Title page

Title: Coexisting of anti-Ro52 autoantibodies on anti-MDA5 autoantibodies-positive dermatomyositis is highly associated with rapidly progressive interstitial lung disease and mortality risk

Chengyin Lv[a #], Hanxiao You[a #], Lingxiao Xu[a], Lei Wang[a], Fenghong Yuan[b], Ju Li[c], Min Wu[d], Shiliang Zhou[d], Zhanyun Da[e], Jie Qian[e], Hua Wei[f], Wei Yan[f], Lei Zhou[g], Yan Wang[g], Songlou Yin[h], Dongmei Zhou[h], Jian Wu[i], Yan Lu[j], Dinglei Su[j], Zhichun Liu[k], Lin Liu[l], Longxin Ma[m], Xiaoyan Xu[n], Yinshan Zang[o], Huijie Liu[p], Tianli Ren[q], Fang Wang[r], Miaojia Zhang[a *], Wenfeng Tan[a *]

[a]Department of Rheumatology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China;
[b]Department of Rheumatology and Immunology, the Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, Jiangsu, China;
[c]Department of Rheumatology, Huai'an First People's Hospital, Huai'an, Jiangsu, China;
[d]Department of Rheumatology, The First People's Hospital of Changzhou, Changzhou, Jiangsu, China;
[e]Department of Rheumatology, Affiliated Hospital of Nantong University, Nantong, Jiangsu, China;
[f]Department of Rheumatology, Northern Jiangsu People's Hospital, Yangzhou, Jiangsu, China;
[g]Department of Rheumatology, Changzhou No.2 People's Hospital, Changzhou, Jiangsu, China;
[h]Department of Rheumatology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China;
[i]Department of Rheumatology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China;
[j]Department of Rheumatology, Jiangsu Province Hospital of Chinese Medicine, Nanjing, Jiangsu, China;
[j]Department of Rheumatology, Nanjing First Hospital, Nanjing, Jiangsu, China;
[k]Department of Rheumatology, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China;
[l]Department of Rheumatology, Xuzhou Central Hospital, Xuzhou, Jiangsu, China;
[m]Department of Rheumatology, Yancheng No.1 People's Hospital, Yancheng, Jiangsu, China;
[n]Department of Rheumatology, Zhongda Hospital Southeast University, Nanjing, Jiangsu, China;
[o]Department of Rheumatology, The Affiliated Suqian First People's Hospital of Nanjing Medical University, Suqian, Jiangsu, China;
[p]Department of Rheumatology, The First People's Hospital of Lianyungang, Lianyungang, Jiangsu, China;
[q]Department of Rheumatology, Wuxi No.2 People's Hospital, Wuxi, Jiangsu, China;
[r]Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, China,

Nanjing, Jiangsu, China

#Chengyin Lv and Hanxiao You are joint first authors and contributed equally to this work.
*Wenfeng Tan and Miaojia Zhang contributed equally to this work.
Correspondence to Dr. Wenfeng Tan and Miaojia Zhang, Department of Rheumatology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China;
tw2006@njmu.edu.cn, mjzhang@njmu.edu.cn
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Summary at a Glance

RP-ILD is a frequent and life-threatening complication in anti-MDA5+ DM. We investigated the association of anti-Ro52 antibodies with clinical characteristics and prognosis in anti-MDA5+ DM patients. Anti-Ro52 antibodies are highly prevalent in anti-MDA5+ DM patients and their coexistence correlates with a higher rate of RP-ILD and mortality.

Abstract

Background and objective: Interstitial lung disease (ILD) is a common extramuscular complication contributing to significant morbidity and mortality in anti-melanoma differentiation associated gene 5 positive dermatomyositis (anti-MDA5+ DM). We conducted this study to investigate the association of anti-Ro52 antibodies with clinical characteristics and prognosis in anti-MDA5+ DM patients.

Methods: We assessed a cohort of 246 patients with anti-MDA5+ DM. To calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for 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 *p* value <0.05 was considered to indicate statistical significance.

Results: 246 anti-MDA5+ DM patients were enrolled, 70 cases male, with an average age of 53.10±12.35 years. Anti-Ro52 coexisted in 64.22% (158/246) patients. Anti-Ro52 autoantibodies positive anti-MDA5+ DM patients had a higher rate of RP-ILD (log-rank $p<0.001$) and a higher mortality rate (log-rank $p=0.010$). For anti-MDA5+ DM patients with positive anti-Ro52 antibodies, patients with a short disease course, and high inflammation are at increased risk of RP-ILD and death. The appearance of the active rash is an independent protective factor of death.

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

Keywords: Dermatomyositis, MDA5, anti-Ro52, Interstitial lung disease, RP-ILD

Running Head: RPILD in anti-MDA5+ DM

Introduction

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 extra muscular complication contributing to significant morbidity and mortality in DM. Myositis-specific autoantibodies permit the delineation of homogenous subgroups of DM[2]. Anti-melanoma differentiation-associated gene 5 (MDA5) antibodies are frequently related to the presence of a DM skin rash, polyarthralgia and ILD but absence the clinical signs of myositis[3].

As a severe subtype of inflammatory myopathy, anti-MDA5+ DM has attracted attention in recent years due to up to one-half of those developing 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 anti-MDA5+ DM patients are necessary to improve the management of this potentially severe disease.

