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ORIGINAL ARTICLE
Heart Failure

Prognostic Impact of Myocardial Interstitial Fibrosis in Non-Ischemic Heart Failure

– Comparison Between Preserved and Reduced Ejection Fraction Heart Failure –

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Background: Although myocardial fibrosis plays an important role in the progression of heart failure (HF), its prognostic impact still remains to be clarified.

Methods and Results: A total of 172 consecutive patients with chronic HF, who underwent cardiac catheterization and endomyocardial biopsy between January 2001 and September 2008, were examined. They were divided into 2 groups: HF with preserved ejection fraction (HFPEF; left ventricular ejection fraction [LVEF] ≥50%, n=81); and HF with reduced LVEF (HFREF; LVEF <50%, n=91). The collagen volume fraction (CVF) in biopsy samples was calculated and its prognostic impact examined. Mean follow-up in the HFPEF and the HFREF groups was 41±33 months and 41±26 months, respectively. Although CVF was similar between the 2 groups (1.83±1.54% vs. 2.07±2.35%), CVF was significantly correlated with LV end-diastolic pressure in the HFREF group but not in the HFPEF group. When HF stage was adjusted, the long-term prognosis was comparable between the 2 groups. When the patients were divided into 2 groups according to median CVF, however, severe fibrosis was a significant predictor for all-cause death (P=0.014) and cardiac events (P=0.02) in the HFREF, but not in the HFPEF group.

Conclusions: Myocardial fibrosis evaluated on biopsy samples is a useful indicator for long-term survival, suggesting that it may be an important therapeutic target as well. (*Circ J* 2011; **75:** 2605–2613)

Key Words: Collagen volume fraction; Ejection fraction; Fibrosis; Heart failure; Prognosis

Myocardial extracellular matrix (ECM) plays an important role in maintaining the structure of myocytes and blood vessels to strengthen myocardial tissue.[1,2] Myocardial collagen is the major constituent of ECM, and myocardial collagen volume is an important determinant of ventricular remodeling that affects ventricular functions.[3] It has previously been demonstrated that myocardial collagen content is correlated with left ventricular (LV) stiffness in patients with heart failure (HF),[4,5] and that the extent of myocardial collagen is correlated with a reduction in LV ejection fraction (LVEF) and is involved in the process of LV dilatation and progression of HF.[6,7] Furthermore, the presence of excessive collagen fibers may induce fatal ventricular arrhythmia.[8] Thus, it is important to estimate the extent of myocardial interstitial fibrosis in order to determine prognosis in HF patients.

Cardiovascular magnetic resonance imaging (MRI) is a useful tool to evaluate myocardial fibrosis that can be used to estimate the prognosis of HF patients by evaluation of LV midwall fibrosis using late gadolinium enhancement.[9] Indeed, MRI can detect and quantify regional myocardial fibrosis in a ventricle but not diffuse myocardial fibrosis.[10] Although serum levels of collagen synthesis markers (eg, procollagen type Ⅲ amino-terminal peptide, PIIINP) may be useful to estimate the prognosis of HF patients,[11–13] those markers may reflect systemic fibrosis.[14,15] Indeed, little is known about the relationship between the prognosis of HF patients and the extent of myocardial fibrosis calculated directly from biopsy specimens in HF patients. In the present study, we thus examined whether collagen volume fraction (CVF) obtained from LV endomyocardial biopsy samples has a prognostic impact in HF patients with or without systolic dysfunction.

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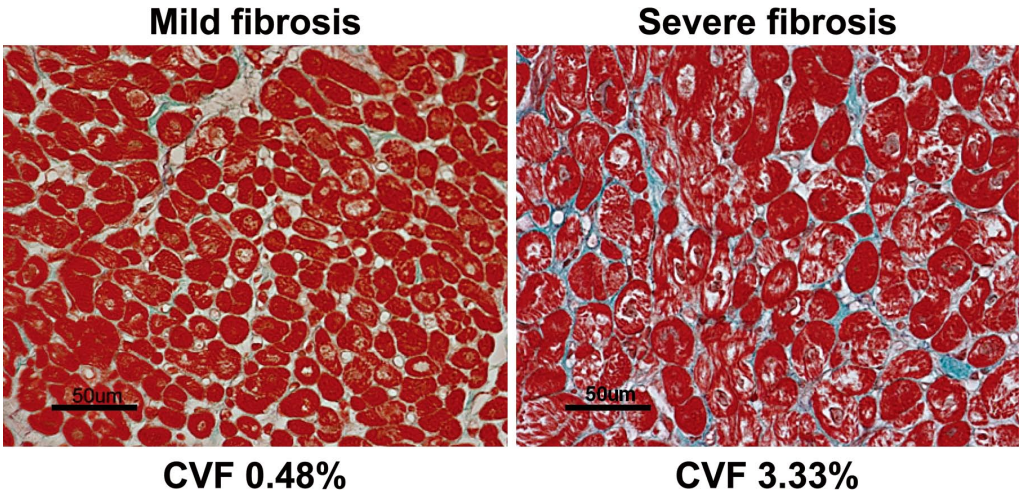

Figure 1. Representative histology of the (**Left**) mild and (**Right**) severe fibrosis groups. Myocardial fibrosis, stained in blue by Elastica–Masson staining. CVF, collagen volume fraction. Scale bar, 50 μm.

