version 22, 2013) and R statistical software (http://www.r-project.org/, version 3.4.4) were used. Normal distribution was assessed by the Kolmogorov–Smirnov test; variables with normal distribution were presented as means ± standard deviations, while those with non-normal distribution as medians and interquartile range. As the assumption of normality for biomarker levels was not met, NT-proBNP, hs-TnT and sST2 were ln-transformed. Mean differences among groups were evaluated through the unpaired Student T test or the Mann Whitney U test, as appropriate. Categorical variables were compared by the chi-square test with Yates correction. The beta coefficients were computed at multivariate linear regression analysis. At multivariate analysis, multicollinearity was searched by calculating the variance inflation factor. In each age category, the additive prognostic value of sST2 to NT-proBNP and hs-TnT was evaluated. The best cut-off at receiver operating characteristics analysis was searched through the Youden method. The Fine–Gray model was used to account for mutually exclusive endpoints; non-cardiovascular death was considered as competing risk for cardiovascular death, and all-cause death for HF hospitalization. The net reclassification improvement (with risk categories set at <10%, 10–30% and >30%) and the integrated discrimination improvement were calculated to assess reclassification. Two-tailed P-values <0.05 were considered statistically significant.

Table 1 Patient characteristics

	Patients	<60 years	60–69 years	70–79 years	≥80 years	*P*-value
Patients, *n* (%)	5301	1332 (25)	1628 (31)	1662 (31)	679 (13)	
Male sex, *n* (%)	3969 (75)	1064 (80)	1277 (78)	1212 (73)	416 (61)	<0.001
Ethnic group, *n* (%)						
Caucasian	4026 (76)	771 (58)	1232 (76)	1442 (87)	581 (86)	<0.001
Afro-American	208 (4)	110 (8)	62 (4)	31 (2)	5 (1)	
Asian	1057 (20)	445 (33)	333 (21)	187 (11)	92 (14)	
Other	10 (0)	6 (1)	1 (0)	2 (0)	1 (0)	
Age, years	66 (57–74)	52 (47–56)	64 (62–67)	74 (72–77)	83 (81–85)	<0.001
Ischaemic HF, *n* (%)	3399 (64)	626 (47)	1138 (70)	1188 (72)	447 (66)	<0.001
LVEF, %	28 (21–46)	26 (20–34)	27 821–35)	29 (23–36)	35 (25–46)	<0.001
LVEF <40%, 40–49%, ≥50%, *n* (%)	4420, 439, 442 (83, 8, 8)	1164, 94, 74 (87, 7, 6)	1411, 108, 109 (87, 7, 6)	1381, 146, 135 (83, 9, 8)	464, 91, 124 (68, 13, 18)	<0.001
NYHA class I–II/III–IV, *n* (%)	3000/2284 (57/43)	964/361 (72/27)	934/691 (57/42)	809/850 (49/51)	293/382 (43/56)	<0.001
BMI, kg/m^2	26.4 (23.5–29.7)	28.2 (24.9–31.9)	26.3 (23.5–29.5)	25.5 (22.9–28.4)	24.2 (21.9–26.9)	<0.001
eGFR, mL/min/1–73 m^2	58 (44–70)	66 (54–79)	57 (46–68)	53 (40–65)	47 (36–59)	<0.001
CKD stage 3–5, *n* (%)	2934 (55)	476 (36)	827 (51)	1105 (67)	526 (78)	<0.001
Anaemia, *n* (%)	1476 (28)	323 (28)	407 (36)	510 (46)	236 (47)	<0.001
Hypertension, *n* (%)	3373 (74)	692 (52)	1068 (66)	1130 (68)	483 (71)	<0.001
Atrial fibrillation, *n* (%)	1043 (20)	138 (10)	292 (18)	399 (24)	214 (32)	<0.001
Diabetes, *n* (%)	1661 (21)	473 (36)	566 (35)	434 (26)	188 (28)	<0.001
COPD, *n* (%)	604 (11)	120 (9)	177 (11)	216 (13)	91 (13)	<0.001
NT-proBNP, ng/L	1564 (593–3622)	810 (272–2008)	1313 (544–3236)	2090 (971–4314)	3618 (1660–7180)	<0.001
hs-TnT, ng/L	21 (11–39)	14 (7–29)	20 (11–37)	24 (15–41)	33 (20–59)	<0.001
sST2, ng/L	29 (22–43)	29 (21–41)	29 (21–42)	30 (22–44)	36 (26–51)	<0.001
Therapies[a], *n* (%)						
ACEi/ARB	4524 (85)	1183 (89)	1377 (85)	1404 (50)	560 (83)	<0.001
Beta-blockers	3594 (68)	918 (69)	1119 (69)	1093 (66)	464 (68)	0.198
MRA	1625 (31)	423 (32)	489 (30)	505 (30)	208 (31)	0.775

Because of non-normal distribution, continuous variables are expressed as median (interquartile range).
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HF, heart failure; hs-TnT, high-sensitivity troponin T; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; sST2, soluble suppression of tumorigenesis-2.
[a]Therapies prescribed after baseline evaluation are reported.

