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Estimating systemic fibrosis by combining galectin-3 and ST2 provides powerful risk stratification value for patients after acute decompensated heart failure

Chao-Hung Wang[1], Ning-I Yang[1], Min-Hui Liu[1, 2], Kuang-Hung Hsu[3], Li-Tang Kuo[1]

[1]Heart Failure Research Center, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung, Chang Gung University College of Medicine, Taoyuan, Taiwan
[2]Department of Nursing, National Yang-Ming University, Taipei, Taiwan
[3]Laboratory for Epidemiology, Department of Health Care Management, Chang Gung University College, Taoyuan, Taiwan

Abstract

Background: *Two fibrosis biomarkers, galectin-3 (Gal-3) and suppression of tumorigenicity 2 (ST2), provide prognostic value additive to natriuretic peptides and traditional risk factors in patients with heart failure (HF). However, it is to be investigated whether their combined measurement before discharge provides incremental risk stratification for patients after acute HF.*

Methods: *A total of 344 patients with acute HF were analyzed with Gal-3, and ST2 measured. Patients were prospectively followed for 3.7 ± 1.3 years for deaths, and composite events (death/HF-related re-hospitalizations).*

Results: *The levels of Gal-3 and ST2 were only slightly related ($r = 0.20$, $p < 0.001$). The medians of Gal-3 and ST2 were 18 ng/mL and 32.4 ng/mL, respectively. These biomarkers compensated each other and characterized patients with different risk factors. According to the cutoff at median values, patients were separated into four subgroups based on high and low Gal-3 (HG and LG, respectively) and ST2 levels (HS and LS, respectively). Kaplan-Meier survival curves showed that HGHS powerfully identified patients at risk of mortality (Log rank = = 21.27, $p < 0.001$). In multivariable analysis, combined log(Gal-3) and log(ST2) was an independent predictor. For composite events, Kaplan-Meier survival curves showed a lower event-free survival rate in the HGHS subgroup compared to others (Log rank = 34.62, $p < 0.001$; HGHS vs. HGLS, Log rank = 4.00, $p = 0.045$). In multivariable analysis, combined log(Gal-3) and log(ST2) was also an independent predictor.*

Conclusions: *Combination of biomarkers involving heterogeneous fibrosis pathways may identify patients with high systemic fibrosis, providing powerful risk stratification value.* (Cardiol J 2016; 23, 5: 563–572)

Key words: galectin-3, suppression of tumorigenicity 2 (ST2), heart failure, prognosis

Address for correspondence: Chao-Hung Wang, MD, PhD, Heart Failure Research Center, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, 222 Mai Chin Road, Keelung, Taiwan, tel: 886 2 24313131, ext. 2245, fax: 886 2 24314906, e-mail: bearty54@gmail.com

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Introduction

Heart failure (HF) is a complex clinical syndrome that represents the end stage of various cardiac diseases. In the past few decades, substantial advances have been made in understanding its underlying pathophysiology and hemodynamics, and in the development of novel pharmaceuticals and interventional therapies. Nevertheless, short- and long-term HF-related re-hospitalization and mortality remain high, and demand substantial amounts of healthcare resources [1]. The limited effectiveness of current treatment strategies indicates the need for better assessment tools for mechanistical phenotyping and risk stratification.

Cardiac fibrosis plays a pivotal role in the outcome of HF. Recently, two biomarkers indicating cardiac fibrosis, namely galectin-3 (Gal-3) and suppression of tumorigenicity 2 (ST2), have been recognized for providing risk stratification in addition to B-type natriuretic peptide (BNP) and N terminal-proBNP (NT-proBNP) [2]. Galectin-3, a β-galactoside-binding lectin, was noted to be higher in the serum of patients with acute decompensated HF, and is independent of NT-proBNP in predicting short-term mortality [3–5]. ST2 is a protein of the interleukin-1 receptor family. Elevated serum levels of soluble ST2 are also associated with adverse outcomes in patients with acute decompensated or chronic HF [6–8].

Although these two biomarkers demonstrated a prognostic value independent of natriuretic peptides, they actually estimate global fibrosis through different mechanisms. Given the importance of assessing fibrosis in HF, combining Gal-3 and ST2 may probably provide more information for patients at risk. The correlation between these two biomarkers in acute HF is not well established. Based on their distinct characteristics, it is clinically applicable to know whether they can compensate each other in the scenario of estimating acute HF patients before discharge.

Methods

Patients and study design

The study consecutively enrolled patients hospitalized for acute de novo or decompensated chronic HF from October 15, 2008 to July 15, 2013. Enrollment criteria included patients (1) with typical signs and symptoms of HF and New York Heart Association (NYHA) functional classification II to IV, who were hospitalized due to acute cardiogenic pulmonary congestion based on chest X-rays (grade ≥ I according to the classification by Battler et al. [9]) after non-cardiogenic causes had been excluded; (2) with structural abnormalities documented by echocardiograms; and (3) between 20 and 85 years of age. Exclusion criteria included (1) having a disorder other than HF that might compromise survival within the next 6 months; (2) having been bedridden for > 3 months; (3) having a serum creatinine of ≥ 3 mg/dL; (4) having undergone dialysis within the previous 2 weeks; (5) having severe coronary artery disease without complete revascularization therapy; and (6) being pregnant. Informed consent was obtained from all the patients. The study was designed and carried out in accordance with the principles of the Declaration of Helsinki and with approval from the Ethics Review Board of Chang Gung Memorial Hospital.

