Journal of Inflammation Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Liver Involvement is Associated with Higher Risk of Rapidly Progressive Interstitial Lung Disease and Mortality in Anti-Melanoma Differentiation-Associated Gene 5 Antibody- Positive Dermatomyositis

Xin Yang[1], Xiaoguang Cui[1], Xiao Yang[2], Bincheng Ren[1], Xiaojing Cheng[1], Xinrui Zhao[1], Shanshan Liu[1], Tian Tian[1], Hui Zhao[1], Li Qu[1], Xueyi Li[1]

[1]Department of Rheumatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, People's Republic of China; [2]Scientific Research Center and Precision Medical Institute, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, People's Republic of China

Correspondence: Xueyi Li, Department of Rheumatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, People's Republic of China, Tel +86 13992891987, Fax +86 02987679832, Email li-xueyi@139.com

Purpose: This study aimed to assess liver involvement and investigate its correlation with rapidly progressive interstitial lung disease (RP-ILD) and mortality in anti-melanoma differentiation-associated gene 5 antibody-positive (anti-MDA5 positive) DM patients.

Patients and Methods: This retrospective study included 159 patients diagnosed with anti-MDA5 positive DM or anti-synthetase syndrome (ASyS). Clinical features and laboratory findings were compared between patients with anti-MDA5 positive DM and patients with ASyS. In the anti-MDA5 positive DM cohort, clinical features and laboratory findings between patients with liver involvement and without liver involvement were further compared. The effects of liver involvement on the overall survival (OS) and development of RP-ILD were also analyzed using Kaplan–Meier method and Cox regression analysis.

Results: Levels of serum aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γGT) and alkaline phosphatase (ALP) were all significantly higher in patients with anti-MDA5 positive DM than those in patients with ASyS. In our cohort of anti-MDA5 positive DM patents, 31 patients (34.4%) were complicated with liver involvement. Survival analysis revealed that serum ferritin >1030.0 ng/mL ($p<0.001$), ALT >103.0 U/l ($p<0.001$), AST >49.0 U/l ($p<0.001$), γGT >82.0 U/l ($p<0.001$), ALP >133.0 U/l ($p<0.001$), lactate dehydrogenase (LDH)>474.0 U/l ($p<0.001$), plasma albumin (ALB) <35.7 g/l ($p<0.001$) and direct bilirubin (DBIL) >2.80 μmol/l ($p=0.002$) predicted poor prognosis. The incidence of RP-ILD increased remarkably in patients with liver involvement compared to patients without liver involvement (58.1% vs 22.0%, $p=0.001$). Multivariate analysis revealed that elevated serum ALT level was an independent risk factor for mortality (HR 6.0, 95% CI 2.3, 16.2, $p<0.001$) and RP-ILD (HR 5.9, 95% CI 2.2, 15.9, $p<0.001$) in anti-MDA5 positive DM patents.

Conclusion: Liver involvement is common in patients with anti-MDA5 positive DM. Elevated serum ALT level was an independent risk factor for RP-ILD and mortality in patients with anti-MDA5 positive DM.

Keywords: anti-melanoma differentiation-associated gene 5 antibody, liver involvement, ferritin, macrophage, interstitial lung disease, dermatomyositis

Introduction

Idiopathic inflammatory myopathy (IIM) constitutes a large spectrum of autoimmune disorders, which can be classified into several groups: dermatomyositis (DM), anti-synthetase syndrome (ASyS), immune-mediated necrotizing myopathy, inclusion body myositis, polymyositis (PM) and overlap myositis[1].

Received: 8 February 2024
Accepted: 7 May 2024
Published: 17 May 2024

A growing body of evidence indicated that myositis-specific antibodies have been associated with distinctive clinical phenotypes.[2] RNA helicase encoded by anti-melanoma differentiation-associated gene 5 (MDA5) was identified as the major autoantigen of anti-MDA5 antibody.[3] MDA5 is a cytosolic protein, essential for antiviral host immune responses, which functions as a virus RNA sensor.[4] Anti MDA5 antibody (anti-MDA5) positive DM is characterized by clinically amyopathic DM and rapidly progressive interstitial lung disease (RP-ILD), which is correlated with an aggressive course and poor prognosis. Skin ulceration and palmar papules are typical skin features of anti-MDA5 positive DM.[5,6] The prevalence of ILD reported in anti-MDA5 positive DM ranged from 50 to 100%.[7]

Anti-MDA5 positive DM has been frequently reported to be refractory to common combined immunosuppressive therapies, and the 6-month mortality of RP-ILD was reported to be as high as 50%.[8] Consequently, exploring the characteristics and risk factors for RP-ILD and mortality is still urgently needed.