Results

Patient population

Patients (*n* = 5301) were aged 66 (57–74) years, and 3969 (75%) were men. Three quarters of patients were of Caucasian ethnicity (*n* = 4026, 76%), followed by Asian individuals (*n* = 1057, 20%), and Afro-Americans (*n* = 208, 4%) (*Table 1*). HF had an ischaemic aetiology in 3399 patients (64%). The majority of patients had HF with reduced ejection fraction (HFrEF; *n* = 4420, 83%); patients with HF and mid-range or preserved ejection fraction (HFmrEF/HFpEF) were 439 (8%) and 442 (8%), respectively. NT-proBNP, hs-TnT, and sST2 levels were 1564 (593–3622) ng/L, 21 (11–39) ng/L, and 29 (22–43) ng/mL, respectively.

Patients were stratified into the following age categories: <60 years (*n* = 1332, 25%), 60–69 years (*n* = 1628, 31%), 70–79 years (*n* = 1662, 31%), and ≥80 years (*n* = 679, 13%). As reported in *Table 1*, patient characteristics differed across age categories under many respects. Most notably, the percentages of women and patients with HFmrEF or HFpEF increased with age, as well as the prevalence of stage 3–5 CKD, severity of New York Heart Association (NYHA) symptom classification, and the overall burden of co-morbidities.

Age and heart failure biomarkers

Circulating levels of all biomarkers increased with age, with significant differences across age categories; nonetheless, sST2 displayed a much less prominent elevation than NT-proBNP and hs-TnT (*Table 1* and *Figure 1*). Similar results were found when considering separately patients with HFrEF, HFmrEF ($P < 0.001$ for all biomarkers), and HFpEF ($P < 0.001$ for NT-proBNP and hs-TnT, $P = 0.002$ for sST2), or men and women ($P < 0.001$ for all biomarkers).

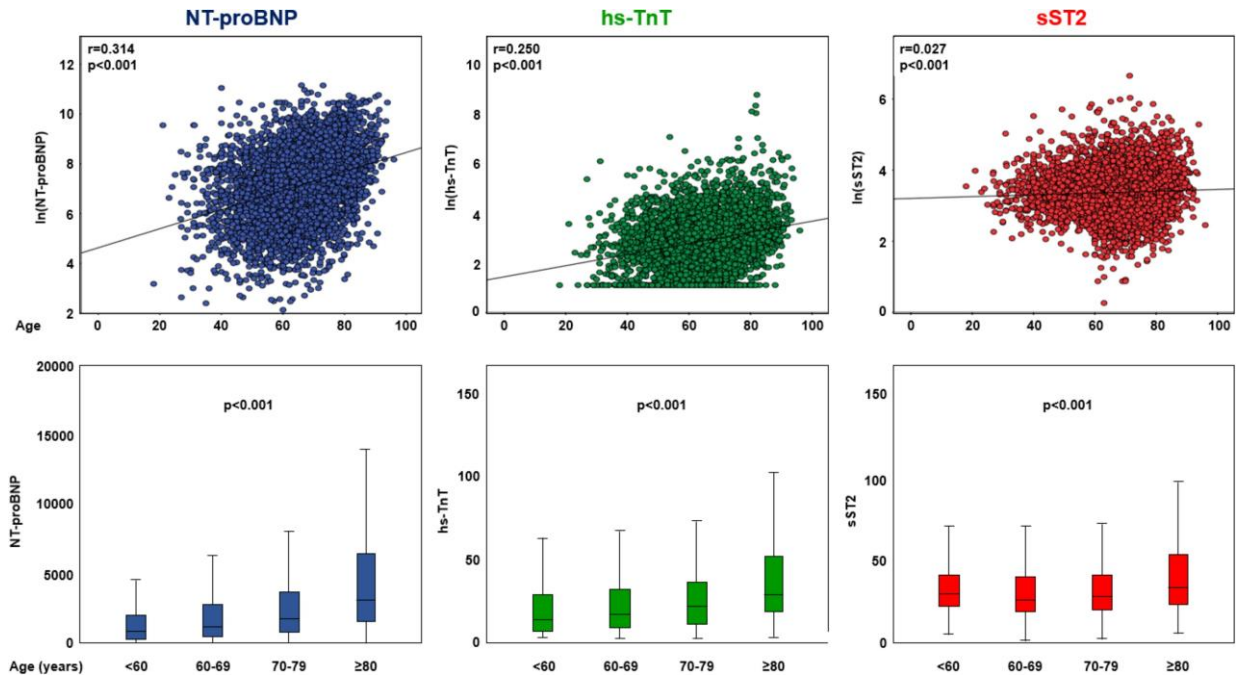

Figure 1 Age and circulating biomarkers. (*Upper panels*) Correlations between age and heart failure biomarker levels in the whole population. (*Lower panels*) Biomarker levels across age categories. hs-TnT, high-sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.

In the whole population, age displayed stronger correlations with NT-proBNP or hs-TnT (r = 0.314 and r = 0.250, respectively; both P <0.001) than sST2 (r = 0.027; P = 0.007) (*Figure 1*). The same conclusions were reached in both men and women (online supplementary *Figure S1*), and across LVEF categories (online supplementary *Figure S2*).

