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ORIGINAL RESEARCH ARTICLE

Long-term prognostic value of ultrastructural features in dilated cardiomyopathy: comparison with cardiac magnetic resonance

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Abstract

Aims This study aims to determine the implications associated with long-term prognosis of heart failure (HF) in patients with dilated cardiomyopathy (DCM) presenting initially as decompensated HF. We stratified the phase of DCM patients without late gadolinium enhancement (LGE) based on ultrastructural changes in cardiomyocytes.

Methods and results Left ventricular (LV) endomyocardial biopsy was performed in 55 consecutive DCM patients with initial decompensated HF. Ultrastructural changes in cardiomyocytes detected by electron microscopy were compared with data including LGE with cardiac magnetic resonance and HF recurrence. Of the 55 DCM patients, 24 (44%) showed LGE, and 26 (47%) showed recurrence decompensated HF, while 23 patients (42%) showed autophagic vacuoles in cardiomyocytes by electron microscopy. Multivariate analysis identified atrial fibrillation [hazard ratio (HR), 3.40; 95% confidence interval (CI), 1.45–7.98], haemoglobin level (HR, 0.82; 95% CI, 0.68–0.99), beta-blocker use (HR, 0.18; 95% CI, 0.05–0.74), and autophagic vacuoles (HR, 0.25; 95% CI, 0.09–0.65) as predictors of HF recurrence in the total patient population. In patients without LGE, only autophagic vacuoles were independent predictors of readmission because of HF (HR, 0.29; 95% CI, 0.09–0.90). In patients with LGE, atrial fibrillation (HR, 19.10; 95% CI, 2.97–123.09), and mid-linear LGE (HR, 12.96; 95% CI, 2.02–82.94) were independent predictors of readmission because of HF.

Conclusions In DCM patients with LGE, characterised by progression of LV remodelling, the LGE pattern was a predictor of HF recurrence, whereas in patients without LGE, absence of autophagic vacuoles was a predictor of HF recurrence.

Keywords Autophagy; Cardiac magnetic resonance; Dilated cardiomyopathy; Heart failure; Late gadolinium enhancement; Left ventricular reverse remodelling

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Introduction

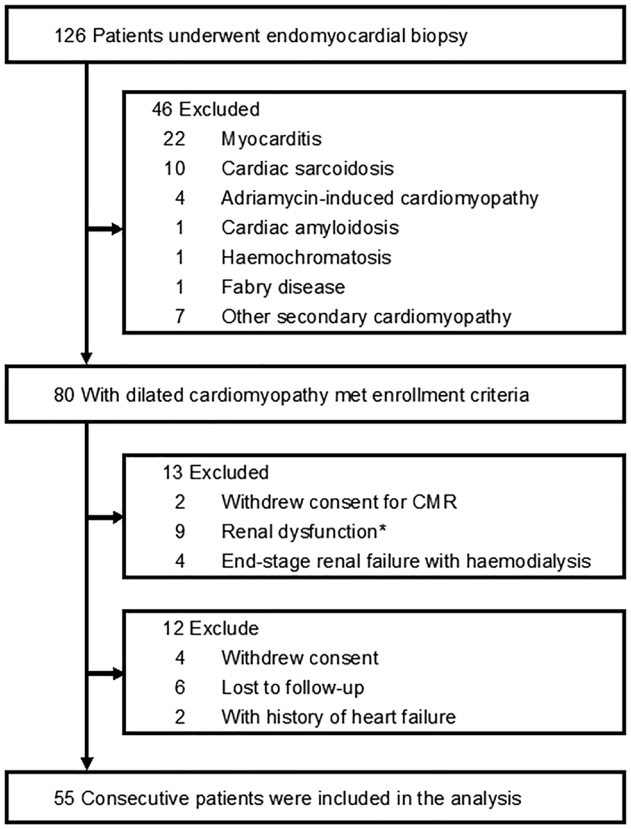
Dilated cardiomyopathy (DCM) is a heart disease characterised by enlarged ventricles and severe systolic dysfunction.[1] Consequently, DCM is a major cause of heart failure (HF) and increases the affected individual's risk of morbidity and mortality. Appropriate treatments for HF such as renin–angiotensin system inhibitors and beta blockers can improve left ventricular (LV) function and prognosis in some patients with DCM.[2] In such cases, LV reverse remodelling (LVRR) could be used a surrogate marker of therapeutic success by indicating the gradual process of LV function recovery.[2]

Late gadolinium enhancement (LGE) on cardiac magnetic resonance (CMR) imaging is a useful modality for detecting the site and distribution of myocardial oedema,[3] fibrosis, and scarring related to the severity of DCM.[4–6] These data also suggested that LGE-CMR can predict mortality, need

for transplantation, HF recurrence, and LVRR.[4,5] Nevertheless, some patients with DCM do not show LGE, especially if they presented initially with decompensated HF,[4,7] and the clinical and pathological characteristics of such patients need to be investigated further.

