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Serum Brain-Derived Neurotrophic Factor Level Predicts Adverse Clinical Outcomes in Patients with Heart Failure

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) is involved in cardiovascular diseases as well as skeletal muscle energy metabolism and depression. We investigated whether serum BDNF level was associated with prognosis in patients with heart failure (HF).

Methods and Results: We measured the serum BDNF level in 58 patients with HF (59.2±13.7 yrs old, New York Heart Association class I–III) at baseline, and adverse events including all cardiac death and HF rehospitalization were recorded during the median follow-up of 20.3 months. In a univariate analysis, serum BDNF levels were significantly associated with peak oxygen capacity (β -coefficient 0.547, $P=0.003$), anaerobic threshold (β -coefficient 0.929, $P=0.004$), and log minute ventilation/carbon dioxide production slope (β -coefficient -10.15 , $P=0.005$), but not PHQ-9 scores (β -coefficient -0.099 , $P=0.586$). A multivariate analysis demonstrated that serum BDNF level was an independent prognostic factor of adverse events (hazard ratio 0.41, 95% confidence interval 0.20–0.84, $P=0.003$). The receiver operating characteristic curve demonstrated that low levels of BDNF (<17.4 ng/mL) were associated with higher rates of adverse events compared to high levels of BDNF (≥ 17.4 ng/mL) (log-rank test, $P<0.001$).

Conclusions: Decreased serum BDNF levels were significantly associated with adverse outcomes in HF patients, suggesting that these levels can be a useful prognostic biomarker.

45 **Key Words:** heart failure, brain-derived neurotrophic factor, exercise capacity,

46 prognosis

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Heart failure (HF) remains highly prevalent and is characterized by repeat hospitalization with a heavy health burden, and it is associated with excess morbidity and mortality.¹ Lowered exercise capacity is well known to be closely related to the poor prognosis in patients with HF.^{2,3} Various skeletal muscle abnormalities including a transition of myofibers from type I to type II, a reduction in muscular strength, muscle atrophy, and impaired energy metabolism each play an important role as a determinant factor of lowered exercise capacity in individuals with HF.⁴ Cytokines and growth factors were recently reported to be secreted by skeletal muscle and to regulate skeletal muscle mass or function.⁵ Such factors may be associated with skeletal muscle abnormalities and exercise capacity in HF patients, and they may be potential biomarkers predicting the severity and prognosis of HF.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which regulates various neurotrophic functions including neuroregeneration, neuroprotection, and synaptic plasticity.⁶ BDNF was discovered in the brain and has been shown to be linked to the pathophysiology of psychiatric disorders including major depression and dementia.⁷⁻⁹ In addition, BDNF expression is reported to be upregulated in ischemic heart and to protect the heart against ischemic injury.¹⁰ Indeed, a low plasma BDNF level was shown to predict poor prognosis in patients with angina pectoris.¹¹

Interestingly, BDNF can also be produced in skeletal muscle,¹² and exercise training can increase its serum levels.¹³ We recently reported that the serum BDNF level was decreased in HF patients compared to normal subjects and that it was positively correlated with their peak oxygen capacity (peak VO₂).¹⁴ Since the peak VO₂ is a strong prognostic marker of HF,² these findings raise the possibility that BDNF may be

involved in lowered exercise capacity and may help predict the prognosis of HF patients. However, it has not been determined whether the serum BDNF level can provide prognostic information in HF patients. In the present study, we determined whether the serum BDNF level could predict the prognosis including all cardiac death and HF readmission during a median follow-up of 20.3 months in HF patients. We also investigated whether BDNF levels were associated with depressive symptoms, because depression is highly prevalent and associated with adverse outcomes in HF patients.¹⁵

METHODS

Patients

The present study enrolled a total of 88 consecutive patients with HF who were admitted to Hokkaido University Hospital between April 2012 and June 2013. Inclusion criteria were: (1) age 20 to 75 yrs; (2) with HF symptoms defined by Framingham criteria; (3) New York Heart Association (NYHA) functional class I–III on optimized pharmacotherapy; (4) absence of the following HF etiologies: severe valve disease, congenital disease, pericardial disease, and pulmonary artery embolism; and (5) absence of the following comorbidities: active infectious disease, cancer, and renal failure requiring dialysis. HF was diagnosed on the basis of standard criteria and the presence of systolic or diastolic functional impairment by echocardiography according to the American College of Cardiology Foundation/American Heart Association Task Force on Practice guidelines by two or more cardiologists.¹⁶

