

[ORIGINAL ARTICLE]

Anti-mitochondrial Antibodies in Patients with Dilated Cardiomyopathy

Tetsuro Yokokawa^{1,2}, Akiomi Yoshihisa^{1,3}, Tomofumi Misaka^{1,3}, Takamasa Sato¹, Takashi Kaneshiro¹, Masayoshi Oikawa¹, Atsushi Kobayashi¹, Takayoshi Yamaki¹, Hiroyuki Kunii¹ and Yasuchika Takeishi¹

Abstract:

Objective It has been reported that anti-mitochondrial antibodies (AMAs) recognize mitochondrial antigens and are associated with some diseases involving multiple organs, such as primary biliary cholangitis, Sjögren syndrome, Hashimoto's thyroiditis, systemic sclerosis, interstitial pneumoniae, dilated cardiomyopathy, and tubulointerstitial nephritis. In the current study, we examined the prevalence of AMAs in patients with dilated cardiomyopathy (DCM) and their clinical characteristics.

Methods We enrolled 270 patients with DCM. We measured serum AMAs and analyzed the associated factors. Out of the 270 patients, positive AMAs were detected in 3 patients (1.1%; mean age, 68 years old; 2 men). These three patients had a significantly higher prevalence of primary biliary cholangitis and myopathy and levels of alanine alkaline phosphatase than those who were negative for said antibodies. There were no significant differences in the levels of B-type natriuretic peptide, aspartate transaminase, and left ventricular ejection fraction between these groups of patients. During the follow-up period, two of the three patients died due to respiratory failure. The other patient survived but experienced type II respiratory failure.

Conclusion The prevalence of AMAs in 270 DCM patients was only 1.1%, and these patients suffered from respiratory failure.

Key words: anti-mitochondrial antibodies, dilated cardiomyopathy, primary biliary cholangitis

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Introduction

Mitochondria are organelles that contribute to the production of respiratory adenosine triphosphate (ATP), which is found in eukaryotes (1, 2). Mitochondria are also integrated into the intracellular signaling pathways and contribute to the cellular functions. Anti-mitochondrial antibodies (AMAs) recognize mitochondrial antigens and are associated with several diseases that involve multiple organs, including primary biliary cholangitis, Sjögren syndrome, Hashimoto's thyroiditis, systemic sclerosis, interstitial pneumoniae, dilated cardiomyopathy (DCM), and tubulointerstitial nephritis (3-8). Regarding cardiovascular diseases, AMAs are also

related to cardiomyopathies, myocarditis, arrhythmias, and pulmonary hypertension (4, 9-11).

Cardiomyopathy and arrhythmias have been reported to occur in 2.9% and 3.6% of patients with primary biliary cholangitis, respectively (12). Cardiac involvement in patients with primary biliary cholangitis has been reported to be related to a poor outcome (12). In addition, 33% to 71% of patients with AMA-associated myopathy have been reported to have cardiomyopathies and/or arrhythmias (4, 9). Furthermore, a higher prevalence of supraventricular arrhythmias has been detected in AMA-positive patients than in those who were AMA-negative (13). Cardiac manifestations, such as cardiomyopathy, in patients with AMAs have been suggested; however, the prevalence and significance of

¹Department of Cardiovascular Medicine, Fukushima Medical University, Japan, ²Department of Pulmonary Hypertension, Fukushima Medical University, Japan and ³Department of Advanced Cardiac Therapeutics, Fukushima Medical University, Japan

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Correspondence to Dr. Akiomi Yoshihisa, yoshihis@fmu.ac.jp

AMAs in patients with DCM has not been fully investigated.

Therefore, in the current study, we examined the prevalence of AMAs in DCM patients and their clinical characteristics.

