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Original article

Prognostic impact of transcardiac gradient of follistatin-like 1 reflecting hemodynamics in patients with dilated cardiomyopathy

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ABSTRACT

Background: Follistatin-like 1 (FSTL1) is a myocyte-secreted glycoprotein that could play a role in myocardial maintenance in response to harmful stimuli. We investigated the association between serum FSTL1 levels, especially focused on transcardiac gradient and the hemodynamics, to explore the prognostic impact of FSTL1 levels in patients with dilated cardiomyopathy (DCM).

Methods: Thirty-two ambulatory patients with DCM (23 men; mean age 59 years) were prospectively enrolled. Blood samples were simultaneously collected from the aortic root (Ao), coronary sinus (CS), as well as from the peripheral vein during cardiac catheterization in stable conditions. The transcardiac gradient of FSTL1 was calculated by the difference between serum FSTL1 levels of CS and Ao ($FSTL1_{CS-Ao}$). Patients were divided into two groups based on the median of $FSTL1_{CS-Ao}$: Low $FSTL1_{CS-Ao}$ group, <0 ng/mL; High $FSTL1_{CS-Ao}$ group, ≥0 ng/mL. Cardiac events were defined as a composite of cardiac deaths and hospitalizations for worsening heart failure.

Results: Mean left ventricular ejection fraction and median plasma B-type natriuretic peptide levels were 30.9% and 92.3 pg/mL, respectively. $FSTL1_{CS-Ao}$ was negatively correlated with pulmonary capillary wedge pressure (r = -0.400, p = 0.023). Kaplan-Meier survival analysis showed that event-free survival rate was significantly lower in the Low $FSTL1_{CS-Ao}$ group than in the High $FSTL1_{CS-Ao}$ group (p = 0.013). Cox regression analyses revealed that the transcardiac gradient of FSTL1 was an independent predictor for cardiac events. Receiver operating characteristic curve analysis showed that the cut-off value of $FSTL1_{CS-Ao}$ for the prediction of cardiac events was -4.09 ng/mL with sensitivity of 82% and specificity of 86% (area under the curve, 0.87).

Conclusions: Fifty percent of patients had negative transcardiac gradient of FSTL1. Reduced transcardiac gradient of FSTL1 might be a novel prognostic predictor in DCM patients with impaired hemodynamics.

Introduction

Dilated cardiomyopathy (DCM) is characterized by progressive left ventricular (LV) dilation and reduced LV contractility [1,2]. The mortality in DCM patients is high, with about two-thirds of patients dying within 2 years [3]. Several reports have highlighted that the prognosis of DCM has greatly improved in the past 30 years [4–6]; however, DCM remains a disease with high mortality and re-hospitalization rates for worsening heart failure [7,8]. Therefore, it is important to identify DCM patients with poor prognosis and introduce optimal medical treatment early.

Multiple biomarkers, including B-type natriuretic peptide (BNP), are currently available for diagnosis, management, and risk stratification of heart failure [9]. However, some of these biomarkers are also secreted from various tissues other than the heart, so previous studies have measured transcardiac gradient of these biomark-

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ers by collecting blood samples from the aortic root (Ao) and coronary sinus (CS) to assess their dynamics through the heart [10–13]. Follistatin-like 1 (FSTL1) is a glycoprotein secreted during myocardial stress, including heart failure and myocardial infarction [14,15]. Several basic research reports have shown the role of FSTL1 as a cardiokine, i.e. a physiologically active substance secreted by cardiac myocytes. Previously, we demonstrated that FSTL1 secreted by cardiomyocytes functions as an autocrine/paracrine regulatory factor that antagonizes myocyte hypertrophic growth and the loss of ventricular performance in response to pressure overload [16]. In addition, we found that FSTL1 improved cardiac function and injury in animal models of ischemia/reperfusion by inhibiting myocardial apoptosis and inflammatory response [17]. Seki et al. reported that FSTL1 could alter myocardial and systemic substrate energy metabolism and had cardioprotective effects [18]. Clinical research showed that the myocardial expression levels of FSTL1 protein are higher in patients with DCM or ischemic cardiomyopathy than in healthy subjects [14]. Furthermore, in patients with end-stage DCM, higher expression of FSTL1 in the LV myocardium at the initiation of LV assist device (LVAD) support correlated with a higher LV ejection fraction (LVEF) after support [19]. However, to date, no report has investigated the association between FSTL1 levels, especially regarding transcardiac gradient, and the hemodynamics or prognosis in DCM patients. Thus, we sought to elucidate (1) the association between FSTL1 levels and hemodynamics assessed by cardiac catheterization and (2) the prognostic impact of FSTL1 levels in DCM patients.

Materials and methods

Study population

Thirty-two consecutive DCM patients at Nagoya University Hospital were enrolled in this prospective study. DCM was defined on the basis of the presence of LVEF <50% in the absence of coronary artery disease, primary valvular heart disease, or secondary cardiomyopathy caused by any known systemic condition. The patients underwent echocardiography, cardiac catheterization with blood sample collection for evaluating FSTL1 levels, and endomyocardial biopsies. This study protocol complied with the Declaration of Helsinki and was approved by the Ethics Review Board of our institute (approval number 2006–0359–4). Written informed consent was obtained from all participating subjects.

Echocardiography

Two-dimensional and Doppler echocardiography were performed by experienced sonographers using a Vivid 7 ultrasound system (GE Healthcare, Chicago, IL, USA) with a 2.5 MHz phased-array transducer. LV wall thickness, LV end-diastolic diameter, LV end-systolic diameter, and left atrial diameter were measured from 2D imaging according to the recommendations of the American Society of Echocardiography [20]. The LV mass was calculated by the standard cube formula [21]. LV mass index was calculated by dividing LV mass by the body surface area. Early diastolic transmitral flow velocity/early diastolic annular velocity ratio (E/e') was also calculated.