As myositis-associated autoantibodies, anti-Ro52 antibodies are frequently present in inflammatory myositis, particularly in anti-aminoacyl tRNA synthetase autoantibodies positive patients[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-positive juvenile myositis, the frequency of anti-Ro52 antibodies is significantly increased and linked to the presence of ILD and poorer prognosis[14]. Recently study indicated that the coexistence of anti-Ro52 and anti-MDA5 antibodies correlates with an increased frequency of RP-ILD and higher mortality in clinical amyopathic dermatomyositis (CADM)[15], which comprises 83 anti-MDA5+ patients. Nevertheless, it is also reported that anti-Ro52 antibodies positive groups showed a progressive stabilization or improvement in ILD[10-12].

Given the previous studies were described in a relatively small sample size, 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 anti-MDA5+ DM patients.

Methods

Patients

All 246 anti-MDA5+ DM patients used in the current study came from Nanjing Medical University myositis associated ILD contort (NNMI) from March 2019 to February 2021. NNMI is a multicenter, retrospective cohort consisting of ten tertiary hospitals in East China. We examined the medical record of all patients who met the diagnosis of DM, based on Bohan/Peter criteria in 1975 or Sontheimer's criteria[16]. The course of the disease is defined as the time interval between the time of onset and the time of enrollment. The baseline time is defined as the time of the diagnosis of DM. The follow-up time is defined as the time interval between the enrollment time and the last follow-up time. Clinical parameter of all subjects was collected in detail, including the general information (such as age, gender and the time since the first symptoms appeared), clinical manifestation (including myasthenia (defined as proximal muscle weakness), active rash, periungual erythema, arthritis, mechanic's hand, skin ulcer and interstitial lung disease). Laboratory indicators were collected at the same time, including alanine transaminase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), creatine kinase (CK), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum ferritin (SF), anti-nuclear antibodies (ANA), anti-Ro52 antibodies, anti-aminoacyl tRNA synthetase autoantibodies and anti-MDA5 antibodies. Anti-Ro-52 and MDA5 antibodies were measured using an immunoblotting method (EUROLINE, EUROIMMUN AG, Germany) by the same central lab. The ANA was detected via indirect immunofluorescence (IIF) using HEp-2 cell substrates. If a patient develops RP-ILD or dies during follow-up, it will be recorded as an endpoint event for poor outcomes. RP-ILD was defined as the presence of progressive dyspnea and progressive hypoxemia, and a worsening of interstitial change on the chest computed tomography (CT) within one month from the onset of respiratory symptoms[17,18]. All-cause death event was recorded. The Ethics Committee approved this study of the First Affiliated Hospital of Nanjing Medical University (ID: 2020-SR-265). The data are anonymous, and the requirement for informed consent was therefore waived.

Statistical analysis

Continuous variables are presented as mean and standard deviation (SD) for normally distributed data and median and interquartile range (P25, P75) for all other data. The Kolmogorov-Smirnov test was used to test for normal distribution. Categorical variables are presented as numbers (percentages). We compared the parameters between the anti-Ro52 antibodies negative and positive groups. Student t-tests or Mann-Whitney U tests were used to evaluate the association between normally distributed variables and endpoint events. The Chi-square test or Fisher's exact test was used to evaluate for categorical variables, as appropriate. To calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for RP-ILD and death in anti-Ro52 antibodies positive patients 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 two-tailed and defined as significant if the value was <0.05. SPSS software, v. 23 (Chicago, IL, USA) was used for all of the statistical descriptions, analyses and inferences.

Results

Demographics of anti-MDA5-positive DM patients with anti-Ro52 autoantibodies

There were 246 anti-MDA5+ DM patients enrolled in this retrospective clinical study. Seventy cases (28.46%) were male, and the average age of all subjects was 53.10 ± 12.35 years, with a median disease course of 2 months and a median follow-up period of 12 months (Table 1). No patients underwent lung transplantation.

In this cohort, anti-Ro52 coexisted in 64.22% (158/246) patients, higher than ANA (52.85%) and anti-aminoacyl tRNA synthetase autoantibodies (6.10%). We compared the clinical characteristics of anti-MDA5+ DM patients with and without anti-Ro52. As shown in Table 1, the presence of anti-Ro52 autoantibodies in anti-MDA5+ DM is associated with a shorter disease course (p < 0.001), high levels of LDH (p = 0.03) and ESR (p = 0.02). Patients with anti-Ro52 positive trend to have a higher rate of

myasthenia and higher levels of liver/ muscle enzyme concentrations but did not reach statistical significance. There were no significant differences in gender, age, active rash, arthritis, mechanic's hand and other lab abnormality between patients with and without anti-Ro52 autoantibodies.