Patients were excluded when they had any of the following within 2 wks prior to the study; changes in NYHA functional class, changes in HF medications, or the administration of any intravenous medication for HF. In addition, patients were

excluded if they: had signs of or a past history of a psychiatric disorder such as major depressive disorder, schizophrenic disorder, and organic brain disorders; were taking anti-depressant medications; had had a stroke within the past 3 months; or were unable to perform a maximal exercise test due to a neurological deficit.

According to these inclusion and exclusion criteria, we excluded patients with NYHA IV (n=10), severe valvular heart disease (n=6), major depression (n=4), active infection (n=3), stroke with significant neurological deficit (n=1), acute pulmonary embolism (n=1), cancer (n=1), and renal failure (n=4). A final total of 58 patients were enrolled in the present study. The protocol was approved by the Medical Ethics Committee of Hokkaido University Hospital, and written informed consent was obtained from all participating subjects.

Baseline Patient Characteristics

Each patient's body weight and height were measured, and his or her body mass index (BMI, body weight/[height]², kg/m²) was calculated because circulating BDNF in patients with newly diagnosed type 2 diabetes showed a positive correlation with BMI.¹⁷ Ischemic heart disease was diagnosed based on coronary artery disease by angiography, hypertensive heart disease based on left ventricular hypertrophy with hypertension, valvular heart disease based on moderate valve disease by echocardiography, and dilated cardiomyopathy based on both left ventricular dilation and dysfunction without any cause.

Hypertension, diabetes mellitus (DM), past history of stroke, chronic obstructive pulmonary disease (COPD), sleep apnea syndrome, and paroxysmal or chronic atrial fibrillation were determined based on the patient's medical records. Depressive

symptoms were assessed using the Patient Health Questionnaire (PHQ-9). The PHQ-9 is a nine-item self-report measure of depressive symptoms using 0-to-3 scales, established for the screening of depression in various populations.¹⁸

The left ventricular (LV) end-diastolic dimension (EDD) was measured in the parasternal long axis view by transthoracic echocardiography. The LV ejection fraction (LVEF) was calculated from the apical four- and two- chamber views according to the biplane Simpson's method.¹⁹ Cardiopulmonary exercise testing was performed using an upright electromechanical bicycle ergometer (Aerobike 75XLII, Combi Wellness, Tokyo) with a ramp protocol as described.²⁰ Briefly, after 3 min of unloaded cycling, the exercise load was increased in 10–15 Watt/min increments to symptom-limited maximal work. Minute ventilation (VE), oxygen capacity (VO₂), and carbon dioxide production (VCO₂) were measured continuously throughout the exercise period using a 280E Aero-monitor (Aeromonitor AE-300S, Minato Medical Science, Osaka, Japan). Peak VO₂ was defined as the maximal VO₂ attained during exercise. The anaerobic threshold (AT) was determined by the V-slope method as described.²¹ VE and VCO₂ responses throughout the exercise were used to calculate the VE/VCO₂ slope via a least squares linear regression ($y = mx + b$; $m = \text{slope}$).²²

Serum BDNF Levels and Biochemistry

Peripheral venous blood samples were collected in serum tubes from all subjects between 6:00 and 9:00 am before cardiopulmonary exercise testing. All samples were allowed to clot before being centrifuged at 1000g for 15 min and were stored at –80 °C until analysis. Serum BDNF levels were determined by a commercially available enzyme immunoassay kit (R&D System, Minneapolis, MN, USA) according to the

manufacturer's protocol as described,²³ its detection limit was 20 pg/mL. To ensure accurate measurements, all of the samples were analyzed in duplicate by investigators blinded to clinical information. The inter- and intra-assay coefficients of variation (CV) were 6.1% and 2.3%, respectively.