Materials and Methods

Study population

We prospectively included 270 consecutive hospitalized patients with DCM from Fukushima Medical University Hospital between January 2010 and October 2018. The diagnosis of DCM was based on the current guidelines as well as scientific statements regarding cardiomyopathy by experienced cardiologists (14-17). The baseline characteristics; comorbidities; history of pacemaker, implantable cardioverter defibrillator (ICD), and cardiac resynchronization therapy; laboratory data; echocardiographic data; medications; and clinical courses were collected at the time of enrollment in this study. The history of interstitial pneumonia, primary biliary cholangitis, and myopathy were also investigated, as these are all AMA-associated diseases (3, 4, 6). Anemia was defined as hemoglobin values of <12.0 g/dL in women and <13.0 g/dL in men. Chronic kidney disease was defined as an estimated glomerular filtration rate of <60 mL/min/1.73 m². The patients were followed up for occurrence of cardiac death, non-cardiac death, and all-cause death. Cardiac death was classified by independent experienced cardiologists as death related to the heart, such as worsened heart failure, ventricular fibrillation, or ventricular tachycardia documented by electrocardiography or implantable devices, acute coronary syndrome, or sudden cardiac death. Non-cardiac death included death due to respiratory failure, stroke infection, sepsis, cancer, digestive hemorrhaging, or other reasons.

The investigation conformed to the principles outlined in the Declaration of Helsinki, and the study protocol was approved by the Ethical Committee of Fukushima Medical University. Written informed consent was obtained from all patients.

The analysis of AMAs and anti-mitochondrial M2 antibodies (AMA M2)

Blood samples were collected for the measurement of AMAs and AMA M2 at the time of admission. The AMAs were analyzed in all patients by an indirect immunofluorescence method. The AMA M2 antibodies were analyzed by a fluorescence-enzyme immunoassay only in AMA-positive patients. These measurements were performed by BML (Tokyo, Japan), who were blind to the patients' information.

Echocardiography

Echocardiography was performed by experienced echocardiographers using standard techniques (18). Two-dimensional echocardiographic images were obtained from

the parasternal long and short axes, apical long axis, and apical four-chamber views. The following echocardiographic parameters were investigated: left ventricular end-diastolic diameter, left ventricular ejection fraction, tricuspid regurgitation pressure gradient, and right ventricular fractional area change. The left ventricular ejection fraction was calculated using Simpson's method in a four-chamber view. The right ventricular fractional area change, defined as (end diastolic area - end systolic area) / end diastolic area ×100, was a measure of the right ventricular systolic function (19).

Statistical analyses

Data were analyzed using the Statistical Package for Social Sciences version 26 software program (SPSS, Chicago, USA). Continuous data are expressed as mean ± standard deviation (SD), and skewed data are presented as the median and interquartile range. Categorical variables are expressed as numbers and percentages. The statistical significance of differences was analyzed using Student's *t*-test for parametric continuous variables and the Mann-Whitney U-test for nonparametric continuous variables. Categorical variables were compared using the chi-square test or Fisher's exact test. A *p* value of <0.05 was considered statistically significant for all comparisons.

Results

Out of the 270 patients, 3 (1.1%; mean age, 68 years old; 2 men) were positive for AMAs. These three patients underwent an AMA M2 analysis, for which they were also positive. We divided the patients into two groups according to presence or absence of AMAs for comparisons (Table 1). In the baseline characteristics, the AMA-positive patients had a significantly higher prevalence of primary biliary cholangitis and myopathy and higher levels of alanine alkaline phosphatase than the AMA-negative patients. There were no significant differences in the prevalence of atrial fibrillation or intestinal pneumonia nor in the levels of aspartate aminotransaminase, estimated glomerular filtration rate, B-type natriuretic peptide, left ventricular ejection fraction, tricuspid regurgitation pressure gradient, or right ventricular fractional area change between the groups.

During the median follow-up period of 1,435 days, 49 cardiac deaths, 23 non-cardiac deaths, and 2 deaths of unknown causes occurred (Table 1). Of the cardiac deaths, 36 were from heart failure, 9 were from sudden death, and 4 were from lethal arrhythmia. Of the non-cardiac deaths, 12 were from cancer, 7 were from respiratory failure, 2 were from kidney failure, and 1 each was from brain infarction and sepsis. Two of the three AMA-positive patients died due to non-cardiac death related to respiratory failure.