Endomyocardial biopsy (EMB) is valuable for detecting myocardial diseases, with subsequent electron microscopy allowing detailed analysis of the myocardial cellular degeneration.[8–10] No prognostic factor has been identified using light microscopy evaluation of EMB samples,[6,9] except that CD163-positive macrophages were associated with poor outcome in DCM.[11] We previously showed that ultrastructural changes in cardiomyocytes, such as myofilament changes[9] or autophagic vacuoles,[10] could predict prognosis in DCM patients with initial decompensated HF. Herein, we therefore aimed to determine the implications associated with prognosis of HF and LVRR in DCM patients presenting initially with decompensated HF, through stratifying those without LGE based on the ultrastructural findings of EMB samples.

Figure 1 Derivation of the study cohort. *Renal dysfunction was classified as glomerular filtration rate < 60 mL/min/1.73 m^2.



Methods

Study population

This prospective, longitudinal study enrolled 55 Japanese patients with idiopathic DCM presenting initially with decompensated HF. All patients underwent EMB from the LV and enhanced CMR during the period from January 2006 to December 2011 at the Nippon Medical School Hospital (*Figure 1*). Patients with secondary (metabolic, drug induced, or inflammatory) cardiomyopathies, myocarditis, or neuromuscular disorders or congenital, ischemic, or severe valvular heart disease were excluded from the study. Patients with a history of symptoms from HF lasting longer than 6 months were also excluded.[12] All patients enrolled in the study had systolic dysfunction (LV ejection fraction<50%) without significant coronary artery stenosis, as assessed by coronary angiography. Written informed consent was obtained from all remaining patients prior to their inclusion in the study. The study protocol was approved by the committee overseeing clinical research at our institution and was performed in accordance with the Declaration of Helsinki.

Clinical data collection including definition of left ventricular reverse remodelling and endomyocardial biopsy

On admission, all patients underwent routine analysis by electrocardiogram, laboratory determination of serum brain natriuretic peptide levels using a commercially available immunoassay kit (Shionogi Inc., Osaka, Japan) and trans-thoracic echocardiography to obtain morphological and functional information. Two-dimensional, M-mode, and colour Doppler imaging was also performed according to the standardised methods of the American Society of Echocardiography.[13] LVRR was defined as an increase in LVEF from ≥10% to a final value of >35%, accompanied by a decrease in LV end-diastolic dimension ≥10% as assessed by echocardiography at 24 months.[2] Right and left catheterisation was performed together with EMB as soon as cardiac catheterisation can be performed, at least, within 2 weeks. The EMB was performed under radiographic guidance with continuous electrocardiographic monitoring. Tissue samples were collected from the LV infero-posterior wall using a 7-Fr bioptome (Cordis; Johnson & Johnson Co, New Brunswick, NJ) by retrograde approach. The type of medication prescribed on discharge for the treatment of HF was recorded.

Cardiac magnetic resonance imaging

Cardiac magnetic resonance was performed as soon as possible after EMB (the time interval between EMB and

CMR was 9.2 ± 5.4 days; the minimum and maximum were 1 and 24 days, respectively). The electrocardiography-gated CMR protocol proceeded with breath holding as previously described[14] using of 1.5-T and 3.0-T Achieva imaging equipment (Philips Healthcare, Best, the Netherlands). A steady-state, free precession sequence was applied for cine CMR. LGE images of the myocardium were acquired 10 min after the intravenous administration of gadolinium-based contrast agents at 0.15 mmol/kg. Two experienced independent observers (M. T. and Y. A.) who were blinded to patient outcomes evaluated the CMR images. LV end-diastolic volume, LVEF, and LV mass were acquired from two-dimensional cine images in the short-axis view, then LGE-CMR images were regionally analysed by dividing the LV myocardium into 17 segments.[7] An example of mid-linear LGE is shown in *Figure 2A*.

Tissue preparation, morphometries, and ultrastructural evaluation

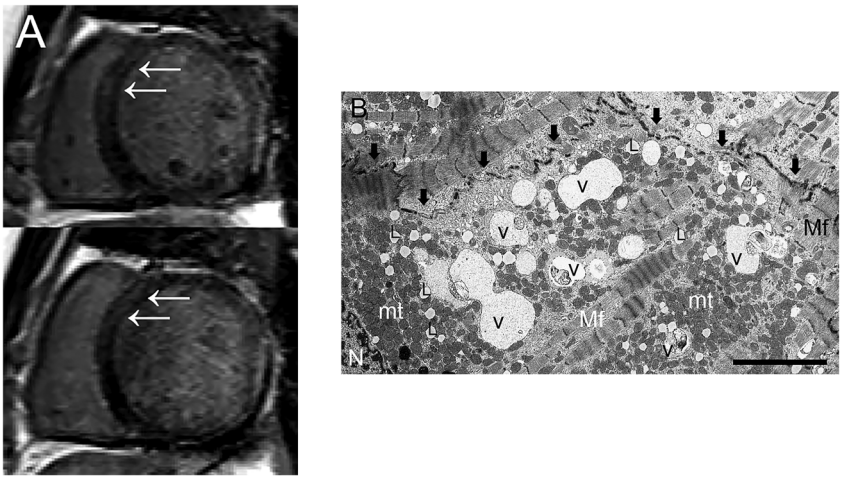
Biopsy specimens for light microscopy analysis were fixed in 20% neutral-buffered formalin, embedded in paraffin and cut into 3 μm thick sections. Serial sections were stained using the haematoxylin and eosin and Elastica–Masson–Goldner methods.