Plasma B-type natriuretic peptide (BNP) was measured by a chemiluminescence immunoassay (CLIA) Kit (Architect BNP-JP; normal reference values, 18.4 pg/mL) in an automated analyzer (Architect, Abbott Japan, Tokyo). The Architect BNP assay was validated as described.²⁴ The limit of detection (LOD) of the assay was 5.8 pg/mL. The within-run and total CVs were 2.4 and 3.4%, respectively. Linearity was acceptable with recoveries within 5.0% of the target values over a concentration range of 429–2894 pg/mL. Our comparison of the Architect BNP-JP assay results with those of another chemiluminescent enzyme immunoassay (CLEIA) (MI02 Shionogi BNP kit; Shionogi, Japan) by the Passing-Bablok method resulted in slopes ranging from 1.00 and correlation coefficients of 0.98. The estimated glomerular filtration rate (eGFR) was calculated from the serum creatinine values and age using the Japanese equation as follows:²⁵

$$\text{eGFR} = 194 \times (\text{serum creatinine in mg/dL})^{-1.094} \times (\text{age in yrs})^{-0.287} \times (0.739 \text{ if female}).$$

Procedures and Clinical Follow-up

At enrollment, the patients underwent a complete clinical and physical examination, including the collection of blood samples for the measurement of the eGFR, plasma BNP, and serum BDNF levels. The examination included chest X-ray, transthoracic echocardiography, PHQ-9 score, NYHA functional class determination, and the cardiopulmonary exercise test. The entire protocol was performed at baseline (at

the time of BDNF measurement). All patients were then followed prospectively by regular outpatient visits up to March 2014. The median follow-up period was 20.3 months [interquartile range (IQR), 11.5 to 22.8 months] after the measurement of serum BDNF.

Clinical information about major adverse events including all cardiac deaths or rehospitalization due to worsening HF during the follow-up period was provided by the patients' cardiologists without knowledge of the serum BDNF levels. Cardiac death was defined as death from worsening HF or sudden cardiac death, and HF rehospitalization was defined as an unplanned hospital admission requiring intravenous diuretics, vasodilators, or inotropic agent infusion. Cardiac death during the rehospitalization was counted as a single event. Additionally, second or further rehospitalizations were not counted as additional events but rather as a single event at the time of the first rehospitalization.

Statistical Analysis

Continuous variables are expressed as means $\pm$ SD for normally distributed variables, and median and IQR for non-normally distributed variables. Categorical variables are expressed as numbers and percentages. The serum BDNF levels, peak VO_2 , and AT were normally distributed as proven by the Shapiro-Wilk test. In contrast, age, BMI, LVDd, LVEF, VE/VCO_2 slope, eGFR, plasma BNP, and PHQ-9 score were not normally distributed. The VE/VCO_2 slope and plasma BNP values were transformed to logarithm (log) VE/VCO_2 slope and log BNP, respectively. There were three missing data in peak VO_2 , AT, and log VE/VCO_2 slope, respectively, due to the patient's inability to participate in the exercise test.

We used a univariate linear model to determine the correlation between serum BDNF levels and variables. Serum BDNF and plasma BNP levels were compared for their ability to predict adverse outcomes of HF by a receiver operating characteristic curve (ROC) analysis. The optimal ROC curve cut-off value for the prediction of adverse outcomes was chosen as the value maximizing sensitivity plus specificity. To determine whether serum BDNF levels were incremental prognostic markers in addition to plasma BNP, we compared the areas under the ROC curve (AUC) of these variables using the DeLong algorithm.²⁶ DeLong's method is a different approach from Hanley's²⁷ to estimate the area under the ROC curve (c-index) regarding the estimation of variance. Since the variance was derived from the assumption of two underlying negative exponential distributions in Hanley's approach, it is known that Hanley's approach can underestimate the variance when the AUC is close to 0.5 and can overestimate it when the AUC is close to 1.0.²⁷ We thus selected DeLong's approach in the present study.

