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Coexistence of Anti-Ro52 Antibodies in Anti-MDA5 Antibody–Positive Dermatomyositis Is Highly Associated With Rapidly Progressive Interstitial Lung Disease and Mortality Risk

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ABSTRACT. ***Objective.*** Interstitial lung disease (ILD) is a common extramuscular complication contributing to significant morbidity and mortality in patients with dermatomyositis (DM) who are positive for antimelanoma differentiation–associated gene 5 antibody (anti-MDA5+). We conducted this study to investigate the association of anti-Ro52 antibodies with clinical characteristics and prognosis in patients with anti-MDA5+ DM.

Methods. We assessed a cohort of 246 patients with anti-MDA5+ DM. To calculate hazard ratios and 95% CIs for rapidly progressive ILD (RP-ILD) and death while controlling for potential confounders, variables selected by univariate Cox regression analysis were included in a multivariate Cox regression model with the stepwise forward-selection method. A 2-tailed analysis with $P < 0.05$ was considered to be statistically significant.

Results. A total of 246 patients with anti-MDA5+ DM were enrolled; 70 patients were male, and the patient group had an average age of 53.1 (12.4) years. Anti-Ro52 was present in 64.2% (158/246) patients. Patients with anti-MDA5+ DM who were positive for anti-Ro52 had a higher rate of RP-ILD (log-rank $P < 0.001$) and a higher mortality rate (log-rank $P = 0.01$). For patients with anti-MDA5+ DM who were positive for anti-Ro52, those with a short disease course and high inflammation were at increased risk of RP-ILD and death. The appearance of active rash was an independent protective factor of death.

Conclusion. Anti-Ro52 antibodies were highly prevalent in patients with anti-MDA5+ DM, and their coexistence correlated with a higher rate of RP-ILD and mortality. Patients with a short disease course, with increased inflammation, and without rash were more likely to have a poor prognosis.

Key Indexing Terms: anti-Ro52, dermatomyositis, interstitial lung disease, MDA5, RP-ILD

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Dermatomyositis (DM) is a heterogeneous autoimmune disease characterized by inflammation in multiple organ systems, most commonly the hallmark cutaneous manifestations and skeletal muscle weakness.[1] Interstitial lung disease (ILD) is a common extramuscular complication contributing to significant morbidity and mortality in DM. Myositis-specific antibodies permit the delineation of homogenous subgroups of DM.[2] Antimelanoma differentiation–associated gene 5 (MDA5) antibodies are frequently related to the presence of a DM skin rash, polyarthralgia, and ILD, but the clinical signs of myositis are absent.[3]

As a severe subtype of inflammatory myopathy, anti-MDA5 antibody–positive (anti-MDA5+) DM has attracted attention in recent years because up to one-half of patients develop rapidly progressive ILD (RP-ILD) with high mortality, despite aggressive glucocorticoid[4-6] and immunosuppressive therapy.[7-9] Knowledge of the clinical characteristics and early identification of high-risk populations in patients with anti-MDA5+ DM are necessary to improve the management of this potentially severe disease.

As myositis-associated antibodies, anti–52-kDa Ro antigen (Ro52) are frequently present in inflammatory myositis, particularly in patients who are positive for antiaminoacyl tRNA synthetase antibodies (anti-ARS).[10] The clinical and prognostic significance of anti-Ro52 remains controversial. Several studies have reported that anti-Ro52 could be strictly associated with a particularly severe phenotype and the development of ILD in inflammatory myositis.[11-13] In patients with anti-MDA5+ juvenile myositis, the frequency of anti-Ro52 is significantly increased and is linked to the presence of ILD and poorer prognosis.[14] A recent study indicated that the coexistence of anti-Ro52 and anti-MDA5 correlates with an increased frequency of RP-ILD and higher mortality in clinical amyopathic DM (CADM)[15]; the study comprised 83 patients who were anti-MDA5+. Nevertheless, it has also been reported that anti-Ro52–positive groups showed a progressive stabilization or improvement in ILD.[10-12]

Given that the previous studies described had relatively small sample sizes, the purpose of this study was to investigate the association of anti-Ro52 antibodies with clinical characteristics and prognosis in a large cohort of 246 patients with anti-MDA5+ DM.

METHODS

Patients. All 246 patients with anti-MDA5+ DM included in the current study were recruited from the Nanjing Medical University myositis-associated ILD (NMMI) cohort between March 2019 and February 2021. The NMMI is a multicenter, retrospective cohort consisting of 10 tertiary hospitals in East China. We examined the medical records of all patients who met the diagnosis of DM, based on the 1975 criteria by Bohan and Peter[16] or the criteria by Sontheimer.[17] The course of the disease was defined as the interval between the time of onset and the time of enrollment. The baseline time was defined as the time of diagnosis of DM. The follow-up time was defined as the interval between the enrollment time and the last follow-up time.