Characteristics of ILD in anti-MDA5-positive DM patients with anti-Ro52 antibodies

Of those 246 anti-MDA5+ DM patients, ILD is commonly observed in 89.84% (221/246) patients, and RP-ILD occurred in 39.81% (88/221) of these patients. The mortality rate of RP-ILD patients was 45.45% (40/88) and 53.41% (47/88) within three months and six months in our cohort, respectively. Importantly, ILD (92.41% *vs.* 85.23%, $p = 0.074$) and RP-ILD (48.73% *vs.* 12.50%, $p < 0.001$) were more prevalent in patients with anti-Ro52 positive than those without (Table 1). RP-ILD patients with anti-MDA5 and anti-Ro2 antibodies double-positive DM patients had a significantly higher proportion of men (35.06% *vs.* 18.52%, $p = 0.019$) than patients without RP-ILD, with a higher age of 56.88±10.72 ($p = 0.002$) years and a shorter median disease course of 1 month ($p = 0.001$) (Table 2). RP-ILD patients also had higher levels of LDH, ESR, CRP and ferritin levels ($p < 0.05$), and a higher proportion of high titer of anti-MDA5 (+++) antibodies (57.14%, $p = 0.009$).

Prognosis of anti-MDA5-positive DM-ILD patients with anti-Ro52 antibodies

Anti-MDA5+ DM patients with anti-Ro52 antibodies positive had a higher mortality rate than those anti-Ro52 antibody negative patients (29.11% *vs.* 15.91%, $p = 0.021$). Kaplan-Meier survival analysis also revealed that co-occurrence of anti-Ro52 and anti-MDA5 antibodies in DM patients had a higher rate of RP-ILD (log-rank $p < 0.001$; Figure 1A) and higher mortality rate (log-rank $p = 0.010$; Figure 1B). Patients with high titer of anti-MDA5 (++~+++) antibodies had a higher rate of RP-ILD (log-rank $p = 0.006$; Figure 1C) and higher mortality rate (log-rank p=0.049; Figure 1D) than a low titer of anti-MDA5 (+) in anti-MDA5-positive DM-ILD patients with anti-Ro52 antibodies.

Univariate and multivariate analyses were performed to investigate the possible predictive factors of RP-ILD occurrence and death in anti-Ro52 positive patients (Table 2). According to the multivariate cox regression analysis, disease duration≤3 months (HR 4.434, 95% CI = 1.878-10.467, $p = 0.001$) and high CRP level (HR 2.701, 95% CI = 1.580-4.617, $p<0.001$) were independent risk factors of RP-ILD (Table 3); active rash (HR 0.251, 95% CI = 0.093-0.676, $p = 0.006$) was independent protective factor of death, high CK level (HR 2.835, 95% CI = 1.364-5.896, $p = 0.005$), high CRP level (HR 2.166, 95% CI = 1.059-4.428, $p = 0.034$) and RP-ILD (HR 15.719, 95% CI = 3.671-67.317, $p < 0.001$) 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 the coexisting of anti-Ro52 autoantibodies is associated with a high rate of RP-ILD and high mortality in anti-MDA5+ DM patients. Our findings provide direct clinical implication that anti-Ro52 autoantibodies can help to screen individuals who are at increased risk of developing life-threatening RP-ILD in anti-MDA5+ DM patients, especially in those patients who have shorter disease duration, elevated CRP or CK level and without active rash simultaneously.

According to the reported data, approximately 60% to 80% of anti-MDA5+ patients develop ILD, and up to one-half of those develop RP-ILD[6]. Several studies have confirmed the negative prognostic value of anti-MDA5 antibodies titer related to RP-ILD relapses or poor treatment outcome[19–21]. Although aggressive therapies, the mortality rate for anti-MDA5+ DM patients with RP-ILD is 50-70% within six months[22–26]. In our data, RP-ILD occurred in 39.81% (88/221) anti-MDA5+ DM patients with a six-months all-cause mortality rate of 53.41% (47/88). Notably, the coexistence of anti-MDA5 and anti-Ro52 autoantibodies conferred nearly the 4-fold higher incidence of RP-ILD (48.73% *vs.* 12.50%) and 2-fold increased mortality rate (29.11% *vs.* 15.91%) in anti-MDA5+ DM patients, as compared with those without anti-Ro52 autoantibodies. The relationship between anti-Ro52 autoantibodies and ILD

has been reported by *Sabbagh* et al. in juvenile myositis[14]. Antao Xu et al. also reported the prognostic values of anti-Ro52 antibodies in aggressive phenotypes of anti-MDA5+ CADM-ILD[15]. Consistent with these results, we suggest the coexistence of anti-MDA5 and anti-Ro52 autoantibodies increases the likelihood of PR-ILD and poor outcome in anti-MDA5+ DM patients.

How coexistence of anti-MDA5 and anti-Ro52 autoantibodies linked to more aggressive phenotype in anti-MDA5+ DM remains unclear. In our data, we also found the anti-Ro52 autoantibodies positive patients have shorter disease course and higher LDH and ESR levels, suggesting a hyperinflammatory state in these patients at early disease onset. Ro52 (also named as TRIM21) is an E3 ubiquitin ligase[27]. Interestingly, both MDA5 and Ro52 are intracellular proteins and play key roles in the regulation of interferon (IFN) signaling in effective responses to viral infection[27,28]. They also include the upregulation of certain proinflammatory transcription factors such as NF-κb[27]. Given viral infections are believed the eliciting event in the pathogenesis of myositis, concurrent over-expression MDA5 and Ro-52 lead to excessive inflammation and autoimmunity in anti-MDA5+ DM might be an explanation. In addition, why these autoantibodies co-occurrence necessitates further study.We further explored independent risk factors for poor prognosis in those anti-Ro52 positive patients. 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 occur within the first 6-months after disease onset. The disease progression tends to gradually decline in 6-month thereafter. Indeed, a shorter disease duration as a predictor of RPLD may simply mean that patients with RPLD develop it as an early disease manifestation. However, our data at least suggest that the first 6-months after disease onset is a risk window for the poor outcome in anti-MDA5+ DM patients, especially for these patients with that coexistence of anti-Ro52 autoantibodies. Qihua Yang et al. reported that higher CRP levels predict poor outcomes in anti-MDA5 positive patients and initial intensive treatment may improve the prognosis[25]. It may be explained by the high inflammatory status in the early onset and might reduce overtime because of medical intervention.