We calculated Kaplan-Meier survival plots from baseline to the time of all cardiac death or rehospitalization due to worsening HF, and we used the log-rank test to compare the results. Univariate and multivariate analyses with the Cox proportional hazard regression model were used to determine significant predictors of all cardiac death and HF rehospitalization. Variables that were significant with a P -value < 0.01 in the univariate analyses including NYHA, log BNP, and BDNF values were entered into the multivariate Cox proportional hazard analysis which was adjusted for age and gender. Peak VO_2 , AT, and log VE/VCO_2 slope data were excluded from the multivariate analysis to avoid problems related to multicollinearity, because these variables were significantly associated with serum BDNF levels (**Table 1**), consistent

with our previous study.¹⁴ We also selected variables in the multivariate Cox model based on the stepwise forward selection method due to the small number of events in this study. All analyses were performed using JMP 10.0.2 (SAS Institute, Cary, NC). The differences were considered significant when *P*-values were < 0.05.

RESULTS

Patients' Characteristics

The clinical characteristics of the study patients are summarized in **Table 2**. The etiologies of HF were dilated cardiomyopathy in 18 (31%) patients, ischemic heart disease in 17 (29%), hypertensive heart disease in 10 (17%), valvular heart disease in five (8%), and others in the remaining eight (13%) patients. Five patients were in NYHA functional class I, 39 patients in class II, and 14 patients in class III. Hypertension was identified in 18 (31%) patients, DM in 19 (32%), history of stroke in seven (12%), COPD in 8 (13%), sleep apnea syndrome in 8(13%), and paroxysmal or chronic atrial fibrillation in 27 (46%) patients. According to the PHQ-9 scores (≥ 10), four patients (6%) were identified to be in a clinically significant depressive state. The mean LVEF was $34.7 \pm 12.7\%$, and the mean peak VO_2 was 14.0 ± 3.9 mL/kg/min. Angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II type I receptor antagonists (ARBs) were used in 89% of the patients, β -blockers in 96%, and spironolactone in 60%. The mean serum BDNF level in the HF patients was 19.0 ± 5.6 ng/mL.

Serum BDNF Levels Correlate with Exercise Capacity

In the univariate linear model, the patients' serum BDNF levels were significantly associated with their peak VO_2 (β -coefficient 0.547, $P=0.003$), AT (β -coefficient 0.929, $P=0.004$), and log VE/VCO_2 slope (β -coefficient -10.15 , $P=0.005$), but not age, gender, BMI, LVEDD, LVEF, eGFR, and plasma BNP levels (**Table 1**). There was no significant association between serum BDNF levels and depressive status according to PHQ-9 scores (β -coefficient -0.099 , $P=0.586$). Moreover, there was no significant association between serum BDNF levels and the change in peak VO_2 from baseline until the end of their follow-ups (β -coefficient 0.410, $P=0.233$) among the 19 patients who performed the exercise pulmonary test repeatedly after their enrollment.

To assess the role of serum BDNF in adherence to exercise training, we obtained data regarding each patient's participation in cardiac rehabilitation from their exercise diaries. One session of cardiac rehabilitation included 30 min of aerobic exercise by a cycle ergometer. Patients who participated in more than one session per week throughout the follow-up period were defined as adherent in cardiac rehabilitation. As a result, 25 patients (43%) were considered adherent. There was no significant difference in serum BDNF levels between the adherent and non-adherent patients (19.0 ± 5.5 vs. 19.1 ± 5.7 ng/mL, $P=0.945$). In contrast, the patients' serum BDNF levels were negatively associated with the number of sessions completed weekly (β -coefficient -0.548 , $P=0.047$).

Serum BDNF Levels Predict Adverse Outcomes

During the median follow-up of 20.3 months (IQR 11.5–22.8 months), there were 19 (32%) adverse events including eight cardiac deaths and 11 rehospitalizations due to worsening HF. Of these, the death of five patients was due to HF and that of

three patients was sudden death. In addition, three patients (5%) had a new onset of stroke. None of the studied patients was lost within the follow-up period. The variables to predict adverse outcomes were identified by the univariate (**Table 3**) and multivariate Cox proportional hazard analyses (**Table 4**).

In the univariate analysis, serum BDNF was significantly associated with all cardiac death and HF rehospitalization [per SD increase: hazard ratio (HR) 0.47; 95% confidence interval (CI) 0.29–0.75, $P=0.001$]. The NYHA functional class, LVEDD, LVEF, peak VO_2 , AT, log VE/ VCO_2 slope, and log BNP were also related to adverse outcomes. The multivariate analysis revealed that among these variables, the serum BDNF level and log BNP were independent predictors of adverse outcomes. The results based on the stepwise forward selection method showed that the NYHA functional class, log BNP, and BDNF were significant prognostic factors, and these results were consistent with the first model, which included all potential candidates as covariates (**Table 4**).

