Prognostic value of plasma sAXL in patients with heart failure: insights from the DRAGON-HF trial

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Abstract

Background Little is known about the predictive value of soluble AXL (sAXL) in heart failure (HF). This study aimed to describe the prognostic value of plasma sAXL in patients with symptomatic HF.
Methods This is a multicentre observational prospective cohort study (Registration No. NCT03727828). Plasma sAXL were measured on admission. The primary endpoint is a composite of cardiovascular mortality and HF rehospitalization. Associations between plasma sAXL levels and clinical endpoints are described using Cox regression models and Kaplan–Meier methods.
Results A total of 1030 symptomatic HF patients were enrolled in the study; the mean age (65% men) was 71 ± 12 years, with a median follow-up of 32 months (IQR: 26–41 months). The mean baseline sAXL levels were 20.03 ± 6.74 ng/mL. Plasma sAXL positively associated with NYHA classification and negatively associated with left ventricular ejection fraction (both $P < 0.001$). Cox regression showed that 1-SD increment of sAXL was associated with primary endpoint [HR (CI): 1.128 (1.024–1.242)], cardiovascular mortality [1.112 (1.032–1.198)], all-cause mortality [1.142 (1.057–1.234)], and HF rehospitalization [1.122 (1.030–1.224)] after adjustment for potential confounders including NT-proBNP. Kaplan–Meier curves revealed that patients with the highest sAXL levels were at the highest risk of primary endpoint events, cardiovascular mortality, and all-cause mortality (all P values < 0.001). Furthermore, both Kaplan–Meier method and Categorical analysis demonstrated that the combined use of sAXL and NT-proBNP were more likely to predict all-cause or cardiovascular mortality (both $P < 0.001$). Similar results were observed when separating patients with respect to left ventricular ejection fraction, namely, in HFrEF, HFmrEF, and HFpEF groups.
Conclusions Plasma sAXL concentrations are of great importance in predicting clinical outcomes in HF patients, independent of NT-proBNP, suggesting that sAXL is a promising prognostic marker for further study.

Introduction

Heart failure (HF), which constitutes the end stage of many heart diseases, is a serious syndrome with substantial morbidity, mortality, and frequent hospitalizations. In adults over 20 years old, the prevalence of HF is around 2.2%,[1] and this rate increases dramatically in the elderly, in people with co-morbidities or cardiovascular risk factors, and in the

population suffering from heart diseases such as myocardial infarction.[2,3] Current guidelines classify HF into mainly three types based on the left ventricular ejection fraction (LVEF), that is, HF with reduced ejection fraction (HFrEF), HF with mid-range ejection fraction (HFmrEF), and HF with preserved ejection fraction (HFpEF).[4] Although the evidence-based management of HF has improved in recent years, much is still lacking in the diagnosis and treatment

of HF, thus culminating in the low survival rate of HF patients.

Plasma biomarkers, which may reveal the pathophysiology of HF, play an important role in identifying HF patients and predicting adverse consequence. For example, natriuretic peptides, especially B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), are the central biomarkers in diagnosis and prognosis of HF.[4,5] Although the utility of BNP and NT-proBNP in HF has been well established, the 2013 American Heart Association (AHA) guidelines on HF management also recommended the use of alternative biomarkers for risk stratification in the management of HF.[6] Thus, novel biomarkers that may improve current management of HF patients are necessary to enhance diagnosis and prognosis of HF patients.

In this regard, AXL is a member of the Tyro3, AXL, and MerTK (TAM) subfamily of receptor tyrosine kinases (RTKs), the membrane proteins recognizing extracellular signals leading to cellular responses such as proliferation, arrest, or activation. AXL is highly expressed by cells of vasculature and cardiomyocytes.[7,8] Membrane-bound AXL can be shed by the effects of a disintegrin and metalloproteinase domain-containing protease (ADAM), causing membrane-bound AXL to switch to a soluble form that can be detected in plasma, termed soluble AXL (sAXL).[9] The sAXL is considered as a biomarker of endothelial dysfunction and has been associated with various cardiovascular diseases such as hypertension[10] and myocardial ischaemia.[11] In previous studies, patients with HF had higher values of sAXL levels. Serum sAXL level has also been independently correlated with parameters indicating worse prognosis of HF.[12,13] However, the direct evidence of an association between sAXL level and the prognosis of HF in a large cohort is still lacking. Hence, we conducted this cohort study to elucidate the association between sAXL levels and adverse cardiovascular events and to further evaluate the additive prognosis value of sAXL to NT-proBNP in patients with HF.

Methods

Study population

This study (Diagnostic, Risk Stratification and Prognostic Value of Novel Biomarkers in Patients with Heart Failure, DRAGON-HF trial) is an ongoing multicentre observational cohort study. The present analysis included consecutive patients diagnosed with symptomatic HF in Shanghai Tenth People's Hospital, People's Hospital of Xinjiang Uygur Autonomous Region, Shanghai Municipal Hospital of Traditional Chinese Medicine, and Shanghai East Hospital, from January 2017 to November 2019. The diagnostic criteria of HF in our study are consistent with the criteria recommended by the

European Society of Cardiology.[4] The inclusion criteria were (i) age >18 years old; (ii) Symptomatic (NYHA Classes II–IV) HF (as diagnosed by clinician, radiographic images, or abnormal natriuretic peptide level); and (iii) hospitalized for HF at the inception of the study. The cut-off value of NT-proBNP was 125 pg/mL, which is recommended by the 2016 guideline of European Society of Cardiology for the management of heart failure.[4] The exclusion criteria were (i) life expectancy <1 year due to causes other than HF such as advanced cancer; (ii) cardiac transplantation or revascularization indicated or expected within 6 months; (iii) severe obstructive or restrictive pulmonary disease; (iv) subject unable or unwilling to provide written informed consent; (v) coronary revascularization (percutaneous coronary intervention or bypass surgery) within previous 3 months; (vi) progressive neurological disease; and (vii) pregnancy. This study complied with the Declaration of Helsinki and was approved by the local ethics committee. All participants provided written informed consent. This study was registered in *clinicaltrials.gov* with the registration number of NCT03727828.

Medical history and biochemical parameters

Medical history such as hypertension, diabetes, smoking, etc., were obtained from patients' medical records or a structured questionnaire if the records were lacking. Body measurements such as height and weight were performed by a trained nurse.