Blood sampling and assays

Blood samples were collected at enrollment during hospitalization. The measurement of BNP and other parameters, including estimated glomerular filtration rate (eGFR), hemoglobin, sodium, lipid profile, albumin, and C-reactive protein (CRP), were immediately conducted in the central core laboratory. The serum was stored at –80°C for later measurement of Gal-3 and ST2.

BNP assay

B-type natriuretic peptide was measured with the Triage BNP Test (Biosite, San Diego, CA), which was a fluorescence immunoassay for quantitative determination of plasma BNP. Precision, analytical sensitivity and stability characteristics of the assay were previously described [10].

Gal-3 assay

For Gal-3 measurement, an enzyme-linked fluorescent assay (bioMérieux ref. 411191) on a mini-VIDAS® analyzer (bioMérieux, France) was used. The total coefficient of variation for the assay was < 7%, the linear range was 3.3–100.0 ng/mL, and the limit of detection was 2.4 ng/mL.

ST2 assay

Soluble ST2 analyses were performed using the Presage ST2 assay (Critical Diagnostics, San Diego, CA, USA) at BG Medicine (Waltham, MA, USA). The average intra-assay coefficient of variation for sST2 was 2.7%, a total coefficient of variation amounted to 4.3%, and a limit of detection to 1.8 ng/mL.

Cardiac echocardiography

Echocardiographic images were obtained with patients in the left lateral decubitus position at end-expiration at 2.5 MHz (2-dimensional) (Philips iE33 machine). The left ventricular ejection fraction was calculated using the Simpson method. We assessed the left ventricular end diastolic and end systolic dimensions and other associated anatomical abnormalities, such as valvular lesions using criteria suggested by the American Society of Echocardiography.

Follow-up program

Follow-up data were prospectively obtained every month from hospital records, personal communication with the patients' physicians, telephone interviews, and patients' regular visits to staff physician outpatient clinics. "Re-hospitalization" was defined as HF-related re-hospitalizations. A committee of 3 cardiologists adjudicated all hospitalizations without knowledge of patients' clinical variables to determine whether the events are related to worsening HF. "Death" was also chosen as an endpoint. Deaths include sudden death (unexpected death, witnessed or not), worsening HF-related death (decompensated HF or treatment-resistant HF), and of other cardiovascular origin (acute myocardial infarction [directly related, whether due to mechanic, hemodynamic, or arrhythmic complications]; stroke). Because of the interrelationship of HF with other comorbidities, deaths due to comorbidities such as infection and multi-organ failure were also included). However, deaths due to cancer, surgery, suicide, or traffic accident (not related to heart) were excluded. Death and a composite event of HF-related re-hospitalization and the following death were selected as endpoints in this prognostic study.

Statistical analysis

Results are expressed as mean ± standard deviation for continuous variables and as number (percentage) for categorical variables. When appropriate, data were compared by 2-sample t-tests, χ^2, and one-way ANOVA (subgroup analysis was conducted by Tukey). Pearson's correlation analysis was used to assess the correlation between Gal-3 and ST2. Cox proportional hazard models were used to determine independent predictors of the defined events after controlling for covariates (all parameters with a p value of < 0.01 in the univariate analysis). Hazard ratios (HRs) and 95% confidence intervals (CIs) were also calculated. For interpretation of these HRs, both Gal-3 and ST2 values were normalized by log transformation. All statistical analyses were 2-sided and performed using SPSS software (version 15.0, SPSS, Chicago, IL, USA). A p value of < 0.05 was considered significant.

Results

Baseline characteristics

Of 356 consecutive patients, Gal-3 and ST2 were available for 344, who were analyzed in this study. The baseline characteristics of all the patients are shown in Table 1. Mean patient age was 60.6 years, 140 (40.7%) patients were equal to or older than 65 years old; 70.1% were men; and 50.3% of patients had coronary artery disease. Most of the patients were in NYHA functional class ≥ III (85.5%). Regarding guideline-based medication, the use of angiotensin-converting enzyme inhibitors (ACEI)/angiotensin II receptor blockers (ARBs), and β-blockers at discharge was 85.5% and 71.5%, respectively.

Correlations of Gal-3 ad ST2 to demography and laboratory variables

The levels of Gal-3 were only slightly related to the levels of ST2 ($r = 0.20$, $p < 0.001$) (Fig. 1). The median of Gal-3 and ST2 were 18 ng/mL and 32.4 ng/mL, respectively. Patients with Gal-3 above the median were older, had higher NYHA functional classes, higher incidences of diabetes mellitus and atrial fibrillation, were more frequently using diuretics, had higher levels of total bilirubin, CRP, BNP, and ST2, but lower high density lipoprotein (HDL) cholesterol levels, serum sodium, hemoglobin, albumin, and eGFR (Table 1). Patients with ST2 above the median had a higher heart rate, higher levels of total bilirubin, BNP and Gal-3, were less frequently using β-blockers, and had lower levels of total cholesterol, HDL and low density lipoprotein (LDL) cholesterol, sodium, hemoglobin, albumin, and eGFR.