Liver injury and elevated liver enzymes in anti-MDA5 positive DM patients were reported in many studies.[4,9–11] Jiang et al reported that 36.4% patients with anti-MDA5 positive DM presented with liver involvement.[9] However, studies focusing on the impact of liver involvement on RP-ILD and mortality are rare.

Analogous to anti-MDA5 positive DM, ASyS patients were also characterized by high frequency of ILD. About 50.67–100% ASyS patients have been shown to be complicated with ILD.[12] ASyS is characterized by the presence of autoantibodies directed against aminoacyl transfer RNA synthetase. Clinical features of ASyS consist of myositis, skin lesions, ILD, arthritis, Raynaud phenomenon, fever of unknown origin, and "mechanic's hand".[12,13]

In the present study, we retrospectively analyzed 159 patients with anti-MDA5 positive DM or ASyS. We compared the clinical features and laboratory findings between anti-MDA5 positive DM patients and ASyS patients. Furthermore, we investigated the prevalence of liver involvement in patients with anti MDA5 positive DM, and explore whether liver involvement has an impact on RP-ILD and overall survival (OS).

Materials and Methods

Study Design

This was a retrospective single-center study. We analyzed the clinical data of 159 consecutive patients with anti-MDA5 positive DM or ASyS hospitalized in the Department of Rheumatology at the Second Affiliated Hospital of Xi'an Jiaotong University from October 2017 to June 2023. The patients were followed up until September 2023. Survival status was confirmed by hospital records or the follow-up calls. All patients were screened for a panel of myositis-specific antibodies and myositis-associated antibodies (anti-OJ, EJ, PL7, PL12, SRP, JO-1, MDA5, TIF1-γ, Mi-2α, Mi-2β, Ku, NXP2, PM-Scl75, PM-Scl100, SAE1 and Ro52 antibodies) using a commercial immunoblot assay. A diagnosis of anti-MDA5 positive DM was based on the Bohan and Peter criteria[14,15] or 239th European Neuromuscular Centre (ENMC) criteria.[16] ASyS patients were clinically confirmed as having ASyS according to both the criteria of Connors et al and the criteria of Solomon et al and the presence of anti-tRNA synthetase antibodies.[17]

The presence of ILD was defined as the presence of interstitial changes on high-resolution computerized tomography (HRCT) scanning of the chest. RP-ILD was characterized by progressive dyspnea and hypoxemia, with a worsening of radiologic changes of interstitial lung inflammation within 3 months after the onset of respiratory symptoms.[18] Clinical, demographic, and laboratory data were collected by reviewing the electronic medical record system. Fatty liver was defined by ultrasonographic detection of hepatic steatosis. Patients were screened for Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection by testing HBV surface antigen and HCV antibody, respectively.

The normal range for alanine transaminase (ALT) is ≤30 U/l. We defined liver involvement as ALT level >60 U/l (more than 2 times the upper limit of the normal range).

The study was approved by the medical ethics committee of The Second Affiliated Hospital of Xi'an Jiaotong University (ID: 2023304) and performed in accordance with the Declaration of Helsinki. Because of its retrospective design, patient informed consent was waived by the above-mentioned ethics committee. The data was anonymized and maintained with confidentiality.

Statistical Analyses

Continuous variables were described as median (first and third quartiles). Comparison of continuous variables was performed by Mann–Whitney *U*-test. Categorical variables were presented as numbers and percentages. Comparison of categorical variables were assessed by the Chi-squared test or Fisher's exact test. The "Surv_cutpoint" function of the R package "survminer" was used to determine the optimal cutoff points for continuous parameter, and then each continuous parameter was divided into a categorical variable. Kaplan–Meier method was performed to analyze the OS, and Log rank tests were used to compare survival curves. Univariate and multivariate analyses of the risk factors for mortality and RP-ILD were performed using Cox regression analysis. Two-tailed P-values of <0.05 were considered statistically significant. All statistical analyses were performed with R statistical package version 4.2.3.