Cardiac catheterization

Right heart catheterization at rest was performed to measure pulmonary capillary wedge pressure, pulmonary artery pressure, right ventricular pressure, right atrial pressure, and the cardiac index using a Swan-Ganz thermodilution catheter (Edwards Life Science Co., Irvine, CA, USA). Coronary angiography was performed to exclude ischemic heart disease. For 27 patients with consent, endomyocardial biopsy was also performed from the right ventricular septum to evaluate FSTL1 expression in the myocardium. To investigate whether FSTL1 expression was associated with myocardial pathological findings, the biopsy samples were stained with a collagen-specific dye–picrosirius red stain–and the collagen volume fraction (CVF) was analyzed using BZ-X800 (KEYENCE Co. Ltd., Osaka, Japan), as previously reported [22]. CVF was calculated by dividing the area of collagen stained red with picrosirius red stain by the total myocardial area. All samples were analyzed by another investigator who was blinded to the clinical information.

FSTL1 measurement

Blood samples were collected at rest in the supine position from Ao and CS using catheters, as well as from the peripheral vein (PV) during cardiac catheterization, to assess the serum FSTL1 levels of each position. Serum FSTL1 levels were measured by the enzyme-linked immunosorbent assay kit for humans (Cloud-Clone Corp., Houston, TX, USA), as previously described [23]. The intra-assay and inter-assay coefficients of variation were less than 10% and 12%, respectively. Albumin, creatinine, uric acid, lipid fractions, electrolytes, HbA1c, BNP, and troponin T were measured using PV blood samples. The estimated glomerular filtration rate (eGFR) was calculated using an equation proposed by the Japanese Society of Nephrology: eGFR (mL/min/1.73 m^2) = 194 × [age (years)] - 0.287 × [serum creatinine (mg/dL)] - 1.094 [× 0.739 for females] [24].

Quantification of mRNA levels in myocardium

Gene expression levels were quantified by real-time polymerase chain reaction (PCR). Total ribonucleic acid (RNA) was extracted from human heart tissues using RNeasy® Micro Kit (Qiagen, Hilden, Germany). RNA which had an OD260/280 ratio of 1.8 or greater was used for reverse transcription reaction. cDNA was produced from 0.5 μg total RNA using a ReverTra Ace® qPCR RT Kit (Toyobo, Osaka, Japan) [25]. PCR was performed with a Bio-Rad real-time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using THUNDERBIRD SYBR qPCR Mix (Toyobo) as a double-standard DNA-specific dye. Primers were 5′-TGCTCAACATCTCCCCCTTCTC-3′ and 5′-ACCAAATCCCATATCCTCGTCC-3′ for human 36B4, and 5′-CCTGTGTGTGGCAGTAATGG-3′ and 5′-TCTCTTTGCAGTGTCCATCG-3′ for human FSTL1.

Clinical follow-up

Because FSTL1 is secreted by the heart and plays a cardioprotective role, not only in the myocardium but also in various tissues such as skeletal muscle, the transcardiac gradient of FSTL1 was calculated by the difference between CS and Ao ($FSTL1_{CS-Ao}$), as described in previous studies [10–13]. Patients were divided into two groups according to the median of their $FSTL1_{CS-Ao}$ levels (Low $FSTL1_{CS-Ao}$ group, <0 ng/mL and High $FSTL1_{CS-Ao}$ group, ≥0 ng/mL). Data on clinical events were collected from the medical records or via telephone interview. The primary endpoint of this study was the occurrence of a cardiac event, which was defined as a composite of cardiac deaths and unexpected hospitalizations for worsening heart failure. Cardiac death was defined as sudden death without a comorbidity and death due to heart failure. Hospitalizations for worsening heart failure was defined as an event that required intravenous diuretics or inotropes to treat heart failure exacerbation.

Statistics

Data are presented as mean ± standard deviation or median with 25th and 75th percentiles. The chi-square test was used to