Methods

The ethics committees of Tohoku University Hospital approved the study protocol and all patients provided written informed consent.

Subjects

We examined 172 consecutive patients with chronic HF enrolled in the Tohoku University Hospital database, and who underwent cardiac catheterization and endomyocardial biopsy to determine the etiology of HF between January 2001 and September 2008. We performed endomyocardial biopsy in all HF patients with suspected cardiomyopathy but we did not perform the procedure in those who had apparent ischemic or valvular heart disease documented on echocardiography and/or cardiac catheterization.

For each patient, we collected clinical, hemodynamic, biochemistry and prognostic data and analyzed endomyocardial biopsy samples.

Definition of HF

In the present study, we included patients in stage B, C and D, according to the chronic HF ACC/AHA 2005 guidelines. According to the ESC 2007 HF guideline, we also divided them into 2 groups: HF with preserved ejection fraction (HFPEF; LVEF ≥50%, n=81) and HF with reduced LVEF (HFREF; LVEF <50%, n=91).

Data Collection

Baseline demographic data, hemodynamic data obtained via catheterization, stage of HF, medications and comorbidities (hypertension, diabetes mellitus, hyperlipidemia, and atrial fibrillation) were obtained based on medical records. The hemodynamic parameters measured via cardiac catheterization included LVEF, LV end-diastolic volume index (LVEDVI), mean aortic pressure, LV end-diastolic pressure (LVEDP), mean pulmonary artery pressure, pulmonary capillary wedge pressure (PCWP) and cardiac index. Before cardiac catheterization, we measured serum levels of hemoglobin, brain natriuretic peptide (BNP), creatinine and high-sensitivity C-reactive protein and estimated creatinine clearance using the Cockroft-Gault formula.

The primary endpoints included all-cause death, and the secondary combined endpoints included cardiovascular death, sudden death and admission for worsening of HF. Follow-up data were obtained from the database.

Quantitative Morphometry of Biopsy Samples

Trans-venous endomyocardial biopsy samples were obtained from the interventricular septum using 6-Fr Biotom (Cordis, Bridgewater, NJ, USA). There were no major complications related to the procedures during the study period. The tissues were immediately fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were stained with hematoxylin–eosin and Elastica–Masson. Images of these sections were acquired with a projection microscope (×400; **Figure 1**). Subsequent image analysis was performed using Macscope 2.5 (Mitani, Fukui, Japan) to determine cardiomyocyte diameter and extent of myocardial interstitial fibrosis, which was expressed as CVF (%). CVF was calculated as the sum of all connective tissue areas divided by the sum of all connective tissue and muscle areas averaged over 2–5 representative fields of the section (mean, 3.6±0.9 fields), where there was no endocardium or blood vessel.[16,17] Myocardial diameter was determined at the nucleus level in 8–15 representative cardiomyocytes (mean, 12.0±2.5 fields) per section, where we also counted the number of inflammatory mononuclear cells in the same fields (mean, 6.0±1.8). This histological evaluation was performed by a well-trained cardiologist without knowledge of which patient provided the tissue sections.

We divided both the HFPEF and HFREF groups into 2 groups using median CVF (HFPEF and HFREF, 1.36% and 1.34%, respectively). We defined mild and severe fibrosis as CVF smaller and greater than the median, respectively (**Figure 1**).

Statistical Analysis

Continuous variables are expressed as mean±SD. Comparisons between 2 groups were conducted using unpaired t-test for continuous variables and chi-squared test for categorical