Blood samples were obtained with EDTA tubes by venipuncture and transported to a central laboratory for further processing according to a standardized protocol. The blood samples were centrifuged at 1700 g for 15 min immediately, and the plasma samples were then separated and preserved at −80°C with RNase/DNase-free tubes for further determination. The concentrations of soluble AXL were determined in plasma using an enzyme-linked immunosorbent assay (ELISA) technique (DY154, R&D system), and final concentrations were obtained from three replicates of each sample. Detection of specifically bound antibody was done through standard colorimetry at 450 nm. Analysts were blinded to patients' characteristics and endpoints. Other biochemical parameters such as NT-proBNP, fasting glucose, lipid profiles, serum creatinine, etc., were measured in laboratories with ISO 9001 international quality certificate in the respective hospitals mentioned.

Follow-ups and clinical endpoints

All participants were followed up every 6 months after the enrolment. During follow-ups, we screened the participants' medical records and then visited or phoned all participants or their contact person to ask or check the information. If

we failed to reach the patient or the contact person, the civil registry was approached to verify the vital status. The primary endpoint was a composite of cardiovascular mortality and HF rehospitalization. Secondary endpoints included cardiovascular mortality, all-cause mortality, and HF rehospitalization. HF rehospitalization was defined based on two criteria: symptoms of cardiac decompensation and treatment with intravenous diuretics. Intravenous diuretics includes the treatment performed in outpatient clinic, emergency department, and in-hospital patients. In cases where the patient had multiple events, the time to first event was counted as clinical outcome. An independent endpoint committee adjudicated all endpoints.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for normally distributed quantitative variables, and median [interquartile range (IQR)] for non-normally distributed quantitative variables. For categorical variables, number and percentage were used in summary statistics. Participants were divided into different groups based on the quantiles of sAXL concentrations, left ventricular ejection fraction (LVEF) levels, and NYHA functional status, respectively. Differences of continuous variables were compared using one-way analysis of variance (ANOVA) with Tukey's test if variables were normally distributed and using Kruskal–Wallis test with Steel–Dwass test if variables were non-normally distributed. Differences of proportions were compared using chi-squared test. Cox regression analyses were performed to assess the associations between sAXL levels and different endpoints. Three models with different adjustment (sensitive analyses) were conducted to verify the independent associations. Next, Kaplan–Meier curve with log-rank test were used to compare the rates of endpoints among participants with different sAXL levels (stratified by the quartiles of sAXL level). To assess the additive prognostic value of sAXL to NT-proBNP in predicting mortality, all patients were divided into four (2 * 2) groups based on the sAXL levels and NT-proBNP values at baseline. The threshold of NT-proBNB was chosen as 1000 pg/mL based on previous studies.[14] Because1000 pg/mL was close to the lower tertile of NT-proBNB level in this population, the lower tertile of sAXL (16.82 ng/mL) was used as the other threshold. For all-cause mortality, cumulative rate of events in each group was calculated and compared with log-rank test. For cardiovascular mortality, the event rates among these four groups were shown in scatterplot graph and were compared using chi-squared test. Finally, subgroup analyses using Cox regression analyses were performed to examine potential different associations between primary endpoint and sAXL level across prespecified subgroups of interest like age, gender, etc. A two-sided P value ≤ 0.05 was considered

statistically significant. All statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., USA)

Results

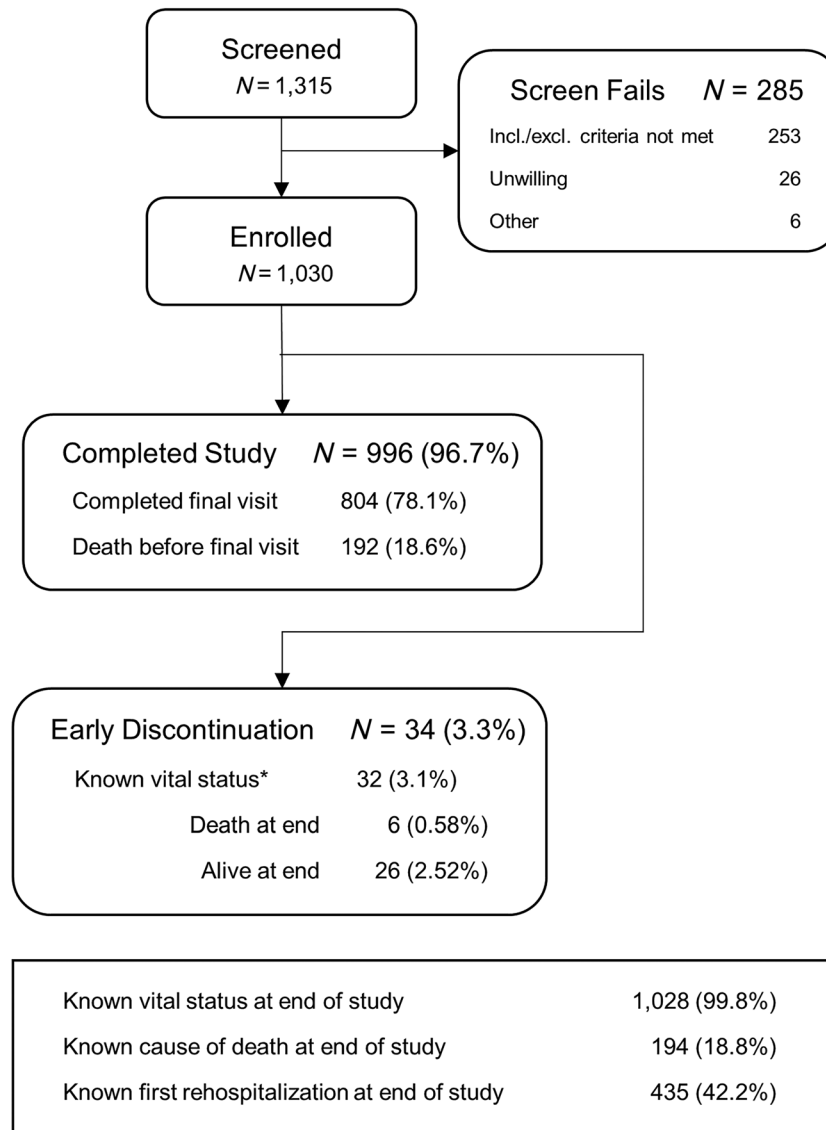## Demographic and clinical characteristics

