Histopathological Study of Specimens Obtained by Left Ventricular Biopsy During Ventriculoplasty for Idiopathic Dilated Cardiomyopathy

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Objective: Pathological changes in the myocardium in idiopathic dilated cardiomyopathy (DCM) are usually studied using endomyocardial biopsy specimens, but the relationship between pathological changes in the myocardium and clinical findings is unclear. The goal of the study was to examine correlations between clinical findings and histopathological findings in specimens of the left ventricular myocardium collected during left ventriculoplasty in DCM patients.
Methods: The subjects were 20 DCM patients (17 males and 3 females; mean age: 59 ± 14 years old) who underwent left ventriculoplasty, including 16 cases of overlapping ventriculoplasty (OLVP) and 4 of papillary muscle approximation (PMA) with left ventricular incision. Preoperative age, sex, The New York Heart Association (NYHA) classification, the brain natriuretic peptide (BNP) level, cardiothoracic ratio (CTR), echocardiographic data, history of diabetes mellitus, drug history of spironolactone, ACE inhibitor, ARB, and β-blocker were used as clinical findings. Histopathological scores were determined for each patient and semi-quantitative data for hypertrophy, attenuation, vacuolation and fibrosis were obtained.
Results: A significant correlation was found between age and interstitial fibrosis. A significant inverse correlation was found between left ventricular diastolic diameter (LVDd) in echocardiographic data and interstitial fibrosis. There were no other significant relation between histopathological scores and clinical findings. Conclusion: From this study, we found that interstitial fibrous increased with aging and more dilated LVDd had less interstitial fibrosis. It is concluded that the kinetics of myocardial fibrosis with remodeling might be variable and histopathological findings does not reflect the clinical and hemodynamic changes in DCM patients. Further morphological data are needed to verify this result.

Key words: dilated cardiomyopathy (DCM), transmural specimen of the left ventricular myocardium, overlapping ventriculoplasty (OLVP), papillary muscles approximation (PMA), histopathological score

INTRODUCTION

The relationship between histopathological findings in idiopathic dilated cardiomyopathy (DCM) and cardiac function has been studied in specimens obtained by endomyocardial biopsy, but there are few studies of transmural myocardial specimens due to the difficulty of this biopsy. A specimen obtained by endomyocardial biopsy is only a small portion of the whole myocardium, and this makes it difficult to evaluate cardiac function with certainty and to determine the appropriate therapeutic strategy based on histopathological results. During left ventriculoplasty, a transmural specimen of the left ventricular anterior myocardium can be collected in the region of the left ventricular incision and the whole myocardium can be evaluated histopathologically with high reproducibility, unlike in endomyocardial biopsy. This approach may allow clarification of the relationship between pathological changes in the myocardium and clinical findings, which is a contentious issue. Therefore, in the current study, specimens of the left ventricular myocardium of DCM patients were collected during left ventriculoplasty and correlations between histopathological findings and clinical findings including cardiac hemodynamic parameters were examined.

METHODS

Patient Population

The subjects were 20 DCM patients who underwent left ventriculoplasty between September, 2001 and September, 2006. The procedures comprised 16 cases of overlapping ventriculoplasty (OLVP) and 4 of lone papillary muscle approximation (PMA) with left ventricular incision. Diagnosis of DCM was made based on criteria established by the study group for

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Table 1 Clinical characteristics of the patients

patient characteristics		echocardiographic data	
N	20	EF %	28±9
Male	17 (85%)	FS %	13±5
Age	59±14	LVDd mm	74±7
NYHA class		LVDs mm	67±8
II	1 (5%)	IVST mm	9±2
III	7 (35%)	LVPWT mm	9±2
IV	12 (60%)		
CTR %	61±7		
BNP pg/mL	1139±890		
Diabetes mellitus	7 (35%)		
Dialysis	2 (10%)		
spironolactone	11 (55%)		
ACE-I/ARB	13 (65%)		
Beta blocker	10 (50%)		
Cardiac death	4 (20%)		