All photomicrographs were taken at ×200 magnification, including the whole tissue section, using the NIS-Elements Documentation System (Version 3.22; Nikon Instruments, Tokyo, Japan) and a digital microscope camera system (DS-Ri1, Nikon). Parameters were calculated using ImageJ analysis software (Version 1.43). The following three parameters were evaluated by morphometry: (i) the short diameter of cardiomyocytes, (ii) nucleus diameter of cardiomyocytes, and (iii) proportion of fibrosis (%*F*).[15] The short diameter of cardiomyocytes at the nuclear level and of nuclei was measured in 75 cells. Adobe Photoshop software version CS4 (Adobe Systems Inc., San Jose, CA) was used to identify red and green in Elastica–Masson–Goldner-stained sections, and the ratio of the fibrotic area in the myocardium was calculated using the following formula:[15] $\%F$ = (area of fibrosis/area of fibrosis + myocardium) × 100.

For electron microscopy analysis, pieces of EMB material were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. Samples were dehydrated in a graded series of ethanol and embedded in Epok 812 (Ernest F. Fullam, Schenectady, NY). Ultrathin sections were cut on an ultramicrotome with a diamond knife, stained with uranyl acetate and lead citrate, and examined under an electron microscope (JEOL-1010; JEOL, Tokyo, Japan) at 80 keV. A minimum of 50 cardiomyocytes was examined in each sample.

Ultrastructural variables such as myofilament changes[9] and autophagic vacuoles[10] were classified as positive (when identified in the cytoplasm of cardiomyocytes) or negative. Four of the authors evaluated all electron microscopy results for EMB samples (T. S., S. S., A. A., and Y. S.), with each sample examined three times in random order; these examiners were blinded to the clinical background of the patient. Any discrepancies in the ultrastructural evaluations were decided by consensus. Autophagic vacuoles are structures enclosed by a double membrane and filled with degenerated organelles. An example of myofilament changes and autophagic vacuoles is shown in *Figure 2B*.

Figure 2 A 29-year-old male patient with dilated cardiomyopathy. (A) Cardiac magnetic resonance showed mid-linear late gadolinium enhancement on the ventricular septum (white arrow). (B) Electron microscopy showed several different sized autophagic vacuoles (v) in a degenerated cardiomyocyte (surrounded by black arrow). Scale bar is 5 μm. L, lipid droplet; Mf, myofilament; mt, mitochondrial area; N, nuclear.



Follow-up and endpoints

Patients were observed from the time of cardiac catheterisation (including EMB) until death or readmission because of HF, or until February 2019. Follow-up information was obtained during routine visits and by telephone contact with the patients or their physicians. The primary endpoint was defined as a composite of death or readmission because of HF recurrence.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation for data with normal distribution, or median (interquartile range) for data with skewed distribution, as determined by the Shapiro–Wilk test. Categorical variables were compared using the χ^2 test or Fisher's exact test, and continuous variables were evaluated by Mann–Whitney's *U* test. Kaplan–Meier survival curves were calculated for the presence and absence of LVRR, LGE, myofilament changes, and autophagic vacuoles. The log-rank test was used to compare mortality and incidence rates of readmission because of HF. Univariate logistic regression analysis was performed to detect the candidate predictive factors related to LVRR, and univariate Cox regression analysis was used to identify candidate predictors of a composite of death and readmission because of recurrent HF; variables with $P < 0.1$ on univariate analysis were included in the multivariate model. Statistical analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL), and $P < 0.05$ was considered significant.

Results

Patient characteristics, magnetic resonance imaging findings, and ultrastructural features

Table 1 summarised the baseline clinical, histopathological, and ultrastructural characteristics of the patients. Initially, ECG revealed 16 (29%) with atrial fibrillation and seven (13%) with a QRS duration >300 ms. LGE was seen in 24 of 55 patients (44%), of which 13 (54% of the 24 with LGE) were identified as mid-linear LGE. Four of the patients with mid-linear LGE had a wide QRS duration, but there were no patients without mid-linear LGE and with wide QRS duration ($P = 0.026$). The electron microscopy revealed myofilament changes in 40 patients (73%) and autophagic vacuoles in 23 patients (42%).