Clinical variables for all subjects were collected in detail, including general information (ie, age, sex, and time since the first symptoms appeared) and clinical manifestation (ie, myasthenia, defined as proximal muscle weakness; active rash; periungual erythema; arthritis; mechanic's hands; skin ulcer; and ILD). Laboratory indicators were collected at the same time, including alanine transaminase, aspartate aminotransferase, lactate dehydrogenase (LDH), creatine kinase (CK), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum ferritin, antinuclear antibodies (ANAs), anti-Ro52, anti-ARS, and anti-MDA5. Anti-Ro52 and MDA5 were measured using the EUROLINE immunoblotting method (EUROIMMUN Medizinische Labordiagnostika AG) by the same central lab. ANAs were detected by indirect immunofluorescence using HEp-2 cell substrates.

If a patient developed RP-ILD or died during follow-up, it would be recorded as an endpoint event for poor outcomes. RP-ILD was defined as the presence of progressive dyspnea and progressive hypoxemia as well as worsening of interstitial change as determined by chest computed tomography (CT) within 1 month from the onset of respiratory symptoms.[8,18] All-cause death events were recorded. This study was approved by the Ethical Committee of the First Affiliated Hospital of Nanjing Medical University (ID: 2020-SR-265). The data are anonymous; therefore, the requirement for informed consent was waived.

Statistical analysis. Continuous variables are presented as mean and SD for normally distributed data and median and IQR for all other data. The Kolmogorov-Smirnov test was used to test for normal distribution. Categorical variables are presented as numbers and percentages. We compared the variables between the anti-Ro52–negative and –positive groups. To evaluate the association between normally distributed variables and endpoint events, *t* tests or Mann-Whitney *U* tests were used. The chi-square test or Fisher exact test was used to evaluate categorical variables, as appropriate. To calculate hazard ratios (HRs) and 95% CIs for RP-ILD and death in patients who are positive for anti-Ro52, while controlling for potential confounders, variables selected by univariate Cox regression analysis ($P < 0.10$) were included in a multivariate Cox regression model with the stepwise forward-selection method. The *P* value was 2-tailed and defined as significant if $P < 0.05$. SPSS software (version 23; IBM Corp.) was used for all of the statistical descriptions, analyses, and inferences.

RESULTS

Demographics of patients with anti-MDA5+ DM with anti-Ro52. There were 246 patients with anti-MDA5+ DM enrolled in this retrospective clinical study. In total, 70 patients (28.5%) were male, and the average age of all subjects was 53.1 (SD 12.4) years. Patients' median disease course was 2 (IQR 1-5) months, and their median follow-up period was 12 (IQR 3-14) months (Table 1). No patients underwent lung transplantation.

In this cohort of 246 patients, anti-Ro52 was present in 64.2% (n = 158) of patients, higher than in patients with ANAs (n = 130, 52.9%) and anti-ARS (n = 15, 6.1%). We compared the clinical characteristics of patients with anti-MDA5+ DM with and without anti-Ro52. As shown in Table 1, the presence of anti-Ro52 in patients with anti-MDA5+ DM was associated with a shorter disease course ($P < 0.001$), high levels of LDH ($P = 0.03$), and high ESR ($P = 0.02$). Patients with anti-Ro52

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Table 1. Clinical characteristics of patients with anti-MDA5 autoantibody–positive DM with or without anti-Ro52 autoantibodies.