The detailed clinical courses of the three patients with AMAs are shown below.

• Case 1

A woman in her 60s suffered from complete atrioven-

Table 1. Baseline Characteristics.

	AMA-positive (n=3)	AMA-negative (n=267)	p value
Age, years	68±13	60±15	0.356
Female	1 (33.3)	76 (28.5)	0.636
Body mass index, kg/m ²	20±2	24±4	0.101
NYHA, I/II/III/IV	1/2/0/0	57/136/45/29	0.759
Co-morbidities			
Atrial fibrillation	2 (66.7)	104 (38.9)	0.341
Diabetes mellitus	1 (33.3)	108 (40.4)	0.643
Hypertension	1 (33.3)	152 (56.9)	0.400
Intestinal pneumonia	1 (33.3)	6 (2.2)	0.076
Chronic obstructive pulmonary disease	0 (0)	53 (19.9)	0.518
Primary biliary cholangitis	2 (66.7)	1 (0.4)	<0.001
Myopathy	1 (33.3)	0 (0)	0.011
Chronic kidney disease	1 (33.3)	144 (54)	0.444
Anemia	2 (66.7)	92 (34.4)	0.278
Laboratory data			
Total bilirubin, mg/dL	0.8±0.1	1.1±0.8	0.458
Aspartate aminotransferase, U/L	29±7	59±220	0.811
Alanine aminotransferase, U/L	23±17	54±190	0.776
Alkaline phosphatase, U/L	559±457	262±121	<0.001
γ-glutamyl transpeptidase, U/L	96±127	79±96	0.757
eGFR, mL/min/1.73 cm ²	68±57	58±22	0.423
Creatine kinase, U/L	123±31	127±142	0.967
B-type natriuretic peptide*, pg/mL	318 (195–483)	330 (112–719)	0.793
Echocardiography			
Left ventricular diastolic diameter, %	60±5	61±9	0.732
Left ventricular systolic diameter, %	50±7	52±10	0.735
Left ventricular ejection fraction, mm	44±7	35±14	0.157
TRPG, mmHg	29±8	25±11	0.494
RV-FAC, %	42±11	39±14	0.608
Medications/ Treatments			
Renin-angiotensin system inhibitor	3 (100.0)	242 (90.3)	0.737
Beta-blocker	3 (100.0)	259 (97.0)	0.913
Diuretics	3 (100.0)	208 (77.9)	0.476
Inotropes	1 (33.3)	82 (30.7)	0.669
Amiodarone	2 (66.7)	1 (33.3)	0.395
Prednisolone	1 (33.3)	4 (1.5)	0.055
Pacemaker	1 (33.3)	24 (9.0)	0.254
Implantable cardioverter defibrillator	1 (33.3)	106 (39.7)	0.654
Cardiac resynchronization therapy	1 (33.3)	96 (35.6)	0.711
Prognosis			
Cardiac death	0 (0)	49 (18.4)	0.547
Non-cardiac death	2 (66.7)	21 (7.9)	0.020
All-cause death	2 (66.7)	72 (27.0)	0.183

Numbers are expressed as number, number (%), mean±SD, or *Median (IQR)

AMA: anti-mitochondrial antibodies, NYHA: New York Heart Association, eGFR: estimated glomerular filtration ratio, TRPG: Tricuspid regurgitation pressure gradient, RV-FAC: right ventricular fractional change, IQR: interquartile range

tricular block and had a pacemaker implanted. Ten years later, she was admitted to the hospital with heart failure and diagnosed with DCM. Coronary angiography revealed no significant stenosis (Fig. 1a, b). The patient underwent cardiac resynchronization therapy pacemaker (CRTP) implantation, which was an upgrade from the patient's pacemaker. Six years later, she was referred to our hospital for exchange

of the CRTP.