NYHA class; New York Heart Association functional classification, CTR; cardiothoracic ratio, BNP; brain natriuretic peptide, ACE-I; angiotensin-converting enzyme inhibitor, ARB; angiotensin receptor antagonist, EF; ejection fraction, FS; fractional shortening, LVDd; left ventricular end-diastolic diameter, LVDs; left ventricular end-systolic diameter, IVST; interventricular septal thickness, LVPWT; left ventricular posterior wall thickness.

idiopathic cardiomyopathy, which is a target disease in the Intractable Disease Research Program of the Ministry of Health, Labour and Welfare [1]. Idiopathic cardiomyopathy associated with myocardial stunning and dilated left ventricular lumen, ischemic, valvular, hypertensive, inflammatory or metabolic myocardial disease were excluded. Consequently, all patients in whom a significant stenosis was found in preoperative coronary angiography were excluded. Informed consent was obtained from each patient or a responsible relative.

Clinical characteristics of the patients

Table 1 shows the clinical characteristics of the patients. We studied 17 males and 3 females; mean age: 59 ± 14 years old. The following clinical findings were determined in the 20 subjects.: NYHA functional classification (class II in 1, class III in 7 and class IV in 12), the level of brain natriuretic peptide (BNP: 1139 ± 890 pg/mL) in a blood test, cardiothoracic ratio (CTR: 61 ± 7%), the history of diabetes mellitus (DM: N = 7), the use of spironolactone (N = 11), the use of angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists (ACE-I/ARB: N = 13), Beta blocker (N = 10), and echocardiographic data as follows; ejection fraction (EF: 28 ± 9%), left ventricular end-diastolic diameter (LVDd: 74 ± 7 mm), left ventricular end-systolic diameter (LVDs: 67 ± 8 mm), interventricular septal thickness (IVST: 9 ± 2 mm), left ventricular posterior wall thickness (LVPWT: 9 ± 2 mm). These clinical findings were evaluated immediately before surgery. Four patients died of impaired cardiac function in the follow-up period. The causes of death were 3 congestive heart failure (1 year after operation in all), and 1 uncontrollable ventricular tachycardia (6 months after operation).

Echocardiography

Two-dimensional (2D), M-mode and the apical 4-chamber view transthoracic echocardiography were obtained before surgery. Wall thickness and LV diameter were derived from M-mode echocardiograms. Ejection fraction (EF) recordings were performed in the 2-D and the apical 4-chamber view by modified Simpson method and took the average of both data.

Biopsy and Semi-quantitative morphometry

A myocardial specimen was collected from all subjects at the incision site of the left ventricle (approximately 10 cm from the cardiac apex of the left ventricular anterior wall to the diagonal branch and the anterior descending artery) (Fig. 1). The specimen was fixed in formalin and sectioned, and the tissue sections were subjected to HE, Masson's Trichrome, PTAH and Azan Mallory staining. The endocardium and epicardium were removed from the left ventricular myocardium and the remaining tissue was equally divided into three layers: the outer layer, stratum compactum and subendocardial layer. Five points were selected randomly from each layer and the tissue was evaluated semi-quantitatively using a 100-power microscope. The four major histopathological findings of DCM (Fig. 2−a, b, c, d), hypertrophy, attenuation, vacuolation and fibrosis were evaluated on a 4-point scale (0 to 3+). This evaluation was performed by a cardiac pathologist who was blinded to the clinical data. Each layer was graded and the scores for the three layers were summed to give the histopathological score (Tables 2−a, b).

Statistical analysis

Spearman rank correlation coefficients were calculated to test the association between selected histopathological variables (hypertrophy, attenuation, vacuolation and fibrosis) and clinical findings (age, NYHA classification, BNP, CTR, echocardiographic data) were analyzed by Spearman's test. Differences of histopathological scores between two groups (male or female, with or without the history of DM, the use of spironolactone, ACE-I or ARB, Beta blocker and