	Total, N = 246	Anti-Ro52 Autoantibody		*P*
		Negative, n = 88	Positive, n = 158	
Male	70 (28.5)	28 (31.8)	42 (26.6)	0.38
Age, yrs, mean (SD)	53.1 (12.4)	51.64 (13.1)	53.92 (11.9)	0.17
Age ≥ 55 yrs	111 (45.1)	40 (45.5)	71 (44.9)	0.94
Disease course, mos	2 (1-5)	3 (2-7)	2 (1-3)	**< 0.001**
Disease duration ≤ 3 mos	169 (68.7)	49 (55.7)	120 (76.0)	**0.001**
Follow-up periods, mos	12 (3-14)	12 (6.0-23.5)	9 (3-12)	**< 0.001**
Myasthenia	112 (45.5)	39 (44.3)	73 (46.2)	0.78
Active rash	229 (93.1)	83 (94.3)	146 (92.4)	0.57
Gottron papule	168 (68.3)	59 (67.1)	109 (67.0)	0.75
Heliotrope rash	140 (56.9)	50 (56.8)	90 (57.0)	0.98
V sign	89 (36.2)	34 (38.6)	55 (34.8)	0.55
Shawl sign	55 (22.4)	21 (23.9)	34 (21.5)	0.67
Periungual erythematosus	52 (21.1)	18 (20.5)	34 (21.5)	0.85
Arthritis	90 (36.6)	34 (38.6)	56 (35.4)	0.62
Mechanic's hands	67 (27.2)	26 (29.6)	41 (26.0)	0.54
Superficial erosion and ulcer	34 (13.8)	13 (14.8)	21 (13.3)	0.75
ALT, units/L	46 (29.0-84.2)	46 (31-77)	46 (25.9-94.5)	0.92
AST, units/L	52 (32.5-82.8)	51 (30.0-82.1)	53 (34-83)	0.39
LDH, units/L	333 (253.5-428.5)	312.50 (224-415)	352 (264.5-460.7)	**0.03**
CK, units/L	61 (36.0-142.5)	57.50 (38.0-114.8)	68 (35.0-167.0)	0.44
ESR, mm/h	37.55 (23.0-56.0)	31 (19.5-53.5)	39 (26.9-57.0)	**0.02**
CRP, mg/L	5.58 (3.0-12.1)	5.18 (3.0-11.5)	5.66 (3.1-15.5)	0.49
Ferritin, ng/mL	860.9 (343.7-1500.0)	775.3 (343.7-1491.4)	904.70 (343.5-1500.0)	0.51
ANA positive	130 (52.9)	35 (39.8)	95 (60.1)	**0.002**
Anti-ARS	15 (6.1)	1 (1.1)	14 (8.9)	**0.02**
Anti-MDA5 autoantibody titer				0.62
+	72 (29.3)	29 (33.0)	43 (27.2)	
++	46 (18.7)	15 (17.1)	31 (19.6)	
+++	128 (52.0)	44 (50.0)	84 (53.2)	
ILD	221 (89.8)	75 (85.2)	146 (92.4)	0.07
RP-ILD	88 (35.8)	11 (12.5)	77 (48.7)	**< 0.001**
Death	60 (24.4)	14 (15.9)	46 (29.1)	**0.02**

Values are n (%) or median (IQR) unless otherwise indicated. Values in bold are statistically significant at $P < 0.05$ (compared between the anti-Ro52–negative group and the anti-Ro52–positive group). ALT: alanine transaminase; ANA: antinuclear antibody; anti-ARS: anti–aminoacyl tRNA synthetase antibody; AST: aspartate aminotransferase; CK: creatine kinase; CRP: C-reactive protein; DM: dermatomyositis; ESR: erythrocyte sedimentation rate; ILD: interstitial lung disease; LDH: lactate dehydrogenase; MDA5: melanoma differentiation–associated gene 5; Ro52: 52-kDa Ro antigen; RP-ILD: rapidly progressive interstitial lung disease.

tended to have a higher rate of myasthenia and higher liver and muscle enzyme concentrations, but these did not reach statistical significance. There were no significant differences in sex, age, active rash, arthritis, mechanic's hands, or other lab abnormalities between patients with anti-Ro52 and those without.

Characteristics of ILD in patients with anti-MDA5+ DM with anti-Ro52. Of the 246 patients with anti-MDA5+ DM, ILD was commonly observed in 89.8% (n = 221) of patients, and RP-ILD occurred in 35.8% (88/246) of these patients. The mortality rate of patients with RP-ILD was 45.5% (40/88) within 3 months and 53.4% (47/88) within 6 months in our cohort. Importantly, ILD (92.4% vs 85.2%; $P = 0.07$) and RP-ILD (48.7% vs 12.5%; $P < 0.001$) were more prevalent in patients with anti-Ro52 than in those without, respectively (Table 1). The patient group with anti-MDA5+ DM and anti-Ro52 who had RP-ILD comprised a significantly higher proportion of men (35.1%) as compared to these patients without RP-ILD (18.5%; $P = 0.02$). They also had a higher mean age of 56.9 (SD 10.7) years ($P = 0.002$) and a shorter median disease course of 1 (IQR 1-3) month ($P = 0.001$; Table 2). Patients with RP-ILD also had higher LDH levels, ESR, CRP levels, and ferritin levels ($P < 0.05$ for all), and a higher proportion of them (57.1%) had a high titer (+++) of anti-MDA5 ($P = 0.009$).

Prognosis of patients with anti-MDA5+ DM with anti-Ro52. Patients with anti-MDA5+ DM with anti-Ro52 had a higher mortality rate than patients who were negative for anti-Ro52 (29.1% vs 15.9%; $P = 0.02$; Table 1). Kaplan-Meier survival analysis also revealed that co-occurrence of anti-Ro52 and anti-MDA5 in patients with DM resulted in a higher rate of RP-ILD (log-rank $P < 0.001$; Figure 1A) and a higher mortality rate (log-rank $P = 0.01$; Figure 1B). Patients with anti-MDA5+ DM-ILD with anti-Ro52 who had a high titer of anti-MDA5

Table 2. Clinical characteristics of patients with anti-MDA5 autoantibody–positive DM who are positive for anti-Ro52 autoantibodies with or without RP-ILD, and survivors versus nonsurvivors.