At univariable linear regression analysis, beta coefficients were higher for age as a predictor of NT-proBNP or hs-TnT than sST2 (*Table 2*). In a multivariable model including available baseline patient characteristics (including LVEF categories and sex), age displayed an independent association with both NT-proBNP and hs-TnT, but not with sST2 (*Table 2*).

Soluble ST2 and outcome across age categories

In the whole population, 542 patients (10%) experienced all-cause death at 1 year, and 1360 (26%) at 5 years. Out of 5235 patients with available data, 396 (8%) and 947 (18%) died because of cardiovascular causes at 1 and 5 years, respectively. Finally, 203 (4%), 437, 650, and 1027 out of 5162 (4%, 8%, 13%, and 19%, respectively) were hospitalized at least once for HF at 1, 3, 6, and 12 months, respectively.

The number of events in the four age categories is reported in online supplementary *Table S1*. The area under the curve (AUC) values for outcome prediction were higher for hs-TnT than NT-proBNP and sST2 (online supplementary *Table S2*). Similar AUC values were found in the HFrEF subgroup (online supplementary *Table S2*), while patients with HFmrEF and HFpEF were not analysed separately due to their limited numbers.

In the whole population, the best NT-proBNP and hs-TnT cut-offs increased with age, while sST2 cut-offs were less influenced by age (*Figure 2*; online supplementary *Tables S3* and *S4*); for example the best cut-offs for all-cause 1-year death in patients aged <60 years and ≥ 80 years increased from 1640 to 3328 ng/L NT-proBNP, and from 17 to 34 ng/L hs-TnT, while sST2 values were very similar (38 vs. 41 ng/mL).

When stratifying patients according to the age- and endpoint-specific cut-offs of the three biomarkers, this stratification yielded independent prognostic significance over absolute NT-proBNP levels alone, and improved risk prediction for most endpoints (online supplementary *Table S5*). A refinement in risk prediction was observed even when this stratification was performed in addition to both NT-proBNP and hs-TnT (*Table 3*). Patients with the three biomarkers ≥ cut-off had constantly a worse prognosis than those with all biomarkers < cut-off, particularly for HF hospitalization, and even of those with both NT-proBNP and hs-TnT ≥ cut-off (*Figure 3*; online supplementary *Table S6*).

Prognostic value of biomarkers: influence of age and other patient variables

As an additional analysis, continuous values of the three HF biomarkers were evaluated as outcome predictors. Three prognostic models were defined, including: (i) age; (ii) age, LVEF

Table 2 Predictors of biomarker levels

	NT-proBNP		hs-TnT		sST2	
	Beta coefficient	***P*-value**	**Beta coefficient**	***P*-value**	**Beta coefficient**	***P*-value**
Unadjusted analysis						
Age	0.330	<0.001	0.262	<0.001	0.049	<0.001
Adjusted analysis						
Age	0.223	<0.001	0.273	0.005	–	0.065
Female sex	–	0.488	−0.134	<0.001	−0.128	<0.001
Ethnic group	0.146	<0.001	0.292	<0.001	0.228	<0.001
Ischaemic HF	–	0.673	–	0.206	–	0.135
LVEF category	−0.147	<0.001	−0.038	0.027	0.044	0.021
NYHA class I–II vs. III–IV	0.140	<0.001	0.133	<0.001	0.169	<0.001
BMI	−0.215	<0.001	–	0.125	−0.043	0.016
eGFR	−0.201	<0.001	−0.214	<0.001	−0.079	<0.001
Anaemia	0.081	<0.001	0.051	0.001	–	0.094
Hypertension	–	0.079	0.064	<0.001	–	0.186
Atrial fibrillation	0.170	<0.001	0.033	0.035	0.116	<0.001
Diabetes	–	0.154	0.167	<0.001	0.074	<0.001
COPD	–	0.799	0.053	<0.001	–	0.229
Beta-blockers	0.092	<0.001	–	0.894	–	0.110
ACEi/ARB	–	0.259	–	0.729	–	0.052
MRA	0.119	<0.001	0.085	<0.001	0.094	<0.001

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HF, heart failure; hs-TnT, high-sensitivity 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 of NT-proBNP, hs-TnT, and sST2 were available for all patients; for each patient, a single measurement of each biomarker was considered. Biomarker values were ln-transformed.
The variable 'ethnic group' was computed as 1 = Caucasian, 2 = Afro-American, 3 = Asian, 4 = other.

categories (HFrEF, HFmrEF, HFpEF), and ethnic group (Caucasian, Asian, Afro-American, other ethnicity); (iii) the variables in model 2 together with all other available variables. With single exceptions, NT-proBNP, hs-TnT and sST2 independently predicted all endpoints (1- and 5-year all-cause and cardiovascular deaths, 1-, 3-, 6-, and 12-month HF hospitalizations) (*Table 4*).