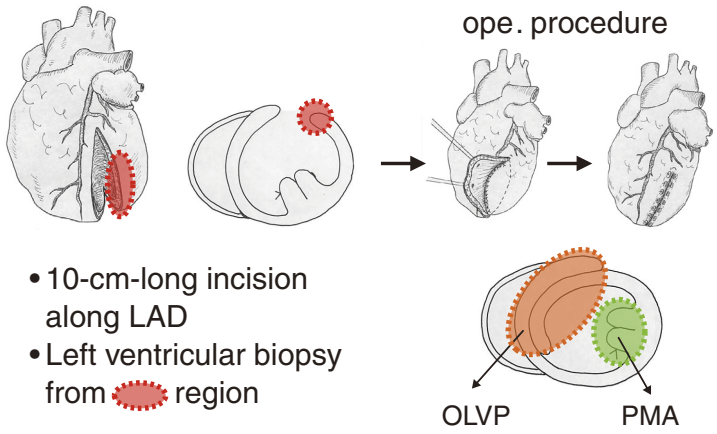

Fig. 1 Left Ventricular Biopsy during Ventriculoplasty
A myocardial specimen was collected from all subjects at the incision site of the left ventricle (approximately 10 cm from the cardiac apex of the left ventricular **anterior** wall to the diagonal branch and the anterior descending artery).

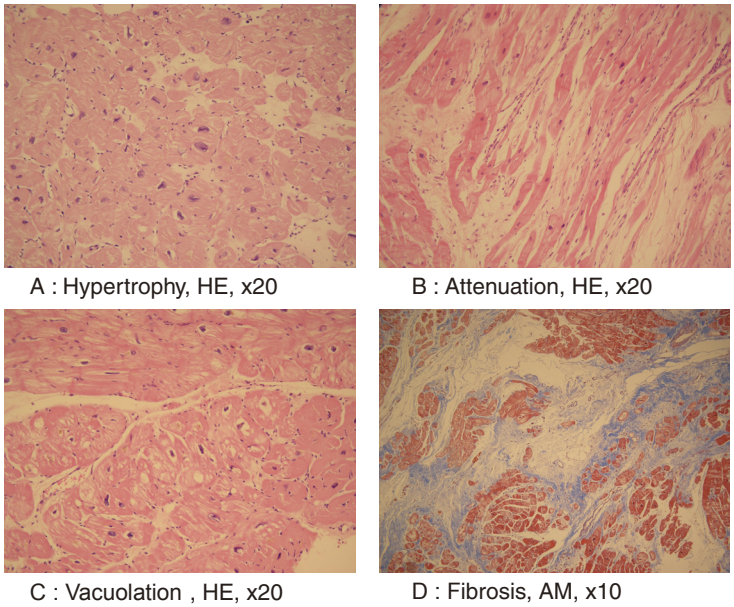

Fig. 2 Histopathological findings of DCM
A: Myocardial hypertrophy
Myocardium showing increased length of cytoplasmas with hyperchromatic and bizarre nuclei.
B: Myocardial attenuation
Myocardium showing slender cytoplasmas, abnormally branching, and disappearances. Interstitial fibrosis also is found.
C: Myocardial vacuolation
Myocardium containing vacuolations in their cytoplasmas.
D: Myocardial fibrosis
Mallory Azan stain showing fibrosis around myocardium as a blue color.

Table 2–a Histopathological Scores

	hypertrophy				attenuation			
case	out.	com.	subend.	totall	out.	com.	subend.	totall
A	++	++	+++	7	+	+	+	3
B	++	++	++	6	+	+	+	3
C	+	++	+	4	++	++	+	5
D	++	++	+	5	+	+	+	3
E	+++	++	+	6	++	+	+	4
F	+	+	+	3	+	+	+	3
G	++	++	++	6	+	+	-	2
H	++	++	++	6	+	+	+	3
I	+	++	++	5	+	+	+	3
J	++	++	++	6	+	+	+	3
K	++	+	+	4	++	+	+	4
L	+	+	+	3	+	+	+	3
M	++	++	++	6	+	+	+	3
N	+++	++	+	6	-	+	-	1
O	++	++	++	6	+++	+	+	5
P	+++	++	+	6	++	+	+	4
Q	++	+++	++	7	++	+	+	4
R	++	++	+	5	+	++	+	4
S	+	-	+	2	++	+	+	4
T	++	++	+	5	-	++	+	3

out; the outer layer, com; stratum compactum,
subend; subendocardial layer.