On admission, laboratory findings revealed an elevated B-type natriuretic peptide level of 648.1 pg/mL and an alkaline phosphatase level of 256 U/L. Laboratory data and treatments are shown in Table 2. Electrocardiography showed a pacing rhythm and heart rate of 46 bpm, and chest X-ray showed no sign of congestion and a cardiothoracic ratio of

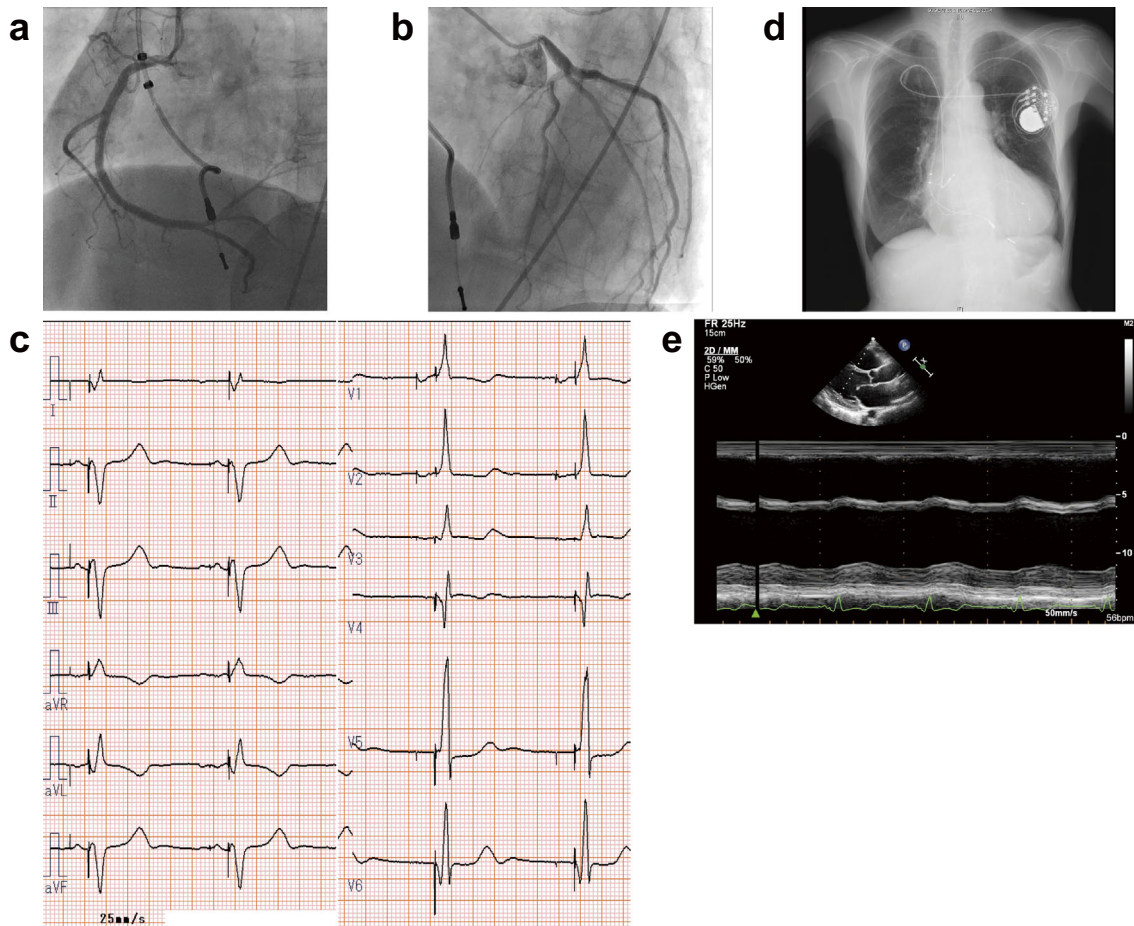


Figure 1. a, b: Coronary angiography revealed no significant stenosis. c: Electrocardiography showed a pacing rhythm and a heart rate of 46 bpm. d: Chest X-ray showed no sign of congestion and a cardiothoracic ratio of 66.5% with a cardiac resynchronization therapy pacemaker. e: Echocardiography detected a reduced ejection fraction and a large left ventricular diastolic diameter.