Comparison of Serum BDNF with Plasma BNP as a Prognostic Indicator

The ROC curves of serum BDNF values and plasma BNP concentrations for the prediction of all cardiac death or HF rehospitalization are shown in **Figure 1**. The AUC of the serum BDNF levels for the prediction of adverse events was 0.798 (95% CI, 0.641–0.897, $P<0.001$), whereas that of the plasma BNP concentration was 0.827 (95% CI, 0.694–0.910, $P<0.001$). There was no significant difference in AUC between serum BDNF and plasma BNP (difference of AUC -0.03 , 95% CI -0.192 – 0.133 , $P=0.721$). The serum BDNF level 17.4 ng/mL and the BNP level 246.7 pg/mL were defined as the optimal cutoff points for discriminating adverse outcomes. The best-

performing value of serum BDNF (17.4 ng/mL) to predict adverse events was associated with 75% sensitivity, 79% specificity, 60% positive predictive values and 85% negative predictive values. This tended to be a slightly weaker performance than that observed for plasma BNP with 85% sensitivity, 71% specificity, 60% positive predictive value, and 90% negative predictive value. Adverse events occurred significantly more frequently in the low (< 17.4 ng/mL) BDNF group compared to the high (≥ 17.4 ng/mL) BDNF group (66% vs. 16%, $P < 0.001$) (**Fig. 2**).

DISCUSSION

The major finding of the present study was that lower serum BDNF levels were associated with a higher incidence of adverse outcomes including all cardiac death and HF rehospitalization among HF patients. Importantly, this is the first report to demonstrate that the serum BDNF level was an independent prognostic factor for adverse events by a multivariate Cox proportional hazard analysis.

Role of BDNF in Lowered Exercise Capacity in HF Patients

The present results showed a significant positive association between the serum BDNF level and exercise capacity measured as the peak VO_2 , AT, and VE/VCO_2 slope. These findings were consistent with those of our previous study.¹⁴ Qualitative abnormalities in skeletal muscles' energy metabolism are well known to determine the exercise capacity in HF patients.^{2,4} BDNF was shown to be produced in skeletal muscle, and it is increased by muscle contractions to enhance fat oxidation in an AMPK-dependent fashion, which can regulate glucose and fatty acid metabolism.^{12,28} In addition, we recently demonstrated that intramyocellular lipid is increased in the

skeletal muscle of patients with dilated cardiomyopathy with lowered exercise capacity.²⁹

These findings suggest that serum BDNF can be a useful marker for exercise capacity in HF patients, reflecting impaired fatty acid metabolism in the skeletal muscle. In the present study, the change in peak VO₂ from baseline did not correlate with the serum BDNF level in the limited patients, but further study is needed to determine whether serum BDNF predicts the future exercise capacity in HF patients. In contrast, there was a negative association between serum BDNF levels and the number of completed exercise sessions, suggesting that patients with low BDNF levels may need more intensive exercise training than those with high BDNF levels.

Predictive Value of Serum BDNF for Prognosis in HF Patients

Our results demonstrated the role of serum BDNF as a prognostic marker in HF. Jiang et al. reported that multiple cardiovascular risk factors were associated with the plasma BDNF level in patients with angina pectoris, and that low plasma BDNF was related to future coronary events and mortality in these patients.¹¹ To our knowledge, the present study is the first to demonstrate the predictive role of BDNF in HF. Since we found that serum BDNF levels were associated with cardiopulmonary exercise variables, these prognostic values of BDNF may be partly explained by a surrogate marker for exercise capacity. Novel biomarkers reflective of ventricular remodeling and fibrosis have been reported as an incremental prognostic value;³⁰ however, potential biomarkers that reflect skeletal muscle abnormalities and exercise capacity have not been investigated, to our knowledge.