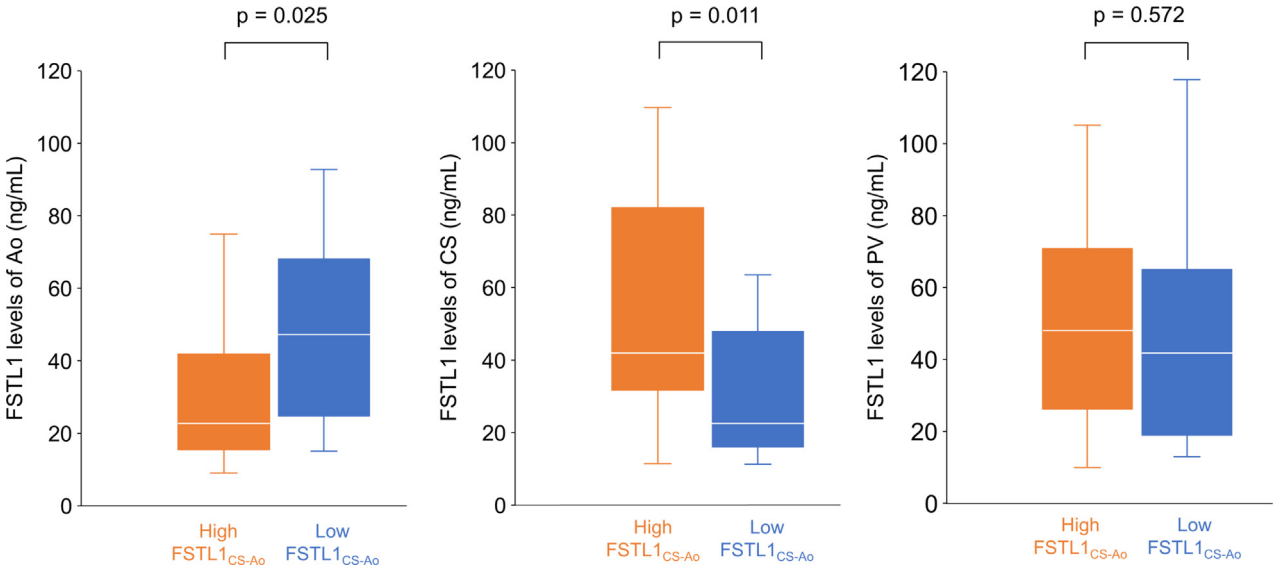

Fig. 1. Serum FSTL1 levels at each position. In the Low $FSTL1_{CS-Ao}$ group, the serum FSTL1 levels of Ao were significantly higher than those of the High $FSTL1_{CS-Ao}$ group and the serum FSTL1 levels of CS were significantly lower than those of the High $FSTL1_{CS-Ao}$ group, whereas the FSTL1 levels of PV did not differ significantly between the two groups.
FSTL1, follistatin-like 1; Ao, aortic root; CS, coronary sinus; PV, peripheral vein; $FSTL1_{CS-Ao}$, transcardiac gradient of FSTL1 levels as calculated by the difference between CS and Ao.

compare categorical variables. The distribution of continuous variables was examined by the Shapiro-Wilk test. Student's t-test was used to compare continuous variables with a normal distribution. The Mann-Whitney U test was conducted to compare continuous variables with a skewed distribution. Event-free survival curves were drawn according to the Kaplan-Meier method and analyzed by the log-rank test. To determine the independent predictors of cardiac events, statistically significant variables ($p<0.05$) in the univariate analysis were included in the multivariate analysis of Cox proportional-hazards model with a 95% confidence interval (CI). Statistical tests were conducted using the JMP software (version 14.2; SAS Institute Inc., Cary, NC, USA). A value of $p<0.05$ was considered to indicate statistical significance.

Results

Baseline characteristics

Thirty-two ambulatory patients (23 men; mean age 59 ± 11 years) with DCM were analyzed in this study. Heart failure in most of the patients was classified as New York Heart Association (NYHA) functional class I (38%) or II (56%). Mean serum FSTL1 levels of Ao, CS, and PV were 39.9 ± 22.7 ng/mL, 42.2 ± 27.0 ng/mL, and 47.8 ± 28.4 ng/mL, respectively. The baseline clinical characteristics of each group are summarized in **Table 1**. Age, sex, body mass index, NYHA functional class, and medical histories, except for hypertension, were not significantly different between the two groups. There was no significant difference in the use of beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and mineralocorticoid receptor antagonists. In the Low $FSTL1_{CS-Ao}$ group, the serum FSTL1 levels of Ao were significantly higher than those of the High $FSTL1_{CS-Ao}$ group (High $FSTL1_{CS-Ao}$ group, 31.0 ± 18.9 ng/mL vs Low $FSTL1_{CS-Ao}$ group, 48.8 ± 23.2 ng/mL, $p = 0.025$) and the serum FSTL1 levels of CS were significantly lower than those of the High $FSTL1_{CS-Ao}$ group (High $FSTL1_{CS-Ao}$ group, 54.4 ± 29.7 ng/mL vs Low $FSTL1_{CS-Ao}$ group, 30.0 ± 17.4 ng/mL, $p = 0.011$), whereas the FSTL1 levels of PV did not differ significantly between the two groups (**Fig. 1**). No significant difference was found between the two groups in other laboratory data, including BNP and troponin T, and in echocardiographic data.

Transcardiac FSTL1 levels and hemodynamics

Regarding hemodynamic data, the Low $FSTL1_{CS-Ao}$ group had a higher pulmonary artery pressure and right atrial pressure (**Table 1**). Pulmonary capillary wedge pressure tended to be higher in the Low $FSTL1_{CS-Ao}$ group. In addition, $FSTL1_{CS-Ao}$ levels were inversely correlated with pulmonary capillary wedge pressure ($r = -0.40$, $p = 0.023$), mean pulmonary artery pressure ($r = -0.40$, $p = 0.023$), and right atrial pressure ($r = -0.41$, $p = 0.019$). However, there was no correlation between $FSTL1_{CS-Ao}$ levels and cardiac index ($r = -0.059$, $p = 0.747$) (**Fig. 2**).

Transcardiac FSTL1 levels and prognosis

All patients were followed up by cardiology sub-specialists. The follow-up period was 4.69 ± 2.80 years, and 11 cardiac events occurred during the follow-up period (unexpected hospitalization due to worsening heart failure, $n = 10$; cardiac death, $n = 1$). Thus, the rate of cardiac events was 34.4% in our cohort. The transcardiac gradient of FSTL1 was significantly lower in patients with cardiac events than those without, whereas the FSTL1 levels of PV and known risk factors for heart failure (e.g. BNP, troponin T, atrial fibrillation, and hemoglobin) did not differ significantly between patients with and without cardiac events. On the other hand, the FSTL1 levels of Ao, PCWP, mean PAP, and RAP for patients with cardiac events were higher than those without (**Online Table 1**). Kaplan-Meier survival analysis revealed that the Low $FSTL1_{CS-Ao}$ group had a low event-free survival rate compared to the High $FSTL1_{CS-Ao}$ group (log-rank test, $p = 0.013$) (**Fig. 3**). We performed a Cox regression analysis; beta-blocker was excluded from this analysis because no events had occurred in patients without beta-blockers. Univariate Cox regression analysis for the primary endpoint showed that Low $FSTL1_{CS-Ao}$ group, NYHA functional class I, history of prior heart failure hospitalization, and total cholesterol were significantly associated with cardiac events. Multivariate Cox regression analyses with Low $FSTL1_{CS-Ao}$ group revealed that Low

Table 1
Baseline clinical characteristics of the study population.