Table 2-b Histopathological Scores

	vacuolation				fibrosis			
case	out.	com.	subend.	totall	out.	com.	subend.	totall
A	++	++	++	6	+	+++	++	6
B	+	+	++	4	-	++	+	3
C	+	+	-	2	++	+++	++	7
D	+++	+++	++	8	+	+	-	2
E	+++	+++	++	8	+++	+++	++	8
F	++	+	+	4	+	+	+	3
G	+	++	+	4	-	-	-	0
H	+	-	-	1	++	++	+	5
I	+	++	+	4	+	++	-	3
J	+	+	++	4	++	+	+	4
K	++	+	+	4	-	+	+	2
L	++	+	++	5	-	-	-	0
M	++	+++	+	6	+	+++	+	5
N	-	+	++	3	-	-	+	1
O	-	-	+	1	-	-	+	1
P	+	+	++	4	-	+	-	1
Q	++	+++	+	6	-	++	++	4
R	+	+	+	3	+	+++	+	5
S	++	+	+	4	+	+	+	3
T	+	++	++	5	+	++	+	4

out; the outer layer, com; stratum compactum,
subend; subendocardial layer.

cardiac death) were assessed by means of the Mann-Whitney's U-test. All tests were two-tailed; statiscal significance was inferred for comparisons at $p < 0.05$ (calculation with SPSS ver. 16).

RESULTS

The interstitial fibrosis had moderate but statistically significant association with LVDd (Spearman rank correlation coefficients (r) = -0.571, p = 0.008, Fig. 3) and with age (r = 0.597, p = 0.005, Fig. 4), but had no association with the preoperative NYHA functional classification, the level of BNP, CTR, or the echocardiographical data as follows; EF, LVDs, IVST, LVPWT. None of the other pathological scores (hypertrophy, attenuation or vacuolation) was associated with the selected clinical or cardiac hemodynamic parameters. Patients with the history of DM, the use of spironolactone, ACE-I or ARB, or beta blocker had no significantly better or worse histopathological scores than those without these characteristics (Table 3).

DISCUSSION

The relationship between histopathological findings in idiopathic dilated cardiomyopathy (DCM) and clinical cardiac function has been examined using specimens obtained by endomyocardial biopsy, but there are few studies of transmural myocardial specimens. Endomyocardial biopsy is considered to be less reproducible due to the reduced specimen volume compared with evaluation of histopathological changes with transmural myocardial specimens obtained by intraoperative biopsy during left ventriculoplasty in DCM patients. In endomyocardial biopsy, specimens are collected usually from the right ventricle, based on the hypothesis that DCM is a diffuse myocardial disease and that biopsy data for the right heart is sufficient. However, Yonesaka *et al*. [2] studied autopsy hearts and showed that the histology varied more than expected and that the difference between the right and left ventricles was not negligible, and Schwarz *et al*. [3] also found significant sampling errors in evaluation of fibrosis. In the current study, the surgical procedures used were OLVP and PMA, as invented by Matsui *et al*. [4-5], and a specimen was collected at the incision site of the left ventricle, approximately 10 cm from the cardiac apex of the left ventricular anterior wall to the diagonal branch and the anterior descending artery. The reproducibility of specimen collection from the left ventricular myocardium, the main region of cardiac contraction, is extremely high and the accuracy of histopathological evaluation is higher than for endomyocardial biopsy, making this approach more useful for evaluation of cardiac function.