Intriguingly, our results showed that the appearance of the active rash is a protective factor for death. The presence of active rash determined 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, Jinghao Lu et al. reported that palmar erythema and palmar papules were associated with DM-related acute/subacute ILD[29]. The difference in research results may be due to the distribution and type of the rash. In the future, it is necessary to expand the sample size and to further explore the relationship between different types of rash and prognosis.

Our research had several limitations. The main limitation of our study is the fact that it is an observational, nonrandomized study with its inherent limitations. Another shortcoming is the lack of pulmonary function data in our research. Because the cohort data comes from multiple centers, we cannot collect the raw data of chest CT imaging and cannot perform CT scoring. Medical intervention may also have a particular impact on the outcome of this study. The patient's 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, RCT studies may be used to analyze the impact of treatment on prognosis. Despite these confounders, the results were as expected.

Conclusions

In conclusion, the anti-Ro52 antibodies are associated with RP-ILD and poor prognosis in anti-MDA5+ DM patients, especially those with a short disease course and high inflammation condition. Thus, testing anti-Ro52 autoantibodies have important value in early diagnosis, evaluation, prognosis monitoring. Our clinical evidence suggests 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 the mechanism.

List of abbreviations: Interstitial lung disease (ILD); mortality in anti-melanoma differentiation associated gene five dermatomyositis (anti-MDA5+ DM); hazard ratios (HRs); confidence intervals (CI); rapidly progressive ILD (RP-ILD); clinical amyopathic dermatomyositis (CADM); alanine transaminase (ALT); aspartate aminotransferase (AST); lactic dehydrogenase (LDH); creatine kinase (CK); erythrocyte sedimentation rate (ESR); C-reactive protein (CRP); serum ferritin (SF); anti-nuclear antibodies (ANA).

Figure Legends

Figure 1A. Difference of cumulative no-RP-ILD rate of anti-MDA5+ DM patients with versus without anti-Ro52 autoantibodies. Patients with anti- Ro52 antibodies positive had a significant higher rate of RP-ILD (log-rank p <0.001).

Figure 1B. Difference of cumulative survival rate anti-MDA5+ DM patients with versus without anti-Ro52 autoantibodies. Patients with anti- Ro52 antibodies positive had a significant higher mortality rate (log-rank p =0.010).

Figure 1C. Difference of cumulative no-RP-ILD rate of high titer of anti-MDA5 (++~+++) antibodies patients versus low titer of anti-MDA5 (+) antibodies in anti-MDA5-positive DM-ILD patients with anti-Ro52 antibodies. Patients with high titer of anti-MDA5 (++~+++) antibodies had a significant higher rate of RP-ILD (log-rank p =0.006).

Figure 1D. Difference of cumulative survival rate of high titer of anti-MDA5 (++~+++) antibodies patients versus low titer of anti-MDA5 (+) antibodies in anti-MDA5-positive DM-ILD patients with anti-Ro52 antibodies. Patients with high titer of anti-MDA5 (++~+++) antibodies had a significant higher mortality rate (log-rank p =0.049).

Declarations

Table and figure counts: 3 tables and 1 figure

Ethical approval information: This study was approved by the Ethical Committee of the First Affiliated Hospital of Nanjing Medical University: 2020-SR-265.

Informed consent: This study was approved by the ethics committee. The data are anonymous, and the requirement for informed consent was therefore waived.

Data statement: Data are available from the corresponding author on reasonable request.

Conflicts of interest: The authors have no conflicts of interest to declare.

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Author Contribution : The conception and design of the correspondence, acquisition of data, and analysis and interpretation of data: WT, CL, HY, LX and WL; Involved in care of the patient, and acquisition of data: FY, JL, MW, SZ, ZD, JQ, HW, WY, LZ, YW, SY, DZ, JW, YL, DS, ZL, LL, LM, XX, YZ, HL, TR, FW, MZ and WT; Drafting the article: CL, HY and WT; Revising it critically for important intellectual content: CL, HY, WL, LX, MZ and WT; Final approval of the version to be submitted: all authors; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: all authors.