Predictors of left ventricular reverse remodelling

Table 1 also summarises the transitions in TTE measurements. LVRR was recognised in 25 patients (45%) at 24 months following admission, and 22 patients (40%) recovered their LVEF by more than 50%. By the log-rank test, LVRR had a significant effect on prognosis, with an event-free survival rate with/without LVRR of 68/28% ($P = 0.008$, *Figure 3A*). The univariate and multivariate analyses for predictors of LVRR identified increased body mass index [odds ratio (OR), 1.24; 95% confidence interval (CI), 1.04–1.48], autophagic vacuoles (OR, 5.42; 95% CI, 1.15–25.56), and LGE (OR, 0.17; 95% CI, 0.04–0.79) as independent predictors of LVRR (*Table 2*).

Outcomes

After a maximum follow-up period of 13.5 years (the mean follow-up period was 8.6 ± 2.3 years), nine patients (16%) had died (seven cardiac-related deaths and two deaths because of malignancy), whereas 26 (47%) had reached the composite HF endpoint of HF death or readmission because of HF recurrence. Kaplan–Meier survival curves for readmission because of decompensated HF in patients with/without LGE, myofilament changes and autophagic vacuoles are shown in *Figure 3B–D*. By the log-rank test, neither presence of LGE nor myofilament changes showed a significant association with prognosis, with event-free survival rates with/without LGE of 54/52% ($P = 0.916$) and with/without myofilament changes of 53/53% ($P = 0.609$); however, in patients with myofilament changes, the group with autophagic vacuoles showed a significantly higher rate of event-free survival than the group without autophagic vacuoles (70% vs. 29%, respectively; $P = 0.003$). Among LGE, myofilament changes and autophagic vacuole, none of the parameters showed a significant difference with respect to all-cause death.

Predictors of events

Results of candidate univariate and multivariate analyses to predict HF recurrence in the total population are given in *Table 2*. In patients with LGE, atrial fibrillation (HR, 19.10; 95% CI, 2.97–123.09) and mid-linear LGE (HR, 12.96; 95% CI, 2.02–82.94) were independent predictors of readmission because of HF. In patients without LGE, multivariate analysis identified only absence of autophagic vacuoles as independent predictors of readmission because of HF (HR, 0.29; 95% CI, 0.09–0.90).

Discussion

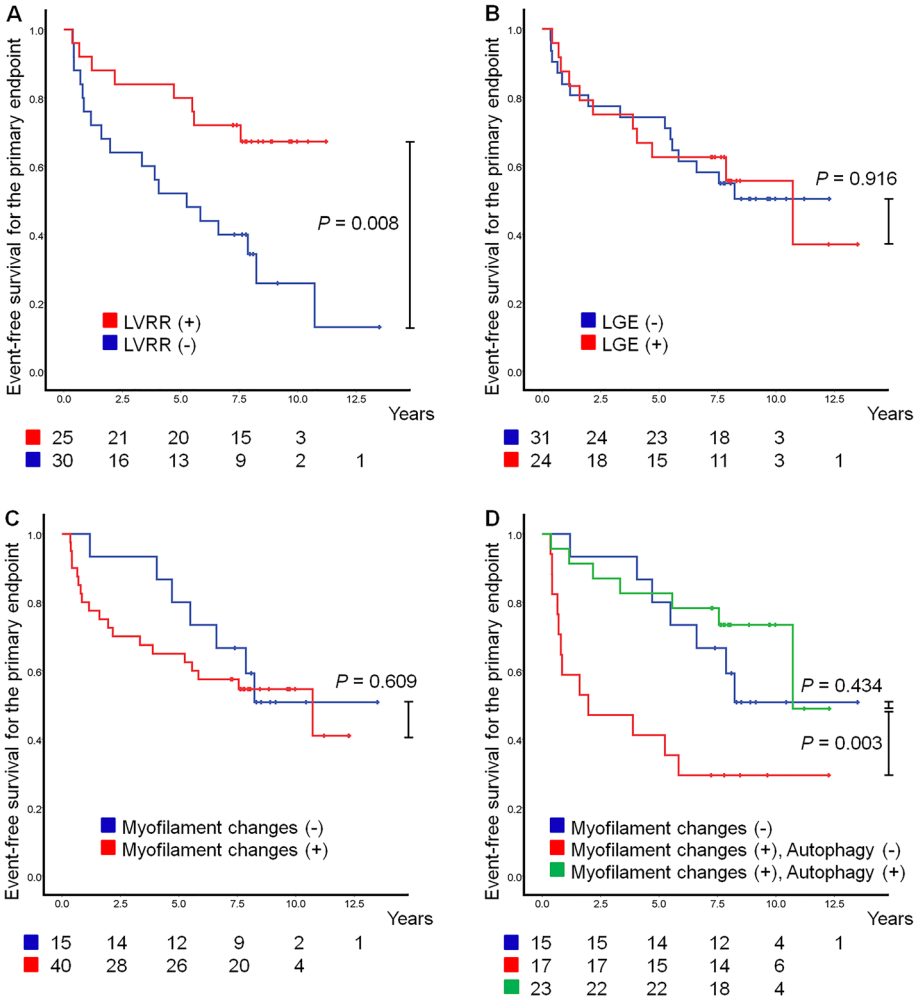
The present study concluded that absence of autophagic vacuoles was a predictor of recurrent decompensated HF in DCM patients with HF initially, while absence of LGE and presence of autophagic vacuoles were predictors of LVRR. Absence of