These two biomarkers of fibrosis compensated each other. Compared to ST2, Gal-3 was better associated with age, functional classes and CRP levels, presence of diabetes mellitus and atrial fibrillation, as well as using diuretics. Compared to Gal-3, ST2 had a better ability to identify patients with higher heart rate, lower total and LDL cholesterol, and without using β-blockers.

Differences between subgroups defined by Gal-3 and ST2 levels

As a next step, based on the cutoff at the median values of Gal-3 and ST2, patients were separated into four subgroups, including high

Table 1. Demographic and clinical baseline characteristics by median values of galectin-3 and ST2.

	All (n = 344)	Galectin-3			ST2		
		≥ 18 ng/mL (n = 172)	< 18 ng/mL (n = 172)	P	≥ 32.4 ng/mL (n = 172)	< 32.4 ng/mL (n = 172)	P
Age [years]	60.6 ± 13.4	63.1 ± 13.3	58.1 ± 12.9	0.001	60.8 ± 13.7	60.3 ± 13.1	0.760
Male	241 (70.1%)	119 (69.2%)	122 (70.9%)	0.814	123 (70.1%)	118 (68.6%)	0.638
LVEF [%]	37.0 ± 15.5	35.5 ± 15.2	38.5 ± 15.7	0.079	36.2 ± 15.4	37.9 ± 15.7	0.318
Acute	228 (66.3%)	108 (62.8%)	120 (69.8%)	0.210	109 (63.4%)	119 (69.2%)	0.305
NYHA class:				< 0.001			0.092
II	50 (14.5%)	12 (7.0%)	38 (22.1%)		18 (10.5%)	32 (18.6%)	
III	282 (82.0%)	152 (88.4%)	130 (75.6%)		147 (85.5%)	135 (78.5%)	
IV	12 (3.5%)	8 (4.7%)	4 (2.3%)		7 (4.1%)	5 (2.9%)	
BP [mm Hg]:							
Systolic	123.4 ± 18.6	125.4 ± 20.1	121.5 ± 16.8	0.054	124.7 ± 19.8	122.2 ± 17.4	0.217
Diastolic	74.1 ± 12.8	74.3 ± 13.0	73.9 ± 12.7	0.791	74.3 ± 13.4	73.9 ± 12.3	0.747
Heart rate [bpm]	77.9 ± 12.3	78.9 ± 12.7	76.9 ± 11.9	0.132	79.8 ± 12.4	75.9 ± 11.9	0.003
Co-morbidity:							
Diabetes mellitus	144 (41.9%)	84 (48.8%)	60 (34.9%)	0.012	79 (45.9%)	65 (37.8%)	0.155
Hypertension	231 (67.2%)	123 (71.5%)	108 (62.8%)	0.108	114 (66.3%)	117 (68.0%)	0.818
Atrial fibrillation	92 (26.7%)	55 (32.0%)	37 (21.5%)	0.038	54 (31.4%)	38 (22.1%)	0.067
COPD	37 (10.8%)	22 (12.8%)	15 (8.7%)	0.296	21 (12.2%)	16 (9.3%)	0.487
Ischemic	173 (50.3%)	86 (50.0%)	87 (50.6%)	1.0	82 (47.7%)	91 (52.9%)	0.388
BMI [kg/m^2]	25.0 ± 5.7	25.0 ± 5.5	25.0 ± 5.9	0.973	24.9 ± 6.3	25.1 ± 5.1	0.815
Medication:							
ACEI or ARB	294 (85.5%)	148 (86.0%)	146 (84.9%)	0.879	142 (82.6 %)	152 (88.4 %)	0.168
Beta-blocker	246 (71.5%)	119 (69.2%)	127 (73.8%)	0.403	113 (65.7%)	133 (77.3%)	0.023
Digoxin	92 (26.7%)	51 (29.7%)	41 (23.8%)	0.273	52 (30.2%)	40 (23.3%)	0.180
Diuretic	206 (59.9%)	116 (67.4%)	90 (52.3%)	0.006	110 (64.0%)	96 (55.8%)	0.153
Laboratory data:							
Cholesterol [mg/dL]	176 ± 46	172.7 ± 46.7	179.7 ± 45.6	0.161	168.3 ± 45.3	184.2 ± 45.8	0.001
Triglyceride [mg/dL]	133 ± 110	133.4 ± 112.2	132.6 ± 108.4	0.947	122.2 ± 78.1	143.7 ± 134.1	0.070
LDL-C [mg/dL]	112 ± 40	112.5 ± 39.3	112.4 ± 41.3	0.982	107.4 ± 40.2	117.6 ± 39.7	0.019
HDL-C [mg/dL]	38 ± 16	35.4 ± 11.2	41.7 ± 20.1	< 0.001	36.4 ± 12.6	40.7 ± 19.6	0.017
Serum sodium [mEq/L]	138.7 ± 3.8	138.3 ± 4.4	139.1 ± 3.1	0.048	138.3 ± 4.1	139.2 ± 3.5	0.041
Hemoglobin [g/dL]	13.4 ± 1.9	13.2 ± 2.1	13.7 ± 1.8	0.006	13.2 ± 2.0	13.7 ± 1.9	0.035
Total bilirubin [mg/dL]	1.1 ± 0.6	1.2 ± 0.8	0.9 ± 0.5	0.001	1.2 ± 0.8	0.9 ± 04	< 0.001
Albumin [g/dL]	3.6 ± 0.5	3.4 ± 0.5	3.7 ± 0.5	< 0.001	3.5 ± 0.5	3.6 ± 0.5	0.002
eGFR [mL/min/ /1.73 m^2]	69.3 ± 26.8	56.7 ± 25.2	81.0 ± 23.2	< 0.001	65.8 ± 27.6	72.8 ± 25.7	0.015
C-reactive protein [mg/L]	21.9 ± 37.4	27.0 ± 44.4	16.7 ± 27.9	0.011	23.2 ± 38.5	20.6 ± 36.3	0.515
B-type natriuretic peptide [pg/mL]	635 ± 749	843.3 ± 901.0	427.0 ± 478.8	< 0.001	772.4 ± 848.8	497.9 ± 608.2	0.001
Galectin 3 [ng/mL]	22.8 ± 16.9	32.5 ± 19.6	13.2 ± 3.0	< 0.001	24.9 ± 18.0	20.8 ± 15.6	0.022
ST2 [ng/mL]	48.3 ± 46.1	57.6 ± 52.5	39.0 ± 36.7	< 0.001	76.9 ± 50.7	19.7 ± 7.3	< 0.001

