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Original article

Low positive titer of anti-melanoma differentiation-associated gene 5 antibody is not associated with a poor long-term outcome of interstitial lung disease in patients with dermatomyositis

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ABSTRACT

Background: Anti-melanoma differentiation-associated gene 5 antibody (anti-MDA5-Ab) is associated with fatal rapidly progressive interstitial lung disease (RP-ILD) in patients with dermatomyositis (DM). We attempted to clarify whether anti-MDA5-Ab is associated with long-term outcomes in patients with DM-ILD.

Methods: Thirty-six patients with DM-ILD were retrospectively analyzed for their serum anti-MDA5-Ab by using an enzyme-linked immunosorbent assay. We analyzed the association between clinical parameters, including the serum levels of anti-MDA5-Ab and ferritin.

Abbreviations: ARS-Ab, aminoacyl-transfer RNA synthetase antibody; AUC, area under the curve; CADM, clinically amyopathic dermatomyositis; CI, confidence interval; CRP, C-reactive protein; $\%D_{LCO}$, diffusing capacity of the lungs for carbon monoxide, predicted; DM-ILD, dermatomyositis-interstitial lung disease; ELISA, enzyme-linked immunosorbent assay; %FVC, forced vital capacity, predicted; HRCT, high-resolution computed tomography; IP, immunoprecipitation; KL-6, Krebs von den Lungen 6; LDH, lactate dehydrogenase; MDA5-Ab, melanoma differentiation-associated gene 5 antibody; NPV, negative predictive value; NSIP, non-specific interstitial pneumonia; OP, organizing pneumonia; OS, overall survival; PPV, positive predictive value; ROC, receiver operating characteristic; RP-ILD, rapidly progressive interstitial lung disease; RR, relative risk; UIP, usual interstitial pneumonia

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Results: Fourteen patients (39%) were positive for anti-MDA5-Ab. The serum levels of anti-MDA5-Ab and ferritin in 7 patients with acute death were higher than those in the surviving patients. An "unclassifiable pattern" on chest computed tomography and the development of RP-ILD were also prognostic markers. The serum levels of anti-MDA5-Ab and ferritin (cut-off levels, 100 IU/mL and 899 ng/mL, respectively) were markers predictive of acute death, showing good sensitivity (86% and 83%) and specificity (97% and 100%). All 7 patients with acute death developed RP-ILD and were positive for anti-MDA5-Ab, including 6 patients with a high titer ($\geq$ 100 IU/mL), whereas only 2 patients (29%) developed RP-ILD among the 7 survivors with a low titer of anti-MDA5-Ab ($<$ 100 IU/mL). In contrast, a low positive titer of anti-MDA5-Ab was not associated with changes in pulmonary function for 2 years.

Conclusions: Although a high serum titer of anti-MDA5-Ab ($\geq$100 IU/mL) is associated with acute death via the development of RP-ILD, outcomes in the chronic phase for patients with a low titer of anti-MDA5-Ab ($<$ 100 IU/mL) were similar to those of patients without anti-MDA5-Ab.

1. Introduction

Interstitial lung disease (ILD) is a common involvement in dermatomyositis (DM) [1]. A phenotype of DM patients with definite cutaneous manifestations but lacking clinically significant muscle involvement is defined as having clinically amyopathic DM (CADM). CADM-ILD patients frequently develop fatal rapidly progressive ILD (RP-ILD) [2–4].

Melanoma differentiation-associated gene 5 (MDA5) is a protein that acts as a sensor for RNA viruses in the innate immune system [5]. The anti-CADM-140 antibody (Ab), initially identified using protein immunoprecipitation (IP), was later found to recognize the MDA5 antigen [3]. The fact that anti-MDA5-Ab is associated with CADM and/or RP-ILD is of clinical importance [2–4,6–9]. DM-ILD patients with anti-MDA5-Ab were reported to have a worse prognosis, compared with anti-aminoacyl-transfer RNA synthetase antibody (anti-ARS-Ab) [9]. However, we and other groups previously reported that DM-ILD patients with anti-MDA5-Ab who survived for 3 months from ILD onset or after commencing therapy did not develop fatal respiratory failure during long-term [8,9]. The presence of anti-MDA5-Ab is a prognostic marker for developing fatal RP-ILD, especially during the acute phase; however, the clinical significance of this autoantibody in the chronic phase of DM-ILD is unclear [3]. Sato et al. suggested that a high anti-MDA5-Ab titer measured using an enzyme-linked immunosorbent assay (ELISA) was associated with the development of RP-ILD and a poor outcome in DM-ILD patients [3,4,6,7]. ELISA showed high sensitivity and specificity (85% and 100%) when the cut-off level was set at 8 IU/mL and IP was set as the reference [3]. We attempted to identify whether a high anti-MDA5-Ab titer in DM-ILD patients was indeed associated with acute death, and whether a low positive titer was associated with the long-term outcome.