As shown in *Figure 1*, a total of 1315 HF patients were screened initially, and 1030 participants (mean age 71 ± 12 years) were recruited. Nine hundred and ninety-six participants (96.7%) completed this study. For participants who discontinued before final visit, we worked with the local government to obtain the updated vital status of those participants, and 1028 participants (99.8%) were with known vital status at the end of study. Of all these HF patients, 673 (65.3%) were men, 237 (23.0%) were smokers, 772 (75.0%) were with coronary heart diseases, 690 (67.0%) were hypertensive patients, and 358 (34.8%) were diabetic patients. The mean left ventricular mass index was 114 ± 36 g/m^2, and the mean LVEF was 47 ± 13%. The median baseline NT-proBNP was 1484.0 pg/mL (IQR: 676.8–3828.0 pg/mL). The mean baseline sAXL level was 20.03 ± 6.74 ng/mL. The median sAXL level was 18.44 ng/mL (8.07 ≤ Q1 < 15.86 ng/mL; 15.86 ≤ Q2 < 18.44 ng/mL; 18.44 ≤ Q3 < 21.94 ng/mL; 21.94 ≤ Q4 < 54.41 ng/mL). Other information about baseline characteristics like NYHA classifications, treatment at discharge, and biochemical parameters were shown in *Table 1*. The further comparison of oral medication at discharge among patients with different LVEF ranges is shown in *Table S1*.

We further divided all participants into four subgroups according to the quartiles of baseline sAXL levels (subgroups Q1–Q4). In ANOVA analysis for linear trend test, sAXL levels were positively associated with NT-proBNP levels (P < 0.001) and negatively associated with LVEF (P < 0.001). Besides, patients with higher sAXL level were associated with higher diastolic blood pressure (P < 0.01, ANOVA analysis for linear trend test, similarly hereinafter), faster heart rate (P < 0.01), higher left ventricular mass index (P < 0.001), lower estimated glomerular filtration rate (eGFR) and worse kidney function (P < 0.001) and higher prevalence of myocardial infarction (P < 0.001). As for other biochemical parameters related to cardiac function, sAXL levels were associated with higher levels of C-reactive protein (CRP), alanine aminotransferase (ALT), and high-sensitivity troponin T (hs-cTnT) (all P < 0.001).

Association between sAXL and cardiac function

To further study the relationship between sAXL levels and cardiac function, patients were divided into three groups based on LVEF and NYHA classification, respectively (see

Figure 1 Flow chart of the study cohort. This study screened 1315 patients with heart failure, and 1030 were enrolled. Of them, 996 participants completed final visit. For the other 34 participants, we contacted the local Civil Affairs Bureau and Police Station to know the vital status and potential cause of death of these participants. One thousand and twenty-eight participants were with known vital status.

Screened
N = 1,315

Screen Fails	*N* = 285
Incl./excl. criteria not met	253
Unwilling	26
Other	6

Enrolled
N = 1,030

Completed Study	*N* = 996 (96.7%)
Completed final visit	804 (78.1%)
Death before final visit	192 (18.6%)

Early Discontinuation	*N* = 34 (3.3%)
Known vital status*	32 (3.1%)
Death at end	6 (0.58%)
Alive at end	26 (2.52%)

Known vital status at end of study	1,028 (99.8%)
Known cause of death at end of study	194 (18.8%)
Known first rehospitalization at end of study	435 (42.2%)

* We cooperated with local Civil Affairs Bureau and Police Station to know the vital status and potential cause of death of the participants who early discontinued from this study.

Table 2). There are 519 (50.4%) patients with HFpEF (LVEF ≥50%), 177 (17.2%) patients with HFmrEF (LVEF between 40% and 49%), and 315 (30.6%) patients with HFrEF (LVEF <40%). The baseline plasma sAXL level in HFrEF patients was significantly greater than HFpEF patients ($P < 0.001$). However, compared with HFmrEF patients, the difference of sAXL levels was not statistically significant ($P = 0.15$). The ANOVA analysis for linear trend test showed that sAXL level was negatively associated with LVEF level ($P < 0.001$). The conventional biomarker of HF, NT-proBNP, was significantly associated with LVEF in ANOVA analysis for linear trend test.

NT-proBNP level was the highest in patients with LVEF <40% among these three groups stratified by LVEF (≥50%, 40–49%, <40%). To verify the association between sAXL levels and ventricular function, we then divided all participants into three groups based on NYHA classification (II, III, IV). Similar results were observed. Patients with NYHA Class IV had higher plasma sAXL and NT-proBNP levels than those with NYHA Class II ($P < 0.001$) or Class III ($P < 0.001$). In ANOVA analysis for linear trend test, both plasma sAXL and NT-proBNP levels increased with the elevation of NYHA classification ($P < 0.001$).

Table 1 Baseline characteristics according to the overall sample (n = 1030) and quartiles of baseline sAXL level