ACEI — angiotensin-converting enzyme inhibitor; ARB — angiotensin receptor blocker; BMI — body mass index; BP — blood pressure; COPD — chronic obstructive pulmonary disease; chronic kidney disease, estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2; HDL-C — high density lipoprotein-cholesterol; LDL-C — low density lipoprotein-cholesterol; LVEF — left ventricular ejection fraction; NYHA — New York Heart Association

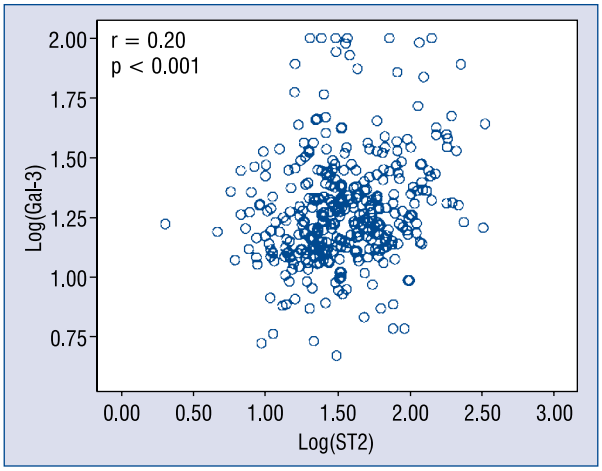

Figure 1. Correlations between galectin-3 (Gal-3) and suppression of tumorigenicity 2 (ST2). Log transformation is performed for Gal-3 and ST2 [log(Gal-3) and log(ST2), respectively].

Gal-3 and high ST2 (HGHS), high Gal-3 and low ST2 (HGLS), low Gal-3 and high ST2 (LGHS), and low Gal-3 and low ST2 (LGLS). Table 2 shows the differences in demography and laboratory data between the four subgroups. Compared to patients in the LGLS subgroup, those in the HGHS subgroup were older, had higher NYHA functional classes and heart rate, higher incidences of diabetes mellitus and atrial fibrillation, were more frequently using diuretics, had higher levels of total bilirubin, and BNP, but lower total and HDL cholesterol levels, hemoglobin, albumin, and eGFR.

Death

During a mean follow-up period of 3.7 ± 1.3 years, there were 90 (26.2%) deaths, including 50 (55.6%) sudden deaths and 40 deaths due to other reasons. In a univariate analysis, associates of death included age, a history of previous HF, functional class, hemoglobin, albumin, eGFR, log(BNP), log(Gal-3), log(ST2), and combined log(Gal-3) and log(ST2) (Table 3). Patients with decompensated HF (with previous HF history) might have had renal fibrosis resulting in worse outcomes compared to those with acute HF (de novo). Gal-3 is closely related to renal fibrosis. Kaplan-Meier curves show that patients with decompensated HF and elevated Gal-3 had worse outcomes than others (Log rank = 16.72, p = 0.001) (Fig. 2A). Survival was further analyzed according to the four subgroups based on Gal-3 and ST2 levels (Kaplan-Meier survival curves in Figure 3A). Patients in the HGHS subgroup had significantly higher accumulated mortality rate than other subgroups (Log rank = 21.27, p < 0.001). In a multivariable analysis, combined log(Gal-3) and log(ST2) was an independent predictor (Table 3).