66.5% with a CRTP (Fig. 1c, d). Echocardiography detected a reduced ejection fraction of 39.0% and a large left ventricular diastolic diameter of 59.6 mm (Fig. 1e, Table 2). Exchange surgery of the CRTP was successfully performed, and the patient was discharged. At 586 days after this hospitalization, the patient died of respiratory failure due to pneumonia.

• Case 2

A man in his sixties was diagnosed with DCM. His blood test revealed an elevated creatinine kinase level and positive results of anti-nuclear antibody, anti-centromere antibody, and AMA M2. He was diagnosed with systemic scleroderma, dermatomyositis, and primary biliary cholangitis, and medical treatment with prednisolone and ciclosporin was initiated.

Three years later, a Holter echocardiogram detected frequent multifocal premature ventricular contractions of 27,072 beats per day (percentage of premature ventricular contractions per total beats: 30%). The patient was admitted to our hospital for radiofrequency catheter ablation of premature ventricular contractions. Radiofrequency catheter ablation for the premature ventricular contractions was per-

formed, but the patient refused implantation of a cardiac device.

On admission, laboratory findings revealed an elevated B-type natriuretic peptide level of 318.3 pg/mL and an elevated alkaline phosphatase level of 336 U/L. Laboratory data and treatment are shown in Table 2. Electrocardiography showed a sinus rhythm, premature ventricular contraction, and a heart rate of 64 bpm, and chest X-ray showed slight congestion and a cardiothoracic ratio of 59.7% (Fig. 2a, b). Echocardiography detected a reduced left ventricular ejection fraction of 41.2% and a large left ventricular diastolic diameter of 65.3 mm (Fig. 2c, Table 2). Cardiac magnetic resonance imaging revealed late gadolinium enhancement in the left ventricular lateral wall (Fig. 2d). Spirometry showed a reduced percentage of predicted vital capacity of 61.3% and a normal percentage of predicted forced expiratory volume in 1 second of 90.0%. In addition, he was diagnosed with type II respiratory failure and received non-invasive positive pressure ventilation therapy at home. During the follow-up period of 1,391 days, he experienced worsening heart failure once, but he survived.

Table 2. Characteristics in Each Patient with Anti-mitochondrial Antibodies on Admission.

	Case 1	Case 2	Case 3
Laboratory data			
Total bilirubin, mg/dL	0.8	0.9	0.7
Aspartate aminotransferase, U/L	28	23	36
Alanine aminotransferase, U/L	11	15	43
Alkaline phosphatase, U/L	256	336	1,084
γ -glutamyl transpeptidase, U/L	28	18	243
eGFR, mL/min/1.73 cm ²	13	66	126
Creatine kinase, U/L	147	88	135
B-type natriuretic peptide, pg/mL	648.1	318.3	71.7
IgM, mg/dL	-	148	-
Echocardiography			
Left ventricular diastolic diameter, %	59.6	65.3	55.2
Left ventricular systolic diameter, %	51.3	56.4	43.4
Left ventricular ejection fraction, mm	39.0	41.2	52.0
TRPG, mmHg	36.0	29.0	20.6
RV-FAC, %	42.1	54.2	31.6
Medications/ Treatments			
Renin-angiotensin system inhibitor	+	+	+
Beta-blocker	+	+	+
Diuretics	+	+	+
Inotropes	-	+	-
Amiodarone	+	+	-
Prednisolone	-	+	-
Pacemaker	+	-	-
Implantable cardioverter defibrillator	-	-	+
Cardiac resynchronization therapy	+	-	-

eGFR: estimated glomerular filtration ratio, TRPG: Tricuspid regurgitation pressure gradient, RV-FAC: right ventricular fractional change, +: use, -: no use.