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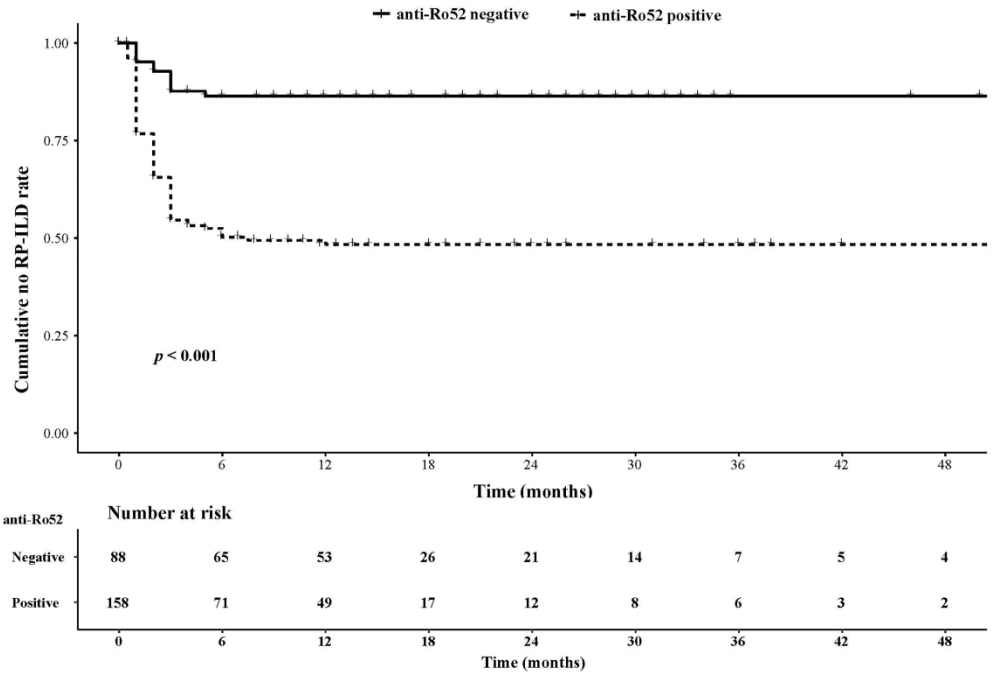

Figure 1A. Difference of cumulative no-RP-ILD rate of anti-MDA5+ DM patients with versus without anti-Ro52 autoantibodies. Patients with anti- Ro52 antibodies positive had a significant higher rate of RP-ILD (log-rank p <0.001).

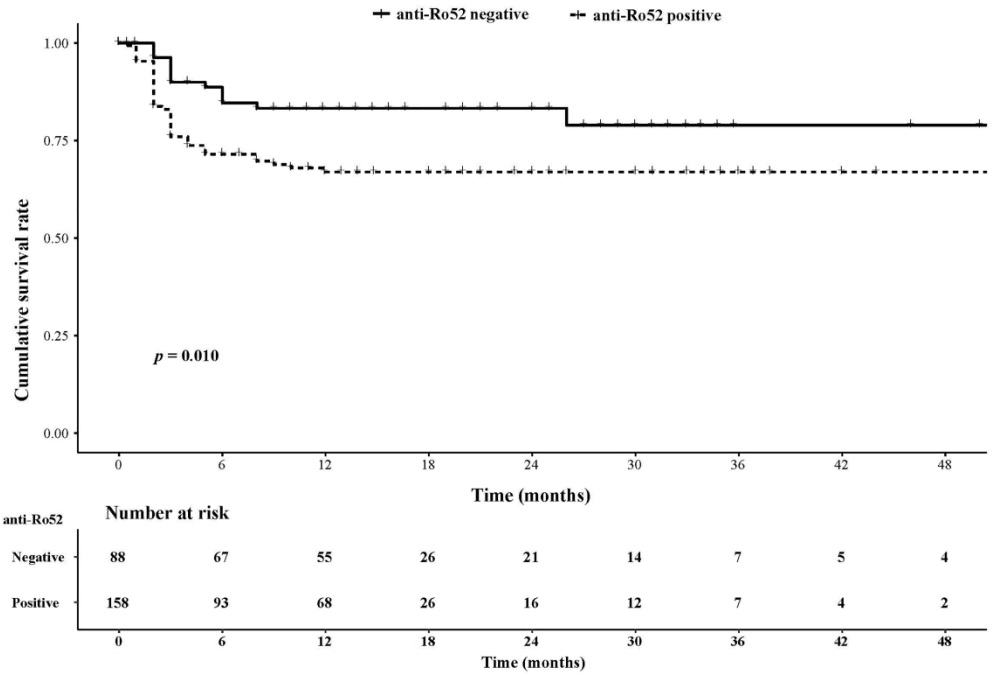

Figure 1B. Difference of cumulative survival rate anti-MDA5+ DM patients with versus without anti-Ro52 autoantibodies. Patients with anti- Ro52 antibodies positive had a significant higher mortality rate (log-rank p =0.010).