Results

Comparison of Clinical Characteristics and Laboratory Features Between Patients with Anti-MDA5 Positive DM and ASyS

A total of 159 patients diagnosed with anti-MDA5 positive DM or ASyS were enrolled between 2017.10.1 and 2023.6.30. There were 93 patients diagnosed with anti-MDA5 positive DM and 66 patients diagnosed with ASyS, with a median follow-up time of 380 days and 598 days, respectively. Among the anti-MDA5 positive DM patients, all patients met the 239th ENMC criteria. Overall, 31, 30 and 19 patients met the possible DM criteria, the probable DM criteria and the definite DM criteria by the Bohan/Peter criteria, respectively.

Comparisons of the demographic features, clinical features and laboratory findings on admission between patients with anti-MDA5 positive DM and patients with ASyS are summarized in Table 1. Anti-MDA5 positive DM group was composed of 61 women and 32 men, with a median age of 52 years. Among them, 77.4% (n=72) patients were treatment-naive (no prior treatment with glucocorticoids (GCs) or immunosuppressants). As expected, anti-MDA5 positive DM group had a greater frequency of heliotrope rash (43.0% vs 10.6%, p<0.001), Gottron's sign (63.4% vs 25.7%, p<0.001) and skin ulceration (27.9% vs 1.5%, p<0.001) than ASyS group.

Table 1 Comparison of Clinical Features Between Anti-MDA5 Positive DM and ASyS

	Anti-MDA5 Positive DM (n=93)	**ASyS (n=66)**	**P-value**
Gender, female, n (%)	61, 65.6%	49, 74.2%	0.322
Age at diagnosis, years, median (IQR)	52.0 (40.0, 58.0)	52.0 (45.0, 62.0)	0.360
Duration of disease, days, median (IQR),	92.0 (59.0, 153.0)	62.5 (31.0, 205.5)	0.780
Heliotrope rash, n (%)	40, 43.0%	7, 10.6%	<0.001
Gottron's sign, n (%)	59, 63.4%	17, 25.7%	<0.001
Skin ulceration, n (%)	26, 27.9%	1, 1.5%	<0.001
Muscle pain, n (%)	37, 39.8%	19, 28.8%	0.207
Muscle weakness, n (%)	54, 58.1%	28, 42.4%	0.075
Dysphagia, n (%)	5, 5.4%	6, 9.1%	0.528
ILD, n (%)	83, 89.2%	55, 83.3%	0.397
Tumor, n (%)	4, 4.3%	5, 7.5%	0.491
Ferritin, ng/mL, median (IQR)	835.5 (485.8, 1297.3)	165.0 (70.3, 429.9)	<0.001
ESR, mm/h, median (IQR)	34.5 (21.0,56.3)	28.0 (17.5, 51.0)	0.260
CRP (mg/dl), median (IQR)	3.2 (3.2,13.2)	7.1 (3.2, 19.6)	0.055
CK, U/l, median (IQR)	83.0 (47.0, 150.0)	152.5 (65.0, 475.3)	0.002
LDH, U/l, median (IQR)	326.0 (274.0, 449.0)	290.5 (230.3, 456.5)	0.087
AST, U/l, median (IQR)	60.0 (39.3, 94.8)	28.0 (21.0, 55.0)	<0.001
ALT, U/l, median (IQR)	41.5 (26.0, 86.8)	26.0 (14.0, 42.0)	<0.001

(*Continued*)

Table 1 (Continued).

	Anti-MDA5 Positive DM (n=93)	ASyS (n=66)	P-value
γGT, U/l, median (IQR)	48.0 (28.0, 94.0)	19.0 (12.0, 31.0)	<0.001
ALP, U/l, median (IQR)	81.0 (65.0, 104.0)	67.0 (54.0, 85.0)	<0.001
ALB, g/l, median (IQR)	33.6(29.4, 36.9)	34.0 (29.1, 36.9)	0.740
WBC, 10^9/L, median (IQR)	4.2 (3.3,5.7)	7.5 (5.7, 9.3)	<0.001
ANA titer≥ 1:320, n (%)	6, 7.6%	21, 34.4%	<0.001
Anti-Ro52, n (%)	55, 59.1%	52, 78.8%	0.015
Liver involvement	31, 34.4%	8, 12.3%	0.003
Treatment-naïve	72, 77.4%	57, 86.3%	0.225
Deceased patients	29, 31.2%	1, 1.5%	<0.001

Notes: Continuous variables are presented as median (interquartile range). Categorical variables were presented as n, %. Statistical significance: $p < 0.05$.