Discussion

In a cohort of 5301 patients with chronic HF, circulating sST2 levels were influenced by age to a lesser extent than NT-proBNP and hs-TnT. Accordingly, the best cut-offs of NT-proBNP and hs-TnT for the prediction of 1-year and 5-year all-cause and cardiovascular mortality and 1- to 12-month HF hospitalization tended to increase with age, while the best sST2 cut-offs did not. Patient classification according to the age-specific cut-offs of the three biomarkers refined risk prediction over NT-proBNP levels, as well as the combination of NT-proBNP and hs-TnT. The three biomarkers yielded independent prognostic significance in models including age, sex, LVEF categories, ethnicity, and other characteristics such as therapies for neurohormonal modulation.

The main stimulus to natriuretic peptide release is pressure and/or volume overload, which increases left ventricular wall tension,[16] while cardiac troponins are released mostly upon cardiomyocyte necrosis.[17] By contrast, extracardiac tissues are a significant source of circulating sST2,[18] whose levels reflect both the activation of inflammatory and profibrotic pathways and haemodynamic overload,[19] which are important determinants of disease progression in HF. This may explain the strong, independent prognostic value of sST2 for all-cause and cardiovascular mortality and HF hospitalization.[20]

Across age categories, sST2 remained much more stable than NT-proBNP or hs-TnT, despite a progressive increase in disease severity in parallel with age, as demonstrated by the rising proportions of patients in NYHA class III or IV (27% <60 years to 56% in the ≥80 years; *Table 1*), with a pattern of NYHA class increase similar to the one observed in cohort studies.[21] Accordingly, age predicted NT-proBNP and hs-TnT regardless of other variables including NYHA class, while it did not predict sST2. Even though this dataset allows to gain only limited insight on the reasons of this different relationship with age, some mechanisms can be proposed. The prevalence of CKD stages 3–5 increased from 36% in patients aged <60 years to 78% in those aged ≥80 years. Natriuretic peptides are excreted to a significant extent by the kidneys, and their circulating levels increase in patients with CKD.[22] It is more controversial as to whether or not cardiac troponins are cleared by the kidneys, but patients with CKD tend to display higher troponin levels.[23,24] By contrast, the influence of renal function on sST2 is thought to be less important.[25–27] Accordingly, in the present study eGFR independently predicted NT-proBNP and

Table 3 Added prognostic value of patient stratification based on age-specific cut-offs vs. absolute N-terminal pro-B-type natriuretic peptide and high-sensitivity troponin T

	Years			
	<60	**60–69**	**70–79**	**≥80**
All-cause death				
1 year				
P-value	0.073	0.121	0.025	0.069
IDI	0.008 (0.003–0.013), *P* = 0.002	0.012 (0.006–0.019), *P* <0.001	0.014 (0.006–0.021), *P* <0.001	0.020 (0.008–0.031), *P* = 0.001
NRI	0.221 (0.007–0.435), *P* = 0.043	0.1474 (−0.0211–0.3158), *P* = 0.086	0.190 (0.036–0.343), *P* = 0.015	0.387 (0.197–0.576), *P* <0.001
5 years				
P-value	0.041	0.011	<0.001	0.020
IDI	0.012 (0.005–0.018), *P* <0.001	0.008 (0.004–0.013), *P* <0.001	0.017 (0.010–0.023), *P* <0.001	0.029 (0.017–0.041), *P* <0.001
NRI	0.225 (0.068–0.382), *P* = 0.005	0.286 (0.172–0.400), *P* <0.001	0.237 (0.134–0.340), *P* <0.001	0.223 (0.077–0.369), *P* = 0.003
CV death				
1 year				
P-value	0.449	0.017	0.049	0.149
IDI	0.011 (0.005–0.017), *P* = 0.001	0.017 (0.009–0.025), *P* <0.001	0.012 (0.004–0.020), *P* = 0.005	0.017 (0.006–0.028), *P* = 0.003
NRI	0.269 (0.025–0.514), *P* = 0.030	0.258 (0.061–0.454), *P* = 0.010	0.1345 (−0.043–0.312), *P* = 0.138	0.503 (0.290–0.716), *P* <0.001
5 years				
P-value	0.161	0.064	0.374	0.004
IDI	0.011 (0.005–0.018), *P* = 0.001	0.006 (0.001–0.010), *P* = 0.014	0.005 (0.001–0.009), *P* = 0.007	0.030 (0.017–0.042), *P* <0.001
NRI	0.192 (0.010–0.374), *P* = 0.039	0.465 (0.334–0.597), *P* <0.001	0.183 (0.066–0.299), *P* = 0.002	0.288 (0.130–0.446), *P* <0.001
HF hospitalization				
1 month				
P-value	0.050	0.126	0.182	0.150
IDI	0.012 (0.002–0.023), *P* = 0.019	0.0052(−0.005–0.015), *P* = 0.309	0.015(0.009–0.021), *P* <0.001	0.019 (0.009–0.029), *P* <0.001
NRI	0.569 (0.323–0.814), *P* <0.001	0.520 (0.253–0.786), *P* <0.001	0.611 (0.379–0.843), *P* <0.001	0.902 (0.632–1.171), *P* <0.001
3 months				
P-value	0.006	0.064	0.061	0.005
IDI	0.019 (0.008–0.0300), *P* = 0.001	0.013 (0.004–0.021), *P* = 0.003	0.008 (0.003–0.014), *P* = 0.003	0.034 (0.018–0.050), *P* <0.001
NRI	0.463 (0.268–0.658), *P* <0.001	0.322 (0.129–0.514), *P* = 0.001	0.414 (0.243–0.584), *P* <0.001	0.479 (0.270–0.688), *P* <0.001
6 months				
P-value	0.003	0.007	0.016	0.001
IDI	0.018 (0.008–0.027), *P* <0.001	0.019 (0.005–0.022), *P* = 0.004	0.014 (0.008–0.021), *P* <0.001	0.038 (0.021–0.056), *P* <0.001
NRI	0.454 (0.283–0.625), *P* <0.001	0.326 (0.139–0.526), *P* <0.001	0.269 (0.123–0.414), *P* <0.001	0.442 (0.257–0.627), *P* <0.001
12 months				
P-value	<0.001	0.085	0.144	0.007
IDI	0.025 (0.015–0.035), *P* <0.001	0.008 (0.002–0.013), *P* = 0.007	0.006 (0.002–0.010), *P* = 0.008	0.029 (0.016–0.043), *P* <0.001
NRI	0.315 (0.171–0.459), *P* <0.001	0.225 (0.100–0.350), *P* <0.001	0.216 (0.093–0.338), *P* = 0.001	0.303 (0.139–0.467), *P* <0.001