2. Patients and methods

2.1. Study subjects

We retrospectively reviewed the medical records of 36 consecutive DM-ILD patients who had visited our hospital for the first time between January 2007 and December 2015. DM was diagnosed according to the Japanese criteria for the diagnosis of DM (revised in 2015). ILD was diagnosed on the basis of high-resolution computed tomography (HRCT) and clinical data. Patients with other conditions, such as pulmonary infectious disease or uncontrolled malignant disease, were excluded. The diagnosis of CADM was based on the diagnostic criteria proposed by Sontheimer [10], i.e., DM patients who had typical DM rashes but not clinical evidence of muscle symptoms for more than 6 months. A subset of RP-ILD patients were defined as those presenting with progressive dyspnea and hypoxemia as well as worsening of interstitial change on chest radiography within 1 month from the onset of respiratory symptoms, as described previously [2]. We set the endpoint for evaluating the outcome in the acute phase as mortality within 3 months after commencing therapy, as reported previously [9,11]. Similarly, we set the endpoint for evaluating the outcome in the chronic phase as overall survival (OS) and changes in the forced vital capacity, predicted (%FVC) and diffusing capacity of the lungs for carbon monoxide, predicted (%D_{LCO}) 6, 12, 18, and 24 months after commencing therapy in patients for whom pulmonary function testing were possible.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of our hospital (Approval date: March 31, 2016; Approval number: 15276) and with the 2013 Helsinki Declaration. Informed consent to participate in this study was obtained from all patients.

2.2. Interpretation of HRCT images

The protocol for pretreatment HRCT is described in the supplementary materials as reported previously [12]. HRCT images were interpreted independently by two board-certified experienced radiologists blinded to the clinical information. ILD patterns were classified into definite usual interstitial pneumonia (UIP), possible UIP, nonspecific interstitial pneumonia (NSIP), NSIP with organizing pneumonia (OP), and OP pattern based on the global guideline of idiopathic interstitial pneumonias as described in the

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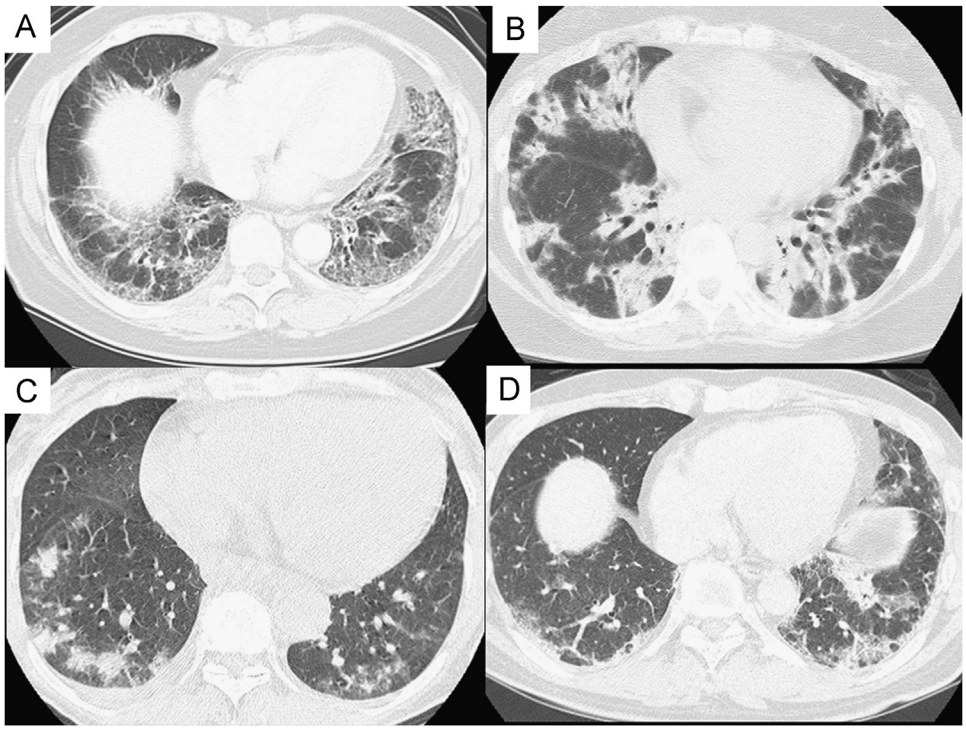

Fig. 1 – HRCT features. Representative HRCT images. (A) An image of the lower lung zones of a 59-year-old woman without anti-ARS-Ab and anti-MDA5-Ab. This was classified as the NSIP pattern showing characteristic ground-glass opacities with dilated air bronchogram (or bronchiologram) showing peribronchovascular and subpleural distribution. (B) An image of the lower lung zones of a 51-year-old woman with anti-Jo-1-Ab and without anti-MDA5-Ab. This was classified as the NSIP with OP pattern showing characteristic consolidations and ground-glass opacities with dilated air bronchogram (or bronchiologram) showing peribronchovascular distribution. (C) An image of the lower lung zones of a 68-year-old woman without anti-MDA5-Ab and anti-ARS-Ab. This was classified as the OP pattern showing patchy consolidations without architectural distortion. (D) An image of the lower lung zones of a 50-year-old man with a high titer of anti-MDA5-Ab without anti-ARS-Ab. This was considered to be the unclassifiable CT pattern with consolidations and ground-glass opacities showing peripheral and subpleural distribution with architectural distortion. HRCT: high-resolution computed tomography; anti-ARS-Ab: anti-aminoacyl-transfer RNA synthetase antibody; anti-MDA5-Ab: melanoma differentiation-associated gene 5 antibody; NSIP: non-specific interstitial pneumonia; OP: organizing pneumonia.

supplementary materials [13,14]. HRCT patterns that could not be otherwise classified were denoted as unclassifiable CT patterns, as reported previously [8,13,14]. Representative HRCT images are shown in Fig. 1.