Variables	Non–RP-ILD, n = 81	RP-ILD, n = 77	*P*	Survival, n = 104	Death, n = 46	*P*
Male	15 (18.5)	27 (35.1)	**0.02**	24 (23.1)	18 (39.1)	**0.04**
Age, yrs, mean (SD)	51.1 (12.3)	56.88 (10.7)	**0.002**	52.18 (11.9)	58.13 (11.5)	**0.005**
Age ≥ 55 yrs	29 (35.8)	42 (54.6)	**0.02**	40 (38.5)	30 (65.2)	**0.002**
Disease course, mos	2 (1-6)	1 (1-3)	**0.001**	1 (2-5)	1 (1-3)	**0.04**
Disease duration ≤ 3 mos	50 (61.7)	70 (90.9)	**< 0.001**	74 (71.12)	41 (89.1)	**0.02**
Follow-up periods, mos	12 (7-14)	3 (2-12)	**< 0.001**	12 (7.3-17.3)	2.25 (2.0-3.3)	**< 0.001**
Myasthenia	39 (48.2)	34 (44.2)	0.62	49 (47.1)	19 (41.3)	0.51
Rash	77 (95.1)	69 (89.6)	0.20	99 (95.2)	39 (84.8)	**0.03**
Gottron papule	57 (70.4)	52 (67.5)	0.70	71 (68.3)	31 (67.4)	0.92
Heliotrope rash	51 (63.0)	39 (50.7)	0.12	61 (58.7)	24 (52.2)	0.46
V sign	28 (34.6)	27 (35.1)	0.95	39 (37.5)	15 (32.6)	0.57
Shawl sign	14 (17.3)	20 (26.0)	0.18	20 (19.2)	12 (26.1)	0.35
Periungual erythematosus	16 (19.8)	18 (23.4)	0.58	21 (20.2)	12 (26.1)	0.42
Arthritis	30 (37.0)	26 (33.8)	0.67	41 (39.4)	11 (23.9)	0.07
Mechanic's hands	22 (27.2)	19 (24.7)	0.72	27 (26.0)	13 (28.3)	0.77
Superficial erosion and ulcer	11 (13.6)	10 (13.0)	0.91	13 (12.5)	8 (17.4)	0.43
ALT, units/L	44 (24.0-95.6)	47.05 (29.0-90.2)	0.92	22 (41.1-91.0)	54 (36.8-118.5)	**0.047**
AST, units/L	49.6 (31.5-77.8)	54.05 (41.3-87.8)	0.07	30.7 (48.0-76.4)	63.5 (47.8-92.0)	**0.002**
LDH, units/L	313.5 (252.0-404.0)	388.5 (282.0-545.3)	**0.01**	249.3 (309.0-393.8)	503.0 (344.8-775.8)	**< 0.001**
CK, units/L	59 (35.0-139.0)	97 (33.0-218.8)	0.27	37 (60.5-133.5)	117 (35.5-316.5)	**0.02**
ESR, mm/h	37 (23.0-54.3)	46 (30.2-61.0)	**0.03**	27 (39-55)	39.5 (23.8-7)	0.62
CRP, mg/L	4.56 (2.5-8.1)	9.48 (3.6-24.7)	**< 0.001**	2.3 (4.7-9.9)	12.0 (3.67-26.80)	**< 0.001**
Ferritin, ng/mL	600.1 (164.0-1343.2)	1200.5 (608.5-1943.1)	**0.001**	191.4 (668.4-1436.8)	1500.0 (800.9-2000.0)	**< 0.001**
ANA positive	49 (60.5)	46 (59.7)	0.92	63 (60.6)	27 (58.7)	0.83
Anti-ARS	10 (12.4)	4 (5.2)	0.11	11 (10.6)	2 (4.4)	0.21
Anti-MDA5 autoantibody titer			**0.009**			0.13
+	30 (37.0)	13 (16.9)		34 (32.7)	8 (17.4)	
++	11 (13.6)	20 (26.0)		20 (19.2)	9 (19.6)	
+++	40 (49.4)	44 (57.1)		50 (48.1)	29 (63.0)	
RP-ILD				30 (28.9)	42 (91.3)	**< 0.001**
Death	4 (4.9)	42 (54.6)	**< 0.001**			
ILD				94 (90.4)	45 (97.8)	0.11

Values are n (%) or median (IQR) unless otherwise indicated. Values in bold are statistically significant at $P < 0.05$. ALT: alanine transaminase; ANA: antinuclear antibody; anti-ARS: anti–aminoacyl tRNA synthetase autoantibody; AST: aspartate aminotransferase; CK: creatine kinase; CRP: C-reactive protein; DM: dermatomyositis; ESR: erythrocyte sedimentation rate; ILD: interstitial lung disease; LDH: lactate dehydrogenase; MDA5: melanoma differentiation–associated gene 5; Ro52: 52-kDa Ro antigen; RP-ILD: rapidly progressive interstitial lung disease.