Even after adjustments for powerful prognostic variables including the NYHA functional class and plasma BNP, we observed that the serum BDNF level was an independent predictor of adverse cardiac events. These findings suggest that measurements of serum BDNF could provide further information about the prognosis in HF. In addition, the optimal cutoff value of BDNF determined by the ROC analysis had a prognostic ability similar to that of BNP, suggesting that serum BDNF could be a novel marker in addition to plasma BNP, which is the first-line biomarker for risk stratification in HF.¹⁶

Role of BDNF in Depressive Symptoms and Adherence to Exercise Training in HF

Circulating and hippocampal BDNF levels in patients with major depression have been reported to be lower and associated with this disease severity.^{31,32} In the present study, however, there was no association between serum BDNF and depressive symptoms according to PHQ-9 scores. This discrepant result may be due to the relatively small number of patients who were in a clinically significant depressive state (6%). On the other hand, higher serum BDNF levels were reported to protect against the future occurrence of dementia in the offspring of participants in the Framingham study.⁹ Cognitive impairment is prevalent among HF patients and is associated with increased mortality risk,³³ and it was also reported to affect poor treatment adherence in HF.³⁴ In the present study, we investigated the role of BDNF in adherence to exercise training, and we found that there was no difference in serum BDNF levels between the adherent and non-adherent patients, suggesting that serum BDNF levels may have a minor role in the adherence to exercise training in HF.

Study Limitations

There are several potential limitations which should be acknowledged in this study. First, the present study was categorized as a prognostic factor research, not prognostic model research and acknowledged the lack of information regarding model validation and calibration as a potential limitation.³⁵ Furthermore, the relatively small number of studied subjects limits the statistical power for detecting the prognostic value of serum BDNF levels. Therefore, a study on a larger scale is warranted to confirm the relationship between worse prognosis and decreased serum BDNF levels in HF patients. Second, an accurate investigation for a new biomarker has never been performed by using specific statistical tests including discrimination, calibration, and reclassification analyses as recommended.^{36,37} Moreover, there was no control group in the present study. Third, there were no data about cognitive function, daily physical activity, or medication adherence. Finally, the sources of serum BDNF are skeletal muscle, heart, and brain, which could not be identified here. Despite these limitations, our observation that serum BDNF can predict the prognosis in HF patients is unique and had not been reported previously.

Conclusions

Decreased serum BDNF levels were related to all cardiac death and HF readmission in HF patients. Serum BDNF may be a promising biomarker to predict the adverse outcomes in HF. Further studies are needed to demonstrate the prognostic incremental value of BDNF compared to the standard cardiovascular biomarkers and to determine the cost-effectiveness of its measurement in patients with HF.

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Disclosures

No conflicts of interest

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521

Table 1. Univariate linear model of serum BDNF in patients with HF

Variable	HF patients (n=58)	
	β -coefficient	P-value
Age, yrs	0.043	0.425
Gender (male=0)	-0.714	0.412
Body mass index, kg/m ²	0.208	0.172
LVEDD, mm	-0.002	0.967
LVEF, %	0.007	0.902
Peak VO ₂ , mL/kg/min	0.547	0.003
AT, mL/kg/min	0.929	0.004
Log VE/VCO ₂ slope	-10.15	0.005
eGFR (mL·min ⁻¹ ·1.73m ⁻²)	0.013	0.523
Log BNP, pg/mL	-1.215	0.047
PHQ-9 score	-0.099	0.586
Change in peak VO ₂ from baseline	0.200	0.233
Exercise session per week	-0.548	0.047

Data are means±SD. HF, heart failure; BDNF, brain-derived neurotrophic factor; NYHA, New York Heart Association; COPD, chronic obstructive pulmonary disease; PHQ-9, patient health questionnaire 9; LV, left ventricular; EDD, end-diastolic diameter; EF, ejection fraction; VO₂, oxygen uptake; AT, anaerobic threshold; VE, minute ventilation; VCO₂, carbon dioxide production; RER, respiratory exchange ratio; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin type I receptor blocker; eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide; BDNF, brain-derived neurotrophic factor.