Table 1. Baseline Subject Characteristics

	HFPEF (n=81)	HFREF (n=91)	P value
Age (years)	54.3±14.1	55.9±12.8	0.429
BMI (kg/m²)	23.4±4.5	23.9±4.2	0.408
Male	54 (67)	66 (73)	0.404
Hypertension	34 (42)	39 (43)	0.962
Diabetes mellitus	9 (11)	13 (14)	0.533
Dyslipidemia	21 (26)	21 (23)	0.631
Sinus rhythm	69 (85)	65 (71)	0.028*
Medication			
ACEI	36 (44)	57 (63)	0.017*
ARB	15 (19)	35 (38)	0.004*
β-blocker	39 (48)	66 (73)	0.001*
Diuretics	13 (16)	41 (45)	<0.001*
Spironolactone	10 (12)	31 (34)	0.001*
Warfarin	12 (15)	46 (51)	<0.001*
Digitalis	7 (9)	28 (31)	<0.001*
CCB	26 (32)	11 (12)	0.001*
Antiplatelet	13 (16)	27 (30)	0.033*
Statin	7 (9)	17 (19)	0.054
Amiodarone	5 (6)	8 (9)	0.514
Stage of heart failure			<0.001*
B	40 (49)	15 (16)	
C	39 (48)	68 (75)	
D	2 (2)	8 (9)	
Laboratory data			
Hemoglobin (g/dl)	13.8±2.0	14.1±1.8	0.277
hsCRP (mg/dl)	0.21±0.46	0.33±0.93	0.294
BNP (pg/ml)	248±342	367±491	0.089
LDL (mg/dl)	110±41	121±39	0.103
HDL (mg/dl)	54.9±24.0	45.1±12.8	0.001*
TG (mg/dl)	131±98	137±75	0.669
Glucose (mg/dl)	106±37	106±21	0.934
CCr (ml/min)	90.1±25.1	88.2±35.4	0.694
Hemodynamic data			
LVEDVI (ml/m²)	75.7±20.4	114.5±35.1	<0.001*
EF (%)	67.8±11.3	35.6±11.0	<0.001*
mAoP (mmHg)	96±15	90±17	0.023*
LVEDP (mmHg)	14±6	13±7	0.420
mPAP (mmHg)	16.7±4.8	19.9±7.7	0.002*
PCWP (mmHg)	9.5±4.2	11.2±6.5	0.050
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.9±0.7	2.6±0.7	0.021*
Morphometric data			
CVF (%)	1.83±1.54	2.07±2.35	0.440
MyD (μm)	19.2±3.2	19.7±2.8	0.362
Inflammatory cell (/field)	4.9±4.9	7.0±6.0	0.015*
All-cause death	0 (0)	9 (10)	0.004*
Cardiac events	4 (5)	15 (16)	0.016*
Cardiac or sudden death	0 (0)	4 (4)	
Admission for HF	4 (5)	11 (12)	

Data given as mean±SD or n (%). *P<0.05, HFPEF vs. HFREF.
HFPEF, heart failure patients with preserved left ventricular ejection fraction; HFREF, heart failure patients with reduced left ventricular ejection fraction; BMI, body mass index; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium cannel blocker; hsCRP, high-sensitivity C-reactive protein; BNP, brain natriuretic peptide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CCr, creatinine clearance; LVEDVI, left ventricular end-diastolic volume index; EF, ejection fraction; mAoP, mean aortic pressure; LVEDP, left ventricular end-diastolic pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CVF, collagen volume fraction; MyD, cardiomyocyte diameter; HF, heart failure.