2.3. *Measurement of autoantibodies*

All serum samples were collected before commencing therapy. The anti-MDA5-Ab titer was measured using an ELISA, as described previously [3]. The cut-off level of the ELISA for anti-MDA5-Ab was 8 IU/mL [3]. Anti-ARS-Ab was screened using the RNA-IP assay, as described previously [15].

2.4. *Statistical analysis*

Data were expressed as the median (25th to 75th percentiles of the interquartile range). Interobserver variations in the ILD patterns of HRCT were analyzed using the kappa statistic on the final diagnosis made prior to agreement by consensus. Differences between multiple groups were analyzed appropriately by using the Wilcoxon rank-sum or Fisher's exact tests. The cut-off levels for various parameters were defined as the optimal value with the highest Youden index on a receiver operating characteristic (ROC) curve generated using logistic regression [12]. $P < 0.05$ was considered to represent statistical significance. All statistical analyses were performed using the JMP 12.0 program (SAS Institute Japan, Tokyo, Japan).

3. Results

3.1. *Characteristics and clinical courses*

The characteristics and clinical courses of the analyzed patients are shown in Table 1. The 36 DM-ILD patients included 14 (39%) CADM patients, 14 (39%) anti-MDA5-Ab-positive patients, and 15 (42%) anti-ARS-Ab-positive patients. One patient was positive for both anti-ARS-Ab and anti-MDA5-Ab, and 8 were negative for both. The antibody subtypes of anti-ARS-Ab-positive patients included anti-PL-7-Ab in 5 patients, anti-EJ-Ab in 4, anti-Jo-1-Ab in 3, anti-PL-12-Ab in 2, and anti-KS-Ab in 1. One of the 36 DM-ILD patients had undergone surgical lung biopsy and was diagnosed as having

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Table 1 – Patient characteristics.

Number of DM-ILD patients	36	**Overall survival** (days)	1070.0 (268.8–1543.3)
Number of CADM-ILD patients	14	**Mortality**	
Age	59.5 (50.3–68.0)	**Within 3 months**	
Gender: Male	13 (36%)	Survivor	29 (81%)
Smoker	11 (31%)	Non-survivor	7 (19%)
No. of RP-ILD	14 (39%)	Cause of death	RP-ILD, 7
Baseline blood data		**For all observation period**	
CRP (mg/dL)	0.51 (0.14–2.3)	Survivor	28 (78%)
KL-6 (IU/mL)	856.0 (568.0–1398.0)	Non-survivor	8 (22%)
LDH (IU/L)	372.0 (264.8–462.8)	Cause of death	RP-ILD, 7; AE, 1
Creatinine kinase (IU/L)	160.5(62.3–704.3)	**Therapy (All course)**	
Ferritin (ng/mL)	357.5 (125.0–802.5)	**Corticosteroid**	34 (94%)
Serum anti-MDA5-Ab and anti-ARS-Ab		**Immunosuppressant**	30 (83%)
With both antibodies	1 (3%)	Tacrolimus	28 (78%)
With anti-MDA5-Ab alone	13 (36%)	Cyclosporin A	13 (36%)
With anti-ARS-Ab alone	14 (39%)	Intravenous cyclophosphamide	9 (25%)
Without either antibody	8 (22%)	Azathioprine	2 (6%)
Titer of anti-MDA5-Ab (IU/mL)	1.3 (0.78–73.3)	Mycophenolate mofetil	1 (3%)
Pulmonary function test (%)			
%FVC	72.3 (64.1–84.1)		
$\%D_{LCO}$	60.8 (46.9–80.0)		
HRCT pattern			
Definite and possible UIP	0		
NSIP	10 (28%)		
NSIP with OP	14 (39%)		
OP	2 (6%)		
Unclassifiable	10 (28%)		

DM-ILD: dermatomyositis-interstitial lung disease; CADM: clinically amyopathic DM; RP-ILD: rapidly progressive ILD; CRP: C-reactive protein; KL-6: Krebs von den Lungen 6; LDH: lactate dehydrogenase; anti-MDA5-Ab: anti-melanoma differentiation-associated gene 5 antibody; anti-ARS-Ab: anti-aminoacyl-transfer RNA synthetase antibody; %FVC: forced vital capacity, predicted; $\%D_{LCO}$: diffusing capacity of the lungs for carbon monoxide, predicted; HRCT: high-resolution computed tomography; UIP: usual interstitial pneumonia; NSIP: non-specific interstitial pneumonia; OP: organizing pneumonia; AE: acute exacerbation.

NSIP. Fourteen of the 36 patients (39%) were diagnosed as having RP-ILD.

Interobserver agreement for the assessment of HRCT patterns was moderate (kappa, 0.60; $P < 0.0001$). The HRCT patterns of ILD among the 36 DM-ILD patients, included NSIP with OP pattern in 14 patients (39%), unclassifiable CT pattern in 10 (28%), NSIP pattern in 10 (28%), and OP pattern in 2 (5%). No patient showed a definite or possible UIP pattern. The HRCT images of anti-MDA5-Ab-positive patients showed the following patterns: unclassifiable CT pattern in 9 patients (64%), NSIP with OP pattern in 4 (29%), and NSIP pattern in 1 (7%). The HRCT images of anti-MDA5-Ab-negative patients showed the following patterns: NSIP with OP pattern in 10 patients (45%), NSIP pattern in 9 (41%), OP pattern in 2 (9%), and unclassifiable CT pattern in 1 (5%). The unclassifiable CT pattern was more frequent in patients positive for anti-MDA5-Ab-positive patients than in anti-MDA5-Ab-negative patients ($P < 0.0001$*).