Variables	All patients (n = 32)	High $FSTL1_{CS-Ao}$ group (n = 16)	Low $FSTL1_{CS-Ao}$ group (n = 16)	p
Age, years	62 (49 – 66)	61 (57 – 65)	62 (43 – 68)	0.865
Male, n (%)	23 (71.9)	12 (75.0)	11 (68.8)	0.694
Body mass index, kg/m^2	22.7 ± 3.6	22.3 ± 3.6	23.2 ± 3.6	0.473
Hypertension, n (%)	7 (21.9)	6 (37.5)	1 (6.3)	0.033
Dyslipidemia, n (%)	11 (34.4)	5 (31.3)	6 (37.5)	0.710
Diabetes, n (%)	7 (21.9)	5 (31.3)	2 (12.5)	0.200
Prior HF hospitalization, n (%)	15 (46.9)	7 (43.8)	8 (50.0)	0.723
Current smoking, n (%)	9 (28.1)	4 (25.0)	5 (31.3)	0.694
Atrial fibrillation, n (%)	8 (25.0)	5 (31.3)	3 (18.8)	0.414
CLBBB, n (%)	2 (6.3)	2 (12.5)	0 (0.0)	0.144
NYHA (I, II, III, IV)	12, 18, 2, 0	5, 10, 1, 0	7, 8, 1, 0	0.758
Medication				
ACE-I/ARB, n (%)	28 (87.5)	15 (93.8)	13 (81.3)	0.285
Beta-blocker, n (%)	30 (93.8)	16 (100)	14 (87.5)	0.144
MRA, n (%)	17 (53.1)	7 (43.8)	10 (62.5)	0.288
Laboratory data				
Albumin, g/dL	3.8 ± 0.4	3.8 ± 0.4	3.8 ± 0.3	1.000
eGFR, mL/min/1.73m^2	63.8 (53.8 – 77.9)	61.6 (57.1 – 70.3)	70.0 (45.8 – 96.5)	0.598
Uric acid, mg/dL	7.0 ± 1.8	7.1 ± 1.6	6.8 ± 2.0	0.660
Na, mEq/L	139.5 (137.0 – 141.0)	139.0 (136.3 – 141.0)	140.0 (137.5 – 141.8)	0.519
Total cholesterol, mg/dL	194.5 ± 36.1	193.5 ± 33.7	195.6 ± 39.4	0.875
LDL-cholesterol, mg/dL	119.9 ± 31.0	120.1 ± 29.5	119.8 ± 33.4	0.978
HDL-cholesterol, mg/dL	50.4 ± 12.9	49.0 ± 12.0	51.9 ± 14.0	0.538
Triglycerides, mg/dL	144.5 (82.3 – 185.0)	103.5 (78.5 – 184.8)	154.5 (87.0 – 185.0)	0.651
HbA1c,%	6.0 (5.7 – 6.3)	6.0 (5.6 – 6.3)	6.0 (5.7 – 6.5)	0.970
Hemoglobin, g/dL	13.9 ± 1.9	13.9 ± 1.4	13.8 ± 2.3	0.896
BNP, pg/mL	92.3 (37.1 – 264.3)	94.1 (59.1 – 236.5)	91.9 (21.7 – 277.5)	1.000
*Troponin T, ng/mL	0.011 (0.006 – 0.017)	0.011 (0.006 – 0.021)	0.010 (0.005 – 0.014)	0.519
Echocardiography				
LVDd, mm	62.1 (54.3 – 65.2)	62.7 (56.8 – 66.4)	60.7 (53.5 – 64.0)	0.346
LVDs, mm	50.8 (45.0 – 54.5)	50.8 (48.0 – 53.8)	51.0 (42.4 – 56.5)	0.895
LVEF,%	30.9 ± 6.8	30.5 ± 7.3	31.4 ± 6.5	0.714
LVMI, g/m^2	122.5 (97.4 – 167.0)	151.8 (102.9 – 186.3)	110.4 (89.7 – 140.8)	0.086
Left atrial diameter, mm	40.4 ± 7.6	39.8 ± 7.5	41.1 ± 7.9	0.634
E/e' ratio	15.2 (10.1 – 21.1)	16.0 (12.8 – 22.0)	12.9 (10.0 – 20.5)	0.462
Catheterization				
Heart rate, beats/minute	77.8 ± 15.0	76.6 ± 15.7	79.0 ± 14.6	0.661
SBP, mmHg	125.6 ± 20.8	122.1 ± 23.6	129.0 ± 17.8	0.359
DBP, mmHg	73.2 ± 12.1	69.4 ± 11.4	77.0 ± 11.8	0.073
PCWP, mmHg	9.5 (6.3 – 12.0)	7.0 (5.0 – 11.0)	11.0 (7.3 – 15.0)	0.082
Mean PAP, mmHg	15.0 (11.3 – 18.8)	12.5 (11.0 – 14.8)	17.5 (15.3 – 20.8)	0.020
Right atrial pressure, mmHg	5.0 (2.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (4.3 – 8.5)	0.003
Cardiac index, L/min/m^2	2.6 ± 0.5	2.5 ± 0.5	2.7 ± 0.5	0.260

Data are mean ± SD or median (interquartile range).
$FTL1_{CS-Ao}$, the difference between serum follistatin-like 1 levels of coronary sinus and those of aortic root; CLBBB, complete left bundle branch block; NYHA, New York Heart Association functional class; HF, heart failure; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; MRA, mineralocorticoid receptor antagonist; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c, glycosylated hemoglobin; BNP, B-type natriuretic peptide; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; E/e' ratio, ratio of early transmitral flow velocity to early diastolic mitral annular velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; PCWP, pulmonary capillary wedge pressure; PAP, pulmonary artery pressure.
*Troponin T (n = 30): High $FSTL1_{CS-Ao}$ group, n = 16; Low $FSTL1_{CS-Ao}$ group, n = 14.