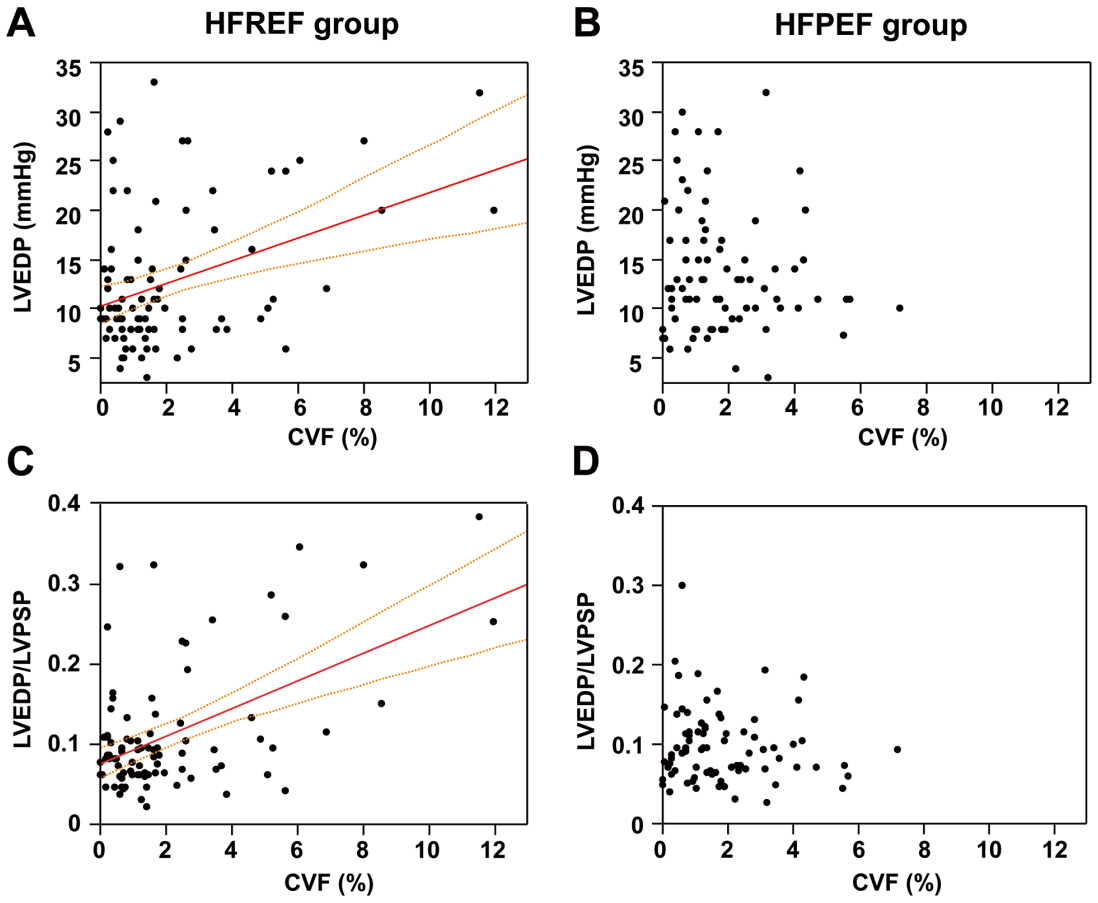

Figure 2. Correlation between left ventricular end-diastolic pressure (LVEDP) and collagen volume fraction (CVF). (**A**) A significant correlation was noted between CVF and LVEDP for patients with heart failure (HF) with reduced left ventricular ejection fraction (HFREF; r=0.387, P<0.001) but not for those with (**B**) HF with preserved left ventricular ejection fraction (HFPEF; r=–0.092, P=0.664). (**C**) LVEDP/left ventricular peak systolic pressure (LVEDP/LVPSP) ratio and CVF were also significantly correlated in the HFREF group (r=0.515, P<0.001), but not in the (**D**) HFPEF group (r=–0.097, P=0.393).

variables. For echocardiographic comparison before and after medical treatment, paired t-test was used. Five-year survival free from all-cause death and that from cardiac events was estimated using the Kaplan-Meier method. We used Cox proportional hazards model to adjust covariates. After comparison of covariates between the mild and severe fibrosis groups, the covariates with P<0.05 were used in the final multivariate models. Furthermore, we evaluated the prognostic value of CVF as a continuous variable. We used the variables with P<0.05 on univariate analysis in the final multivariate models, in which age, cardiac index, LV filling pressure, and stage of HF were controlled for, and we chose the parameters for final models using the step-up method. In these analyses, we used PCWP as a parameter of LV filling pressure, because LVEDP data were lacking in 3 cases. Furthermore, as previously reported,[18] we tested the proportionality assumptions of each parameter of the final models, with P<0.05 indicating non-proportionality. All statistical analysis was performed using JMP 7.0.2 (SAS Institute, Cary, NC, USA) and R 2.8.1(www.r-project.org/). All P-values were 2-sided, and P<0.05 was considered to be statistically significant.

Results

HFPEF Group vs. HFREF Group

All patients were successfully followed up in the present study. Mean follow-up period in the HFPEF and the HFREF groups was 41±33 months and 41±26 months, respectively. The HFREF group was characterized by more advanced stage of HF (Table 1). There were more all-cause deaths and cardiac events in the HFREF group than in the HFPEF group (Table 1). Five-year prognosis was significantly lower in the HFREF group than in the HFPEF group, in terms of survival from all-cause death (P=0.006) and survival from cardiac events (P=0.034). After the adjustment of HF stage of HF, however, there was no significant difference in cardiac events between the 2 groups.

The prevalence of the use of medications for HF at cardiac catheterization, including angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), β-blockers, diuretics, spironolactone and digitalis, was significantly higher in the HFREF than in the HFPEF group (Table 1). In contrast, the use of calcium cannel blockers (CCB) was more common in the HFPEF group (Table 1). The HFREF group had significantly larger LV volume, lower LVEF and lower cardiac index compared with the HFPEF group