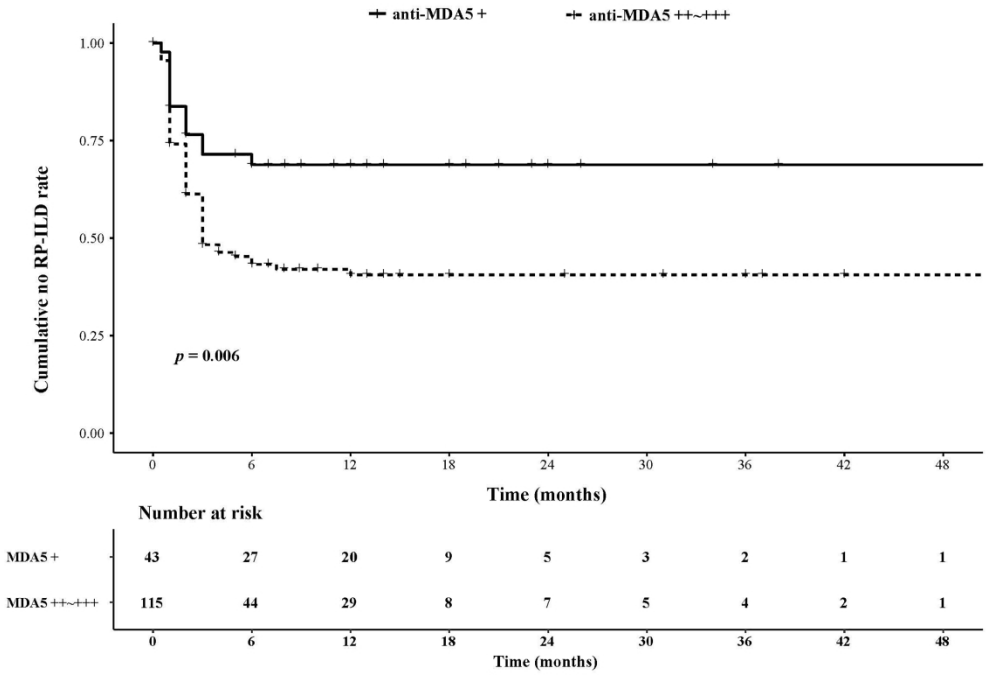

Figure 1C. Difference of cumulative no-RP-ILD rate of high titer of anti-MDA5 (++~+++) antibodies patients versus low titer of anti-MDA5 (+) antibodies in anti-MDA5-positive DM-ILD patients with anti-Ro52 antibodies. Patients with high titer of anti-MDA5 (++~+++) antibodies had a significant higher rate of RP-ILD (log-rank p =0.006).

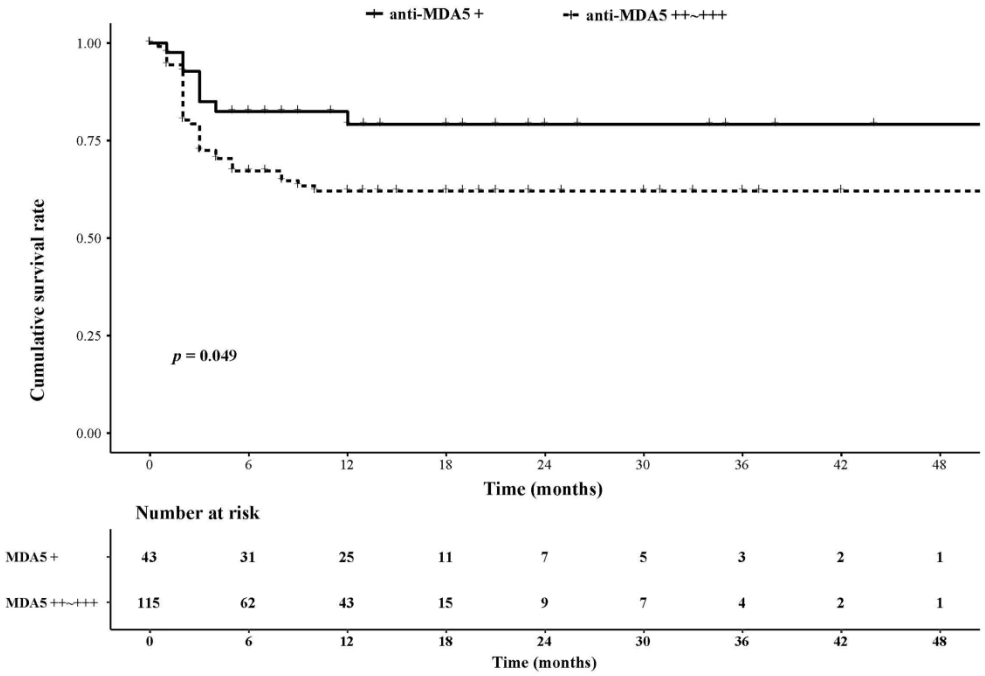

Figure 1D. Difference of cumulative survival rate of high titer of anti-MDA5 (++~+++) antibodies patients versus low titer of anti-MDA5 (+) antibodies in anti-MDA5-positive DM-ILD patients with anti-Ro52 antibodies. Patients with high titer of anti-MDA5 (++~+++) antibodies had a significant higher mortality rate (log-rank p =0.049).