$FSTL1_{CS-Ao}$ group remained an independent predictor for cardiac events (Table 2).

FSTL1 mRNA expression in myocardium and pathological findings

The expression of FSTL1 mRNA in the myocardium in the Low $FSTL1_{CS-Ao}$ group (n = 7) was significantly higher than that in the High $FSTL1_{CS-Ao}$ group (n = 6) (p = 0.018) (Fig. 4**A**). CVF in endomyocardial biopsy samples did not differ between the two groups [High $FSTL1_{CS-Ao}$ group: 7.5 (5.8–9.0)% (n = 12), Low $FSTL1_{CS-Ao}$ group: 7.2 (4.9–12.8)% (n = 15), p = 0.864] (Fig. 4**B**).

Best cut-off of transcardiac FSTL1 level for predicting prognosis

Receiver operating characteristic curve analysis showed that the cut-off value of $FSTL1_{CS-Ao}$ for the prediction of cardiac events was −4.09 ng/mL with sensitivity of 82% and specificity of 86%, and the area under the curve was 0.87 (Fig. 5**A**). The Kaplan-Meier survival curves of two sub-groups divided by this cut-off value is shown in Fig. 5**B**.

Discussion

To the best of our knowledge, this is the first report to elucidate the impact of FSTL1 levels on hemodynamics and prognosis in DCM patients. The main findings of our observational study are summarized as follows: (1) FSTL1 levels were increased during FSTL1 passage through the heart in 50% of the subjects, whereas FSTL1 levels were decreased in the other half of the subjects. Furthermore, transcardiac FSTL1 levels were significantly correlated with right-sided heart pressures, including pulmonary capillary wedge pressure, mean pulmonary artery pressure, and right atrial pressure. (2) Event-free survival rate was significantly lower in the Low $FSTL1_{CS-Ao}$ group than in the High $FSTL1_{CS-Ao}$ group. (3) FSTL1 mRNA expression in the myocardium was significantly higher in the Low $FSTL1_{CS-Ao}$ group than in the High $FSTL1_{CS-Ao}$ group, al-

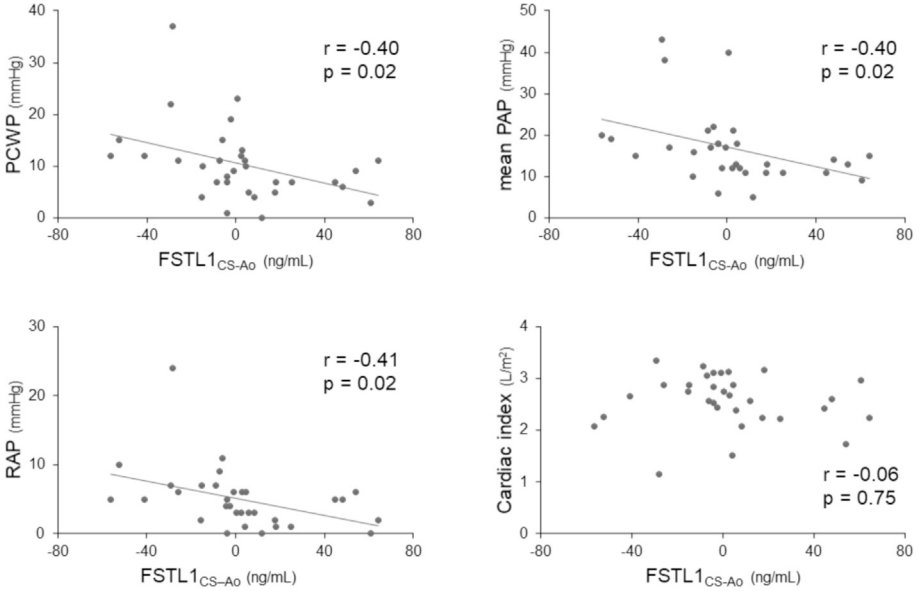

Fig. 2. Correlation between the transcardiac gradient of FSTL1 levels and hemodynamic data. $FSTL1_{CS-Ao}$ levels were inversely correlated with PCWP, mean PAP, and RAP, but not with cardiac index.
FSTL1, follistatin-like 1; CS, coronary sinus; Ao, aortic root; $FSTL1_{CS-Ao,}$ transcardiac gradient of FSTL1 levels as calculated by the difference between CS and Ao; PCWP, pulmonary capillary wedge pressure; PAP, pulmonary artery pressure; RAP, right atrial pressure.