The median observation period from commencing therapy was 1070 (268.8–1543.3) [range, 2–3446] days. Eight (22%) of the 36 DM-ILD patients died during the observation period. Seven patients (19%) died of RP-ILD within 3 months after commencing therapy (acute phase). The remaining anti-MDA5-Ab-negative patient died of respiratory failure due to ILD after the acute phase. Thirty patients (83%) received prednisolone in combination with an immunosuppressant and 4 (11%) received prednisolone alone.

3.2. *Predictors of acute death and OS*

We compared the clinical data between the 29 survivors and 7 non-survivors at 3 months after commencing therapy to clarify the predictors of acute death (Table 2). The serum levels of anti-MDA5-Ab ($P < 0.001$) and ferritin ($P < 0.001$) were higher in patients with acute death than in the surviving patients. The unclassifiable CT pattern and development of RP-ILD were also associated with acute death ($P < 0.05$). The sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), and results of ROC analyses for distinguishing patients with acute death from the surviving patients are shown in Table 3. The optimal cut-off levels of anti-MDA5-Ab and ferritin based on the ROC curves were 100 IU/mL and 899 ng/mL, respectively. The accuracy for predicting acute death was higher for the anti-MDA5-Ab titer (sensitivity, 86%; specificity, 97%; PPV, 86%; and NPV, 97%), ferritin level (sensitivity, 83%; specificity, 100%; PPV, 100%; and NPV, 96%), and development of RP-ILD (sensitivity, 100%; specificity, 76%; PPV, 50%; and NPV, 100%) than for the unclassifiable CT pattern (sensitivity, 71%; specificity, 83%; PPV, 50%; and NPV, 92%). Among the 14 anti-MDA5-Ab-positive patients, we compared the clinical data between the 7 survivors and 7 non-survivors during the acute phase (Table 4). The serum levels of anti-MDA5-Ab, ferritin, and C-reactive protein and the incidence of RP-ILD were all higher in patients with acute death than in the surviving patients (all $P < 0.05$). Acute death patients

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Table 2 – Clinical data for patients with or without acute death within 3 months after commencing therapy.

	Survivor in acute phase	Non-survivor in acute phase	P value
Number of DM-ILD patients	29	7	
Number of CADM-ILD patients	10 (34%)	4 (57%)	NS
Number of RP-ILD patients	7 (24%)	7 (100%)	< 0.001***
Age	57.0 (50.0–68.0)	62.0 (53.0–72.0)	NS
Gender; Male	10 (34%)	3 (43%)	NS
Smoker	10 (34%)	1 (14%)	NS
Baseline blood test			
CRP (mg/dL)	0.50 (0.13–1.7)	1.8 (0.25–2.5)	NS
KL-6 (IU/mL)	1021.0 (535.5–1477.0)	693.5 (536.0–875.5)	NS
LDH (IU/L)	356.0 (258.0–460.5)	426.0 (276.0–484.0)	NS
Creatinine kinase (IU/L)	155.0 (62.0–753.0)	191.0 (72.0–395.0)	NS
Ferritin (ng/mL)	157.9 (113.5–436.5)	1370.3 (768.8–2177.8)	< 0.001***
Positivity for anti-MDA5-Ab	7 (24%)	7 (100%)	< 0.001***
Titer of anti-MDA5-Ab	0.98 (0.77–10.6)	311.9 (102.9–603.2)	< 0.001***
High titer (≥ 100 IU/mL)	1 (3%)	6 (83%)	
Low titer (< 100 IU/mL)	6 (21%)	1 (17%)	
Pulmonary function test (%)			
%FVC	72.8 (62.6–86.2)	64.7 (64.4–76.0)	NS
$\%D_{LCO}$	63.3 (46.9–82.4)	52.8 (36.9–57.6)	NS
HRCT patterns			
Definite and possible UIP	0	0	
NSIP	10 (34%)	0	
NSIP with OP	12 (41%)	2 (29%)	
OP	2 (7%)	0	
Unclassifiable	5 (2%)	5 (71%)	< 0.05*

DM-ILD: dermatomyositis-interstitial lung disease; CADM: clinically amyopathic DM; RP-ILD: rapidly progressive ILD; CRP: C-reactive protein; KL-6: Krebs von den Lungen 6; LDH: lactate dehydrogenase; anti-MDA5-Ab: anti-melanoma differentiation-associated gene 5 antibody; %FVC: forced vital capacity, predicted; $\%D_{LCO}$: diffusing capacity of the lungs for carbon monoxide, predicted; HRCT: high-resolution computed tomography; UIP: usual interstitial pneumonia; NSIP: non-specific interstitial pneumonia; OP: organizing pneumonia; NS: not significant. *P < 0.05 and ***P < 0.001 were considered to represent statistical significance.

Table 3 – Accuracy of the predictors of acute death in DM-ILD patients.