Table 1 Patient characteristics and outcomes of morphometry

Variable	All patients (n = 55)	LGE (−) (n = 31)	LGE (+) (n = 24)	P-value
Clinical characteristics				
Age (years)	55.5 ± 13.1	53.4 ± 14.9	58.2 ± 10.1	0.197
Male gender	44 (80%)	26 (84%)	18 (75%)	0.415
Body mass index (kg/m^2)	23.6 (21.6–27.5)	24.4 (20.9–26.6)	23.5 (21.8–29.1)	0.722
Systolic blood pressure (mmHg)	131.9 ± 33.9	132.8 ± 39.9	130.8 ± 24.8	0.593
Diastolic blood pressure (mmHg)	79.0 ± 21.2	79.8 ± 23.4	78.0 ± 18.2	0.926
Heart rate (b.p.m.)	90.1 ± 29.4	99.8 ± 31.0	77.5 ± 22.0	0.005
QRS duration >300 ms	7 (15%)	3 (10%)	4 (17%)	0.686
NYHA class III and IV	27 (49%)	17 (55%)	10 (42%)	0.333
Family history of DCM	10 (18%)	5 (16%)	5 (21%)	0.654
Atrial fibrillation	16 (29%)	8 (26%)	8 (33%)	0.542
Hypertension	29 (53%)	18 (58%)	11 (46%)	0.368
Diabetes	20 (36%)	10 (32%)	10 (42%)	0.472
Renal dysfunction[a]	9 (16%)	8 (26%)	1 (4%)	0.031
Clinical chemistry				
B-type natriuretic peptide (pg/mL)	729.2 (306.5-1416.7)	766.5 (439.6–1734.5)	392.8 (236.8–1029.3)	0.023
C-reactive protein (mg/dL)	0.2 (0.1–0.8)	0.4 (0.1–0.9)	0.2 (0.1–0.4)	0.039
Fasting blood sugar (mg/dL)	102.0 (93.5–113.5)	104.0 (92.0–117.0)	101.5 (96.8–109.8)	0.905
HbA1c (%)	5.7 (5.4–6.1)	5.6 (5.4–5.9)	5.8 (5.5–6.3)	0.104
Creatinine (mg/dL)	0.9 (0.7–1.0)	0.9 (0.7–1.2)	0.9 (0.7–1.0)	0.593
Haemoglobin (g/dL)	14.3 ± 2.0	14.3 ± 2.1	14.4 ± 1.9	0.959
Total bilirubin (mg/dL)	0.8 (0.6–1.2)	0.8 (0.5–1.2)	0.8 (0.7–1.1)	0.745
Echocardiaographic data at admission				
Left atrial dimension (mm)	44.7 ± 6.2	44.6 ± 6.1	44.9 ± 6.4	0.818
LV ejection fraction (%)	30.3 ± 12.3	28.4 ± 11.4	32.9 ± 13.3	0.197
LV diastolic dimension (mm)	64.0 ± 8.4	65.9 ± 8.4	61.5 ± 7.9	0.083
LV systolic dimension (mm)	54.6 ± 9.9	57.0 ± 9.5	51.6 ± 9.8	0.035
Interventricular septum thickness (mm)	9.7 ± 2.1	9.9 ± 2.3	9.5 ± 1.7	0.552
Posterior wall thickness (mm)	9.0 (7.5–10.0)	10.0 (8.5–10.5)	8.0 (7.0–9.3)	0.027
Echocardiographic data at 24 months after admission				
Left atrial dimension (mm)	39.3 ± 6.5*	37.7 ± 6.2*	44.4 ± 6.5‡	0.037
LV ejection fraction (%)	47.7 ± 15.1*	49.5 ± 14.5*	45.3 ± 15.9***	0.398
LV diastolic dimension (mm)	55.5 ± 8.6*	55.1 ± 9.3*	56.1 ± 7.7†	0.581
LV systolic dimension (mm)	42.1 ± 10.6*	41.4 ± 11.2*	43.0 ± 9.9‡	0.609
Interventricular septum thickness (mm)	10.0 (9.0–11.0)	10.0 (9.0–11.0)	10.0 (8.0–10.0)	0.323
Posterior wall thickness (mm)	9.0 (8.0–10.0)	9.0 (8.0–10.0)	8.0 (7.0–9.0)	0.167
LV reverse remodelling	25 (45%)	18 (58%)	7 (29%)	0.045
Cardiac magnetic resonance data				
LGE no. of segments	0.0 (0.0–2.0)	N/A	2 (2–3.25)	–
Mid-linear LGE	13 (24%)	N/A	13 (54%)	–
LV end-diastolic volume index (mL/m^2)	243.6 ± 67.5	265.6 ± 68.7	220.6 ± 59.2	0.218
LV ejection fraction (%)	20.5 ± 7.9	20.0 ± 8.9	21.3 ± 6.7	0.083
LV mass index (g/m^2)	164.9 ± 50.1	171.7 ± 51.8	156.1 ± 47.4	0.197
Medication on admission				
ACEI or ARB	5 (9%)	1 (3%)	4 (17%)	0.156
Medication in follow-up period				
ACEI or ARB	48 (87%)	25 (81%)	23 (95%)	0.122
Beta blockers	52 (95%)	31 (100%)	21 (88%)	0.077
Diuretics	43 (78%)	26 (84%)	17 (71%)	0.246
Aldosterone receptor antagonists	41 (75%)	24 (77%)	17 (71%)	0.578
Outcome of morphometry				
Cellular diameter (μm)	18.0 ± 1.7	17.7 ± 2.1	18.4 ± 1.0	0.095
Nuclear diameter (μm)	8.2 ± 0.9	8.3 ± 1.1	8.2 ± 0.6	0.456
Proportion of fibrosis (%)	13.1 ± 8.0	15.0 ± 8.1	10.6 ± 7.3	0.025
Ultrastructural variables				
Myofilament changes	40 (73%)	23 (74%)	17 (71%)	0.781
Autophagic vacuoles	23 (42%)	15 (48%)	8 (33%)	0.262

ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; DCM, dilated cardiomyopathy; LGE, late gadolinium enhancement; LV, left ventricular; NYHA, New York Heart Association; N/A, not applicable.
Data are given as either mean ± standard deviation, median (interquartile range), or number of patients, with percentages in parentheses, as appropriate.
[a]Renal dysfunction was classified as glomerular filtration rate < 60 mL/min/1.73 m^2.
*The significant differences compared with the data at admission $P < 0.001$.
**The significant differences compared with the data at admission $P = 0.020$.
***The significant differences compared with the data at admission $P = 0.007$.
†The significant differences compared with the data at admission $P = 0.021$.
‡The significant differences compared with the data at admission $P = 0.005$.

Figure 3 Kaplan–Meier survival curves in total population. (A) Left ventricular reverse remodelling (LVRR) and no recurrence of heart failure (HF). (B) Late gadolinium enhancement (LGE) in cardiac magnetic resonance imaging and no HF recurrence. (C) Myofilament changes in cardiomyocytes and no HF recurrence. (D) Autophagic vacuoles in cardiomyocytes and no HF recurrence.



autophagic vacuoles was also a predictor of HF recurrence in DCM patients without LGE. In contrast, mid-linear LGE was a predictor of HF recurrence in patients with LGE.

Myocardial fibrosis in patient with DCM is divided into two types, reactive (intercellular) and reparative (replacement) fibrosis.[16] Collagenous fibrous tissue usually wraps around the cardiomyocyte in a single layer.[17,18] When myocardium is subjected to mechanical stress because of pressure or volume, the collagenous fibrous tissue can proliferate directly[18] to fill up the intercellular space.[17] Reactive (intercellular) fibrosis then occurs that is so fine and thin that it is not easily detected without using light microscopy or a T1 mapping parameter obtained by CMR.[20] When cardiomyocytes degenerate and drop out, fibrotic tissues take their place and replacement fibrosis occurs that is so large it can be identified by LGE-CMR,[5] a contrast-enhanced method based on tissue differences in gadolinium transition. Gadolinium normally distributes throughout extracellular components including fibrotic tissue and vessels; thus, the concentration of gadolinium contrast agent becomes much higher in areas of myocardial scaring and fibrosis than in normal myocardium.[21]

Several studies have linked LGE in patients with DCM to adverse outcomes such as all-cause death, cardiac transplantation, HF-related mortality and hospitalisation, and sudden cardiac death.[6] Thus, the prognosis is poor for those patients in which fibrosis progresses, and because our DCM patients are admitted with initial decompensated HF, those with LGE are deemed to have a potentially advanced phase of DCM. In this study, mid-linear LGE in CMR was therefore a risk factor for recurrence of decompensated HF.[6] The LV myocardium has different contraction directions on the epicardial and endocardial sides, with the middle layer subject to the strained tension.[22] In the ventricular septum, the myocardium constituting the outside left and right ventricles is in

Table 2 Univariate and multivariate analyses for candidate predictors of left ventricular reverse remodelling