The major microscopic histopathological changes in DCM are cardiomyocyte hypertrophy corresponding to an increased heart weight, vacuolation associated with fibrosing lesions, and attenuated cardiomyocytes [6]. Based on these characteristics, findings for hypertrophy, attenuation, vacuolation and fibrosis were established as histopathological endpoints. The histopathological changes in DCM are non-specific, and other diseases with similar histological findings must be excluded in diagnosis of DCM [2]. Fuda *et al*. [7] studied the incidence of various lesions in biopsy specimens obtained from dilated cardiomyopathy heart (53 cases), hypertrophic cardiomyopathy heart (50 cases), and the right heart in cases with a chronically stressed right ventricle (72 cases) including valvular heart disease and congenital heart disease. Cardiomyocyte hypertrophy, variation in cell size, deformed and enlarged nuclei, cardiomyocyte lysis to loss, and interstitial fibrosis were often found in DCM patients, but

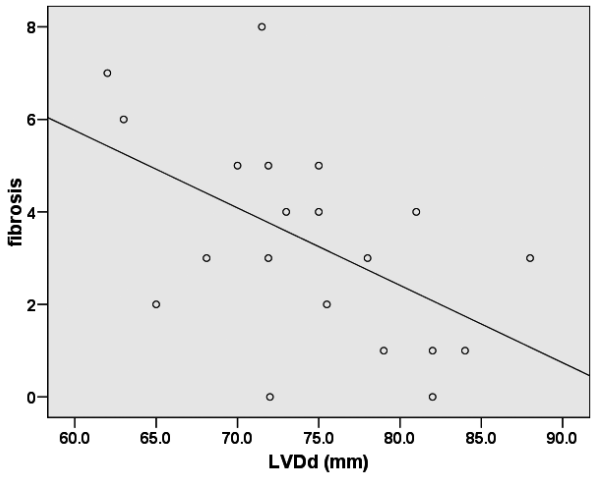

Fig. 3 Fibrosis & LVDd
LVDd had significant inverse correlation with interstitial fibrosis.

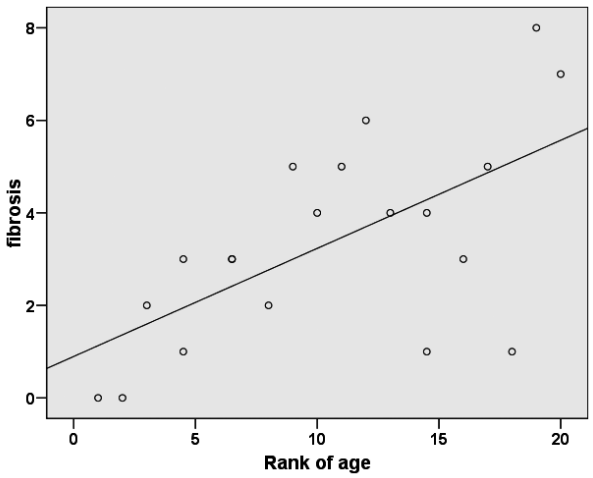

Fig. 4 Fibrosis & Age
Age had significant correlation with interstitial fibrosis. Age data are shown in rank.

Table 3 Histopathological scores in patients with or without the history of DM, the use of spironolactone, ACE-I/ARB or beta blocker, or cardiac death
The results are expressed as median (25%, 75%) of each histopathological scores.

	Diabetes Mellitus		spironolactone		ACE-I/ARB		Beta blocker		Cardiac death	
	-	+	-	+	-	+	-	+	-	+
hypertrophy	6 (4-6)	6 (5-6)	6 (3.5-6)	6 (5-6)	5 (3-6)	6 (5-6)	5 (3-6)	6 (5-6.25)	6 (4.25-6)	5 (4.25-5.75)
attenuation	3 (3-4)	4 (3-4)	3 (3-4)	3 (3-4)	4 (3-4)	3 (3-4)	3 (3-4)	3 (3-4.25)	3 (3-4)	3.5 (1.5-4.75)
vacuolation	4 (3.5-5.5)	4 (3-8)	4 (4-6)	4 (2-5)	4 (4-5)	4 (2.5-6)	4 (3.75-5.25)	4 (2.75-6)	4 (4-6)	3 (2.25-4.5)
fibrosis	3 (1-4.5)	4 (2-5)	3 (1-4.5)	3 (1-5)	2 (0-3)	4 (2.5-5)	3 (1.5-5)	3.5 (1-4.5)	3 (1.25-4.75)	4.5 (1.75-6.5)

were also observed in the other two groups, suggesting that these characteristics are not specific to DCM.