• Case 3

A man in his 40s was diagnosed with primary biliary cholangitis. Two years later, he complained of palpitations and was diagnosed with DCM and ventricular tachycardia. Coronary angiography revealed no significant stenosis (Fig. 3a, b), and a myocardial biopsy showed no sign of secondary cardiomyopathies, such as sarcoidosis or amyloidosis. The patient was implanted with an ICD.

Seven years later, he was admitted to our hospital for replacement of his ICD. On admission, laboratory findings revealed an elevated B-type natriuretic peptide level of 71.7 pg/mL and an elevated alkaline phosphatase level of 1,084 U/L. Laboratory data and treatments are shown in Table 2.

Electrocardiography showed a pacing rhythm and heart rate of 59 bpm, and chest X-ray showed slight congestion and a cardiothoracic ratio of 64.9% with an ICD (Fig. 3c, d). Under guideline-based treatment, echocardiography detected a preserved ejection fraction of 52.0% and a slightly large left ventricular diastolic diameter of 55.2 mm (Fig. 3e, Table 2). Replacement surgery of an ICD was performed, and he was discharged. at 1,573 days after the hospitalization, the patient died of respiratory failure due to interstitial pneumonia.

Discussion

This study demonstrated a 1.1% prevalence of AMAs among 270 DCM patients. In addition, two of the three patients with AMAs died due to respiratory failure, and the remaining patient with AMAs had type II respiratory failure.

AMAs are typically found in patients with primary biliary cholangitis (6, 20). Primary biliary cholangitis is a chronic inflammatory autoimmune liver disease and can overlap with several autoimmune diseases, such as Sjögren syndrome, systemic sclerosis, systemic lupus erythematosus, autoimmune hepatitis, rheumatoid arthritis, psoriasis, chronic thyroiditis, and/or inflammatory bowel disease (7, 20-26). Other AMA-related extrahepatic phenotypes have been reported to manifest in the heart, lung, kidney, and skeletal muscles (3-5, 27). The following cardiac manifestations, considered to be rare AMA-associated complications, have been previously reported: atrial fibrillation, atrial flutter, sick sinus syndrome, paroxysmal supraventricular tachycardia, complete atrioventricular block, ventricular tachycardia, pulmonary hypertension, cardiomyopathy, and myocarditis (10-13, 28, 29). Regarding the prevalence of AMAs, AMA-M7 has been reported to be detected in 31% of con-