Characteristics	Overall sample	Q1(n = 258)	Q2(n = 257)	Q3(n = 257)	Q4(n = 258)	P	P for trend
Age, years	71 ± 12	71 ± 11	71 ± 11	72 ± 12	70 ± 13	0.626	0.983
Male	673(65.3%)	151(58.5%)	169(65.8%)	177(68.9%)	176(68.2%)	0.053	0.015
Smoking status	237(23.0%)	47(18.2%)	57(22.2%)	53(20.6%)	80(31.0%)	0.004	0.002
Drinking status	59(5.7%)	11(4.2%)	14(5.4%)	15(5.8%)	19(7.4%)	0.504	0.134
BMI, kg/m²	24.4 ± 3.8	24.8 ± 3.3	24.0 ± 4.0	24.4 ± 4.2	24.3 ± 3.7	0.252	0.538
SBP, mmHg	136 ± 24	137 ± 21	133 ± 23	136 ± 24	138 ± 27	0.099	0.464
DBP, mmHg	77 ± 15	76 ± 13	74 ± 14	78 ± 16	79 ± 16	0.001	0.001
Heart rate, beats/min	82 ± 18	79 ± 18	82 ± 19	81 ± 17	85 ± 19	0.004	0.003
LVMI, g/m²	114 ± 36	100 ± 28	118 ± 36	119 ± 39	118 ± 36	<0.001	<0.001
Coronary heart disease	772(75.0%)	197(76.4%)	179(69.6%)	188(73.2%)	208(80.6%)	0.030	0.177
Myocardial infarction	260(25.2%)	50(19.4%)	60(23.3%)	56(21.8%)	94(36.4%)	<0.001	<0.001
Hypertension	690(67.0%)	174(67.4%)	165(64.2%)	176(68.5%)	175(67.8%)	0.737	0.679
Diabetes mellitus	358(34.8%)	84(32.6%)	83(32.3%)	86(33.5%)	105(40.7%)	0.142	0.054
HF history							
NYHA classification						<0.001	<0.001
II	662(64.3%)	208(91.2%)	162(63.0%)	155(60.3%)	137(53.1%)		
III	301(29.2%)	49(19.0%)	82(31.9%)	84(32.7%)	86(33.3%)		
IV	67(6.5%)	1(0.4%)	13(5.1%)	18(7.0%)	35(13.6%)		
LVEF, %	47 ± 13	55 ± 10	47 ± 13	46 ± 13	41 ± 13	<0.001	<0.001
Treatment at discharge							
ACEi/ARB	576(55.9%)	154(59.7%)	152(59.1%)	145(56.4%)	125(48.4%)	0.038	0.008
β-Blocker	758(73.6%)	176(68.2%)	190(73.9%)	191(74.3%)	201(77.9%)	0.094	0.016
Diuretic	526(51.1%)	80(31.0%)	127(49.4%)	159(61.9%)	160(62.0%)	<0.001	<0.001
Spirolactone	476(46.2%)	73(28.3%)	122(47.5%)	138(53.7%)	143(55.4%)	<0.001	<0.001
Statins	834(81.0%)	217(84.1%)	210(81.7%)	196(76.3%)	211(81.8%)	0.136	0.256
Nitrates	308(29.9%)	70(27.1%)	97(37.7%)	83(32.3%)	58(22.5%)	0.001	0.128
Ivabradine	33(3.2%)	2(0.8%)	7(2.7%)	7(2.7%)	17(6.6%)	0.002	<0.001
Laboratory measurements							
Hb, g/L	128 ± 19	130 ± 17	129 ± 20	127 ± 20	127 ± 20	0.170	0.039
CRP, mg/L	3.6(3.0, 12.7)	3.0(2.5, 4.3)	3.8(3.0, 10.0)	4.4(3.0, 18.2)	7.4(3.0, 24.8)	<0.001	<0.001
ALT, U/L	22.3(13.9, 36.2)	21.6(13.0, 33.8)	21.2(13.8, 33.4)	23.8(13.7, 38.4)	24.2(15.2, 39.5)	0.040	0.001
eGFR, mL/min/1.73m²	72.3(54.2, 8.4)	82.2(67.4, 7.6)	70.9(55.1, 85.7)	64.6(48.7, 83.0)	66.0(45.3, 87.9)	<0.001	<0.001
LDL, mmol/L	2.1 ± 1.0	2.1 ± 1.1	2.1 ± 0.9	2.1 ± 0.9	2.2 ± 1.0	0.896	0.488
HbA1C, %	6.8 ± 1.5	6.7 ± 1.5	6.8 ± 1.5	6.7 ± 1.5	7.0 ± 1.6	0.115	0.089
hs-cTnT, ng/mL	0.030 (0.015, 0.096)	0.015 (0.010, 0.026)	0.028 (0.016, 0.065)	0.043 (0.020, 0.180)	0.054 (0.022, 0.490)	<0.001	<0.001
NT-proBNP, pg/mL	1484.0 (676.8, 3828.0)	544.9 (318.1, 1011.0)	1479.0 (819.5, 3000.0)	2274.0 (1216.0, 5632.0)	3445.0 (1272.0, 7952.0)	<0.001	<0.001

ALT, alanine transaminase; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HbA1C, glycosylated haemoglobin; HF, heart failure; hs-cTnT, high-sensitivity cardiac troponin; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; Q, quartile; SBP, systolic blood pressure.
8.07 ≤Q1 < 15.86 ng/mL; 15.86 ≤ Q2 < 18.44 ng/mL; 18.44 ≤ Q3 < 21.94 ng/mL; 21.94 ≤ Q4 < 54.41 ng/mL. Values presented as mean ± SD, median (IQR), or n (%). P value for differences between quartiles of baseline sAXL level.

Table 2 sAXL and NT-proBNP levels in patients with different HF characteristics

	n	sAXL(ng/mL)	NT-proBNP(pg/mL)
LVEF			
≥50%	519	18.26 ± 5.50	1051.0 (545.7, 2300.0)
40–49%	177	21.09 ± 7.29	1291.5 (568.45, 3608.5)
<40%	315	22.29 ± 7.41	3351.0 (1462.0, 7572.0)
P*		<0.001	<0.001
P#		0.147	<0.001
Overall *P* value		<0.001	<0.001
P for trend		<0.001	<0.001
NYHA			
II	662	19.32 ± 6.65	1082.0 (557.8, 2385.0)
III	301	20.65 ± 6.47	2635.0 (1149.0, 6796.0)
IV	67	24.30 ± 7.09	5331.0 (3106.0, 13104.0)
P*		<0.001	<0.001
P#		<0.001	<0.001
Overall *P* value		<0.001	<0.001
P for trend		<0.001	<0.001

P*: comparison between LVEF≥50% and LVEF <40% or between NYHA Class II and NYHA Class IV. *P*#: comparison between LVEF 40–49% and LVEF <40%, or between NYHA Class III and NYHA Class IV. Other abbreviations as in *Table 1*.

Relationships between sAXL and clinical endpoints

Cox regressions were performed to test if baseline sAXL level is able to predict cardiovascular events (*Table 3*). First, univariate analyses were performed to obtain the crude estimates of the effect of baseline sAXL level (Model 1). Second, analyses were adjusted for age and sex (Model 2) and then additionally adjusted for body mass index, smoking and drinking history, coronary heart disease, hypertension, diabetes mellitus, eGFR, low density lipoprotein cholesterol, LVEF, left ventricular mass index, and baseline NT-proBNP (Model 3).

The results are presented as adjusted hazard ratios (HRs) per 1-SD increase of the biomarker level with 95% confidence intervals (CIs). The median follow-up duration was 32 months (IQR: 2641 months), and 578 (56.12%) patients reached the predefined primary and secondary endpoints.