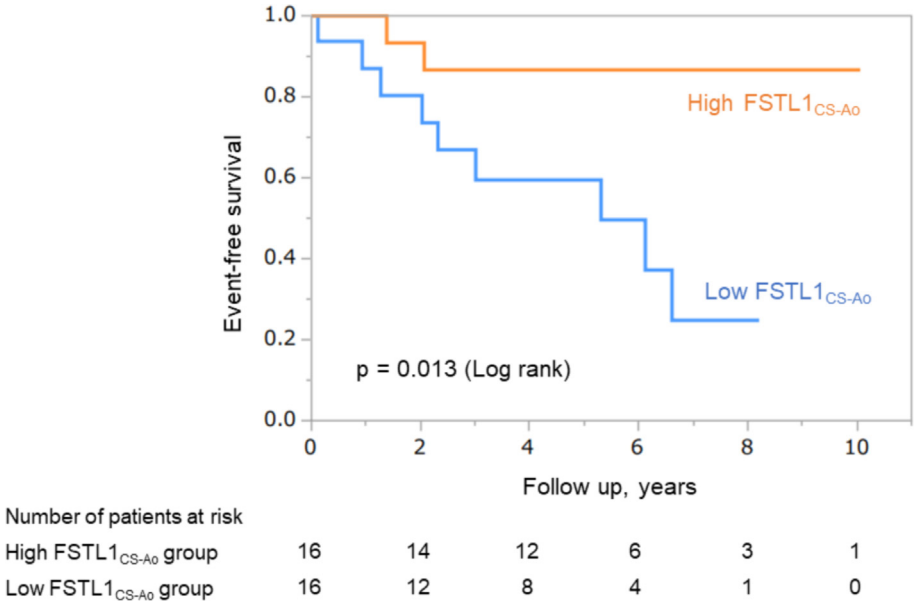

Fig. 3. Kaplan-Meier survival curves for cardiac events. The Low $FSTL1_{CS-Ao}$ group had a low event-free survival rate compared to the High $FSTL1_{CS-Ao}$ group.
FSTL1, follistatin-like 1; CS, coronary sinus; Ao, aortic root; $FSTL1_{CS-Ao,}$ transcardiac gradient of FSTL1 levels as calculated by the difference between CS and Ao.

though there was no difference between the groups in CVF in the myocardium.

Some basic studies have shown that FSTL1 is secreted by various tissues, including the skeletal muscle [26,27]; thus, the FSTL1 levels of each position could include FSTL1 secreted from tissues other than the myocardium. Therefore, we measured the transcardiac gradient of FSTL1 to identify FSTL1 secreted from the myocardium. However, our study found that 50% of the subjects had reduced FSTL1 levels via the heart, which suggested that there was another mechanism of FSTL1 secretion other than that of the heart. A previous study mentioned that a negative transcardiac gradient of a substance might represent the accumulation of the substance in the myocardium during coronary circulation [12]. Pulmonary circulation can also affect the transcardiac gradient of the substance. Therefore, a possible explanation for the reduced FSTL1 levels via the heart is that $FSTL1_{CS-Ao}$ levels show the difference in FSTL1 secretion and deposition or consumption in coronary and pulmonary circulation. Although the dynamics of cardiokine in coronary and pulmonary circulation has not been established, increase in FSTL1 deposition or consumption could exceed that secreted, resulting in the deficiency of cardioprotective FSTL1 in the low $FSTL1_{CS-Ao}$ group.

As for the hemodynamics, $FSTL1_{CS-Ao}$ levels were inversely correlated with pulmonary capillary wedge pressure, pulmonary artery pressure, and right atrial pressure. These results indicate that $FSTL1_{CS-Ao}$ levels may be closely related to intracardiac pressure. A previous study in dog models with heart failure showed that bolus administration of FSTL1 reduced LV end-diastolic pres-

Table 2
Cox regression analysis for cardiac events.

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*	HR	95%CI	*p*
Low $FSTL_{CS-Ao}$ group	2.38	1.21 – 6.09	0.011	3.92	1.75 – 11.75	0.004
Male	1.18	0.34 – 4.04	0.796			
Age	0.96	0.90 – 1.01	0.122			
Body mass index, kg/m^2	1.12	0.93 – 1.34	0.233			
Hypertension	0.90	0.35 – 1.77	0.781			
Dyslipidemia	1.06	0.54 – 1.95	0.848			
Diabetes	1.53	0.70 – 2.99	0.262			
Current smoker	1.38	0.71 – 2.52	0.320			
NYHA	0.49	0.19 – 0.97	0.040	0.41	0.15 – 0.86	0.016
Prior HF hospitalization	2.04	1.09 – 4.10	0.026	1.52	0.71 – 3.51	0.285
Atrial fibrillation	0.55	0.13 – 1.26	0.182			
ACE-I/ARB	1.26	0.55 – 5.40	0.640			
MRA	1.66	0.89 – 3.55	0.113			
Albumin	0.30	0.05 – 1.92	0.200			
eGFR, $mL/min/1.73m^2$	1.00	0.97 – 1.03	0.911			
Uric acid, mg/dL	1.18	0.87 – 1.55	0.279			
Na, mEq/L	0.85	0.70 – 1.04	0.109			
Total cholesterol, mg/dL	0.98	0.97 – 1.00	0.035	0.98	0.95 – 1.00	0.066
Hemoglobin, g/dL	0.99	0.73 – 1.38	0.973			
Log BNP, pg/mL	1.31	0.87 – 1.98	0.190			
LVDd, mm	0.94	0.84 – 1.04	0.241			
LVDs, mm	1.00	0.92 – 1.10	0.945			
LVEF,%	0.94	0.84 – 1.05	0.274			
LVMI, g/m^2	1.00	0.99 – 1.02	0.757			
Left atrial diameter, mm	1.04	0.96 – 1.13	0.354			
E/e' ratio	1.07	0.97 – 1.17	0.160			

HR, hazard ratio; CI, confidence interval; $FTL1_{CS-Ao}$, the difference between serum follistatin-like 1 levels of coronary sinus and those of aortic root; NYHA, New York Heart Association functional class; HF, heart failure; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; MRA, mineralocorticoid receptor antagonist; eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; E/e' ratio, ratio of early transmitral flow velocity to early diastolic mitral annular velocity.