Death or HF-related re-hospitalization

During follow-up there were 64 (18.6%) HF-related re-hospitalizations, and 122 (35.5%) composite events of death/HF-related re-hospitalizations. In a univariate analysis, associates of composite events included age, sex, history of previous HF, functional class, hemoglobin, albumin, eGFR, log(BNP), log(Gal-3), log(ST2), and combined log(Gal-3) and log(ST2) (Table 4). Patients with decompensated HF also had worse outcomes compared to those with acute HF. Kaplan-Meier curves show again that patients with decompensated HF and elevated Gal-3 had the worst outcomes (Log rank = 42.05, p < 0.001) (Fig. 2B). Survival was further analyzed according to the four subgroups (Kaplan-Meier survival curves in Figure 3B). Patients in the HGHS subgroup has significantly higher accumulated mortality rate than all other subgroups (Log rank = 34.62, p < 0.001; HGHS vs. HGLS, Log rank = 4.00, p = 0.045). In multivariable analysis, combined log(Gal-3) and log(ST2) remained an independent predictor (Table 4).

Discussion

In the population of patients with acute HF, combined measurement of Gal-3 and ST2 during the acute stage in the hospital significantly and independently predicted long-term outcomes in terms of death and the composite event of death, as well as HF-related re-hospitalization. Combined measurement provided advanced risk stratification value compared to Gal-3 or ST2 measured alone in both study endpoints.

In patients with chronic HF, Gal-3 has been shown to provide incremental prognostic value over BNP [11–13]. In patients with acute HF, its prognostic value was demonstrated as well in a few studies, most of which, however, had a relatively small sample size or short follow-up period. A few studies in acute HF patients reported that ST2 provided good prognostic values. In a brief communication, Mueller et al. [6] reported that increased ST2 concentrations predicted 1-year mortality in 137 patients with acute destabilized HF. Later on, Pascual-Figal et al. [7] demonstrated that ST2 provided prognostic value in addition to NT-proBNP and high-sensitivity troponin T in 107 patients with acutely decompensated HF. Recently,

Table 2. Demographic and clinical baseline characteristics in different populations defined by the levels of galectin-3 and ST2.

	HGHS (n = 100)	HGLS (n = 72)	LGHS (n = 72)	LGLS (n = 100)	P
Age [years]	64.61 ± 13.26*	60.89 ± 13.17	55.47 ± 12.56*	59.95 ± 13.03	< 0.001
Male	66 (60.0%)	53 (73.6%)	57 (79.2%)	65 (65.0%)	0.152
Left ventricular ejection fraction [%]	36.34 ± 15.31	34.42 ± 15.18	35.93 ± 15.53	40.31 ± 15.62	0.070
Acute	59 (59%)	49 (68.1%)	50 (69.4%)	70 (70%)	0.332
NYHA functional class:					0.004
II	5 (5.0%)**	7 (9.7%)*	13 (18.1%)	25 (25.0%)	
III	90 (90.0%)**	62 (86.7%)*	57 (79.2%)	73 (73.0%)	
IV	5 (5.0%)	3 (4.2%)	2 (2.8%)	2 (2.0%)	
Blood pressure [mm Hg]:					
Systolic	126.76 ± 21.14	123.4 ± 18.68	121.8 ± 17.43	121.3 ± 16.42	0.165
Diastolic	73.24 ± 13.39	75.75 ± 21.14	75.85 ± 13.25	72.54 ± 12.17	0.216
Heart rate [bpm]	80.12 ± 12.08**	77.15 ± 13.38	79.47 ± 12.89*	75.01 ± 10.74	0.016
Co-morbidity:					
Diabetes mellitus	52 (52.0%)*	32 (44.0%)	27 (37.5%)	33 (33.0%)	0.042
Hypertension	70 (70.0%)	53 (73.6%)	44 (61.1%)	64 (64.0%)	0.338
Atrial fibrillation	39 (39.0%)*	16 (22.2%)	15 (20.8%)	22 (22.0%)	0.013
COPD	15 (15.0%)	7 (9.7%)	6 (8.3%)	9 (9.0%)	0.437
Ischemic	48 (48.0%)	38 (52.8%)	34 (47.2%)	53 (53.0%)	0.813
Body mass index [kg/m^2]	24.58 ± 6.1	25.56 ± 4.57	25.41 ± 6.51	24.73 ± 5.47	0.614
Medication					
ACEI or ARB	82 (82.0%)	66 (91.7%)	60 (83.3%)	86 (86.0%)	0.323
Beta-blocker	62 (62.0%)	57 (79.2%)	51 (70.8%)	76 (76.0%)	0.057
Digoxin	36 (36.0%)	15 (20.8%)	16 (22.2%)	25 (25.0%)	0.087
Diuretic	66 (66.0%)**	50 (69.4%)**	44 (61.1%)	46 (46.0%)	0.006
Laboratory data:					
Cholesterol [mg/dL]	164.5 ± 44.0**	184.0 ± 48.2	173.4 ± 46.9	184.2 ± 44.23	0.008
Triglyceride [mg/dL]	126.0 ± 68.2	143.6 ± 153.3	116.9 ± 89.8	143.9 ± 119.1	0.311
LDL-C [mg/dL]	105.3 ± 38.1	122.5 ± 38.9	110.3 ± 42.9	114.0 ± 40.1	0.051
HDL-C [mg/dL]	34.33 ± 10.9**	36.8 ± 11.3**	39.3 ± 14.0	43.45 ± 23.4	0.006
Serum sodium [mEq/L]	137.8 ± 4.3	138.9 ± 4.4	138.8 ± 3.5	139.3 ± 2.6	0.056
Hemoglobin [g/dL]	12.7 ± 2.0**	13.7 ± 2.0	13.9 ± 1.7	13.6 ± 1.8	< 0.001
Total bilirubin [mg/dL]	1.2 ± 0.8**	1.0 ± 0.5	1.0 ± 0.5*	0.8 ± 0.3	< 0.001
Albumin [g/dL]	3.3 ± 0.5**	3.5 ± 0.4**	3.6 ± 0.4	3.7 ± 0.4	< 0.001
eGFR [mL/min/1.73 m^2]	54.6 ± 24.1**	61.8 ± 26.23**	81.3 ± 24.5	80.7 ± 22.3	< 0.001
C-reactive protein [mg/L]	28.7 ± 45.0	24.8 ± 43.9	15.8 ± 25.4	17.6 ± 29.7	0.074
B-type natriuretic peptide [pg/mL]	984.3 ± 957.4**	739.9 ± 750.1**	540.3 ± 522.1	338.8 ± 407.1	< 0.001