	Candidate univariate analyses			Multivariate analyses		
Variable	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Age	0.99	0.95–1.03	0.354			
Male gender	2.32	0.45–10.54	0.278			
Body mass index	1.16	1.01–1.33	0.041	1.24	1.04–1.48	0.020
Systolic blood pressure	1.01	0.99–1.03	0.248			
Diastolic blood pressure	1.01	0.98–1.04	0.476			
Heart rate	1.10	0.98–1.02	0.988			
NYHA class III and IV	0.85	0.28–2.59	0.777			
Family history of DCM	0.28	0.05–1.53	0.140			
Atrial fibrillation	0.33	0.11–1.33	0.129			
Hypertension	0.85	0.28–2.95	0.777			
Diabetes	0.84	0.27–267	0.771			
Renal dysfunction	2.88	0.50–16.48	0.236			
Log_{10} (B-type natriuretic peptide)	4.08	0.95–17.50	0.058	3.14	0.41–24.07	0.271
C-reactive protein	0.94	0.44–2.00	0.874			
Haemoglobin	1.28	0.96–1.73	0.095	1.33	0.89–1.98	0.161
Total bilirubin	1.46	0.55–3.90	0.451			
ACEI or ARB	2.19	0.36–13.21	0.393			
Diuretics	2.86	0.64–12.64	0.618			
Aldosterone receptor antagonists	4.89	1.15–20.73	0.032	5.04	0.89–28.59	0.068
Myofilament changes	0.83	0.25–2.78	0.758			
Autophagic vacuoles	2.79	0.86–9.01	0.087	5.42	1.15–25.56	0.033
Late gadolinium enhancement	0.31	0.09–0.99	0.048	0.17	0.04–0.79	0.024

ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; CI, confidence interval; DCM, dilated cardiomyopathy; LGE, late gadolinium enhancement; NYHA, New York Heart Association.
Because every patient using beta blockers showed left ventricular reverse remodelling, effective analysis for the entire group was impossible.

Table 3 Univariate and multivariate analyses for candidate predictors of readmission because of heart failure recurrence in the total population

	Candidate univariate analyses			Multivariate analyses		
Variables	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value
Age	0.99	0.96–1.02	0.436			
Male gender	1.27	0.47–3.41	0.641			
Body mass index	0.99	0.91–1.08	0.841			
Systolic blood pressure	1.00	0.99–1.01	0.977			
Diastolic blood pressure	1.00	0.98–1.02	0.898			
Heart rate	1.00	0.99–1.02	0.689			
NYHA class III and IV	0.79	0.36–1.71	0.543			
Family history of DCM	1.58	0.63–3.93	0.330			
Atrial fibrillation	2.68	1.22–5.89	0.015	3.40	1.45–7.98	0.005
Hypertension	0.61	0.46–2.15	0.980			
Diabetes	1.63	0.75–3.54	0.213			
Renal dysfunction	1.20	0.45–3.18	0.718			
Log_{10} (B-type natriuretic peptide)	1.26	0.55–2.88	0.593			
C-reactive protein	1.41	0.88–2.26	0.151			
Haemoglobin	0.85	0.70–1.03	0.089	0.82	0.68–0.99	0.035
Total bilirubin	1.19	0.64–2.20	0.589			
Left ventricular ejection fraction	0.99	0.96–1.02	0.619			
ACEI or ARB	0.83	0.28–2.45	0.738			
Beta blockers	0.32	0.09–1.11	0.072	0.18	0.05–0.74	0.017
Diuretics	1.35	0.51–3.59	0.544			
Aldosterone receptor antagonists	0.96	0.41–2.22	0.922			
Myofilament changes	1.25	0.53–2.99	0.610			
Autophagic vacuoles	0.41	0.17–0.97	0.042	0.25	0.09–0.65	0.005
Late gadolinium enhancement	2.08	0.92–4.68	0.077	1.32	0.54–3.25	0.541

ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; CI, confidence interval; DCM, dilated cardiomyopathy; LGE, late gadolinium enhancement; NYHA, New York Heart Association.

contact with each other back to back, and a larger tension is also applied. At these sites, both reactive and replacement fibrosis are detected as mid-linear LGE.[6] In the present study, mid-linear LGE was correlated with wide QRS duration, suggesting damage to the myocardium and fibrosis extensive enough to cause right ventricular and LV dyssynchrony. Myocardial fibrosis is a finding seen in the advanced phase of DCM, while cardiomyocyte degeneration and loss occur prior to fibrosis in patients with DCM.

In the present study, higher frequency of atrial fibrillation was a predictor for HF recurrence, supporting previous findings of HF risk factors.[23] Sustained atrial fibrillation has also been strongly associated with atrial fibrosis,[17] and a recent study indicated that genetic variants of *TTN* could cause atrial fibrillation.[24] These findings suggest that cardiomyocyte degeneration can lead to myocardial fibrosis, although the effect is different between atrial and ventricular muscles.