(++ to +++) had a higher rate of RP-ILD (log-rank $P = 0.006$; Figure 1C) and a higher mortality rate (log-rank $P = 0.049$; Figure 1D) than those with a low titer of anti-MDA5 (+).

Univariate and multivariate analyses were performed to investigate the possible predictive factors of RP-ILD occurrence and death in patients who are positive for anti-Ro52 (Table 2). According to the multivariate Cox regression analysis, disease duration of 3 months or less (HR 4.43, 95% CI 1.88-10.47; $P = 0.001$) and a high CRP level (HR 2.70, 95% CI 1.58-4.62; $P < 0.001$) were independent risk factors of RP-ILD (Table 3). Active rash (HR 0.25, 95% CI 0.09-0.68; $P = 0.006$) was an independent protective factor of death. A high CK level (HR 2.84, 95% CI 1.36-5.90; $P = 0.005$), a high CRP level (HR 2.17, 95% CI 1.06-4.43; $P = 0.03$), and RP-ILD (HR 15.72, 95% CI 3.67-67.32; $P < 0.001$) were independent risk factors of death (Table 3).

DISCUSSION

Lung involvement, especially the development of RP-ILD, is a frequent and potentially life-threatening complication in anti-MDA5+ DM. Our study highlights that in patients with anti-MDA5+ DM, the presence of anti-Ro52 is associated with a high rate of RP-ILD and high mortality. Our findings provide direct clinical evidence that in patients with anti-MDA5+ DM, detection of anti-Ro52 can help screen individuals who are at increased risk of developing life-threatening RP-ILD, especially in those patients who have shorter disease duration, elevated CRP or CK levels, and without active rash.

According to the reported data, approximately 60% to 80% of patients who are anti-MDA5+ develop ILD, and up to one-half of those patients develop RP-ILD.[6] Several studies have confirmed the negative prognostic value of anti-MDA5 titer related to RP-ILD relapses or poor treatment outcome.[19-21]