						ROC analysis	
	Cut-off level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)	P value
Anti-MDA5-Ab	≥ 100 IU/mL	86	97	86	97	97	< 0.01**
Ferritin	≥ 899 ng/mL	83	100	100	96	95	< 0.05*
RP-ILD	RP-ILD	100	76	50	100		
HRCT pattern	Unclassifiable	71	83	50	92		

DM-ILD: dermatomyositis-interstitial lung disease; ROC: receiver operating characteristic; PPV: positive predictive value; NPV: negative predictive value; AUC: area under the curve; Anti-MDA5-Ab: anti-melanoma differentiation-associated gene 5 antibody; RP-ILD; rapidly progressive interstitial lung disease, HRCT; high-resolution computed tomography. *P < 0.05 and **P < 0.01 were considered to represent statistical significance.

were also significantly older than the surviving patients ($P < 0.05$). All 7 acute death patients developed RP-ILD and were positive for anti-MDA5-Ab, including 6 patients with a high titer (≥ 100 IU/mL), whereas among the 7 survivors with a low titer of anti-MDA5-Ab (< 100 IU/mL), only 2 (29%) developed RP-ILD (Fig. 2; Table 4). Among the 29 survivors, only 1 (3%) had a high titer of anti-MDA-5-Ab (330.6 IU/mL) and did not develop RP-ILD.

We analyzed the predictors of OS by using the log-rank test. A higher anti-MDA5-Ab titer (≥ 100 IU/mL; relative risk (RR), 26; 95% confidence interval (CI), 5.7–184.2; $P < 0.0001$), higher ferritin level (≥ 899 ng/mL; RR, 49.6; 95% CI, 7.6–971.5; $P < 0.0001$), unclassifiable CT pattern (RR, 5.3; 95% CI, 1.3–26.1; $P < 0.05$), and development of RP-ILD (RR, 16; 95% CI, 2.8–300.4; $P < 0.001$), which were the predictors of acute death, were also predictors of OS (Fig. 2).

3.3. *Predictors of the outcome of pulmonary function in the chronic phase*

We attempted to clarify the outcome of pulmonary function in 7 anti-MDA5-Ab-positive patients who survived for 3 months after commencing therapy (acute phase). We evaluated the association between prognostic factors such as anti-MDA5-Ab and changes in pulmonary function in the 29 surviving

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Table 4 – Clinical data for 14 patients with anti-MDA5-Ab with or without acute death.

	Survivor in acute phase	Non-survivor in acute phase	*P* value
Number of patients with DM-ILD	7	7	NS
Number of patients with CADM-ILD	5 (71%)	4 (57%)	NS
Number of patients with RP-ILD	2 (29%)	7 (100%)	$< 0.05^{*}$
Age	44.0 (38.0–54.0)	62.0 (53.0–72.0)	$< 0.05^{*}$
Sex: Male	2 (29%)	3 (43%)	NS
Smoker	1 (14%)	1 (14%)	NS
Baseline blood			
CRP (mg/dL)	0.11 (0.040–0.95)	1.8 (0.25–2.5)	$< 0.05^{*}$
KL-6 (IU/mL)	1060.0 (491.0–1398.0)	693.5 (536.0–875.5)	NS
LDH (IU/L)	320.0 (273.0–453.0)	426.0 (276.0–484.0)	NS
Creatinine kinase (IU/L)	94.0 (49.0–324.0)	191.0 (72.0–395.0)	NS
Ferritin (ng/mL)	199.0 (82.0–844.0)	1370.3 (768.8–2177.8)	$< 0.05^{*}$
Titer of anti-MDA5-Ab (IU/mL)	71.1 (45.3–86.9)	311.9 (102.9–603.2)	$< 0.05^{*}$
High titer ($\geq$ 100 IU/mL)	1 (17%)	6 (83%)	
Low titer ($<$ 100 IU/mL)	6 (83%)	1 (17%)	
Pulmonary function test (%)			
%FVC	77.5 (61.3–86.3)	64.7 (64.4–76.0)	NS
$\%D_{LCO}$	66.4 (45.6–84.4)	52.8 (36.9–57.6)	NS
HRCT patterns			
Definite and possible UIP	0	0	
NSIP	1 (14%)	0	
NSIP with OP	2 (29%)	2 (29%)	
OP	0	0	
Unclassifiable	4 (57%)	5 (71%)	NS

MDA5-Ab: melanoma differentiation-associated gene 5 antibody; DM-ILD: dermatomyositis-interstitial lung disease; CADM: clinically amyopathic DM; RP-ILD: rapidly progressive ILD; CRP: C-reactive protein; KL-6: Krebs von den Lungen 6; LDH: lactate dehydrogenase; %FVC: forced vital capacity, predicted; %DLCO: diffusing capacity of the lungs for carbon monoxide, predicted; HRCT: high-resolution computed tomography; UIP: usual interstitial pneumonia; NSIP: non-specific interstitial pneumonia; OP: organizing pneumonia; NS: not significant.
* $P < 0.05$ was considered to represent statistical significance.

patients after the acute phase. The %FVC at 6, 12, 18, and 24 months after commencing therapy was measured in 19, 20, 15, and 16 patients, respectively, and $\%D_{LCO}$ was measured at the same time points in 19, 20, 15, and 15 patients, respectively. Fig. 3 shows the %FVC and $\%D_{LCO}$ of patients with and without anti-MDA5-Ab after commencing therapy. No significant difference was observed in the change in %FVC or $\%D_{LCO}$ between the patients with and without anti-MDA5-Ab at all the measurement time points during the chronic phase. Similarly, neither the unclassifiable CT pattern, high serum ferritin level, nor development of RP-ILD was associated with the changes in %FVC and $\%D_{LCO}$ (data not shown).