Table 1. Clinical characteristics of anti-MDA5+ DM patients with versus without anti-Ro52 autoantibodies

Variables	Total (n=246)	Anti-Ro52 Negative (n=88)	Anti-Ro52 Positive (n=158)	p-value
Male, No. (%)	70 (28.46%)	28 (31.82%)	42 (26.58%)	0.383
Age, mean±SD, years	53.10±12.35	51.64±13.14	53.92±11.86	0.165
Age≥55 years, No. (%)	111 (45.12%)	40 (45.45%)	71 (44.94%)	0.938
Course of the disease, median (range), months	2 (1, 5)	3 (2, 7)	2 (1, 3)	**<0.001**
Disease duration≤3 months	169 (68.7%)	49 (55.68%)	120 (75.95%)	**0.001**
Follow-up periods, median (range), months	12 (3, 14)	12 (6, 23.50)	9 (3, 12)	**<0.001**
Myasthenia, No. (%)	112 (45.53%)	39 (44.32%)	73 (46.20%)	0.776
Active rash, No. (%)	229 (93.09%)	83 (94.32%)	146 (92.41%)	0.571
Gottron papule, No. (%)	168 (68.29%)	59 (67.05%)	109 (68.99%)	0.754
Heliotrope rash, No. (%)	140 (56.91%)	50 (56.82%)	90 (56.96%)	0.983
V sign, No. (%)	89 (36.18%)	34 (38.64%)	55 (34.81%)	0.549
Shawl sign , No. (%)	55 (22.36%)	21 (23.86%)	34 (21.52%)	0.672
Periungual erythematosus, No. (%)	52 (21.14%)	18 (20.45%)	34 (21.52%)	0.845
Arthritis, No. (%)	90 (36.59%)	34 (38.64%)	56 (35.44%)	0.618
Mechanic's hands, No. (%)	67 (27.24%)	26 (29.55%)	41 (25.95%)	0.544
Superficial erosion and ulcer, No.	34 (13.82%)	13 (14.77%)	21 (13.29%)	0.747

(%)				
ALT, median (range), units/L	46 (29, 84.18)	46 (31, 77)	46 (25.90, 94.50)	0.919
AST, median (range), units/L	52 (32.50, 82.80)	51 (30, 82.10)	53 (34, 83)	0.389
LDH, median (range), units/L	333 (253.50, 428.50)	312.50 (224, 415)	352 (264.50, 460.73)	**0.030**
CK, median (range), units/L	61 (36, 142.50)	57.50 (38, 114.75)	68 (35, 167)	0.442
ESR, median (range), mm/h	37.55 (23, 56)	31 (19.50, 53.50)	39 (26.90, 57)	**0.020**
CRP, median (range), mg/L	5.58 (3.02, 12.10)	5.18 (3, 11.50)	5.66 (3.10, 15.50)	0.485
Ferritin, median (range), ng/mL	860.90 (343.73, 1500)	775.25 (343.73, 1491.43)	904.70 (343.50, 1500)	0.505
ANA, positive, No. (%)	130 (52.85%)	35 (39.80%)	95 (60.13%)	**0.002**
Anti-aminoacyl tRNA synthetase autoantibodies, No. (%)	15 (6.10%)	1 (1.13%)	14 (8.86%)	**0.015**
Anti-MDA5 titer				0.623
+	72 (29.27%)	29 (32.95%)	43 (27.22%)	
++	46 (18.70%)	15 (17.05%)	31 (19.62%)	
+++	128 (52.03%)	44 (50%)	84 (53.16%)	
ILD, No. (%)	221 (89.84%)	75 (85.23%)	146 (92.41%)	0.074
RP-ILD, No. (%)	88 (35.77%)	11 (12.50%)	77 (48.73%)	**<0.001**
Death, No. (%)	60 (24.39%)	14 (15.91%)	46 (29.11%)	**0.021**

Values in bold are statistically significant at p<0.05 (compared between anti-Ro52 negative and anti-Ro52 positive

groups).

Table 2. Clinical characteristics of anti-MDA5+ and anti Ro52+ DM patients with versus without RP-ILD, and survivors versus non-survivors

Variables	non-RP-ILD (n=81)	RP-ILD (n=77)	p-value	non-death (n=104)	death (n=46)	p-value
Male, No. (%)	15 (18.52%)	27 (35.06%)	**0.019**	24 (23.08%)	18 (39.13%)	**0.043**
Age, mean±SD, years	51.1±12.25	56.88±10.72	**0.002**	52.18±11.87	58.13±11.51	**0.005**
Age≥55 years, No. (%)	29 (35.80%)	42 (54.55%)	**0.018**	40 (38.46%)	30 (65.22%)	**0.002**
Course of the disease, median (range), months	2 (1, 6)	1 (1, 3)	**0.001**	1 (2, 5)	1 (1, 3)	**0.038**
Disease duration≤3 months	50 (61.73%)	70 (90.91%)	**<0.001**	74 (71.15%)	41 (89.13%)	**0.016**
Follow-up periods, median (range), months	12 (7, 14)	3 (2, 12)	**<0.001**	12 (7.25, 17.25)	2.25 (2, 3.25)	**<0.001**
Myasthenia, No. (%)	39 (48.15%)	34 (44.16%)	0.615	49 (47.12%)	19 (41.30%)	0.510
Rash, No. (%)	77 (95.06%)	69 (89.61%)	0.196	99 (95.19%)	39 (84.78%)	**0.030**
Gottron papule, No. (%)	57 (70.37%)	52 (67.53%)	0.700	71 (68.27%)	31 (67.39%)	0.915