With the 1-SD increase of baseline plasma sAXL level, the risk of primary endpoint events was significantly increased in the univariate Cox regression (Model 1), with the HR of 1.276 [1.183–1.376], $P < 0.001$. After the adjustment for age and sex (Model 2), baseline sAXL level was significantly associated with primary endpoint events [HR: 1.267 (1.174–1.369), $P < 0.001$]. This association was also statistically sig-

Table 3 Hazard ratios for different endpoints per 1-SD increase of the baseline sAXL level

	n	HR(95% CI)	*P* value
Primary endpoint			
Model 1		1.276(1.183–1.376)	<0.001
Model 2		1.267(1.174–1.369)	<0.001
Model 3		1.128(1.024–1.242)	0.015
Number of events/patients	531/1030		
Cardiovascular mortality			
Model 1		1.279(1.204–1.358)	<0.001
Model 2		1.272(1.197–1.352)	<0.001
Model 3		1.112(1.032–1.198)	0.005
Number of events/patients	133/1030		
All-cause mortality			
Model 1		1.284(1.206–1.366)	<0.001
Model 2		1.279(1.201–1.362)	<0.001
Model 3		1.142(1.057–1.234)	<0.001
Number of events/patients	198/1030		
HF rehospitalization			
Model 1		1.239(1.159–1.324)	<0.001
Model 2		1.243(1.162–1.330)	<0.001
Model 3		1.122(1.030–1.224)	0.009
Number of events/patients	435/1030		

CI, confidence interval; HF, heart failure; HR, hazard ratio; SD, standard deviation.
Hazard ratios are related to a 1-SD increase of sAXL at baseline. Model 1 unadjusted; Model 2 adjusted for age and sex; Model 3 adjusted for age, sex, body mass index, smoking and drinking history, coronary heart disease, hypertension, diabetes mellitus, estimated glomerular filtration rate, low density lipoprotein, left ventricular ejection fraction, left ventricular mass index, and baseline NT-proBNP. The primary endpoint included cardiovascular mortality and HF rehospitalization. Other abbreviations as in *Tables 1* and *2*.

nificant after adjustment for potential confounders such as NT-proBNP [HR: 1.128 (1.024–1.242), P = 0.015]. *Figure 2* showed the relative hazard ratio of participants with different sAXL levels. Similar results were also observed between primary endpoint and baseline sAXL levels. sAXL levels were significantly and independently associated with cardiovascular mortality in both univariate analysis (Model 1, P < 0.001) and multivariate analyses (Model 2 and Model 3, both P < 0.01). With respect to all-cause mortality and HF rehospitalization, these associations were still statistically significant in these three Cox regression models. We also performed the Cox regressions in patients with HFrEF, HFmrEF, and HFpEF (*Table S2*). In patients with HFpEF, the risks of all predefined endpoints were significantly increased with the increase of baseline plasma sAXL level. With respect to cardiovascular mortality and all-cause mortality, these associations were still statistically significant in HFrEF patients. However, in HFmrEF patients, the association between baseline sAXL and endpoints did not reach statistical significance. The Cox regression analyses showed that baseline sAXL level was an independent predictor of primary endpoint events, all-cause mortality, HF hospitalization, and cardiovascular mortality, respectively.

The Kaplan–Meier method was used to further analyse the associations between sAXL levels and different endpoints. Patients were divided into four groups based on the quartiles of plasma sAXL level (Q1–Q4). *Figure 3* shows the risk of primary endpoint events (cardiovascular mortality or HF hospitalization) in Q4 group (sAXL >21.94 ng/mL) was about 1.4-fold higher compared with the Q1 group [HR 1.43 (1.23–1.82), P < 0.001]. The risk of cardiovascular mortality of patients in Q4 group was 8.4-fold higher than the patients in Q1 group [Q4 vs Q1, HR 8.40 (5.15–13.70), P < 0.001]. The risk of all-cause mortality in Q4 group also higher than in the Q1 group [HR 5.79 (3.85–8.71), P < 0.001]. However, the risk of HF rehospitalization among Q1–Q4 was not statistic signif-

icant (P ≥ 0.194). The same analyses were performed in HFrEF, HFmrEF, and HFpEF patients, respectively (*Figure S1*). Similar results were observed in these patients. High sAXL level contributes to more cardiovascular mortality and all-cause mortality in all types of HF patients and more primary endpoints in HFrEF patients.

The additional prognostic value of sAXL to NT-proBNP

Patients with NT-proBNP over 1000 pg/mL have been associated with worse clinical outcomes as demonstrated in previous studies.[14] We thus classified patients into low BNP group (NT-proBNP ≤1000 pg/mL) and high BNP group (NT-proBNP >1000 pg/mL). Because 1000 pg/mL is close to the lower tertile of baseline NT-proBNB values, the lower tertile of sAXL (16.82 ng/mL) at baseline was chosen as a threshold to classify patients into low sAXL group (sAxl ≤16.82 ng/mL) and high sAXL group (sAxl >16.82 ng/mL). Therefore, there were four groups (2 * 2), namely, Low sAXL-Low BNP (Low-Low) group, High sAXL-Low BNP (High-Low) group, Low sAXL-High BNP (Low-High) group, and High sAXL-High BNP (High-High) group. Kaplan–Meier curves were used to characterize risks of primary endpoint events, cardiovascular mortality, all-cause mortality, and HF rehospitalization in each group. We further studied the prognostic value of sAXL in addition to NT-proBNP in predicting mortality. As shown in *Figure 4*, compared with the High-High group (reference), all other groups were significantly at lower risks of primary endpoint events, cardiovascular mortality, and all-cause mortality except HF rehospitalization. These results suggested that the combined use of sAXL and NT-proBNP is important in predicting primary endpoint events, cardiovascular mortality, and all-cause mortality in HF patients. Similar results were observed in the categorical analysis related to predefined