*p < 0.05,**p < 0.01 compare to LGLS; HG and LG indicate galectin-3 ≥ 18 ng/mL and < 18 ng/mL, respectively. HS and LS indicate ST2 ≥ 32.4 ng/mL and < 32.4 ng/mL, respectively; ACEI — angiotensin-converting enzyme inhibitor; ARB — angiotensin receptor blocker; COPD — chronic obstructive pulmonary disease; chronic kidney disease, estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m^2; HDL-C — high density lipoprotein-cholesterol; LDL-C — low density lipoprotein-cholesterol; NYHA — New York Heart Association

Lassus et al. [8] conducted an international collaborative study on patients hospitalized for acute HF, and showed that ST2 predicted 30-day and 1-year mortality. However, these data were analyzed from cohorts with relatively short periods of follow-up. Our study, enrolling more patients with a longer period of follow-up, supported the prognostic value of Gal-3 and ST2 in hospitalized HF patients.

Table 3. Cox univariable and multivariable analysis for death.

Item	Univariable		Multivariable	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Age [years]	1.058 (1.039~1.078)	< 0.001	1.050 (1.032~1.069)	< 0.001
Sex	0.730 (0.477~1.117)	0.147		
LVEF [%]	0.991 (0.977~1.005)	0.209		
Acute (de novo)	0.523 (0.349~0.785)	0.002	0.590 (0.388~0.896)	0.013
NYHA class	4.744 (2.693~8.357)	< 0.001	3.482 (1.881~6.445)	< 0.001
Diabetes mellitus	1.302 (0.862~1.967)	0.210		
Hypertension	1.161 (0.741~1.821)	0.515		
Atrial fibrillation	1.395 (0.897~2.168)	0.140		
Cholesterol [mg/dL]	0.996 (0.991~1.001)	0.096		
Serum sodium [mEq/L]	0.977 (0.930~1.026)	0.344		
Hemoglobin [g/dL]	0.840 (0.758~0.930)	0.001	1.003 (0.899~1.119)	0.956
Serum albumin [g/dL]	0.383 (0.261~0.564)	< 0.001	0.573 (0.378~0.870)	0.009
eGFR [mL/min/1.73 m^2]	0.984 (0.975~0.992)	< 0.001	1.000 (0.991~1.008)	0.933
C-reactive protein [mg/L]	1.004 (0.999~1.009)	0.100		
BNP (log)	2.880 (1.884~4.401)	< 0.001	2.090 (1.345~3.246)	0.001
Galectin-3 (log)	5.792 (2.232~12.280)	< 0.001		
ST2 (log)	2.652 (1.473~4.773)	0.001		
Galectin-3 (log)+ST2 (log)	3.057 (2.008~4.654)	< 0.001	2.088 (1.264~3.448)	0.004

BNP — B-type natriuretic peptide; CI — confidence interval; eGFR — estimated glomerular filtration rate; LVEF — left ventricular ejection fraction; NYHA — New York Heart Association; ST2 — suppression of tumorigenicity 2