Table 2. Subject Characteristics vs. Level of Fibrosis

	HFPEF			HFREF		
	Mild fibrosis (n=40)	Severe fibrosis (n=41)	P value	Mild fibrosis (n=46)	Severe fibrosis (n=45)	P value
Age (years)	57±11	52±16	0.082	58±12	54±14	0.21
BMI (kg/m²)	23±5	23±4	0.96	24±4	24±4	0.372
Male	29 (73)	25 (63)	0.238	34 (74)	32 (70)	0.949
Hypertension	13 (33)	21 (53)	0.164	21 (46)	18 (39)	0.739
Diabetes mellitus	5 (13)	4 (10)	0.906	5 (11)	8 (17)	0.521
Dyslipidemia	10 (25)	11 (28)	0.894	12 (26)	9 (20)	0.66
Sinus rhythm	31 (78)	38 (93)	0.281	31 (67)	34 (76)	0.389
Medication						
ACEI	17 (43)	19 (48)	0.941	28 (61)	29 (63)	0.892
ARB	7 (18)	8 (20)	0.874	18 (39)	17 (37)	0.934
β-blocker	19 (48)	20 (50)	0.902	32 (70)	34 (74)	0.685
Diuretics	7 (18)	6 (15)	0.884	17 (37)	24 (52)	0.174
Spironolactone	7 (18)	3 (8)	0.255	16 (35)	15 (33)	0.94
Warfarin	5 (13)	7 (18)	0.862	20 (43)	26 (57)	0.248
Digitalis	5 (13)	2 (5)	0.371	17 (37)	11 (24)	0.287
CCB	14 (35)	12 (30)	0.64	6 (13)	5 (11)	0.969
Antiplatelet	6 (15)	7 (18)	0.884	14 (30)	13 (28)	0.946
Statin	2 (5)	5 (13)	0.491	8 (17)	9 (20)	0.96
Amiodarone	5 (13)	0 (0)	0.053	3 (7)	5 (11)	0.687
Stage of heart failure			0.236			0.577
B	23 (58)	17 (43)		9 (20)	6 (13)	
C	16 (40)	23 (58)		34 (74)	34 (74)	
D	1 (3)	1 (3)		3 (7)	5 (11)	
Laboratory data						
Hemoglobin (g/dl)	14±2	14±2	0.357	14±2	14±2	0.674
hsCRP (mg/dl)	0.16±0.29	0.26±0.58	0.317	0.35±1.12	0.32±0.69	0.888
BNP (pg/ml)	255±377	243±314	0.892	245±347	494±584	0.019*
LDL (mg/dl)	108±38	113±44	0.635	116±38	125±40	0.239
HDL (mg/dl)	52±21	58±26	0.269	45±14	45±11	0.952
TG (mg/dl)	125±75	137±116	0.603	145±86	129±62	0.305
Glucose mg/dl)	112±43	100±29	0.134	104±19	109±23	0.315
CCr (ml/min)	90±25	90±26	0.898	89±30	87±40	0.804
Hemodynamic data						
LVEDVI (ml/m²)	76±18	75±22	0.874	107±28	122±40	0.040*
EF (%)	66±11	69±11	0.275	38±11	33±11	0.053
mAoP (mmHg)	97±16	96±15	0.775	92±15	88±19	0.189
LVEDP (mmHg)	14±7	13±6	0.236	11±6	14±8	0.06
mPAP (mmHg)	17±4	17±5	0.736	19±7	21±9	0.136
PCWP (mmHg)	10±4	9±4	0.926	10±5	12±8	0.161
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.9±0.7	2.9±0.7	0.932	2.7±0.7	2.6±0.6	0.367
Morphometric data						
CVF (%)	0.64±0.41	2.93±1.38	<0.001*	0.61±0.4	3.56±2.58	<0.001*
MyD (μm)	19±1.9	20±4.1	0.287	19±2	20±3	0.055
Inflammatory cell (/field)	4.7±4.6	5.1±5.2	0.696	8±6	6±6	0.116
All-cause death	0 (0)	0 (0)	–	1 (2)	8 (18)	0.013*
Cardiac events	3 (8)	1 (2)	0.293	3 (7)	12 (27)	0.001*
Cardiac or sudden death	0 (0)	0 (0)		1 (2)	3 (7)	
Admission for HF	3 (8)	1 (2)		2 (4)	9 (20)	

Data given as mean±SD or n (%). *P<0.05, mild fibrosis vs. severe fibrosis.
Abbreviations see in Table 1.

(Table 1). Although LVEDP and CVF were comparable between the 2 groups (Table 1), CVF was significantly correlated with LVEDP, and also with LV peak systolic pressure (LVPSP) after adjustment in the HFREF group (Figures 2A,C), but not in the HFPEF group (Figures 2B,C).

Morphometric Variables as Prognostic Indicators

When comparing the mild and the severe fibrosis groups, a statistically significant difference was noted in terms of LVEDVI and BNP in the HFREF group (Table 2), but not in the HFPEF group (Table 2).