Figure 2 Association between sAXL and primary endpoint: continuous. Cox regression was performed to test the association between sAXL and primary endpoint including cardiovascular mortality and heart failure rehospitalization. Increased sAXL levels were associated with increased risk of primary endpoint events [hazard ratio (HR) and 95% confidence interval (CI) per 1 ng/mL increment of sAXL level: 1.018 (1.004–1.033), P < 0.001]. Solid oblique line and dash curves indicates the relative HR and 95% CI. Solid horizontal line indicates the referent HR of 1.00.
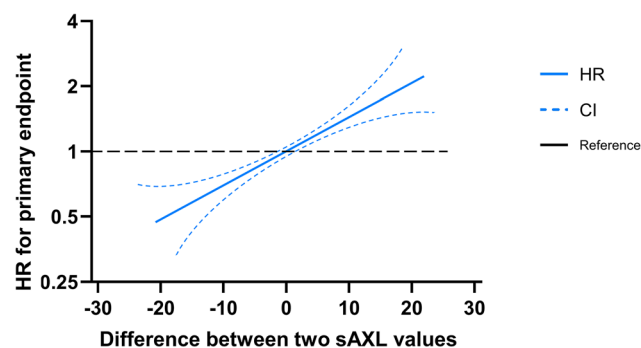
Figure 3 Associations between sAXL levels and different endpoints. All participants were divided into four groups based on the quartiles of sAXL levels (Q1–Q4 group). Kaplan–Meier method was used to analyse the associations between different groups and predefined endpoints. (A) Primary endpoint including cardiovascular mortality and heart failure rehospitalization. (B) Cardiovascular mortality. (C) All-cause mortality. (D) HF rehospitalization.
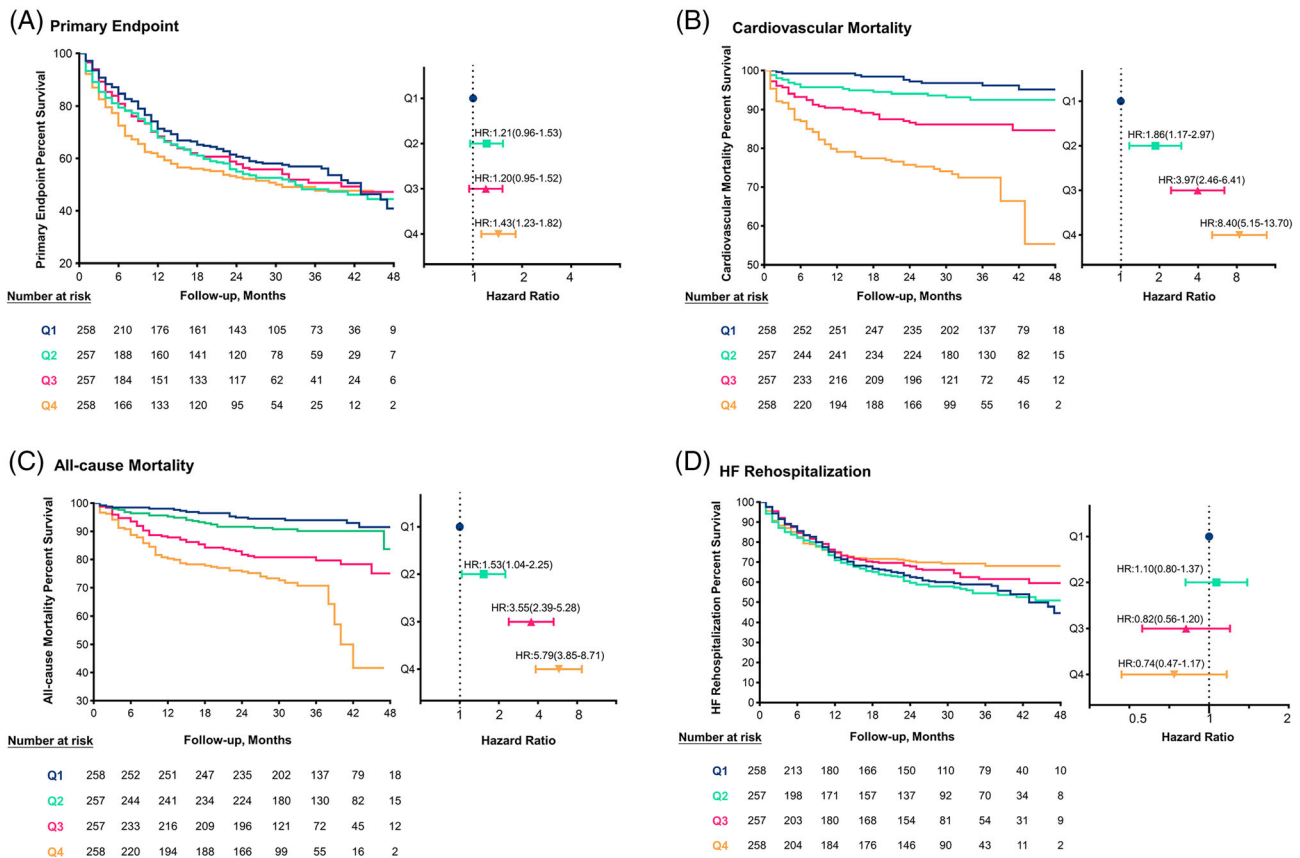
endpoints (*Figure S2*). Compared with High sAXL-High BNP group, patients in Low sAXL-High BNP group were with lower rate of cardiovascular mortality (3.5% vs. 21.1%, *P* < 0.001) and all-cause mortality (8.6% vs. 29.1%, *P* < 0.001), and tended to be at lower risk of primary endpoint (45.7% vs. 54.8%, *P* = 0.08).

Subgroup analyses of the relationship between sAXL and cardiovascular mortality

To examine the relationship between sAXL level and cardiovascular mortality in population with different characteristics, Cox regression analyses were performed in different prespecified subgroups of interest. Participants were divided into two groups according to age (≥75 or <75 years), gender, smoking status, coronary heart disease, hypertension, diabetes, and renal function, respectively. As shown in *Figure 5*, sAXL were significantly associated with primary endpoint in all subgroups, suggesting the consistence of sAXL in patients with different characteristics.

Discussion

Our findings demonstrated that plasma sAXL on admission was closely associated with cardiac function. Importantly, we established that in symptomatic HF patients, plasma sAXL was a strong independent predictor of adverse cardiovascular events that provided incremental prognostic value compared with NT-proBNP.

The understanding of HF has been developed from the syndrome of disordered haemodynamics caused by alterations in the architecture of the heart to a disease that involves intertwined molecular pathways in disarray.[15] Increasing evidence showed that serum biomarkers play important and unique roles in unravelling the pathophysiology of HF. Because the symptoms and signs of HF are often non-specific, major practical guidelines have recognized the utility of recommended biomarkers in the diagnosis and management of HF, especially BNP and NT-proBNP,[4,5] albeit there are limitations with NT-proBNP.