4. Discussion

We confirmed that a high serum level of anti-MDA5-Ab and ferritin and the development of RP-ILD could predict acute death in DM-ILD patients as reported previously [4,6,7]. A previous study suggested that the anti-MDA5-Ab titer was significantly higher in anti-MDA5-Ab-positive DM patients with RP-ILD than in those without RP-ILD [6]. A high anti-MDA5-Ab titer is considered to be associated with acute death via the development of RP-ILD. However, we could not prove that a low titer of anti-MDA5-Ab was a favorable prognostic predictor of acute death, because few patients developed RP-ILD. In our study, the diagnostic accuracy for the serum levels of anti-MDA5-Ab and ferritin exhibited high sensitivity and specificity. Sato et al. suggested that the mean anti-MDA5-Ab titer was significantly higher in survivors ($n = 4$) than in non-survivors ($n = 6$) among anti-MDA5-Ab-positive DM-ILD patients (356.9 vs. 110.3 IU/mL) [4]. The autoantibody titer in survivors with anti-MDA5-Ab was higher in the previous study than in our study (71.1 IU/mL). However, both studies equally noted that a high anti-MDA5-Ab titer ($\geq$ 100 IU/mL) was associated with higher mortality. Analysis involving a larger population will be needed to identify the optimal cut-off level, as the previous and our study both involved small cohorts. Moreover, we suggested that patients with a low titer of anti-MDA5-Ab ($<$ 100 IU/mL) maintained their pulmonary function for 2 years similar to anti-MDA5-Ab-negative patients. A decrease in FVC or D_{LCO} is a well-known significant risk factor for mortality in idiopathic pulmonary fibrosis patients [16,17]. The maintenance of pulmonary function is associated with increased survival and quality of life in ILD patients. Our results suggest that a low titer of anti-MDA5-Ab was not associated with a poor long-term outcome in DM-ILD patients. We have previously reported that 4 of 9 polymyositis/dermatomyositis (PM/DM)-ILD patients with anti-MDA5-Ab died of RP-ILD, whereas in the 5 remaining survivors positive of anti-MDA5-Ab was not associated with the deterioration of pulmonary function for 1 year [9]. Thus, the clinical importance of anti-MDA5-Ab differs between the acute and chronic phases of DM-ILD.

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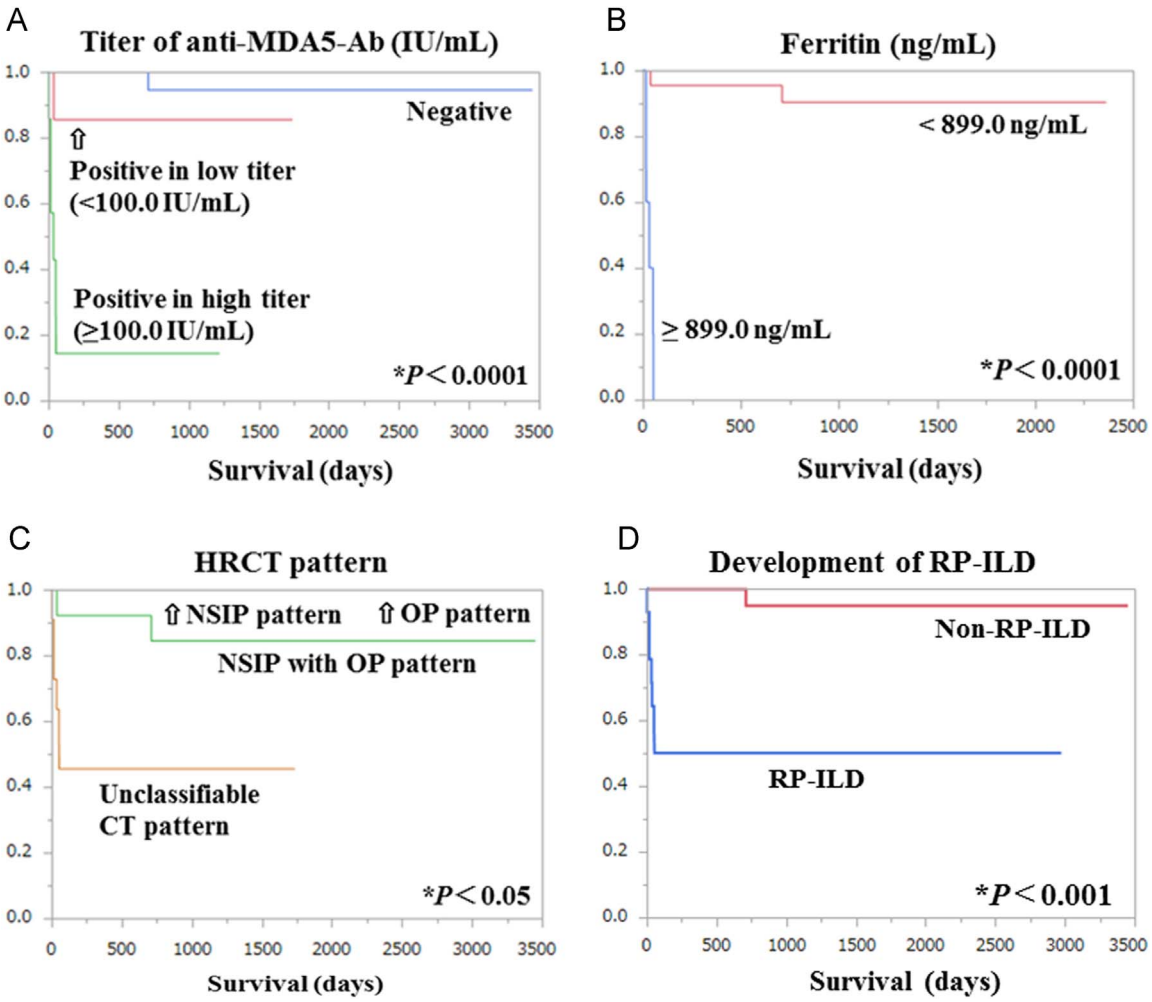