Abbreviations: ASyS, anti-synthetase syndrome; ILD, interstitial lung disease; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ALB, albumin; WBC, White blood cell; ANA, antinuclear antibodies; MDA5, melanoma differentiation-associated gene 5; IQR, interquartile range.

Consistent with previous studies, a significant increase in serum ferritin level was also identified in anti-MDA5 positive DM patients compared to those in ASyS patients (835.5 (485.8, 1297.3) vs 165.0 (70.3, 429.9) ng/mL, p<0.001). As expected, significantly lower serum creatine kinase (CK) level in anti-MDA5 positive DM patients was documented than in patients with ASyS (83.0 (47.0, 150.0) vs 152.5(65.0, 475.3) ng/mL, p=0.002). White blood cell (WBC) counts were also lower in anti-MDA5 positive DM patients when compared with ASyS patients (4.2(3.3,5.7) vs 7.5(5.7, 9.3) ×10^9/L, p<0.001).

Since liver injury was frequently reported in anti-MDA5 positive DM patients in previous study,[10] we comprehensively evaluated liver enzymes in the current study. As shown in Table 1, serum levels of AST (60.0(39.3, 94.8) vs 28.0 (21.0, 55.0) U/l, p<0.001), ALT (41.5(26.0,86.8) vs 26.0(14.0, 42.0) U/l, p<0.001), γGT (48.0(28.0, 94.0) vs 19.0(12.0, 31.0) U/l, p<0.001) and ALP(81.0(65.0, 104.0) vs 67.0 (54.0, 85.0) U/l, p<0.001) were all remarkably higher in patients with anti-MDA5 positive DM patients than those in patients with ASyS, indicating common liver involvement in anti-MDA5 positive DM patients.

The overall mortality of patients with anti-MDA5 positive DM was 29(31.2%), significantly higher than that of patients with ASyS [1(1.5%)]. Survival analysis showed that OS of anti-MDA5 positive DM group was remarkably lower than that of ASyS group (p<0.001, Supplementary Figure 1).

Comparison of Characteristics Between Anti-MDA5 Positive DM Patients with and without Liver Involvement

We defined liver involvement as ALT level >60 U/l (more than 2 times the upper limit of the normal range). After excluding 3 patients whose initial serum ALT was not available, we compared the clinical features of anti-MDA5 positive DM patients with and without liver involvement (Table 2). There were 31 patients in liver involvement group and 59 patients in non-liver involvement group.

We further analyzed R factor to determine the pattern of liver injury in each patient.[19] Among patients with liver involvement, 13.3% had a cholestatic injury pattern, 56.7% had a mixed pattern, 30.0% had a hepatocellular pattern. Serum ferritin level was strikingly higher in patients with liver involvement than in patients without liver involvement (1281.0 (748.0, 1700.5) vs 642.0 (326.9, 1016.0) ng/mL, p=0.005). The mortality of the liver involvement group was significantly higher than that of the non-liver involvement group (48.9% vs 18.6%, p = 0.007). Moreover, the incidence of RP-ILD was higher in patients with liver involvement when compared with patients who did not have liver involvement (58.1% vs 22.0%, p=0.001). Thirty-six patents performed electromyogram (EMG) on admission. MRI data were available in 24 patients. There was no difference between liver involvement group and non-liver involvement group regarding the presence of myogenic

Table 2 Comparison of Characteristics Between Anti MDA5 Positive DM Patients with and without Liver Involvement