Cardiac biopsy and histology are required for registration of heart transplant recipients [1] and these data have allowed correction of diagnoses of DCM to cardiac amyloidosis and sacroidosis [8]. These tests have improved differential diagnosis, and Sekiguchi *et al.* further proposed that careful evaluation and semi-quantitation of nonspecific histopathological changes can be used to predict the prognosis of DCM patients [9–10]. Morimoto *et al.* [11] showed that myocardial biopsy is useful not only in differentiation of DCM from some other myocardial diseases, but also in evaluation of cardiac function and prognosis by semi-quantification of histological findings. They established pathological findings corresponding to reduced ventricular systolic function observed in cardiomyopathy patients, including cardiomyocyte degeneration, lysis and loss; myocardial cord rupture; and interstitial fibrosis, with these three findings obtained from endomyocardial biopsy and scored on a 4-point scale (0 to 3). The total scores for histopathological severity of myocardial stunning showed a significant inverse correlation with the cardiac output coefficient and a significant negative correlation with left ventricular ejection fraction [11]. Also Schwarz *et al.* [3] conducted endomyocardial biopsy in 71 DCM patients and found an inverse correlation between left ventricular ejection fraction and interstitial fibrosis ($r = -0.47$, $p < 0.001$), and Kuhn *et al.* [12] showed a good correlation between semi-quantitative data from electron micrographs in cardiac biopsy and DCM prognosis. In contrast, Baandrup *et al.* [13] suggested that the prognosis of DCM could not be established clearly from semi-quantitative cardiac biopsy data, and Rose *et al.* [14] showed that myocardial lesions are non-specific and that morphological evidence of cardiomyopathy and severity of cardiac dysfunction are only weakly associated in biopsy and autopsy of DCM patients.

We designed and conducted the current study using clinical and hemodynamic parameters that are used clinically to make decisions regarding surgical treatment (i.e., left ventriculoplasty) to verify the relationship between cardiac function and histopathological changes established by endomyocardial biopsy. Our results showed no significant correlation between histopathological changes and clinical and hemodynamic parameters, except for an inverse correlation between LVDd and interstitial fibrosis. As described above, positive correlations between endomyocardial biopsy data and cardiac function have been found in some papers, but these studies have differences from our study in patient background and sampling. Such studies showing a correlation between histopathological changes and cardiac function have included patients with relatively mild DCM, including one in which patients with an EF of 55% or higher accounted for 26% of the subjects [3] and another in which DCM and

hypertrophic cardiomyopathy (HCM) patients were included as subjects [11], with almost all HCM patients having a cardiac output coefficient of 2.2 or more. In contrast, the mean parameters of clinical cardiac function in our patients were NYHA classification class II in 1, class III in 7 and class IV in 12, EF 28 ± 9%, LVDd 74 ± 7 mm, CTR: 61 ± 7% and BNP level 1139 ± 890 pg/mL, indicating inclusion of many severe cases. Our subjects also included many patients with poor cardiac function who are usually candidates for transplantation in other countries. Since such patients generally had severe heart failure that cannot be controlled by drugs, this also indicates that many relatively severe cases were included in this study. Therefore, the almost complete absence of a correlation between histopathological changes and cardiac function may be mainly due to selection of a narrow spectrum of DCM patients who underwent left ventriculoplasty, compared with the wide spectrum of DCM patients examined using endomyocardial biopsy specimens in the previous studies.

The histopathological findings may also differ because of the use of left ventricular biopsy specimens, rather than those from endomyocardial biopsy. A comparison of endomyocardial biopsy specimens indicated that left ventricular lesions were larger than those in the right ventricle [2], and the collective results of many studies suggest that at least 5 and 3 specimens from the right and left ventricles, respectively, are required for an evaluative accuracy similar to that of transmural specimens. In our intraoperative myocardial biopsy, transmural specimens were collected along the left ventricular incision line during left ventriculoplasty (a whole layer of approximately 10 cm from the cardiac apex of the left ventricular anterior wall to the diagonal branch and the anterior descending artery). The specimens were collected from the same region by the same surgeon, which suggests that the results should be highly reproducible, and the increased tissue volume of transmural specimens permits a more detailed histopathological study compared to specimens collected by endomyocardial biopsy. Therefore, we believe that the histopathological evaluation in this study is superior in reproducibility, quality and quantity of findings due to collection of specimens from the left ventricular myocardium, the main region of cardiac contraction, compared to results from endomyocardial biopsy specimens.