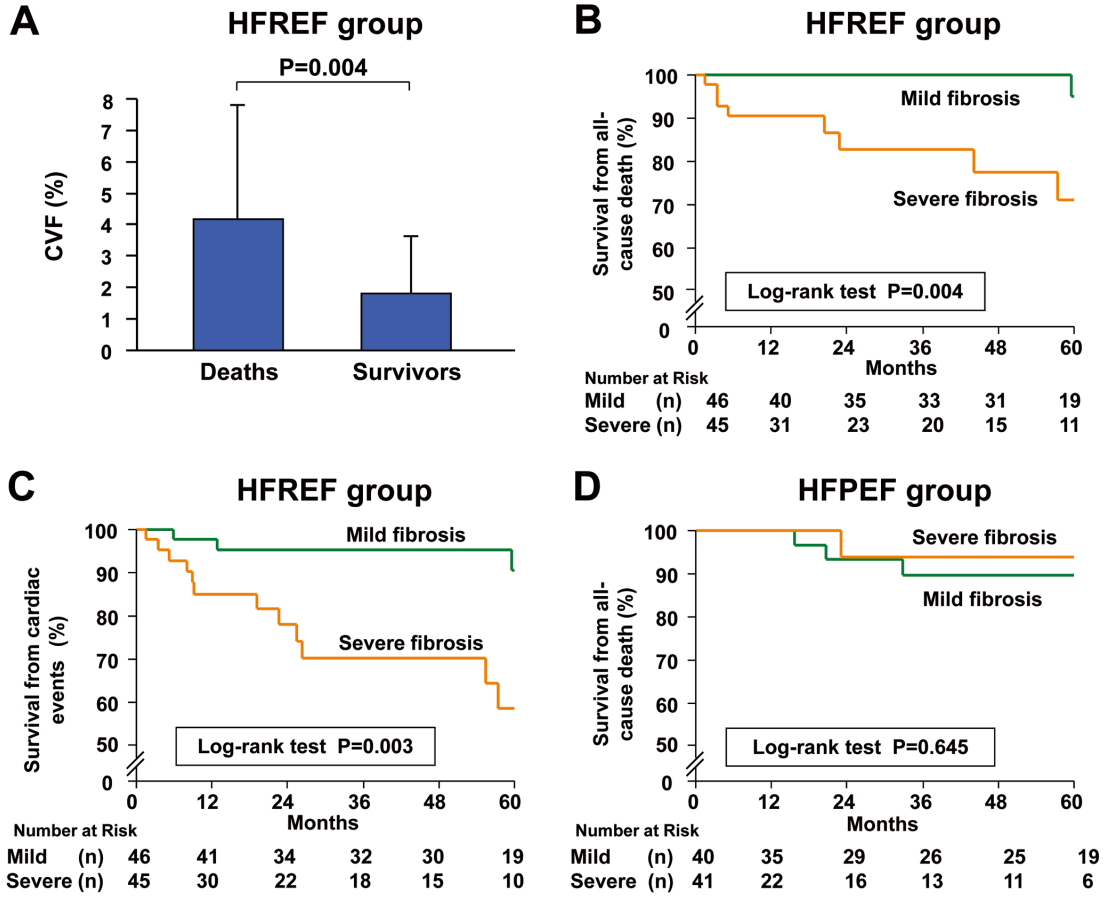

Figure 3. Comparison of survival between the mild and the severe fibrosis groups. (**A**) Collagen volume fraction (CVF) was significantly higher in heart failure (HF) patients who died than in survivors. (**B**,**C**) The mild fibrosis group had better survival from (**B**) all-cause death and (**C**) cardiac events compared with the severe fibrosis group. (**D**) In contrast, no difference was noted between the mild and severe fibrosis groups. HFPEF, heart failure patients with preserved left ventricular ejection fraction; HFREF, heart failure patients with reduced left ventricular ejection fraction.

In the HFREF group, CVF was significantly higher in HF patients who died than in survivors (Figure 3A). Indeed, there were more all-cause deaths and cardiac events in the severe fibrosis group than in the mild fibrosis group (Table 2). Five-year survival from all-cause death was significantly lower in the severe fibrosis group than in the mild fibrosis group (P=0.004; Figure 3B), and was so even after adjustment with the covariate (severe fibrosis vs. mild fibrosis; hazard ratio [HR], 13.5; 95% confidence interval [CI]: 2.01–307, P=0.006). Similarly, survival after cardiac events was significantly lower in the severe fibrosis group than in the mild fibrosis group in the HFREF subjects (P=0.003; Figure 3C), and was so even after adjustment with the covariate (severe fibrosis vs. mild fibrosis; HR, 6.20; 95%CI: 1.52–25.4, P=0.011). In contrast, in the HFPEF group, there was no significant difference in the cardiac events (Table 1) or survival rate (Figure 3D) between the mild and severe fibrosis groups. In the HFREF group, multivariate analysis showed that a 1% elevation of CVF increased the risk of all-cause death and that of cardiac events by 1.50-fold (95%CI: 1.18–1.95, P=0.002) and 1.28-fold (95%CI: 1.07–1.50, P=0.008), respectively (Figure 4). Furthermore, other histological parameters (eg, cardiomyocyte hypertrophy) were not significant predictors in the present study.

Discussion

The novel findings of the present study are as follows: (1) CVF was similar between the HFPEF and HFREF groups; (2) CVF was an independent predictor of all-cause death and cardiac events in the HFREF group but not in the HFPEF group; and (3) CVF was significantly correlated with LVEDP in the HFREF group but not in the HFPEF group. To the best of our knowledge, this is the first report to demonstrate the prognostic impact of CVF in non-ischemic HF patients with systolic dysfunction.