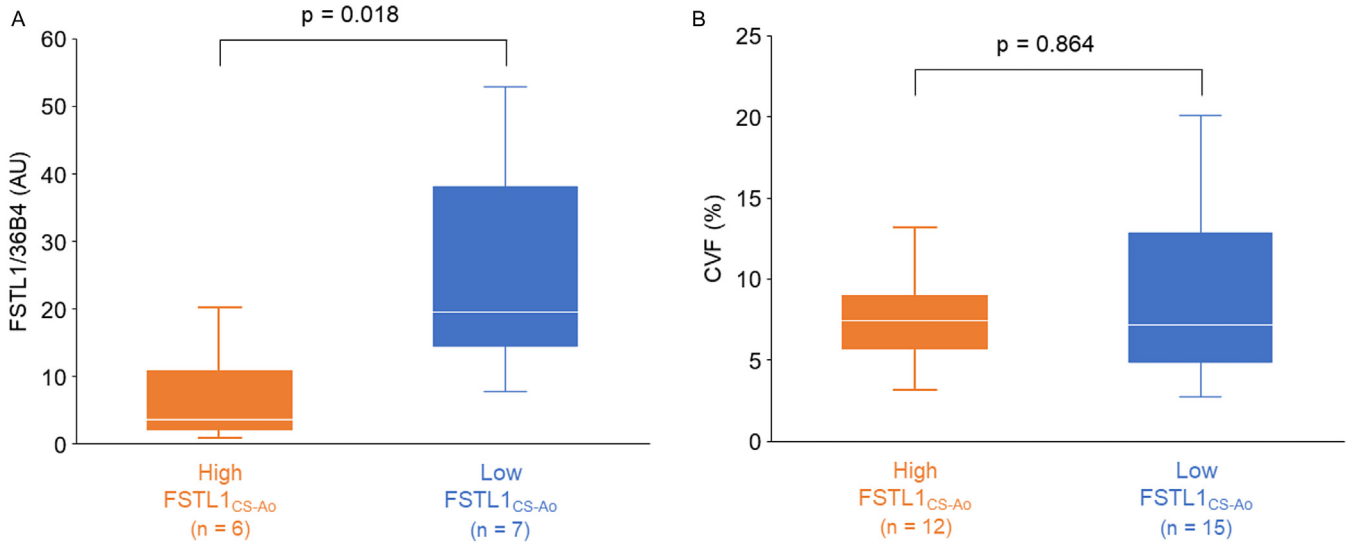

Fig. 4. Expression of FSTL1 mRNA and collagen volume fraction in myocardium. (A) The expression of FSTL1 mRNA in the myocardium in the Low $FSTL1_{CS-Ao}$ group was significantly higher than that in the High $FSTL1_{CS-Ao}$ group. (B) CVF in endomyocardial biopsy samples did not differ between the High and Low $FSTL1_{CS-Ao}$ groups.
FSTL1, follistatin-like 1; CS, coronary sinus; Ao, aortic root; $FSTL1_{CS-Ao,}$ transcardiac gradient of FSTL1 levels as calculated by the difference between CS and Ao; AU, Arbitrary unit; CVF, collagen volume fraction.

sure within 10 min. In addition, maintaining a high blood level of FSTL1 by chronic administration of FSTL1 has been shown to lower LV end-diastolic pressure [18]. High blood levels of FSTL1 may cause a rapid and sustained reduction in intracardiac pressure.

It was reported that serum FSTL1 levels of PV in patients with heart failure with reduced LVEF were strongly associated with LV hypertrophy, a measure of LV remodeling, with a slightly significant association between survival and FSTL1 levels [14]. In contrast, there was no association between FSTL1 levels of PV and the occurrence of cardiac events in our study. In addition, BNP, one of the most routinely used biomarkers in the treatment of heart failure, was also not associated with the occurrence of cardiac events in this study. This discrepancy is probably based on the severity of heart failure in the subjects. While the patients with NYHA functional class III or IV accounted for 45% of the subjects in the previous study, most of the subjects in our study had mild symptoms and only 6.3% were classified as having NYHA III or IV. Contrarily, our survival analysis revealed the association between reduced