We have shown that the age had significant correlation with interstitial fibrosis. Although, there has been the standard consensus of the poor correlation between aging and interstitial fibrosis, several reports indicated the positive correlation between them [15–16]. Klima *et al.* [16] concluded that cardiac interstitial fibrosis develops independently from the clinical conditions (hypertension, congestive heart failure, emphysema, cor pulmonale, and coronary artery disease) and may be considered as a true aging process. It is difficult to determine the interstitial fibrosis of DCM either as a substance of the disease or as a consequence of the remodeling [6]. And there is significant correlation between the period of the remodeling and interstitial fibrosis [6]. Consequently, above-mentioned our result suggests that age reflected disease period.

Although we hypothesized that there are differences of histopathological scores especially in myocardial fibrosis between two groups as follows; with or without the use of spironolactone, ACE-I or ARB, and late cardiac death, our study was in discord with the hypothesis. Spironolactone has been reported to prevent myocardial fibrosis by blocking the effects of aldosterone on the formation of collagen, reduces the risk of both morbidity and death in addition to use of ACE-I among patients with sever heart failure [17]. The roles of the rennin angiotensin aldosterone and sympathoadrenergic systems have been well established in pathophysiology of the remodeling process and chronic heart failure [17, 19]. In hypertensive patients with left ventricular hypertrophy, losartan decreases myocardial collagen content and induces regression of sever myocardial fibrosis. [20–21]. Aoki *et al.* [22] showed that the extent of left ventricular fibrosis is a strong predictor of cardiac death in hemodialysis patients with DCM. Our results are not in line with above accomplished concepts. We consider that the limitation of sampling size influenced to these results. As mentioned above, there is a possibility that the selection of a narrow spectrum of DCM patients and small population suppressed the significancy of histopathological differences between two groups. On the other hand, in the report by Aoki *et al.* [22], 50 patients with a diagnosis of DCM who were not on dialysis was the control group. They reported cumulative survival for cardiac death stratified by the extent of fibrosis. Though the dialysis group with 30% or more fibrosis had a significantly worse survival compared with the control group, there was no significant difference between more and less than 30% in DCM patients as the control group. Furthermore, Terasaki *et al.* [23] analyzed myocardium specimens obtained from 13 DCM patients by partial left ventriculectomy and reported that the severity of interstitial fibrosis (percent fibrosis) did not differ significantly between the three patients who died of cardiac insufficiency after surgery and survivors. These articles support our result regarding fibrosis and outcome of cardiac death.

It is interesting that the only association between parameters of clinical cardiac function and histopathological scores was an inverse correlation between LVDd and the score for interstitial fibrosis. It has been generally considered that DCM patients with dilated LVDd have severe heart failure and show reduced cardiac function with myocardial fibrosis. However, our results indicate that patients with a more dilated LVDd had less interstitial fibrosis. We should note that collagen fiber, rather than elastic fiber, was stained and the severity of interstitial fibrosis was based on collagen fibrosis. Mechanically, the left ventricle may be difficult to dilate due to the stiffness caused by an excess collagen fiber volume, and Timonen *et al.* recently reported that DCM associated with mild to moderate left ventricular failure promoted synthesis of elastic type III collagen and induced degeneration of inelastic type I collagen [24]. These findings suggest that the mechanism of remodeling of collagen fiber may change both quantitatively and qualitatively in myocardial interstitial fibrosis. We considered that the kinetics of myocardial fibrosis with remodeling might

be variable and histopathological findings does not reflect the clinical and hemodynamic changes in DCM patients. Further morphological studies in a larger number of cases are required to confirm this finding, given the relatively narrow population of DCM patients in the current study.

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