	Liver involvement GROUP (n = 31)	Non Liver Involvement Group (n = 59)	P-value
Gender, female, n (%)	19, 61.2%	38, 64.4%	0.951
Age at diagnosis, median (IQR), years	53.0 (33.0, 62.0)	51.0 (40.8, 56.3)	0.400
Duration of disease, median (IQR), days	92.0 (31.0, 120.0)	98.0 (60.8, 181.0)	0.045
Heliotrope rash, n (%)	8, 25.8%	30, 50.8%	0.039
Gottron's sign, n (%)	22, 71.0%	36, 61.0%	0.481
Skin ulceration, n (%)	8, 25.8%	17, 28.8%	0.956
ILD, n (%)	29, 93.5%	51, 86.4%	0.484
Ferritin, ng/mL, median (IQR)	1281.0 (748.0, 1700.5)	642.0 (326.9, 1016)	0.005
ESR, mm/h, median (IQR)	30.5 (16.6, 56.8)	35.0 (22.8, 55.5)	0.210
CRP (mg/dl), median (IQR)	3.2 (3.2, 12)	3.2 (3.2, 16.5)	0.630
CK, U/l, median (IQR)	73.5 (46.3, 157.8)	96.0 (50.0, 149.0)	0.650
LDH, U/l, median (IQR)	331.0 (298.5, 439.5)	318.0 (269.0, 449.0)	0.380
AST, U/l, median (IQR)	102.0 (72.0, 375.0)	46.0 (31.0, 64.0)	<0.001
ALT, U/l, median (IQR)	118.0 (86.0, 163.0)	30.0 (18.0, 42.0)	<0.001
γGT, U/l, median (IQR)	93.0 (59.8, 299.8)	36.0 (24.0, 76.0)	<0.001
ALP, U/l, median (IQR)	116.5 (75.0, 179.0)	77.0 (64.0, 95.0)	0.007
ALB, g/l, median (IQR)	32.2 (29.8, 36.5)	33.9 (29.3, 37.1)	0.490
TBIL, μmol/L, median (IQR)	10.5 (9.0, 14.8)	10.0 (8.2, 12.0)	0.270
DBIL, μmol/L, median (IQR)	2.8 (2.3, 4.0)	2.7 (2.1, 3.3)	0.140
IBIL, μmol/L, median (IQR)	7.4 (6.5, 9.7)	7.3 (5.9, 9.0)	0.540
HBV, n (%)	0, 0%	0, 0%	-
HCV, n (%)	1, 3.6%	0, 0%	0.341
Fatty liver, n (%)	9, 32.1%	12, 21.4%	0.423
Treatment naive, n (%)	24, 77.4%	48, 81.3%	0.868
WBC, 10^9/L, median (IQR)	4.0 (3.5, 5.0)	4.3 (3.2, 6.2)	0.510
RP-ILD, n (%)	18, 58.1%	13, 22.0%	0.001
Deceased, n (%)	15, 48.9%	11, 18.6%	0.007

Notes: Continuous variables are presented as median (interquartile range). Categorical variables were presented as n, %. Statistical significance: $p < 0.05$.

Abbreviations: ILD, interstitial lung disease; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ALB, albumin; WBC, White blood cell; ANA, antinuclear antibodies; MDA5, melanoma differentiation-associated gene 5; RP-ILD, rapidly progressive interstitial lung disease; TBIL, total bilirubin; DBIL, direct bilirubin; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range.

pattern at EMG (62.5% vs 46.2%, p = 0.689) and magnetic resonance imaging (MRI) testing indicative of myositis (87.5% vs 75.0%, p = 0.859). No patient was complicated with HBV infection in anti-MDA5 positive DM. Only one patient was complicated with chronic HCV infection. No differences were observed between patients with liver involvement and patients without liver involvement in terms of incidences of HBV infection, HCV infection and fatty liver (Table 2).

Comparison of Baseline Characteristics of Anti MDA5 Positive DM Patients Between Survivors and Non-survivors

The baseline characteristics between deceased and surviving patients are shown in Table 3. Ferritin level (1385.0.5(925,0, 2189.5) vs 627.5(359.6, 1007.0) ng/mL, p<0.001) were higher in the non-survivor group than in the survivor group. The non-survivors also had higher levels of CK (131.0 (69.8, 279.0) vs 72.0(45.5, 127.0) U/l, p=0.012) and lactate dehydrogenase (LDH) (431.0(316.0, 509.5) vs 307.0 (270.5, 418.5) U/l, p=0.003).

To evaluate liver involvement in anti-MDA5 positive DM patients, we compared liver injury markers between survivors and non-survivors. AST level (77.0 (62.1, 130.5) vs 48.5 (31.8, 83.3) U/l, p <0.001), ALT level (97.0(43.3, 145.8) vs 34.0

Table 3 Comparison of Baseline Characteristics of Anti MDA5 Positive DM Patients Between Survivors and Non-survivors