HFPEF Group vs. HFREF Group

Several studies have shown that the prognosis is comparable between patients with HFPEF and those with HFREF.[19–21] In the present study, the patients with HFPEF had a significantly better prognosis than those with HFREF, but after adjustment for stage of HF, the survival became similar between the 2 groups. In the present study, the 5-year survival rate from all-cause death was better than in the previous study,[22] probably because we followed up the patients monthly to control sodium intake and blood pressure. It has been reported that intensive medical treatment for HF patients with close fol-

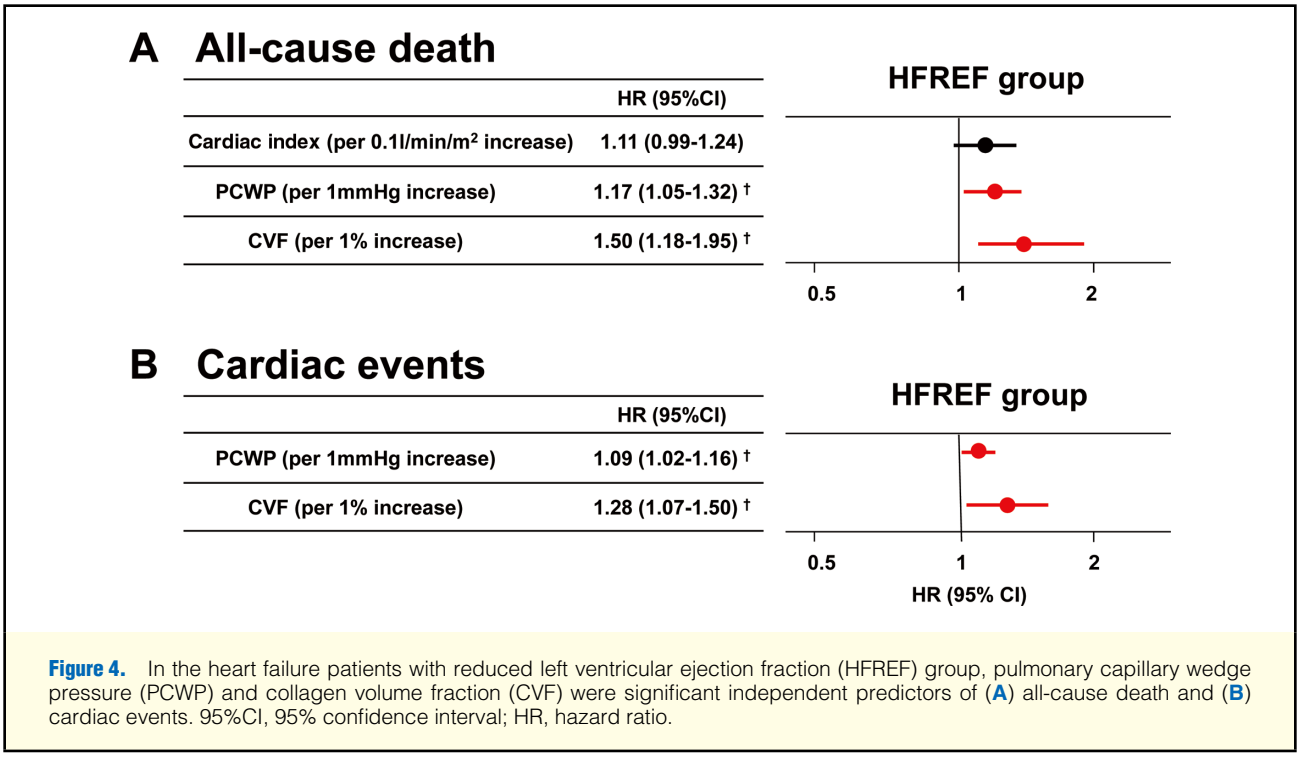

Figure 4. In the heart failure patients with reduced left ventricular ejection fraction (HFREF) group, pulmonary capillary wedge pressure (PCWP) and collagen volume fraction (CVF) were significant independent predictors of (**A**) all-cause death and (**B**) cardiac events. 95%CI, 95% confidence interval; HR, hazard ratio.

low-up can reduce re-admission for HF and cardiac deaths,[23] suggesting that the regular follow-up in the present study was effective to improve the prognosis of the HF patients.

Morphometric Variables and Cardiac Function as Prognostic Indicators

Myocardial fibrillar collagen, the main component of ECM, is a major contributor to myocardial stiffness.[3] In the present study, CVF in the HFPEF and the HFREF groups was 1.83% and 2.07%, respectively, consistent with the previous report.[24]

Recently, degradation of interstitial collagen has been reported in patients with mild to moderate dilated cardiomyopathy (DCM).[25,26] In contrast, marked accumulation of myocardial interstitial fibrosis has also been reported in patients with end-stage HFREF (eg, explanted heart).[27] The present study also demonstrated that CVF was significantly higher in HF patients who died than in survivors and that CVF and LVEDP were significantly correlated in HFREF patients. Taken together, these results suggest that reduction of myocardial interstitial collagen causes LV dilatation complicated with systolic dysfunction in the early stage of HFREF and that the increased myocardial interstitial collagen causes diastolic dysfunction in the advanced stage of HFREF with the resultant poor prognosis.