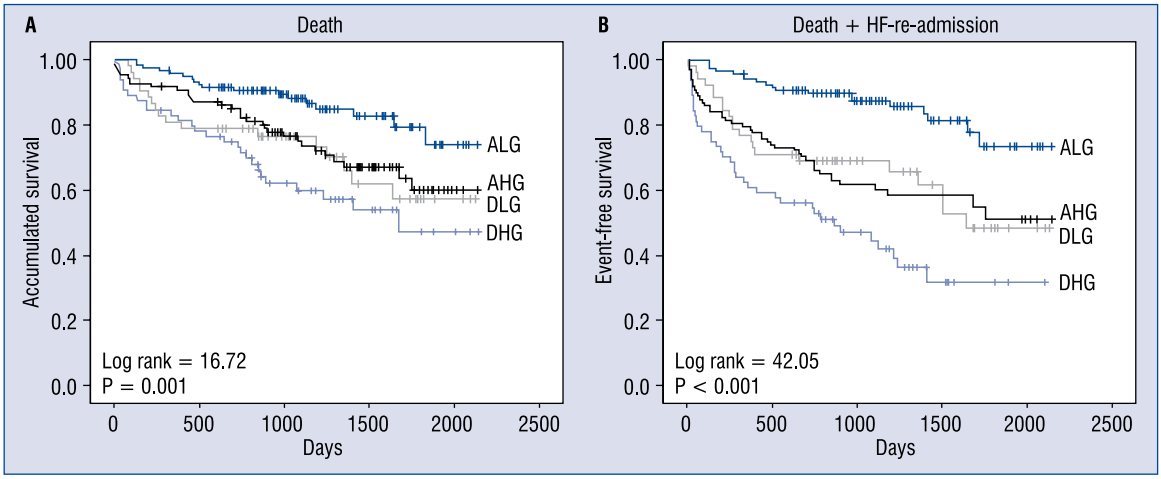

Figure 2. Kaplan-Meier estimates of event risk according to acute/decompensated heart failure (HF) and biomarkers; **A.** Kaplan-Meier curves for 5-year mortality stratified by acute (de novo)/decompensated HF, and galectin-3 levels; **B.** Kaplan-Meier curves for 5-year composite events of mortality or HF-related re-hospitalization stratified by acute/ /decompensated HF, and galectin-3 levels; A and D — acute (de novo) and decompensated HF, respectively; HG and LG — galectin-3 ≥ 18 ng/mL and < 18 ng/mL, respectively.

As shown in our data, although both Gal-3 and ST2 are supposed to estimate the severity of global fibrosis, their correlation is weak. They assess fibrosis by different mechanisms. Gal-3 is expressed by activated macrophages and stimulates cardiac fibroblasts to proliferate and modulate

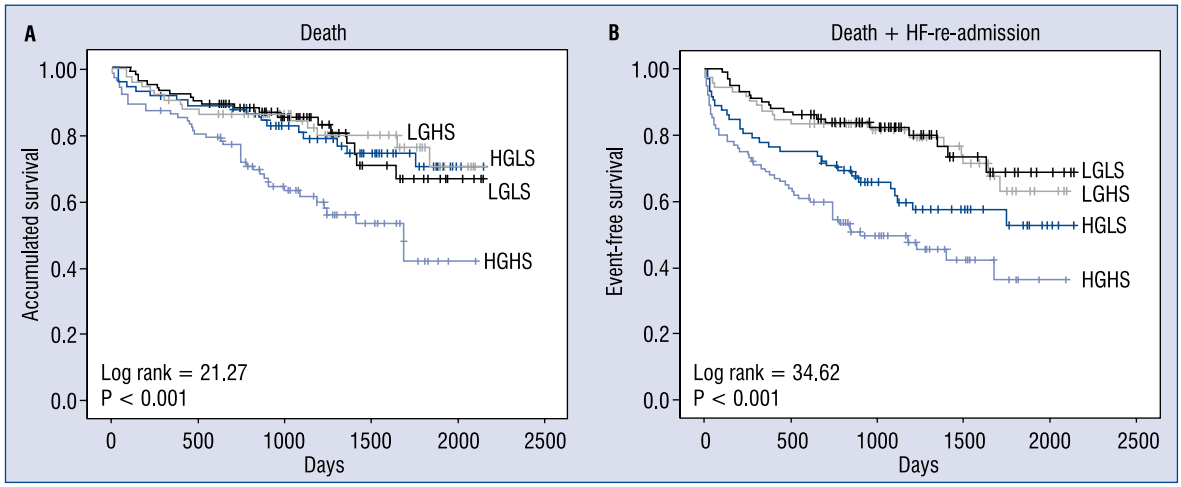

Figure 3. Kaplan-Meier estimates of event risk according to biomarkers; **A.** Kaplan-Meier curves for 5-year mortality stratified by high galectin-3 and high ST2 (HGHS), low galectin-3 and low ST2 (LGLS), HGLS, and LGHS; **B.** Kaplan-Meier curves for 5-year composite events of mortality or heart failure-related re-hospitalization stratified by the same four subgroups; HG and LG — galectin-3 ≥ 18 ng/mL and < 18 ng/mL, respectively; HS and LS — ST2 ≥ 32.4 ng/mL and < 32.4 ng/mL, respectively.

Table 4. Cox univariable and multivariable analysis for heart failure-related re-hospitalization and death.