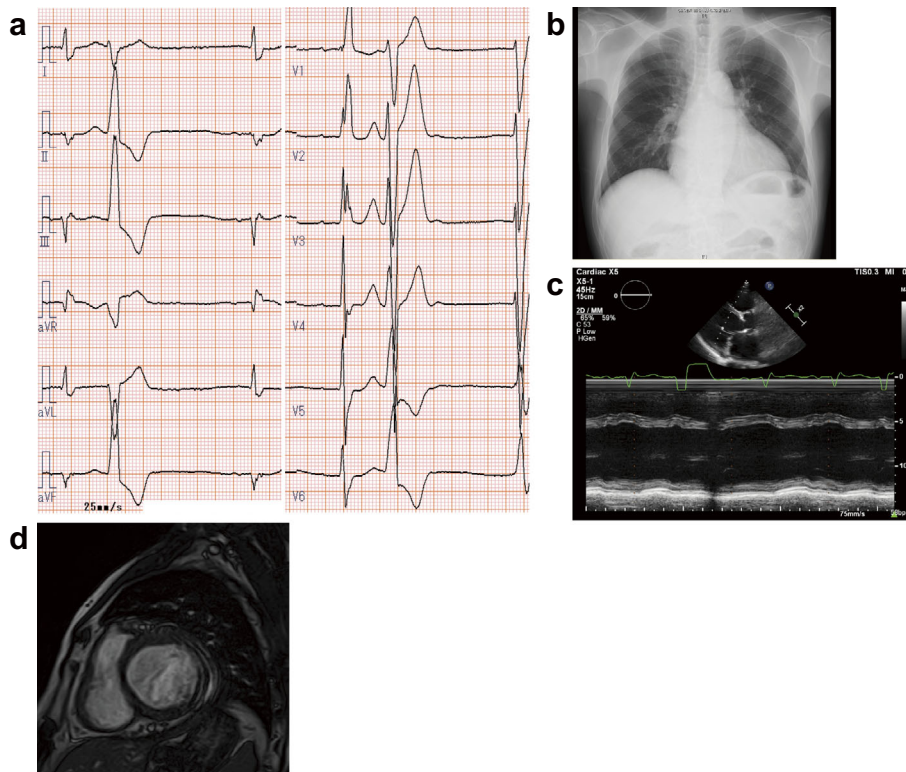


Figure 2. a: Electrocardiography showed a sinus rhythm, premature ventricular contraction, and heart rate of 64 bpm. b: Chest X-ray showed a slight congestion and a cardiothoracic ratio of 59.7%. c: Echocardiography detected a reduced left ventricular ejection fraction and a large left ventricular diastolic diameter. d: Cardiac magnetic resonance imaging revealed late gadolinium enhancement in the left ventricular lateral wall.

gestive or hypertrophic cardiomyopathy patients; in contrast, AMA-M7 was not detected in any other subjects in the same study (30).

In the present study, the prevalence of AMAs was 1.1% in DCM patients (30). We showed that AMA-positive DCM patients were associated with respiratory failure. Some patients with primary biliary cholangitis experience interstitial pneumonia, which might be a respiratory complication in patients with DCM associated with AMAs (3, 8, 31, 32). In the present study, the three AMA-positive patients underwent guideline-based medical treatments for heart failure with renin-angiotensin system inhibitors, beta blockers, amiodarone, implantable cardiac devices (CRTP, ICD), and radiofrequency catheter ablation. These intensive treatments might have improved the cardiac prognosis (e.g. arrhythmia, heart failure) in the current study's three AMA-positive patients with DCM. Non-cardiac events might thus have been involved as the cause of death in the current study's two AMA-positive patients who died.

AMAs are classified from M1 to M9. AMA M2, M4, M8, and M9 have been detected in cases of primary biliary cholangitis (33-36). AMA-M7 has been reported to be associated with cardiomyopathies and myocarditis (37). However, other studies have reported AMA M2 in patients with DCM (4, 28). The present study also identified AMA M2 in all three patients with AMA-positive DCM. One anecdotal

report described the infiltration of CD3-positive T cells of the myocardium in AMA M2-related cardiomyopathy (29). However, another study showed that there were no significant differences in the number of AMA M2-positive primary biliary cholangitis patients with and without cardiac involvement (12). The relationship between AMA M2 and cardiac phenotype needs to be clarified in further studies.

AMA-associated cardiac dysfunction has been reported to be improved after immunosuppressive therapy in anecdotal case reports (28, 29). Complication with cardiomyopathy has been reported in patients with systemic autoimmune diseases (38). Cardiomyopathy in patients with AMAs might be caused by autoimmune inflammatory response (11). There might be a subgroup of DCM that is related to AMAs.

Several limitations associated with the present study warrant mention. First, since the prevalence of AMAs was only 1.1% in 270 DCM patients, this study may have been too weak to perform a full statistical analysis, including Kaplan-Meier and Cox proportional hazard analyses. Second, this study was unable to completely demonstrate the clinical characteristics due to the small number of AMA-positive DCM patients. Further studies with more DCM patients with AMAs are needed in order to confirm the prognosis and characteristics of these patients. Third, since this was an observational study, a causal relationship between AMAs and