Values of independent discrimination improvement (IDI) and net reclassification index (NRI) are reported with the corresponding 95% confidence intervals.
CV, cardiovascular; HF, heart failure.

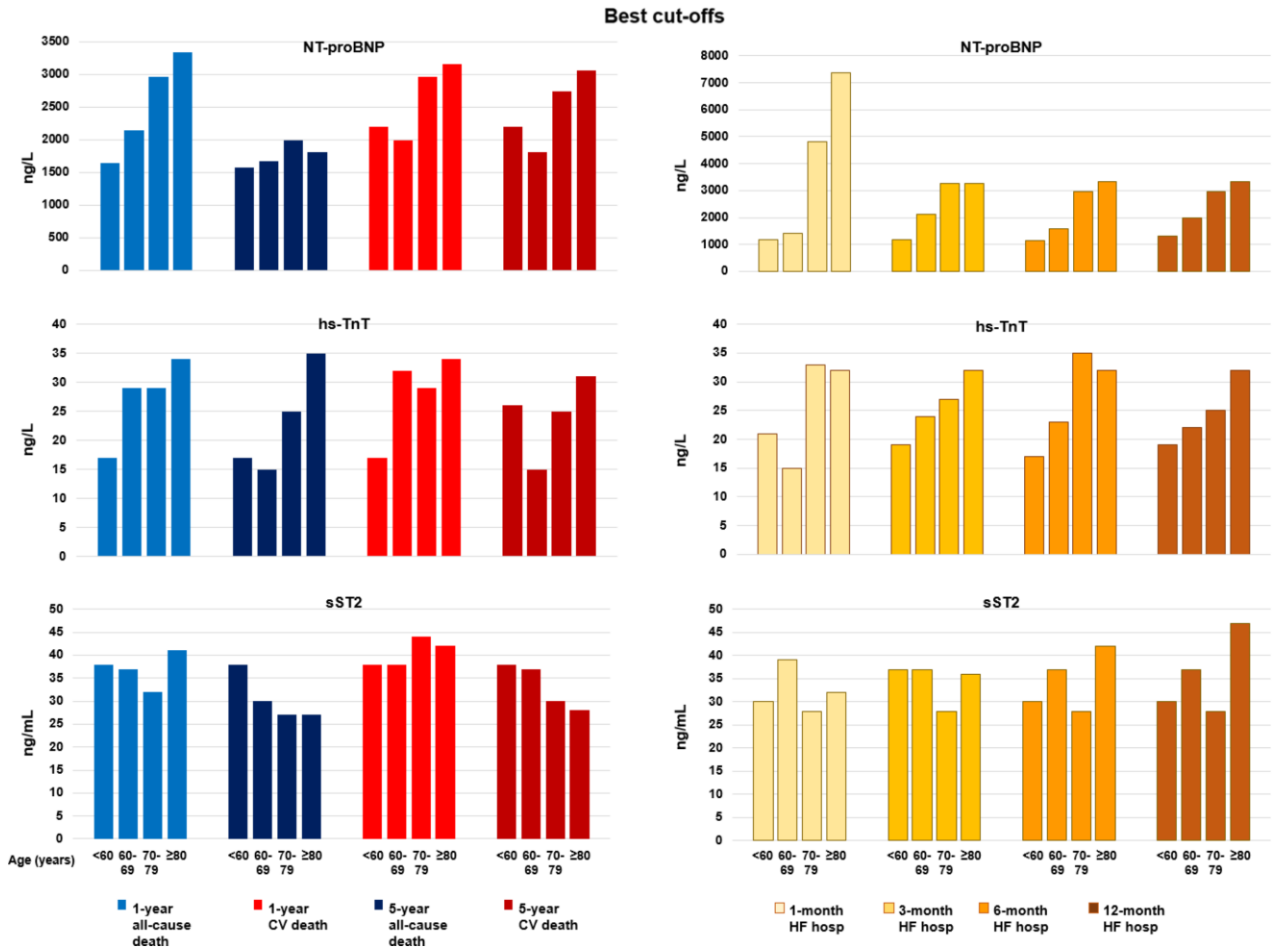

Figure 2 Best cut-offs for the prediction of all-cause and cardiovascular (CV) death and heart failure (HF) hospitalization across age categories. The best cut-off values, with corresponding sensitivity and specificity values, are reported in online supplementary *Table S3*. hs-TnT, high-sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.