Fig. 2 – Survival curves. Survival curves obtained using significant predictors of overall survival in the log-rank test. A, positivity for anti-MDA5-Ab with a high (≥ 100 IU/mL) or low (<100 IU/mL) titer; B, high serum ferritin level (≥ 899 ng/mL or < 899.9 ng/mL); C, ILD pattern on HRCT; D, development of RP-ILD. Anti-MDA5: anti-melanoma differentiation-associated gene 5 antibody; ILD: interstitial lung disease; HRCT: high-resolution computed tomography; RP-ILD: rapidly progressive interstitial lung disease.

A previous report suggested that intensive immunosuppressive therapy, including corticosteroids, calcineurin inhibitors, and intravenous cyclophosphamide, was required for DM-RPILD patients with anti-MDA5-Ab from the early phase of disease [18]. However, such therapy may be associated with severe adverse events, such as infectious diseases. To optimize the therapeutic strategy, a biomarker for predicting acute death is required. A high serum anti-MDA5-Ab titer (≥ 100 IU/mL) as well as a high ferritin level may have some potential as biomarkers to identify patients who require intensive immunosuppressive therapy in the acute phase. Conversely, the presence of this autoantibody at a low titer (<100 IU/mL) may not affect the therapeutic strategy used during the chronic phase. Although anti-MDA5-Ab is a useful prognostic biomarker, it should be noted that its clinical importance differs based on the serum titer.

In anti-MDA5-Ab-positive-DM-ILD patients, the radiological ILD pattern tended to be the unclassifiable CT pattern, which was predominant over the NSIP with or without OP pattern, as reported previously [8]. Moreover, the unclassifiable CT pattern was associated with acute death. In contrast, we and other groups have reported anti-ARS-Ab-positive patients exhibited the radiological NSIP with or without OP pattern [9,19]. The NSIP with or without OP pattern is reportedly associated with a more favorable response to immunosuppressive therapy than other ILD subtypes such as UIP [14]. The HRCT findings in anti-MDA5-Ab-positive patients were distinct from those without anti-MDA5-Ab, including anti-ARS-Ab-positive patients. Thus, in addition to serological data, radiological findings appear valuable for predicting the prognosis of DM-ILD.

The present study had three limitations. First, because it was a single-center study, it involved only a small patient cohort. Second, we were unable to follow changes in the anti-MDA5-Ab titer because the number of serum samples collected was insufficient. However, our study suggested early biomarkers capable of predicting the outcome of DM-ILD. Third, the change in pulmonary function could not be measured in all of the study subjects after commencing therapy. This may have affected our results. However, we

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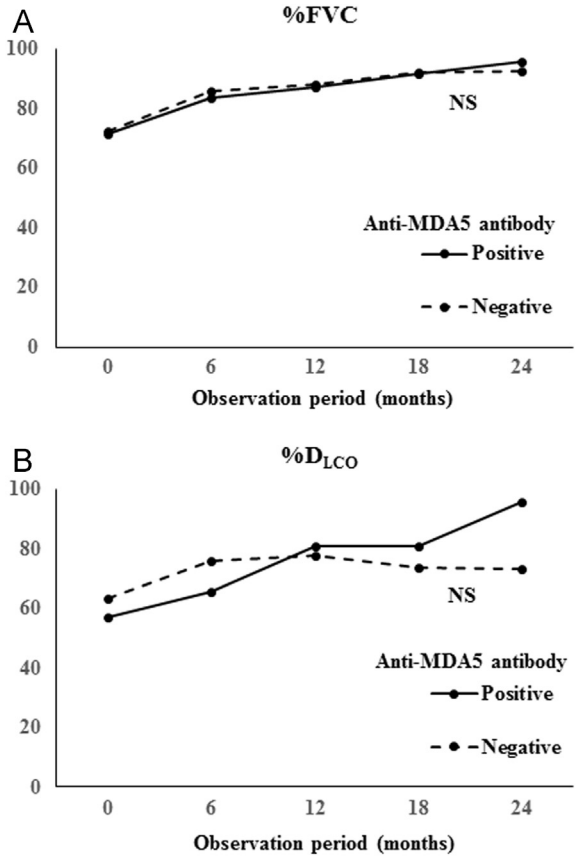

Fig. 3 – Changes in forced vital capacity, predicted (%FVC) and diffusing capacity of the lung for carbon monoxide, predicted (%D_{LCO}) determined every 6 months. Changes in pulmonary function parameters at 6, 12, 18, and 24 months after commencing therapy in groups positive and negative for anti-MDA5-Ab (A, %FVC, B, %D_{LCO}). Anti-MDA5-Ab: anti-melanoma differentiation-associated gene 5 antibody; NS: not significant.

believe that our results are valid, because no significant difference was observed in the pulmonary function measurements between patients with and without the various prognostic factors.