Although cardiac MRI is well established as a method for evaluating cardiac fibrosis, it cannot detect all cases of severe fibrosis, especially in HFREF patients with non-ischemic etiology.[28] It has also been reported that diffuse cardiac fibrosis is not able to be detected on cardiac MRI.[29] Furthermore, a recent study has shown that late gadolinium enhancement does not always indicate the change in myocardial interstitium.[30] Our preliminary data showed that there was no significant difference in CVF between the patients with and those without delayed enhancement on cardiac MRI (unpublished observation). Thus, we consider that the extent of myocardial fibrosis should be evaluated in multiple ways, including on endomyocardial biopsy, MRI and via serum markers of collagen turnover.

It has been reported that HFREF patients with diastolic dysfunction had a worse prognosis than those without it.[31,32] In the present study, elevated LVEDP was significantly related to increased CVF. Therefore, accumulation of myocardial interstitial fibrillar collagen may have caused ventricular diastolic dysfunction in the HFREF group with a resultant poor prognosis. The Randomized Aldactone Evaluation Study (RALES) showed that spironolactone improves prognosis in HF patients.[33] Interestingly, the RALES subanalysis showed that this benefit of spironolactone is noted only in patients with a high level of collagen synthesis marker (PIIINP) but not in those with low PIIINP.[11,33] It has also been shown that spironolactone reduced LV diastolic dysfunction only in DCM patients with increased myocardial fibrosis.[34]

In contrast, HFPEF seems to be a very different condition from HFREF in terms of response to medical treatment. Although ARB and ACEI could decrease myocardial fibrosis in HFPEF,[4,35,36] large clinical trials failed to demonstrate any beneficial effects of ARB or ACEI (eg, irbesartan, candesartan, enalapril, and valsartan) in patients with HFPEF.[37–40] This is consistent with the present finding that no significant correlation was noted between myocardial fibrosis and cardiac events in the HFPEF group, suggesting that the prognostic impact of myocardial fibrosis might be small in HFPEF. It has been previously reported, however, that in approximately 20% of patients with HFPEF, LVEF was significantly decreased during the 3-month follow-up period,[41] which is consistent with the present study, in which LVEF was significantly decreased in 11% of patients with HFPEF during follow-up. Thus, patients with severe myocardial fibrosis should be closely followed up because HFPEF patients with large CVF are at higher risk for disease progression and poor prognosis.

CVF and LVEDP

In the present study a significant but relatively weak correlation was noted between CVF and LVEDP in the HFREF group, probably because 60–70% of the HFREF patients received β-blockers and ACEI and/or ARB, which might have affected the systemic hemodynamics measured during cardiac catheterization.

As mentioned here, we were unable to observe any significant correlation between CVF and LVEDP in the HFPEF group, probably because the HFPEF is associated with heterogeneous diseases, such as hypertensive heart disease, cardiac amyloidosis, early-stage DCM and hypertrophic cardiomyopathy, and could also have been affected by the medical treatment including β-blocker and ACEI and/or ARB.

Study Limitations

Several limitations should be mentioned for the present study. First, we assessed only the collagen content in the myocardium. It was previously reported that not only the quantity but also the quality of collagen are important determinants for myocardial stiffness.[42] Indeed, the ratio of cross-linked collagen (insoluble collagen) to non-cross-linked collagen (soluble collagen) and the type I/type III collagen ratio are important determinants of myocardial stiffness,[17,27,43] and reduction in collagen cross-linking ameliorates myocardial stiffness and ventricular dilatation irrespective of collagen content.[44] Although we did not measure collagen turnover markers that have been established as prognostic in HF patients, it has been reported that there is a significant correlation between CVF and procollagen I carboxy-terminal peptide (PICP), a collagen synthesis marker.[45] Thus, the quality of ventricular fibrosis should be evaluated in biopsy specimens in future studies.

Second, because myocardial fibrosis may exist in a patchy fashion, we obtained at least 3 endomyocardial biopsy samples in each patient and evaluated CVF in as many fields as possible (mean, 3.6±0.9 fields) in order to minimize errors from patchy distribution of myocardial fibrosis in the present study. We still consider that we should evaluate the extent of myocardial fibrosis in multiple ways, including on endomyocardial biopsy, MRI and via serum markers of collagen turnover.

Third, in the present study, the HF subject group might be biased because we included patients who underwent endomyocardial biopsy alone and excluded those with other major causes of HF, such as ischemic heart disease and valvular heart disease. But because we did not include HF patients with valvular or ischemic etiology, we were able to minimize the overestimation of LVEF due to those factors in the present study.

Fourth, the present study was an observational study with a relatively small number of patients, and for reasons of ethics we were unable to perform repetitive myocardial biopsy to evaluate the time-course of HF. Thus, a future study with a large number of patients with a longer follow-up is required to address this issue.

Finally, the relatively small number of events limits the generalization of the present findings. Although we analyzed the present results with several statistical models, we found that the Cox proportional hazard model was the best. Thus, after univariate analysis, we used the Cox proportional hazard model with as small covariates as possible.

In conclusion, we have demonstrated that myocardial CVF evaluated with biopsy samples is a useful predictor for long-term survival in patients with HFREF (but not in those with HFPEF), and may be an important therapeutic target as well.

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Disclosures

None.

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