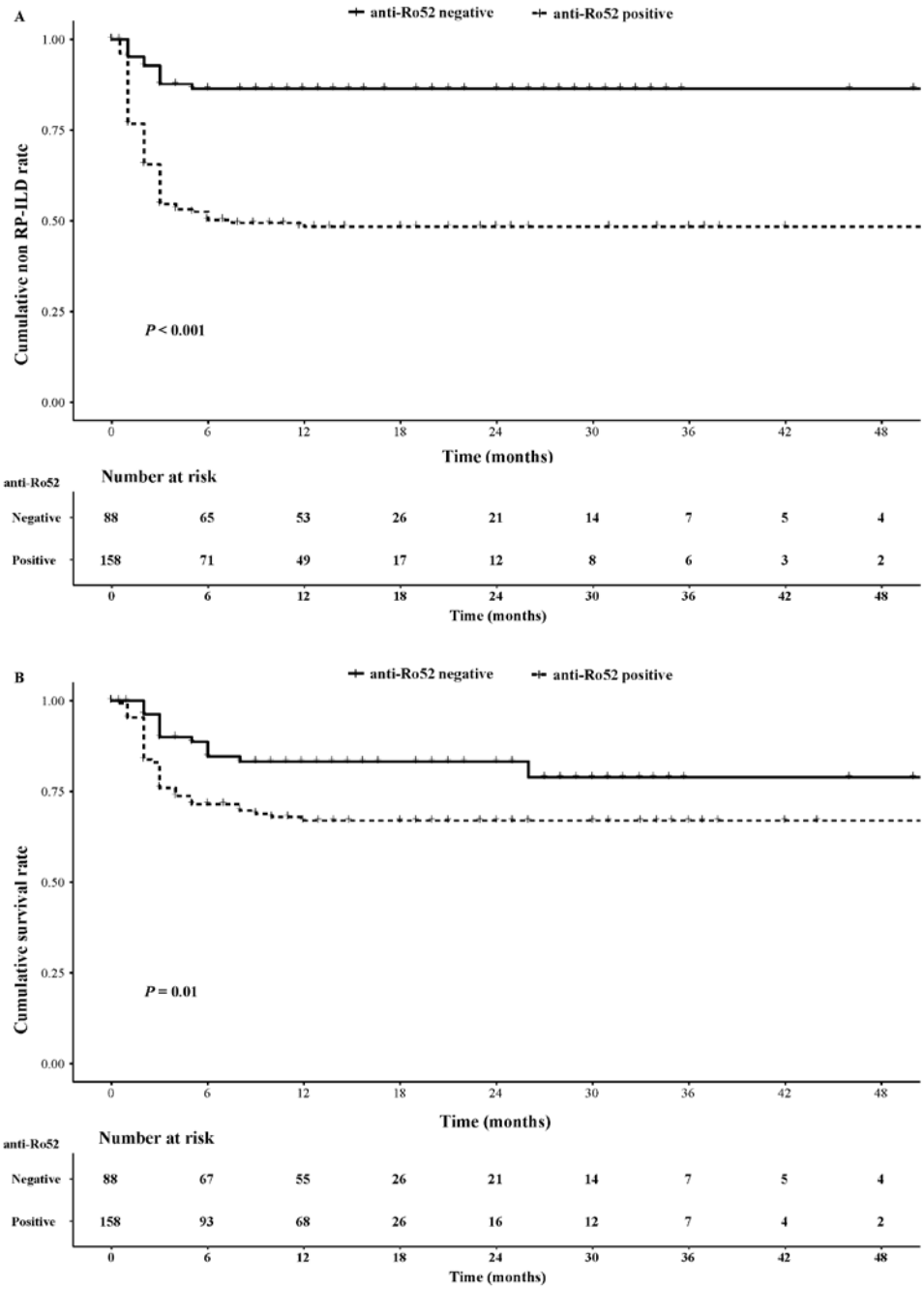

Figure 1A. Difference in cumulative non–RP-ILD rate in patients with DM who are anti-MDA5 positive with or without anti-Ro52 antibodies. Patients with anti-Ro52 had a significantly higher rate of RP-ILD (log-rank $P < 0.001$). DM: dermatomyositis; MDA5: melanoma differentiation–associated gene 5; Ro52: 52-kDa Ro antigen; RP-ILD: rapidly progressive interstitial lung disease. *B.* Difference in cumulative survival rate in patients with DM who are anti-MDA5 positive with or without anti-Ro52 antibodies. Patients with anti-Ro52 had a significantly higher mortality rate (log-rank $P = 0.01$). DM: dermatomyositis; MDA5: melanoma differentiation–associated gene 5; Ro52: 52-kDa Ro antigen.

Even with aggressive therapies, the mortality rate for patients with anti-MDA5+ DM who have RP-ILD is 50% to 70% within 6 months.[2,6,22-24] In our data, RP-ILD occurred in 39.8 (88/221) of patients with anti-MDA5+ DM, with a 6-month all-cause mortality rate of 53.4% (47/88). Notably, in patients with anti-MDA5+ DM, the coexistence of anti-MDA5 and anti-Ro52, compared to patients without anti-Ro52, conferred a nearly 4-fold higher incidence of RP-ILD (48.7% vs 12.5%) and a 2-fold increased mortality rate (29.1% vs 15.9%). The relationship between anti-Ro52 and ILD has been reported by Sabbagh et al[14] in juvenile myositis. Xu et al[15] also reported the prognostic values of anti-Ro52 antibodies in aggressive phenotypes of anti-MDA5+ CADM-ILD. Consistent with these results, we suggest that the coexistence of anti-MDA5 and anti-Ro52