The application of sAXL in clinical practice has been extensively studied in recent years. sAXL values have been associ-

Figure 4 Effect of high sAXL in addition to high NT-proBNP in predicting different endpoints. Patients were divided into four categorical groups, namely, the high sAXL- high NT-proBNP (High-High) group, high sAXL- low NT-proBNP (High-Low) group, low sAXL- high NT-proBNP (Low-High) group, and Low sAXL- low NT-proBNP (Low-Low) group. The threshold of NT-proBNB was 1000 pg/mL (close to the lower tertile of NT-proBNB level), and the threshold of sAXL is 16.82 ng/mL (the lower tertile of sAXL level). Kaplan–Meier curve and hazard ratio together with 95% confidence intervals were shown in each panel. (A) Primary endpoint including cardiovascular mortality and heart failure rehospitalization. (B) Cardiovascular mortality. (C) All-cause mortality. (D) HF rehospitalization.
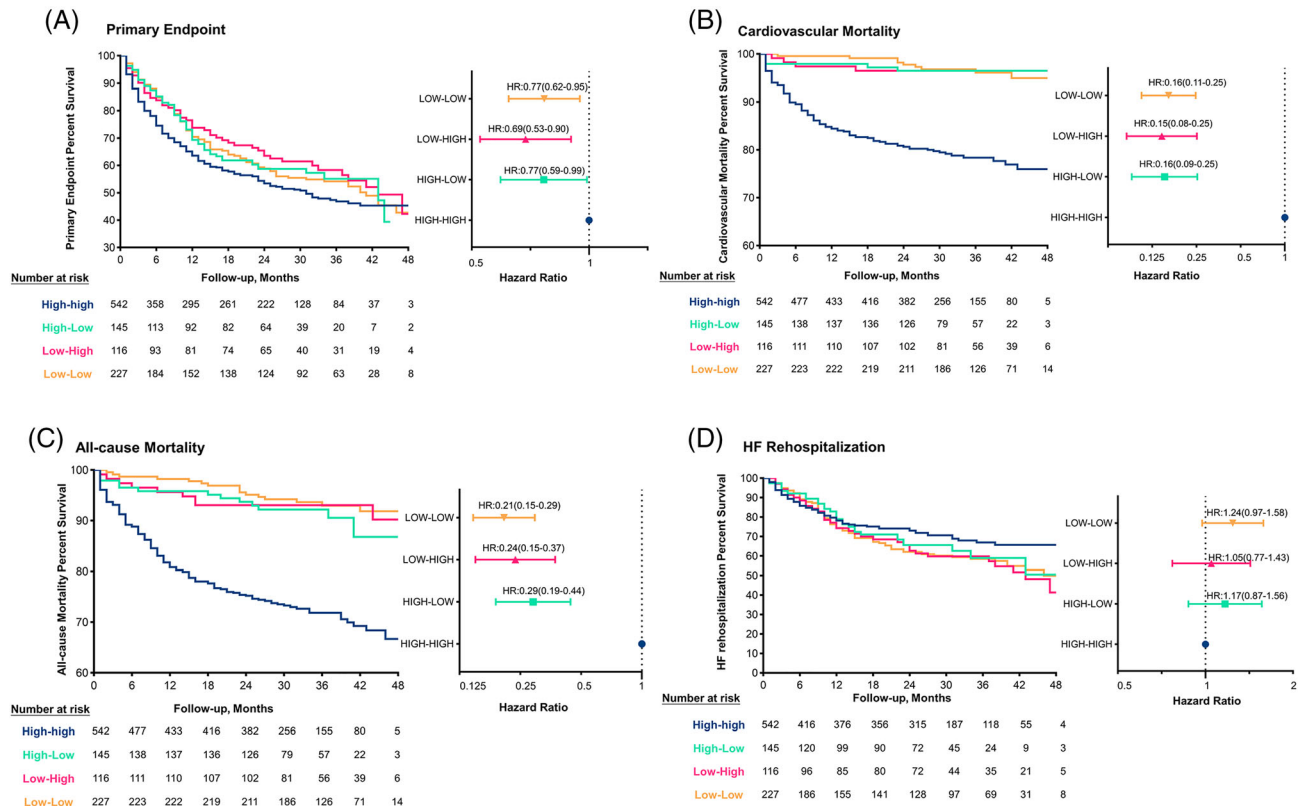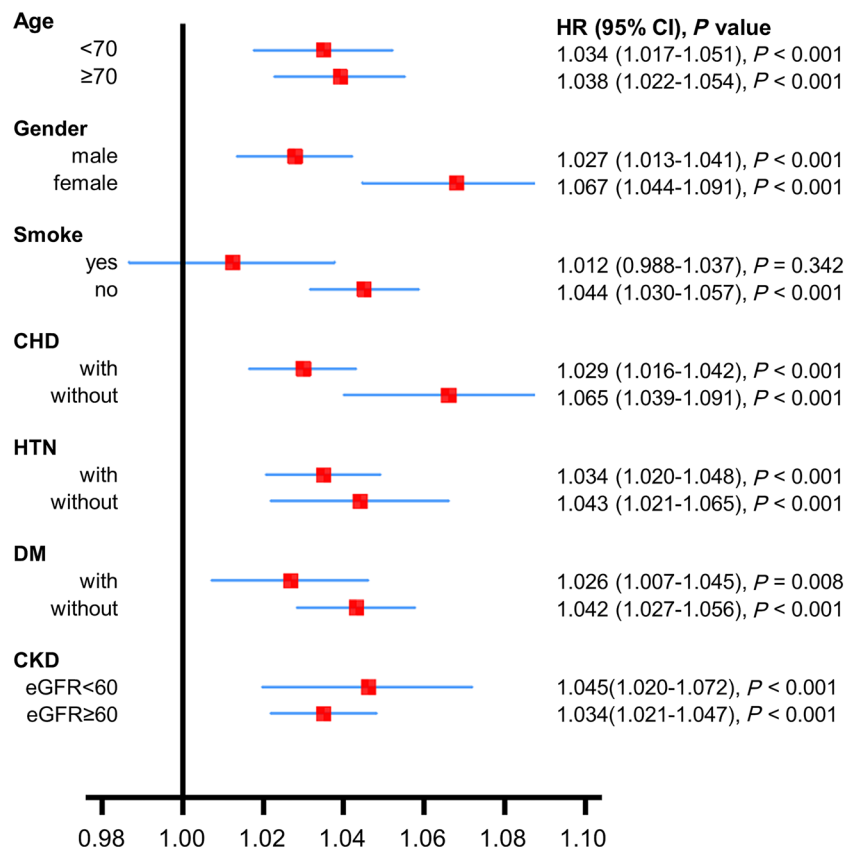

ated with myocardial ischemia and HF.[11] Previous study showed that the myocardium samples from 15 end-stage HF patients had a sixfold increase in AXL, compared with 11 controls from heart donors.[12] Similarly, sAXL levels also significantly increased according to the NYHA classification.[12] In our study, which included over 1000 participants with a mean follow-up of about 3 years, plasma sAXL was also closely associated with cardiac function by LVEF and NYHA classification in HF patients. However, it should be pointed out that the absolute differences of sAXL values among NYHA classifications in our study were not large enough (NYHA II vs. NYHA IV: 19.32 ± 6.65 vs. 24.30 ± 7.09). The overlaps might cause difficulties in the application of sAXL alone in clinical settings. Therefore, the addition of sAXL to NT-proBNP rather than the use of sAXL alone substantially improves the risk stratification and the prediction of death in HF patients.