hs-TnT, but not sST2 (*Table 2*). We may also consider that previous studies have reported limited differences in plasma sST2 between men and women,[28] and between patients with HFrEF or HFmrEF or HFpEF,[29] possibly justifying the relative stability of sST2 across age categories despite marked differences in the proportion of women and patients with HFmrEF or HFpEF (*Table 1*).

While dedicated studies are needed to clarify the mechanisms of the different relationships between age and HF biomarkers, the BIOS cohort represents an ideal platform to search for age-specific cut-offs for risk prediction, and to evaluate the added value of a multi-marker strategy over absolute NT-proBNP levels across age categories. We identified specific cut-offs for each age category (<60, 60–69, 70–79, and ≥80 years), and for each endpoint (1-year and 5-year all-cause and cardiovascular death, and 1-, 3-, 6-, and 12-month HF hospitalization), and we reported that patient classification according to these cut-offs yielded independent prognostic significance over absolute NT-proBNP levels, which are commonly used for risk prediction, according to guideline recommendation.[30] This multi-marker approach sometimes was more predictive than the combination of absolute NT-proBNP and hs-TnT, also improving metrics of risk reclassification. The additive prognostic value for the prediction of short-to-intermediate term HF hospitalization seems particularly interesting, as HF admissions have a negative impact on the quality of life and natural history of the disease,[31] and can often be prevented if subclinical congestion is detected and addressed through appropriate changes in HF medications, lifestyle advice, and close follow-up. Interestingly, absolute levels of all three biomarkers were independent predictors of almost all outcome measures, including HF hospitalization at the different time-points, independent from age, but also from the combination of age, sex, HF category (HFrEF, HFmrEF, HFpEF), and patient ethnicity, and even from other baseline variables including medical therapy for HF. These findings provide a further demonstration of the strong, independent prognostic value of HF biomarkers in chronic HF, and outline that their predictive performance is unaffected by age and other patient characteristics.

Several limitations to this analysis should be acknowledged. First, patients with HFrEF accounted for 83% of the whole population