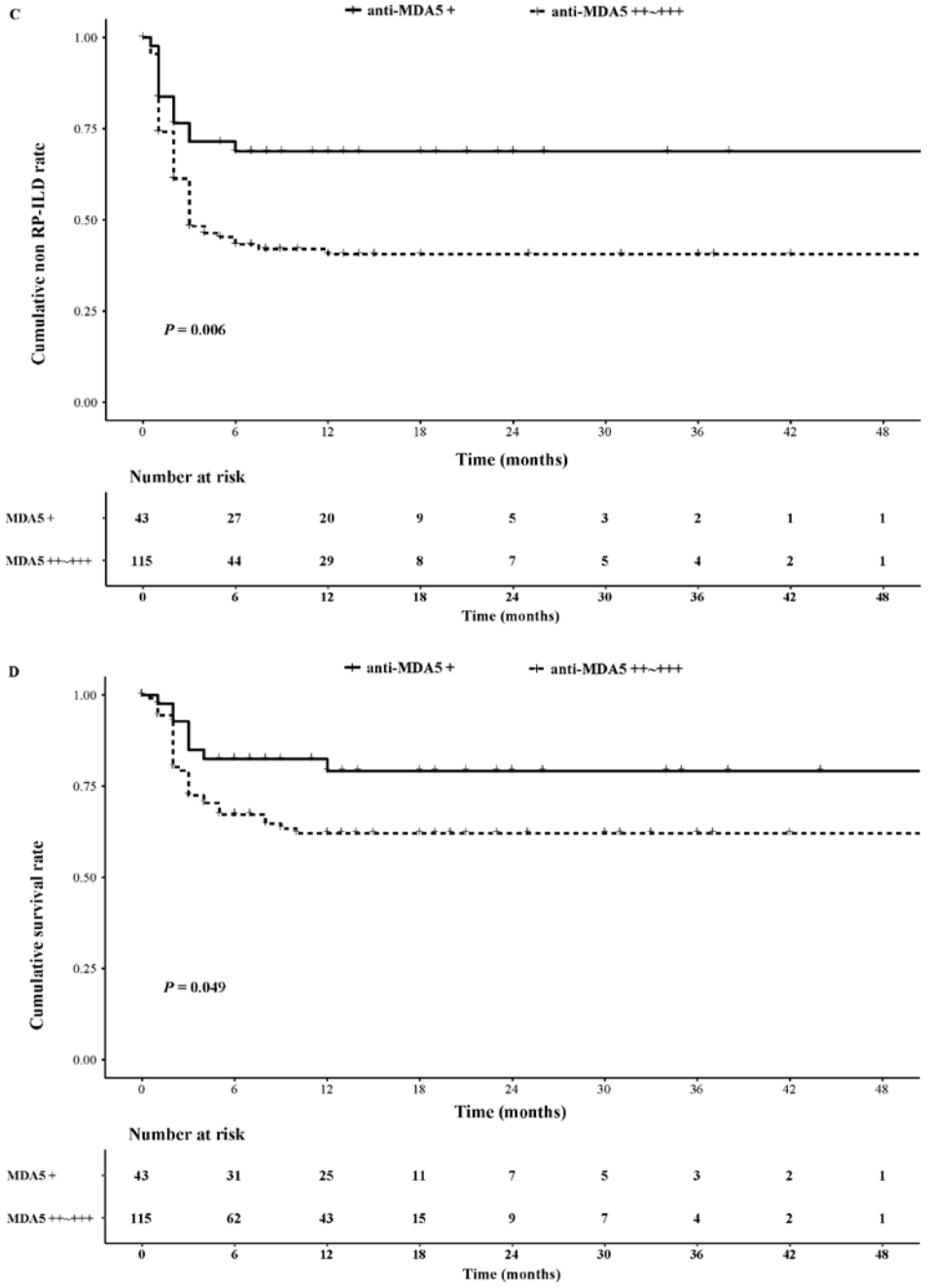

Figure 1C. Difference in cumulative non–RP-ILD rate in patients with DM-ILD who are anti-MDA5 positive and have anti-Ro52 antibodies: comparison between those with a high titer of anti-MDA5 (++ to +++) and those with a low titer of anti-MDA5 (+). Patients with high titer of anti-MDA5 (++ to +++) had a significantly higher rate of RP-ILD (log-rank $P = 0.006$). DM: dermatomyositis; ILD: interstitial lung disease; MDA5: melanoma differentiation–associated gene 5; Ro52: 52-kDa Ro antigen; RP-ILD: rapidly progressive interstitial lung disease. *D.* Difference in cumulative survival rate in patients with DM-ILD who are anti-MDA5 positive and have anti-Ro52 antibodies: comparison between those with a high titer of anti-MDA5 (++ to +++) and those with a low titer of anti-MDA5 (+). Patients with a high titer of anti-MDA5 (++ to +++) had a significantly higher mortality rate (log-rank $P = 0.049$). DM: dermatomyositis; ILD: interstitial lung disease; MDA5: melanoma differentiation–associated gene 5; Ro52: 52-kDa Ro antigen.

increases the likelihood of PR-ILD and poor outcomes in patients with anti-MDA5+ DM.

It remains unclear as to how the coexistence of anti-MDA5 and anti-Ro52 antibodies is linked to a more aggressive phenotype in anti-MDA5+ DM. In our data, we also found that the patients who were positive for anti-Ro52 had a shorter disease course, higher LDH levels, and higher ESR, suggesting a hyperinflammatory state in these patients at early disease onset.

Table 3. Risk factors of RP-ILD and death in patients with DM who are anti-Ro52 autoantibody positive and anti-MDA5 autoantibody positive in univariate and multivariate Cox regression analyses.