532 **Table 2.** Patients' characteristics

	All patients (n=58)
Demographic factors	
Age, yrs (mean±SD)	59.2 ± 13.7
Male, n (%)	44 (75)
Body mass index, kg/m ²	23.4 ± 4.9
Causes of heart failure, n (%)	
Dilated cardiomyopathy	18 (31)
Ischemic heart disease	17 (29)
Hypertensive heart disease	10 (17)
Valvular heart disease	5 (8)
NYHA functional class (I/II/III)	5/39/14
Medical history, n (%)	
Hypertension	18 (31)
Diabetes mellitus	19 (32)
Stroke	7 (12)
Paroxysmal or chronic atrial fibrillation	27 (46)
COPD	8 (13)
Sleep apnea syndrome	8 (13)
PHQ-9 score	3.9 ± 4.2
Echocardiographic parameters	
LV EDD, mm	62.9 ± 11.5
LVEF, %	34.7 ± 12.7
Cardiopulmonary exercise test	
Peak VO ₂ , mL/kg/min	14.0 ± 3.9
AT, mL/kg/min	9.1 ± 2.2
VE/VCO ₂ slope	38.5 ± 8.2
Peak RER	1.23 ± 0.1
Medications, n (%)	
ACE-I or ARB	52 (89)
β-Blockers	56 (96)
Spironolactone	35 (60)
Laboratory tests:	
eGFR, mL·min ⁻¹ ·1.73m ⁻²	55.5 ± 20.3
Plasma BNP, pg/mL	410 ± 646

Serum BDNF, ng/mL

19.0 ± 5.6

Abbreviations as in Table 1.

Table 3. Univariate analysis of predictors of all cardiac death and HF rehospitalization

Variable	HR	95% CI	P-value
Age*	1.00	0.65–1.53	0.982
Gender (male vs. female)	3.35	0.96–21.1	0.057
BMI*	0.91	0.58–1.42	0.685
NYHA functional class (III vs. I/II)	4.36	1.68–13.4	<0.001
COPD	1.41	0.32–4.26	0.594
Sleep apnea syndrome	0.68	0.10–2.36	0.590
PHQ-9	1.04	0.94–1.13	0.324
LVEDD*	1.56	1.00–2.43	0.046
LVEF*	0.53	0.29–0.95	0.021
Peak VO ₂ , mL/kg/min*	0.32	0.16–0.64	<0.001
AT, mL/kg/min*	0.27	0.13–0.59	<0.001
Log VE/VCO ₂ slope*	71.6	5.82–1017	<0.001
ACE-I or ARB	0.11	0.01–2.22	0.121
β-Blockers	0.22	0.06–1.43	0.100
Spironolactone	1.49	0.59–4.21	0.402
Estimated GFR*	0.76	0.41–1.43	0.357
Log BNP*	2.63	1.64–4.21	<0.001
BDNF*	0.47	0.29–0.75	0.001

Abbreviations as in Table 1.

*Per 1 SD increase. HR, hazard ratio; CI, confidence interval; SD, standard deviation.

Table 4. Multivariate analysis of predictors of all cardiac death and HF rehospitalization

Variable	HR	95% CI	P-value
Age*	1.35	0.83-2.20	0.127
Gender (male vs. female)	3.11	0.82-20.2	0.098
NYHA functional class (III vs. I/II)	2.02	0.70-6.03	0.186
Log BNP*	2.54	1.15-5.60	<0.001
BDNF*	0.41	0.20-0.84	0.003

Abbreviations as in Table 1.

*Per 1 SD increase.

Figure Legends

Fig. 1. Predictive ability of serum BDNF and plasma BNP levels for adverse outcomes.

The ROC curve was created to predict all cardiac death and rehospitalization due to worsening of HF based on serum BDNF and plasma BNP levels.

Fig. 2. Kaplan-Meier event-free curves for serum BDNF levels. The event-free rate

from cardiac death and rehospitalization caused by the worsening of heart failure was

compared between patients with high and low serum BDNF levels. The cut-off level of

the serum BDNF concentration (17.4 ng/mL) was determined by the ROC. The

significance of separation between the two groups was examined using a log-rank test at

20.4 months.

Figure 1

	BDNF	BNP
AUC	0.798	0.827
95%CI	0.641-0.897	0.694-0.910
<i>P</i>	<0.001	<0.001

Figure 2