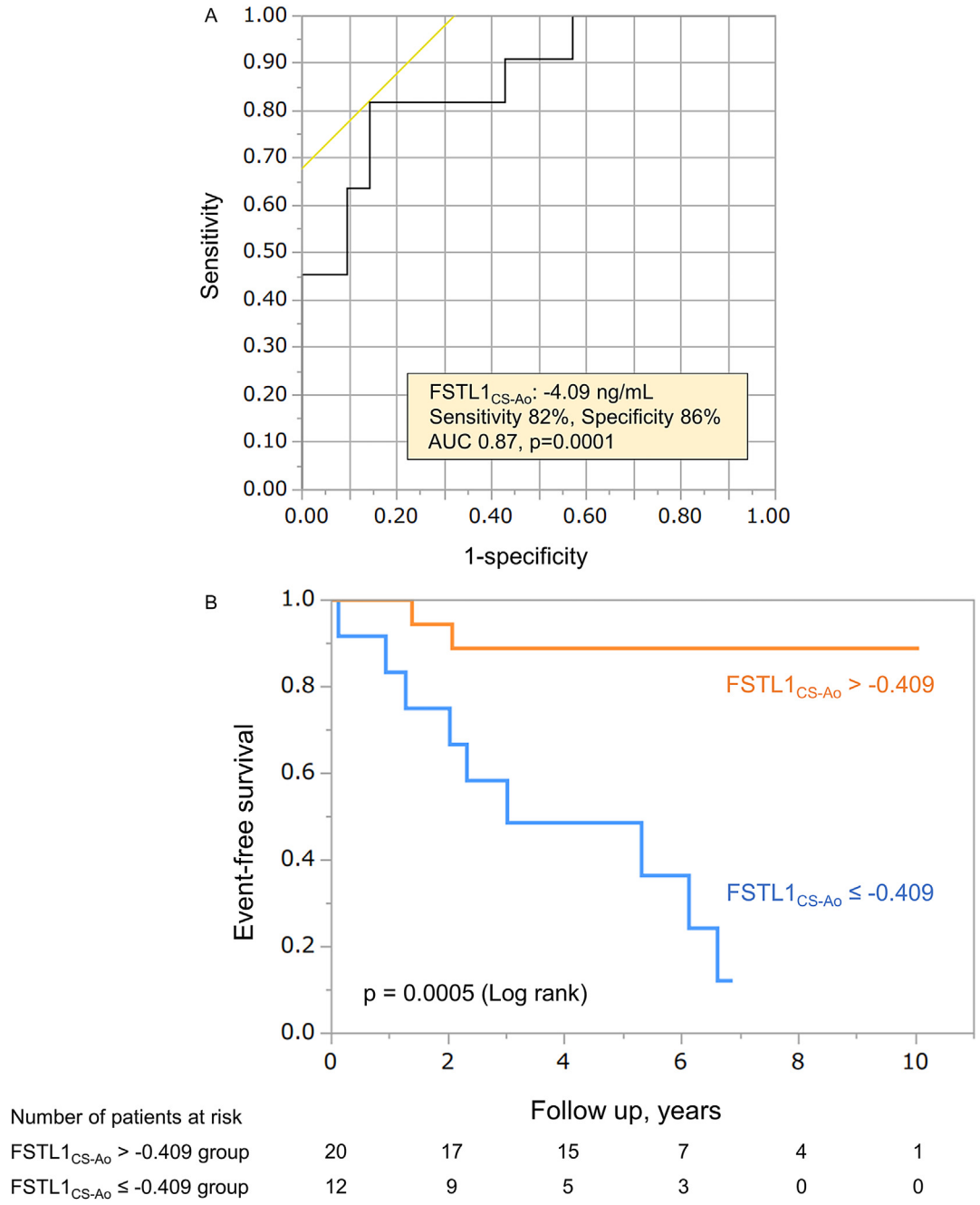

Fig. 5. Best cut-off value of transcardiac FSTL1 level for predicting prognosis. (A) Receiver operating characteristic curve analysis of $FSTL1_{CS-Ao}$ for cardiac events. The best cut-off value of $FSTL1_{CS-Ao}$ for predicting cardiac events was −4.09 ng/mL with sensitivity of 82% and specificity of 86%, and the AUC was 0.87. (B) Survival analysis for cardiac events using best cut-off value. The $FSTL1_{CS-Ao} > -4.09$ ng/mL group had a low event-free survival rate compared to the $FSTL1_{CS-Ao} \leq -4.09$ ng/mL group.
AUC, area under the curve; FSTL1, follistatin-like 1; CS, coronary sinus; Ao, aortic root; $FSTL1_{CS-Ao}$, transcardiac gradient of FSTL1 levels as calculated by the difference between CS and Ao.

transcardiac gradient of FSTL1 and poor prognosis. In DCM patients with mild symptoms, the transcardiac gradient of FSTL1 might be predictive for cardiac events, prior to the FSTL1 level of PV and BNP. The dysregulation of cardioprotective cardiokine FSTL1 might lead to poor cardiac prognosis. It has been reported that expression levels of FSTL1 mRNA were increased in patients with end-stage heart failure and FSTL1 mRNA expression returned to normal in cases of myocardial recovery from heart failure after LVAD implantation. Furthermore, the expression levels of FSTL1 mRNA at the time of LVAD implantation significantly correlated with LVEF after treatment [19]. The hypothesized mechanisms of normalization of FSTL1 mRNA expression in the myocardium after LVAD implantation are described as follows: After LVAD implantation, cardiac stress reflecting LV end-diastolic pressure is decreased due to LV unloading. This results in FSTL1 secretion exceeding the consumption and cardioprotective effects might be manifested. Thus, we hypothesized that FSTL1 mRNA expression in the myocardium was increased in the High $FSTL1_{CS-Ao}$ group, which showed a positive transcardiac gradient of FSTL1. However, the results showed that the FSTL1 mRNA expression in the myocardium was significantly higher in the Low $FSTL1_{CS-Ao}$ group than in the High $FSTL1_{CS-Ao}$ group. A possible explanation may be that in the Low $FSTL1_{CS-Ao}$ group, although the expression of FSTL1 was accelerated, the consumption of FSTL1 was further increased. Thus, the

transcardiac gradient of FSTL1 became negative. Conversely, in the High $FSTL1_{CS-Ao}$ group, although the FSTL1 expression was low, FSTL1 consumption was lower, resulting in a positive transcardiac gradient of FSTL1. However, reverse translational research is necessary to clarify this hypothesis. If it could be demonstrated that the relative deficiency of FSTL1 in the myocardium worsens the prognosis, its supplementation may be a new therapeutic strategy.

Limitations

Several limitations of this study are noteworthy. First, this was a small-scale, single-center, study and limited to Japanese patients. In addition, our study could not show an association between known risk factors for heart failure, such as BNP, troponin T, atrial fibrillation, and hemoglobin, and prognosis. One of the possible causes was that most patients had mild heart failure with NYHA functional class I or II. However, to our knowledge, for the first time in the literature, we reported the cardiodynamics of the cardiokine– FSTL1–which has a positive role in cardioprotection, and showed its association with prognosis at an earlier DCM stage. We believe these results make a relevant contribution to existing knowledge in elucidating the cardiac metabolism of FSTL1. A larger multicenter study is needed to support the results of this study. Second, the methods to measure FSTL1 levels in the Ao and CS need invasive techniques. To apply these findings in clinical settings, it is desirable to identify new surrogate markers using non-invasive modalities that reflect the FSLT1 dynamics in the heart. Third, the study did not provide a sufficient explanation regarding how FSTL1 affected the prognosis of DCM. In addition, we have not directly proved the hypothesis that FSTL1 deposition or consumption is increased. Therefore, a reverse translational research–from bedside to bench–is required to prove the hypothesis. Fourth, we did not measure biomarkers including serum FSTL1 levels of PV at another point in this study. By measuring these biomarkers at two or more points, it may have been possible to show the mechanism by which FSTL1 affects prognosis.

Conclusions

In conclusion, approximately 50% of DCM patients had a negative transcardiac gradient of FSTL1. This may indicate the consumption, as well as secretion of FSTL1, in the myocardium. A negative transcardiac gradient of FSTL1 might be a poor prognostic predictor in DCM patients with impaired hemodynamics.

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Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jjcc.2021.07.005.

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