Macroautophagy (hereafter referred to as autophagy) is a lysosomal degradation pathway involving of bulk protein decomposition.[25] Hypoxia and malnutrition can induce autophagy,[26] and clinically, those situations emerge in HF. The presence of autophagic vacuoles around degenerative myofilaments suggests that autophagy could be partially responsible for the detected changes to cardiomyocytes. In support of this proposal, our previous study revealed that patients showing cardiomyocytes with myofilament changes but without autophagic vacuoles had a poorer prognosis compared with patients with both myofilament changes and autophagic vacuoles.[10] The present study confirmed this previous finding, even in the LGE-negative group, suggesting that absence of LGE indicates that no replacement fibrosis has been initiated because of the loss of cardiomyocytes. Autophagy degrades long-lived proteins to amino acids and removes damaged organelles,[25] and through this process, autophagy could repair degenerated cardiomyocytes or even prevent both the loss of cardiomyocytes and the extension of myocardial fibrosis.

Time-series analyses by CMR imaging revealed that LGE is not always fixed.[4] In DCM patients with LGE, the area of LGE continued to spread as LV contractility decreased. Replacement fibrosis, which is the pathological essence of LGE, then extends into the cardiac wall to eventually cause enlargement of the LV cavities and therefore should become the focus of arrhythmogenicity[6] and inhibition of LVRR.[4] DCM patients with extended LGE, that is, those showing an expanded myocardial fibrosis, were less likely to develop LVRR even once pharmacotherapy was started.[5] Replacement fibrosis in myocardium spreads to fill the site of dropping cardiomyocytes via the actions by fibroblasts[18] and macrophages;[19] however, DCM patients without LGE showed recovery of LV function and cavity size. Following the decision to treat those without LGE at baseline CMR with appropriate oral treatment for HF, none developed LGE at the CMR review 2 years later.[4] If collagen is still immature and not so extended, it will be decomposed by the action of matrix metalloproteinases, thus reducing the area of myocardial fibrosis.[27] Farris *et al*.[19] reported that pro-fibrotic gene expression in both cardiac fibroblasts and macrophages was changed in patients with end-stage HF provided with cardiac unloading by an LV assist device. In the present study, the absence of LGE and the presence of autophagic vacuoles were independent predictors of LVRR. This means that all factors to predict the prognosis of DCM with or without fibrosis correlated with recovery of LV size and function, which is a surrogate marker of therapeutic success in HF. LGE reflects extensive myocardial fibrosis because of cardiomyocytes dropping off, and autophagic vacuoles reflect the protection of cardiomyocytes against dropping. Thus, even patients with DCM who initially present with decompensated HF need to prevent irreversible degeneration of the myocardium to improve their prognosis. Autophagy is a fundamental physiological function possessed by every eukaryotic cell.[25] The present study showed that patients with myofilament changes and without autophagy have poor prognosis. These patients are likely to have some factors that prevent the induction of autophagy, which is therefore a potential therapeutic target for DCM.

Study limitations

The major limitation of this study is that it relies on structural evidence for autophagic vacuoles rather than biochemical analyses. By morphological assessment alone, autophagic flux could not be evaluated in real time; however, autophagic vacuoles can be found easily by electron microscopy. The cycle of autophagy is quick at less than 10 min from start to end. This indicates that autophagy in cardiomyocytes is a common phenomenon that occurs simultaneously and frequently; thus, observation and counting by electron microscopy is an appropriate method for evaluating autophagy.[10] In the present study, the evaluation of autophagy was conducted according to that definition. As a result, the clinical significance of autophagy was not detected in patients with advanced LGE, that is, those with extended fibrosis, and these patients could therefore potentially show progressive myocardial degenerations. Therefore, autophagic vacuolation found by electron microscopy can be a prognostic indicator only in the early phase of DCM, where it is a very sensitive indicator. We thus propose that identifying autophagic vacuoles by electron microscopy should be regarded as an established method.

The number of patients in this study was small, especially patients with LGE. In large populations of patients with DCM who developed initial HF, fibrosis is generally not extended;[4,7] thus, it is necessary to accumulate more LGE-positive cases for effective survival analysis. Patients with advanced myocardial fibrosis at the onset of initial HF can be classified into the following two patterns: (i) myocardial

fibrosis progresses potentially before the onset of decompensated HF, or (ii) myocardial fibrosis proceeds remarkably rapidly following the onset of decompensated HF. The risk analysis for these two groups of DCM patients should be examined in future studies.

The lack of normal controls in this study is another limitation, as it was in our previous studies.[9,10] As the major purpose of this study is to stratify patients with DCM based on ultrastructural findings of EMB samples, we regard normal controls as unnecessary at this stage.

Conclusions

The degree of fibrosis was varied even in the patients with DCM and initially decompensated HF. Autophagic vacuoles in cardiomyocytes could serve as an independent predictor of improved prognosis in both all patients with DCM and DCM patients without LGE as part of their initial decompensated HF. Contrarily, the presence of linear LGE in a middle layer of LV wall that indicated extensive fibrosis could be a predictor of HF recurrence in DCM patients with LGE.

Conflict of interest

None declared.

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