Current studies show that sAXL is increased in HF patients, but the mechanisms are still in discussion. One of the most important links between sAXL and HF is cardiac remodelling. Caldentey *et al*. found that the elevation of sAXL level at any time has an association with left ventricle remodelling, which may be related to a state of persistent inflammation. As a result, sAXL could be identified as an independent predictor of adverse left ventricle remodelling.[13] Given the fact that cardiac remodelling is a determinant of the clinical course of HF,[16] it is predictable that sAXL is of great importance in HF. sAXL may also affect heart failure through (i) the regulation of the immune response, (ii) the promotion of cell proliferation and regeneration, and (iii) the complex role in angiogenesis and fibrosis.[17] Studies about the exact role of sAXL in HF are warranted.

NPs, especially BNP and NT-proBNP, are well recognized as important diagnostic and prognostic biomarker in patients with HF.[2,13,18,19] Although a normal BNP or NT-proBNP values may exclude the incidence of HF, an increase of these NPs be related to a wide variety of cardiac and non-cardiac causes.[6,20] As such, new biomarkers are needed to better stratify the risk of HF patients other than BNP or NT-proBNP. A previous study has shown that sAXL provided additional prognostic value on top of BNP to predict all-cause mortality, admissions for HF or heart transplantation at short-term follow-up.[12] In a recent study, it showed that sAXL, but not tro-

Figure 5 Subgroup analyses of sAXL and cardiovascular mortality. Cox regress analyses were performed in different prespecified subgroups of interest. Participants were divided into two groups according to age (≥75 or <75 years), gender, smoking status, hypertension, coronary heart disease, diabetes, and renal function (eGFR ≥60 mL/min/1.73m^2 or eGFR <60 mL/min/1.73m^2), respectively. sAXL were significantly associated with cardiovascular mortality in all subgroups. CHD, coronary heart disease; CKD, chronic kidney disease; DM, diabetes mellitus; HTN, hypertension.

	HR (95% CI), *P* value
Age	
<70	1.034 (1.017-1.051), *P* < 0.001
≥70	1.038 (1.022-1.054), *P* < 0.001
Gender	
male	1.027 (1.013-1.041), *P* < 0.001
female	1.067 (1.044-1.091), *P* < 0.001
Smoke	
yes	1.012 (0.988-1.037), *P* = 0.342
no	1.044 (1.030-1.057), *P* < 0.001
CHD	
with	1.029 (1.016-1.042), *P* < 0.001
without	1.065 (1.039-1.091), *P* < 0.001
HTN	
with	1.034 (1.020-1.048), *P* < 0.001
without	1.043 (1.021-1.065), *P* < 0.001
DM	
with	1.026 (1.007-1.045), *P* = 0.008
without	1.042 (1.027-1.056), *P* < 0.001
CKD	
eGFR<60	1.045(1.020-1.072), *P* < 0.001
eGFR≥60	1.034(1.021-1.047), *P* < 0.001

ponins, added a greater predictive value for adverse events than high BNP alone in patients with HF.[11] Our study aligned with these prior studies demonstrated that combination of high sAXL and NT-proBNP values was a better predictor of adverse events, especially cardiovascular mortality and all-cause mortality, than the use of BNP alone. The findings suggest that combination of high sAXL and NT-proBNP values rather than NT-proBNP alone may serve as a more accurate predictor of clinical endpoints in HF patients.

Strengths and limitations

One of the major strengths of our study is the large and well-characterized cohort of individuals with symptomatic HF. Another strength of the current study is the quality of DRAGON-HF cohort study, with only two subjects lost to follow-up (<0.2%). The details of deaths were recorded by the police registered residence management system in China, which are used in this study for follow-up of subjects.

However, this study had some limitations. Firstly and most importantly, we only conducted a spot measurement of sAXL at baseline. As such, we cannot analyse how sAXL will change with time or treatment. The lack of repeated measurements during the follow-up lowers the understanding of sAXL. In future follow-up, the repeated sAXL measurements might be performed to reflect the dynamic and progressive nature of the underlying pathophysiological processes of HF more accurately. Secondly, though this is a multicentre cohort study with over 1000 participants in current analysis, this population might not be large enough to represent the average HF patients. Besides, women might be underrepresented because only about 35% participants were women in this study. However, this study is still ongoing, and more participants will be enrolled in the future. Third, some biochemical parameters, especially NT-proBNP, were measured in different labs; we cannot preclude any errors among these measurements.

Conclusions

In summary, we found that elevated plasma levels of sAXL were associated with cardiac function and increased mortal-

ity in patients with symptomatic HF, which provides incremental prognostic value compared with NT-proBNP. Our results indicate that future intervention on AXL signalling may be of clinical interest.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

Supporting information

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

Figure S1. Associations between sAXL levels and different endpoints in patients with HFpEF, HFmrEF, and HFrEF
Kaplan–Meier curve analyses were performed according to the quartiles of sAXL level in patients with HFpEF (LVEF≥50%), HFmrEF (LVEF 40–49%), and HFrEF (LVEF < 40%) to examine the association between sAXL level and pre-defined endpoints. HFpEF, heart failure with preserved ejection fraction; HFmrEF, heart failure with mid-range ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction.
Figure S2. The additional prognostic value of sAXL to NT-proBNP in pre-defined endpoints
Patients were divided into 4 categorical groups (same as in Figure 4). Each point represents one patient. Red points indicate the occurrence of events, and blue points indicate free of events. Compare to High-High group (upper right), patients in Low-High group (low right) were with lower rate of cardiovascular mortality and all-cause mortality.
Table S1. Treatment at Discharge.
Table S2. Hazard Ratios for Different Endpoints per 1-SD Increase of the Baseline sAXL Level in people with different HF characteristics.

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