Variables	Risk Factors of RP-ILD				Risk Factors of Death			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Male	1.73 (1.08-2.76)	**0.02**	–	–	1.91 (1.06-3.46)	**0.03**	–	–
Age ≥ 55 yrs	1.73 (1.10-2.71)	**0.02**	–	–	2.64 (1.44-4.86)	**0.002**	–	–
Disease duration ≤ 3 mo	4.45 (2.04-9.72)	**< 0.001**	4.43 (1.88-10.47)	**0.001**	3.23 (1.27-8.19)	**0.01**	–	–
Active rash	–	–	–	–	0.34 (0.15-0.75)	**0.008**	0.25 (0.09-0.68)	**0.006**
ALT ≥ 50 units/L	–	–	–	–	1.68 (0.94-3.00)	**0.08**	–	–
AST ≥ 40 units/L	1.55 (0.91-2.63)	0.11	–	–	–	–	–	–
LDH ≥ 270 units/L	1.67 (0.93-2.98)	0.09	–	–	4.29 (1.54-11.97)	**0.005**	–	–
CK ≥ 170 units/L	–	–	–	–	2.72 (1.49-4.94)	**0.001**	2.84 (1.36-5.90)	**0.005**
ESR ≥ 20 mm/h	1.38 (0.63-3.02)	0.42	–	–	–	–	–	–
CRP ≥ 8 mg/L	2.37 (1.50-3.74)	**< 0.001**	2.70 (1.58-4.62)	**< 0.001**	3.50 (1.90-6.44)	**< 0.001**	2.17 (1.06-4.43)	**0.03**
Ferritin ≥ 336.2 ng/mL	2.24 (1.06-4.71)	**0.03**	–	–	3.88 (1.19-12.64)	**0.02**	–	–
Anti-MDA5 autoantibody titer								
+	–	–	–	–	–	–	–	–
++	2.50 (1.24-5.03)	**0.01**	–	–	–	–	–	–
+++	2.01 (1.08-3.74)	**0.03**	–	–	–	–	–	–
RP-ILD	–	–	–	–	15.67 (5.58-43.67)	**< 0.001**	15.72 (3.67-67.32)	**< 0.001**

Values in bold are statistically significant at $P < 0.05$. ALT: alanine transaminase; AST: aspartate aminotransferase; CK: creatine kinase; CRP: C-reactive protein; DM: dermatomyositis; ESR: erythrocyte sedimentation rate; HR: hazard ratio; LDH: lactate dehydrogenase; MDA5: melanoma differentiation–associated gene 5; Ro52: 52-kDa Ro antigen; RP-ILD: rapidly progressive interstitial lung disease.

Ro52, also named tripartite motif-containing protein 21, is an E3 ubiquitin ligase.[25] Interestingly, both MDA5 and Ro52 are intracellular proteins and play key roles in the regulation of interferon signaling in effective responses to viral infection.[25,26] They also include the upregulation of certain proinflammatory transcription factors, such as nuclear factor-κB.[25] Given that viral infections are believed to be the eliciting event in the pathogenesis of myositis, the concurrent overexpression of MDA5 and Ro52 leading to excessive inflammation and autoimmunity in anti-MDA5+ DM might be an explanation. In addition, why these antibodies co-occur necessitates further study.

We further explored independent risk factors for poor prognosis in those patients who were positive for anti-Ro52. A shorter disease course and higher CRP levels further increase the risk of RP-ILD, which has also been reported in the previous studies.[6] In our data, RP-ILD and mortality mostly occurred within the first 6 months after disease onset. The disease progression tended to gradually decline in the 6 months thereafter. Indeed, a shorter disease duration as a predictor of RP-ILD may simply mean that patients with RP-ILD develop it as an early disease manifestation. However, our data suggest that the first 6 months after disease onset is a risk window for the poor outcomes seen in patients with anti-MDA5+ DM, especially for those patients with anti-Ro52. Yang et al[6] reported that higher CRP levels predict poor outcomes in patients who are anti-MDA5+, and initial intensive treatment may improve the prognosis. It may be explained by the high inflammatory status during the early onset of the disease, which might decrease over time because of medical intervention.

Intriguingly, our results showed that the appearance of active rash is a protective factor for death. The presence of active rash was related to favorable outcomes. However, the clinical significance of rash has been controversial. It has been reported that facial rash was associated with no ILD development during follow-up in idiopathic inflammatory myositis.[11] In contrast, Lu et al[27] reported that palmar erythema and palmar papules were associated with DM-related acute and subacute ILD. The difference in research results may be due to the distribution and type of rash. In the future, it will be necessary to expand the sample size and to further explore the relationship between different types of rashes and prognoses.

Our research had several limitations. The main limitation of our study is the fact that it was an observational, nonrandomized study with its inherent limitations. Another shortcoming is the lack of pulmonary function data in our research. Because the cohort data came from multiple centers, we were unable to collect the raw chest CT imaging data and could not perform CT scoring. Medical intervention may also have had a particular effect on the outcomes of this study. Patients' treatment protocols will also be adjusted during follow-up, including the dosage of glucocorticoids and the type of immunosuppressant. In addition, there may be some heterogeneity in data and treatment practices between different centers. In the future, randomized controlled trials may be used to analyze the effect of treatment on prognosis. Despite these confounders, the results were as expected.

In conclusion, we found that anti-Ro52 antibodies are associated with RP-ILD and poor prognosis in patients with anti-MDA5+ DM, especially in those with a short disease course and high inflammatory condition. Thus, testing for the presence of anti-Ro52 antibodies has value in early diagnosis, evaluation,

and prognosis monitoring. Our clinical evidence suggests that there might be a complex pathophysiological mechanism behind the co-occurrence of anti-Ro52 and anti-MDA5 antibodies. Further translational and clinical research is needed to explore this mechanism.

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DATA AVAILABILITY

Data are available from the corresponding author upon reasonable request.

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