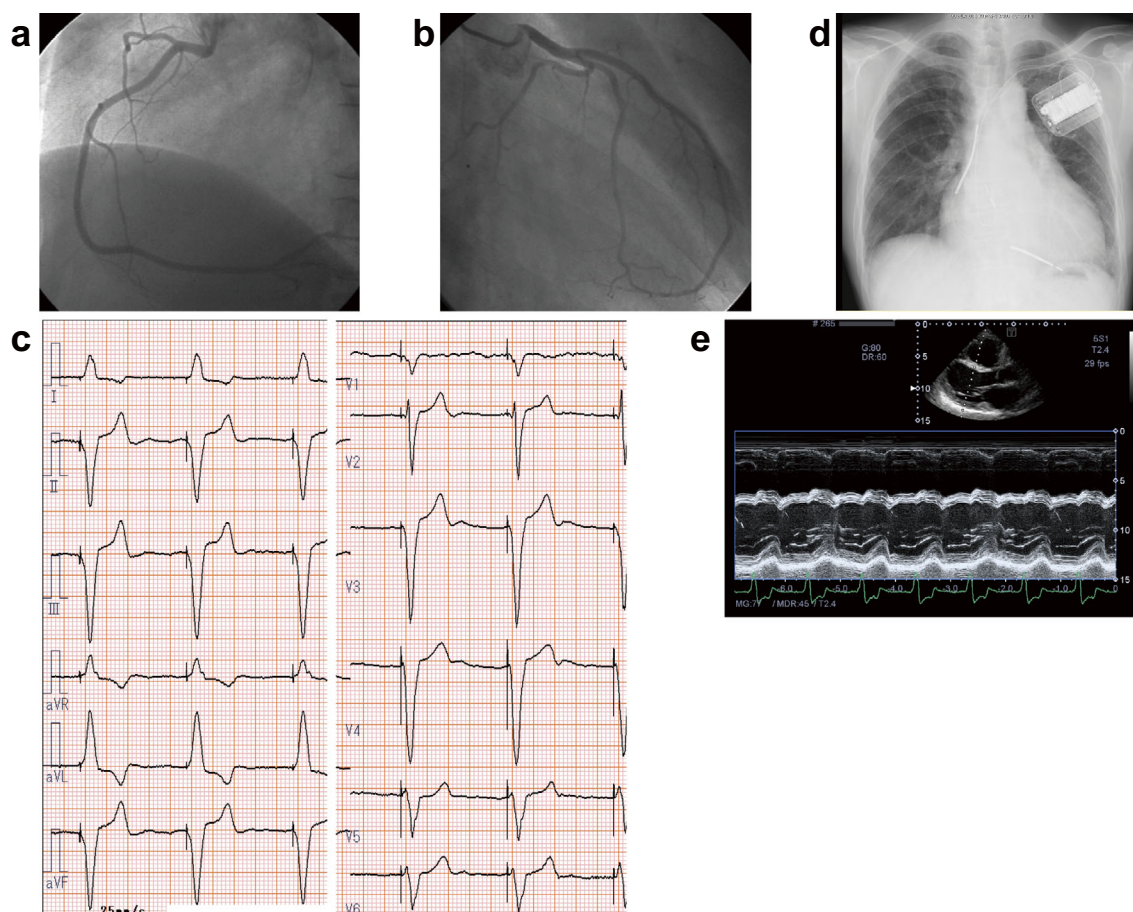


Figure 3. a, b: Coronary angiography revealed no significant stenosis. c: Electrocardiography showed a pacing rhythm and a heart rate of 59 bpm. d: Chest X-ray showed slight congestion and a cardiothoracic ratio of 64.9% with an implantable cardioverter defibrillator. e: Echocardiography detected a preserved ejection fraction and a slight large left ventricular diastolic diameter.

DCM could not be fully explained.

In conclusion, the prevalence of AMAs was only 1.1% in 270 DCM patients, and these patients suffered from respiratory failure. Thus, close attention should be paid to cases of respiratory failure in AMA-positive patients with DCM.

The authors state that they have no Conflict of Interest (COI).

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