Heliotrope rash, No. (%)	51 (62.96%)	39 (50.65%)	0.118	61 (58.65%)	24 (52.17%)	0.460
V sign, No. (%)	28 (34.57%)	27 (35.06%)	0.948	39 (37.50%)	15 (32.61%)	0.565
Shawl sign , No. (%)	14 (17.28%)	20 (25.97%)	0.184	20 (19.23%)	12 (26.09%)	0.345
Periungual erythematosus, No. (%)	16 (19.75%)	18 (23.38%)	0.580	21 (20.19%)	12 (26.09%)	0.422
Arthritis, No. (%)	30 (37.04%)	26 (33.77%)	0.667	41 (39.42%)	11 (23.91%)	0.066
Mechanic's hands, No. (%)	22 (27.16%)	19 (24.68%)	0.722	27 (25.96%)	13 (28.26%)	0.769
Superficial erosion and ulcer, No. (%)	11 (13.58%)	10 (12.99%)	0.913	13 (12.50%)	8 (17.39%)	0.426
ALT, median (range), units/L	44 (23.95, 95.60)	47.05 (29, 90.23)	0.917	22 (41.1, 91)	54 (36.75, 118.50)	**0.047**
AST, median (range), units/L	49.60 (31.50, 77.80)	54.05 (41.25, 87.78)	0.071	30.7 (48, 76.35)	63.50 (47.75, 92.03)	**0.002**
LDH, median (range), units/L	313.50 (252, 404)	388.50 (282, 545.25)	**0.012**	249.3 (309, 393.75)	503 (344.75, 775.75)	**<0.001**
CK, median (range), units/L	59 (35, 139)	96.50 (33,	0.269	37 (60.5,	117 (35.50,	**0.020**

		218.75)		133.5)	316.50)	
ESR, median (range), mm/h	37 (23, 54.25)	46 (30.20, 61)	**0.034**	27 (39, 55)	39.50 (23.83, 71)	0.615
CRP, median (range), mg/L	4.56 (2.50, 8.13)	9.48 (3.57, 24.70)	**<0.001**	2.3 (4.7, 9.89)	12.01 (3.67, 26.80)	**<0.001**
Ferritin, median (range), ng/mL	600.10 (164.03, 1343.15)	1200.49 (608.45, 1943.07)	**0.001**	191.4 (668.4, 1436.8)	1500 (800.90, 2000)	**<0.001**
ANA, positive, No. (%)	49 (60.49%)	46 (59.74%)	0.923	63 (60.58%)	27 (58.70%)	0.828
Anti-aminoacyl tRNA synthetase autoantibodies, No. (%)	10 (12.35%)	4 (5.19%)	0.114	11 (10.58%)	2 (4.35%)	0.211
Anti-MDA5 titer						0.134
+	30 (37.04%)	13 (16.88%)		34 (32.69%)	8 (17.39%)	
++	11 (13.58%)	20 (25.97%)		20 (19.23%)	9 (19.57%)	
+++	40 (49.38%)	44 (57.14%)	**0.009**	50 (48.08%)	29 (63.04%)	
RP-ILD				30 (28.85%)	42 (91.30%)	**<0.001**
Death	4 (4.94%)	42 (54.55%)	**<0.001**	94 (90.38%)	45 (97.83%)	0.107

Values in bold are statistically significant at $p<0.05$.

Table 3. Risk factors of RP-ILD and death in anti-Ro52 positive anti-MDA5+ DM patients in univariate and multivariate cox regression analyses

Variables	Risk factors of RP-ILD				Risk factors of death			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Male	1.725 (1.078, 2.762)	**0.023**			1.91 (1.055, 3.457)	**0.033**		
Age≥55 years	1.727 (1.102, 2.707)	**0.017**			2.643 (1.439, 4.855)	**0.002**		
Disease duration≤3 months	4.453 (2.04, 9.722)	**<0.001**	4.434 (1.878, 10.467)	**0.001**	3.228 (1.273, 8.185)	**0.014**		
Active Rash					0.335 (0.149, 0.751)	**0.008**	0.251 (0.093, 0.676)	**0.006**
ALT≥50 units/L					1.68 (0.939, 3.003)	**0.08**		
AST≥40units/L	1.547 (0.91, 2.628)	0.107						
LDH≥270 units/L	1.666 (0.932, 2.978)	0.085			4.288 (1.536, 11.97)	**0.005**		
CK≥170 units/L					2.717 (1.494, 4.942)	**0.001**	2.835 (1.364, 5.896)	**0.005**
ESR≥20 mm/h	1.383 (0.634,	0.415						

	3.019)							
CRP≥8 mg/L	2.365 (1.497, 3.735)	**<0.001**	2.701 (1.580, 4.617)	**<0.001**	3.501 (1.903, 6.441)	**<0.001**	2.166 (1.059, 4.428)	**0.034**
Ferritin≥336.2 ng/mL	2.237 (1.062, 4.712)	**0.034**			3.879 (1.191, 12.637)	**0.024**		
Anti-MDA5 titer								
+								
++	2.501 (1.242, 5.033)	**0.01**						
+++	2.014 (1.084, 3.742)	**0.027**						
RP-ILD					15.606 (5.578, 43.666)	**<0.001**	15.719 (3.671, 67.317)	**<0.001**
Values in bold are statistically significant at p<0.05.								