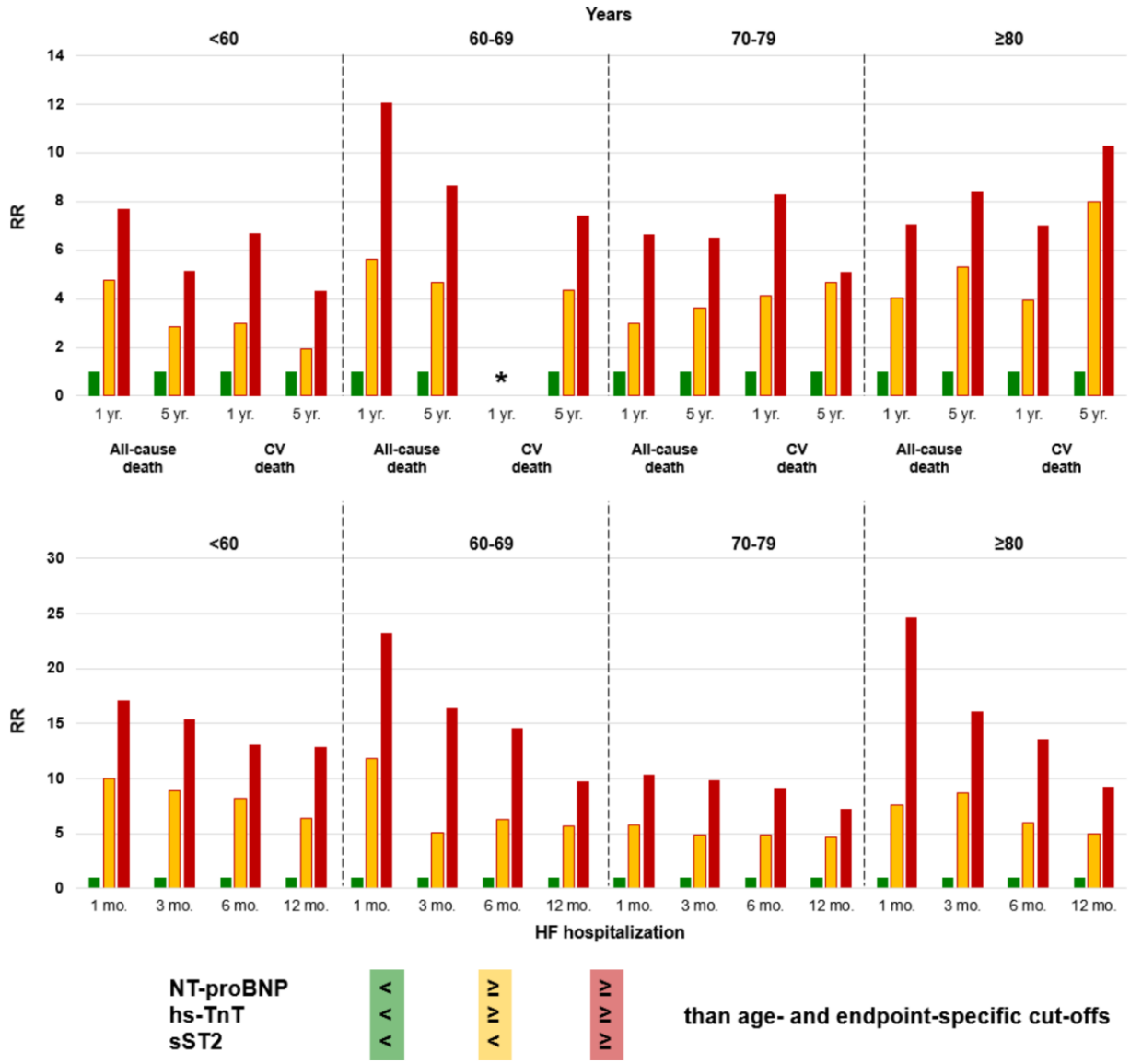

Figure 3 Risk of all-cause and cardiovascular (CV) mortality and heart failure (HF) hospitalization with biomarkers ≥ cut-offs. Patients were stratified according to age- and outcome-specific cut-offs (*Table 3*). Compared with patients with N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity troponin T (hs-TnT), and soluble suppression of tumorigenesis-2 (sST2) < cut-offs [reference category, with relative risk (RR) 1], those with NT-proBNP and hs-TnT ≥ cut-offs had a higher risk, and those with the three biomarkers ≥ cut-offs had an even greater risk. For 1-year CV death in the 60–69-year category (*), RR values could not be computed as no events were observed in the reference category.

as a consequence of the inclusion of several trials (for example, LVEF <40% in the Valsartan Heart Failure Trial,[32] or ≤ 40% in the Controlled Rosuvastatin Multinational Trial in Heart Failure[33]). Therefore, the results on the prognostic role of biomarkers across age categories were mostly driven by HFrEF patients, as indirectly confirmed by the similar AUC values in the whole population and the HFrEF subgroup (online supplementary *Table S2*). Further studies including a larger proportion of patients with HFmrEF and HFpEF are then warranted to gain a deeper insight on the impact of age on the prognostic value of HF biomarkers across LVEF categories. Second, the percentage of female patients was 27%, while study registries suggest a higher proportion of female patients, up to 50%.[21,34] Indeed, patient data for this study derived mainly from clinical trials on HF, where women are traditionally underrepresented, with an average representation of 20%.[35,36] Overall, sex differences in the prognostic value of HF biomarkers could be more accurately searched in real-world HF registries with available biomarker values. Third, the relatively small number of women and patients with non-Caucasian ethnicity across age categories did not allow a reliable assessment of the relative

Table 4 Prognostic value of heart failure biomarkers: effect of age and other patient variables

	All-cause death 1 year			All-cause death 5 years			CV death 1 year			CV death 5 years		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age												
NT-proBNP	1.34	1.22–1.48	<0.001	1.23	1.15–1.32	<0.001	1.34	1.20–1.49	<0.001	1.30	1.21–1.40	<0.001
hs-TnT	1.54	1.38–1.73	<0.001	1.49	1.36–1.62	<0.001	1.53	1.35–1.74	<0.001	1.48	1.32–1.59	<0.001
sST2	1.53	1.30–1.80	<0.001	1.47	1.30–1.66	<0.001	1.29	1.07–1.55	0.008	–	–	0.169
Age, sex, LVEF category, ethnic group												
NT-proBNP	1.37	1.24–1.51	<0.001	1.28	1.19–1.37	<0.001	1.38	1.23–1.55	<0.001	1.36	1.26–1.47	<0.001
hs-TnT	1.57	1.39–1.76	<0.001	1.63	1.49–1.78	<0.001	1.59	1.40–1.82	<0.001	1.59	1.44–1.76	<0.001
sST2	1.58	1.34–1.87	<0.001	1.64	1.45–1.86	<0.001	1.38	1.14–1.68	0.001	1.25	1.09–1.44	0.002
Model 3												
NT-proBNP	1.34	1.16–1.54	<0.001	1.29	1.15–1.43	<0.001	1.30	1.11–1.52	0.001	1.27	1.12–1.43	<0.001
hs-TnT	1.65	1.41–1.93	<0.001	1.59	1.39–1.81	<0.001	1.62	1.37–1.92	<0.001	1.54	1.44–1.77	<0.001
sST2	1.88	1.39–2.37	<0.001	1.61	1.33–1.95	<0.001	1.84	1.42–2.38	<0.001	1.51	1.23–1.86	<0.001