Item	Univariable		Multivariable	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Age [years]	1.044 (1.029~1.060)	< 0.001	1.036 (1.021~1.051)	< 0.001
Sex	0.627 (0.434~0.908)	0.013		
LVEF [%]	0.991 (0.979~1.004)	0.169		
Acute (de novo)	0.456 (0.320~0.651)	< 0.001	0.548 (0.379~0.790)	0.001
NYHA class	3.722 (2.218~6.245)	< 0.001	2.615(1.576~3.352)	< 0.001
Diabetes mellitus	1.416 (0.988~2.031)	0.058		
Hypertension	1.418 (0.946~2.127)	0.091		
Atrial fibrillation	1.351 (0.916~1.992)	0.129		
Cholesterol [mg/dL]	0.997 (0.992~1.001)	0.096		
Serum sodium [mEq/L]	0.972 (0.932~1.014)	0.189		
Hemoglobin [g/dL]	0.791 (0.720~0.870)	< 0.001	0.907 (0.819~1.005)	0.061
Albumin [g/dL]	0.467 (0.339~0.665)	< 0.001	0.801 (0.556~1.153)	0.233
eGFR [mL/min/1.73 m^2]	0.983 (0.976~0.991)	< 0.001	1.000 (0.992~1.008)	0.999
C-reactive protein [mg/L]	1.003 (0.999~1.007)	0.160		
BNP (log)	2.963 (2.035~4.315)	< 0.001	2.299 (1.576~3.352)	0.001
Galectin-3 (log)	5.413 (2.844~10.30)	< 0.001		
ST2 (log)	2.516 (1.489~4.249)	0.001		
Galectin-3 (log)+ST2 (log)	2.875 (1.840~3.795)	< 0.001	2.072 (1.373~3.128)	0.001

BNP — B-type natriuretic peptide; CI — confidence interval; eGFR — estimated glomerular filtration rate; LVEF — left ventricular ejection fraction; NYHA —New York Heart Association; ST2 — suppression of tumorigenicity 2

extracellular matrix formation, followed by adverse cardiac remodeling [14, 15]. Based on these molecular mechanisms, Gal-3 appears to be a direct mediator of fibrosis. Decompensated chronic HF is associated with systemic involvement of fibrosis, especially in renal pathology. Our data showed

a close relationship between Gal-3 levels and eGFR. Moreover, decompensated chronic HF along with elevated Gal-3 levels appear to be at the highest risk of events. Interleukin (IL)-33, a functional ligand of ST2, intensifies antihypertrophic and antifibrotic effects on myocardium. Soluble ST2 acts as a decoy receptor of IL-33 and attenuates its cardioprotective properties [14, 15]. Our clinical data suggest that different biomarkers identify different populations with increased fibrosis activity. Except for a few common prognosis-relevant factors, each marker has its role in discovering other risk factors related to fibrosis and may provide further compensatory information when used together.

Previous studies in ambulatory HF patients revealed that Gal-3 level remained relatively steady and only changed over periods of months [16]. However, some studies have shown rapid dynamic changes of ST2, comparable to BNP [8, 17, 18]. Measuring both biomarkers in the meanwhile before hospital discharge may integrate information by combining the basal fibrosis activity provided by Gal-3 and the acutely increased fibrotic stress provided by ST2. Based on our study, we found that broader coverage of fibrosis defined by either an elevated Gal-3 or ST2 did not give rise to better prognostic evaluation. An increase in one biomarker together with a decrease in the other actually explored the weak prognostic value provided by a single marker alone. This phenomenon was noted especially when estimating the risk of mortality, which could be predicted only in those with an increase in both biomarkers. To predict composite events, there was a gradient trend with the highest risk seen in patients with high Gal-3 and high ST2 levels, followed by those with high Gal-3 but low ST2 levels. Activation of two different pathways associated with fibrosis, as suggested by an elevation in both Gal-3 and ST2, indicates a stronger systemic fibrotic activity.

Limitations of the study

Some limitations should be acknowledged. The present study has a relatively small sample size with various etiologies of HF. To better predict an HF-related event, a recent study performed by Boisot et al. [18] demonstrated that serial measurements of ST2 might be needed. Acute HF patient with ≥ 15.5% decline in ST2 levels during the hospital stay had a lower short-term (90-day) mortality than those with < 15.5% decrease (7% vs. 33%) [18]. Our study cannot answer whether serial measurements of ST2 changes during hospitalization may provide better evaluation than measuring both Gal-3 and ST2 simultaneously, in terms of cost or efficiency. However, serial measurements of both biomarkers in patients while in stable status are believed to better clarify the relationship between these two markers.

On the other hand, kidney dysfunction is a poor prognostic parameter, and may interfere with Gal-3 and ST2 levels. Kidney dysfunction also influences the concentrations of blood albumin and erythropoietin, which is related to hemoglobin. In order to represent the information encountered in the real world and also to diminish the influence of kidney dysfunction on the data analysis, this study excluded patients with a serum creatinine level of ≥ 3 mg/dL, and included eGFR in the panel of multivariable analysis models.

Conclusions

Combination of biomarkers involving heterogeneous fibrosis pathways may identify patients with high systemic fibrosis activity and provide powerful risk stratification value.

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Conflict of interest: None declared

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