5. Conclusions

Although a high serum titer of anti-MDA5-Ab ($\geq$ 100 IU/mL) is associated with acute death via the development of RP-ILD, patients with a low titer of anti-MDA5-Ab ($<$ 100 IU/mL) showed an outcome in the chronic phase similar to that of anti-MDA5-Ab-negative-patients. Anti-MDA5-Ab is a useful biomarker that facilitates the identification of DM-ILD patients who require intensive immunosuppressive therapy in the acute phase, but does not affect the therapeutic strategy applied during the chronic phase.

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Conflict of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.resinv.2018.07.007.

REFERENCES

[1] Fathi M, Dastmalchi M, Rasmussen E, Lundberg IE, Tornling G. Interstitial lung disease, a common manifestation of newly diagnosed polymyositis and dermatomyositis. Ann Rheum Dis 2004;63:297–301.

[2] Sato S, Hirakata M, Kuwana M, Suwa A, Inada S, Mimori T, et al. Autoantibodies to a 140-kd polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. Arthritis Rheum 2005;52:1571–6.

[3] Sato S, Hoshino K, Satoh T, Fujita T, Kawakami Y, Fujita T, et al. RNA helicase encoded by melanoma differentiation–associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: association with rapidly progressive interstitial lung disease. Arthritis Rheum 2009;60:2193–200.

[4] Sato S, Kuwana M, Fujita T, Suzuki Y. Anti-CADM-140/MDA5 autoantibody titer correlates with disease activity and predicts disease outcome in patients with dermatomyositis and rapidly progressive interstitial lung disease. Mod Rheumatol 2013;23:496–502.

[5] Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J Exp Med 2008;205:1601–10.

[6] Sato S, Murakami A, Kuwajima A, Takehara K, Mimori T, Kawakami A, et al. Clinical utility of an enzyme-linked immunosorbent assay for detecting anti-melanoma differentiation-associated gene 5 autoantibodies. PLoS One 2016;11:e0154285.

[7] Gono T, Sato S, Kawaguchi Y, Kuwana M, Hanaoka M, Katsumata Y, et al. Anti-MDA5 antibody, ferritin and IL-18 are useful for the evaluation of response to treatment in interstitial lung disease with anti-MDA5 antibody-positive dermatomyositis. Rheumatology 2012;51:1563–70.

[8] Hozumi H, Fujisawa T, Nakashima R, Johkoh T, Sumikawa H, Murakami A, et al. Comprehensive assessment of myositis-specific autoantibodies in polymyositis/dermatomyositis-associated interstitial lung disease. Respir Med 2016;121:91–9.

[9] Yoshida N, Okamoto M, Kaieda S, Fujimoto K, Ebata T, Tajiri M, et al. Association of anti-aminoacyl-transfer RNA synthetase antibody and anti-melanoma differentiation-associated gene 5 antibody with the therapeutic response of

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polymyositis/dermatomyositis-associated interstitial lung disease. Respir Investig 2017;55:24–32.

[10] Sontheimer RD. Would a new name hasten the acceptance of amyopathic dermatomyositis (dermatomyositis sine myositis) as a distinctive subset within the idiopathic inflammatory dermatomyopathies spectrum of clinical illness? J Am Acad Dermatol 2002;46:626–36.

[11] Kataoka K, Taniguchi H, Kondoh Y, Nishiyama O, Kimura T, Matsuda T, et al. Recombinant human thrombomodulin in acute exacerbation of idiopathic pulmonary fibrosis. Chest 2015;148:436–43.

[12] Tajiri M, Okamoto M, Fujimoto K, Johkoh T, Ono J, Tominaga M, et al. Serum level of periostin can predict long-term outcome of idiopathic pulmonary fibrosis. Respir Investig 2015;53:73–81.

[13] Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788–824.

[14] Travis WD, Costabel U, Hansell DM, King Jr TE, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013;188:733–48.

[15] Forman MS, Nakamura M, Mimori T, Gelpi C, Hardin JA. Detection of antibodies to small nuclear ribonucleoproteins and small cytoplasmic ribonucleoproteins using unlabeled cell extracts. Arthritis Rheum 1985;28:1356–61.

[16] Flaherty KR, Mumford JA, Murray S, Kazerooni EA, Gross BH, Colby TV, et al. Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J Respir Crit Care Med 2003;168:543–8.

[17] Latsi PI, du Bois RM, Nicholson AG, Colby TV, Bisirtzoglou D, Nikolakopoulou A, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med 2003;168:531–7.

[18] Nakashima R, Hosono Y, Mimori T. Clinical significance and new detection system of autoantibodies in myositis with interstitial lung disease. Lupus 2016;25:925–33.

[19] Debray MP, Borie R, Revel MP, Naccache JM, Khalil A, Toper C, et al. Interstitial lung disease in anti-synthetase syndrome: initial and follow-up CT findings. Eur J Radiol 2015;8:516–23.

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