	HFH 1 month			HFH 3 months			HFH 6 months			HFH 12 months		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age												
NT-proBNP	1.41	1.22–1.63	<0.001	1.41	1.27–1.56	<0.001	1.39	1.27–1.52	<0.001	1.41	1.31–1.52	<0.001
hs-TnT	1.49	1.26–1.75	<0.001	1.51	1.34–1.72	<0.001	1.62	1.45–1.81	<0.001	1.63	1.48–1.79	<0.001
sST2	1.81	1.42–2.31	<0.001	1.87	1.57–2.24	<0.001	1.82	1.55–2.12	<0.001	1.48	1.30–1.79	<0.001
Age, sex, LVEF category, ethnic group												
NT-proBNP	1.44	1.23–1.69	<0.001	1.45	1.30–1.62	<0.001	1.43	1.30–1.57	<0.001	1.44	1.33–1.55	<0.001
hs-TnT	1.29	1.07–1.66	0.008	1.36	1.19–1.56	<0.001	1.45	1.29–1.63	<0.001	1.45	1.31–1.60	<0.001
sST2	1.34	1.03–1.75	0.027	1.50	1.24–1.82	<0.001	1.50	1.27–1.77	<0.001	1.23	1.07–1.42	0.004
Model 3												
NT-proBNP	1.44	1.18–1.75	<0.001	1.34	1.16–1.55	<0.001	1.26	1.11–1.42	<0.001	1.27	1.14–1.42	<0.001
hs-TnT	–	–	0.050	1.42	1.20–1.77	<0.001	1.60	1.38–1.85	<0.001	1.62	1.41–1.85	<0.001
sST2	–	–	0.110	1.66	1.31–2.11	<0.001	1.79	1.45–2.22	<0.001	1.78	1.46–2.17	<0.001

CI, confidence interval; CV, cardiovascular; HFH, heart failure hospitalization; HR, hazard ratio; hs-TnT, high-sensitivity troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenesis-2.

Values of NT-proBNP, hs-TnT, and sST2 were available for all patients; for each patient, a single measurement of each biomarker was considered. Biomarker values were ln-transformed.

The variable 'ethnic group' was computed as 1 = Caucasian, 2 = Afro-American, 3 = Asian, 4 = other.

Model 3 included: age, female sex, ethnic group, ischaemic heart failure, LVEF category (heart failure with reduced, mid-range, or preserved ejection fraction), New York Heart Association class I–II vs. III–IV, body mass index, estimated glomerular filtration rate, anaemia, hypertension, atrial fibrillation, diabetes, chronic obstructive pulmonary disease, therapy with beta-blockers, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, mineralocorticoid receptor antagonists.

prognostic performance of the three biomarkers in men vs. women, and Caucasian vs. non-Caucasian ethnicity in the different age categories. Indeed, the AUC values calculated for these patient subsets were probably affected by the highly different patient numbers (online supplementary *Table S7*). Fourth, all studies containing these patient data were published before 2016, thus not considering treatment with sacubitril/valsartan, now prescribed in a significant number of patients with HFrEF. Fifth, the boundaries between age categories were rather arbitrarily set at 60, 70, and 80 years, in order to have an adequate number of patients within each category. Sixth, cut-offs might be much more easily used in current clinical practice than continuous values, but dichotomizing continuous predictors in multiple regression might entail a loss of prognostic information compared to absolute biomarker values,[37] and age-specific cut-offs defined through the Youden method are more influenced by the size and composition of patient groups than continuous biomarker values. Seventh, the best age-specific cut-offs were calculated only in the whole population, instead than in smaller patient subgroups identified by sex, LVEF categories, different ethnicities, etc. Eighth, as stated above, the study design did not allow to define the mechanisms of the different relationships observed between age and HF biomarkers. Finally, limited information was available on patient co-morbidities (particularly chronic inflammatory conditions) potentially affecting sST2 values.

In conclusion, sST2 is less influenced by age than NT-proBNP or hs-TnT; all these biomarkers predict outcome regardless of age. The use of age- and outcome-specific cut-offs of NT-proBNP, hs-TnT and sST2 allows a more accurate risk stratification than NT-proBNP alone, or the combination of NT-proBNP and hs-TnT.

Supplementary Information

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

Figure S1. Relationship between age and circulating levels of heart failure biomarkers among men and women.

Figure S2. Relationship between age and circulating levels of heart failure biomarkers across categories of left ventricular ejection fraction.

Table S1. Number of events at the different timepoints.

Table S2. Area under the curve values of the heart failure biomarkers.

Table S3. Sensitivity and specificity of the best age-specific cut-offs.

Table S4. Best cut-offs for prognostic prediction across age categories.

Table S5. Patient stratification based on age-specific cut-offs: independent prognostic value from plasma N-terminal pro-B-type natriuretic peptide.

Table S6. Relative risks of all-cause and cardiovascular death and heart failure hospitalization according to age-specific cut-offs.

Table S7. Area under the curve values across population subsets defined by age, gender, and ethnicity.

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