	Survivors (n = 64)	Non-Survivors (n = 29)	P- value
Gender, female, n (%)	46, 71.9%	17, 58.6%	0.304
Age at diagnosis, median (IQR), years	49.0 (32.3, 56.0)	55.0 (51.0.0, 64.0)	0.002
Duration of disease, median (IQR), days	120.0 (90.0, 181.0)	31.0 (19.0, 61.0)	<0.001
Heliotrope rash, n (%)	30, 46.9%	10, 34.5%	0.372
Gottron's sign, n (%)	42, 65.6%	17, 58.6%	0.676
Skin ulceration, n (%)	18, 28.1%	8, 27.6%	1.000
Ferritin, ng/mL, median (IQR)	627.5 (359.6, 1007.0)	1385.0 (925.0, 2189.5)	<0.001
ESR, mm/h, median (IQR)	35.0 (21.0, 54.5)	28.0 (22.0, 59.0)	0.660
CRP (mg/dl), median (IQR)	3.2(3.2, 3.2)	17.1(5.3, 44.1)	<0.001
CK, U/l, median (IQR)	72.0 (45.5, 127.0)	131.0 (69.8, 279.0)	0.012
LDH, U/l, median (IQR)	307.0 (270.5, 418.5)	431.0 (316.0, 509.5)	0.003
AST, U/l, median (IQR)	48.5 (31.8, 83.3)	77.0 (62.1, 130.5)	<0.001
ALT, U/l, median (IQR)	34.0 (18.0, 57.8)	97.0 (43.3, 145.8)	<0.001
γGT, U/l, median (IQR)	38.5 (25.0, 88.3)	89.0 (41.0, 239.0)	0.007
ALP, U/l, median (IQR)	79.5 (63.0, 97.8)	90.0.0 (65.0, 140.0)	0.170
ALB, g/l, median (IQR)	34.7 (30.0, 37.7)	29.83 (27.1, 32.9)	0.006
TBIL, μmol/L, median (IQR)	9.9 (8.1, 12.2)	10.6 (9.2, 12.1)	0.190
DBIL, μmol/L, median (IQR)	2.6 (2.0, 3.3)	3.3 (2.7, 4.0)	0.004
IBIL, μmol/L, median (IQR)	7.3 (5.8, 9.2)	7.4 (6.7, 8.9)	0.630
HBV, n (%)	0, 0%	0, 0%	-
HCV, n (%)	0, 0%	1, 3.7%	0.318
Fatty liver, n (%)	16, 26.7%	6, 22.2%	0.861
WBC, 10^9/L, median (IQR)	3.9 (3.3, 5.0)	5.1 (3.2, 8.0)	0.120
Anti-Ro52, n (%)	35, 54.7%	20, 69.0%	0.285
RP-ILD, n (%)	13, 20.3%	19, 65.5%	<0.001

Notes: Continuous variables are presented as median (interquartile range). Categorical variables were presented as n, %. Statistical significance: $p < 0.05$.

Abbreviations: ILD, interstitial lung disease; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ALB, albumin; WBC, White blood cell; ANA, antinuclear antibodies; MDA5, melanoma differentiation-associated gene 5; RP-ILD, rapidly progressive interstitial lung disease; TBIL, total bilirubin; DBIL, direct bilirubin; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range.

(18.0, 57.8) U/l, p<0.001), γGT level (89.0 (41.0, 239.0) vs 38.5(25.0, 88.3) U/l, p=0.007) and direct bilirubin (DBIL) level (3.3 (2.7, 4.0) vs 2.6(2, 3.3) μmol/l, p=0.004) were all increased in non-survivors (Table 3). Also, the level of plasma albumin (ALB) was significantly lower in non-survivors (29.83 (27.1, 32.9) vs 34.7 (30.0, 37.7) g/l, p=0.006).

Correlation Between Liver Enzymes and Serum Ferritin of Anti MDA5 Positive DM Patients

We compared the correlation between liver injury markers and serum ferritin. As shown in Figure 1, There was a significant association between ferritin level and level of ALT (p=0.002), AST (p<0.001), γGT (p<0.001), ALP (p=0.005), DBIL (p=0.001) and LDH (p<0.001), respectively.

Elevated Liver Enzyme Levels Predict RP-ILD and Mortality of Anti MDA5 Positive DM Patients

The standard treatment regimen for anti-MDA5 positive DM has not been established. In our clinical practice, all anti-MDA5 positive DM patients were treated with GCs. Immunosuppressants were simultaneously administered, including hydroxychloroquine, tacrolimus, intravenous cyclophosphamide, or Janus kinase inhibitor.

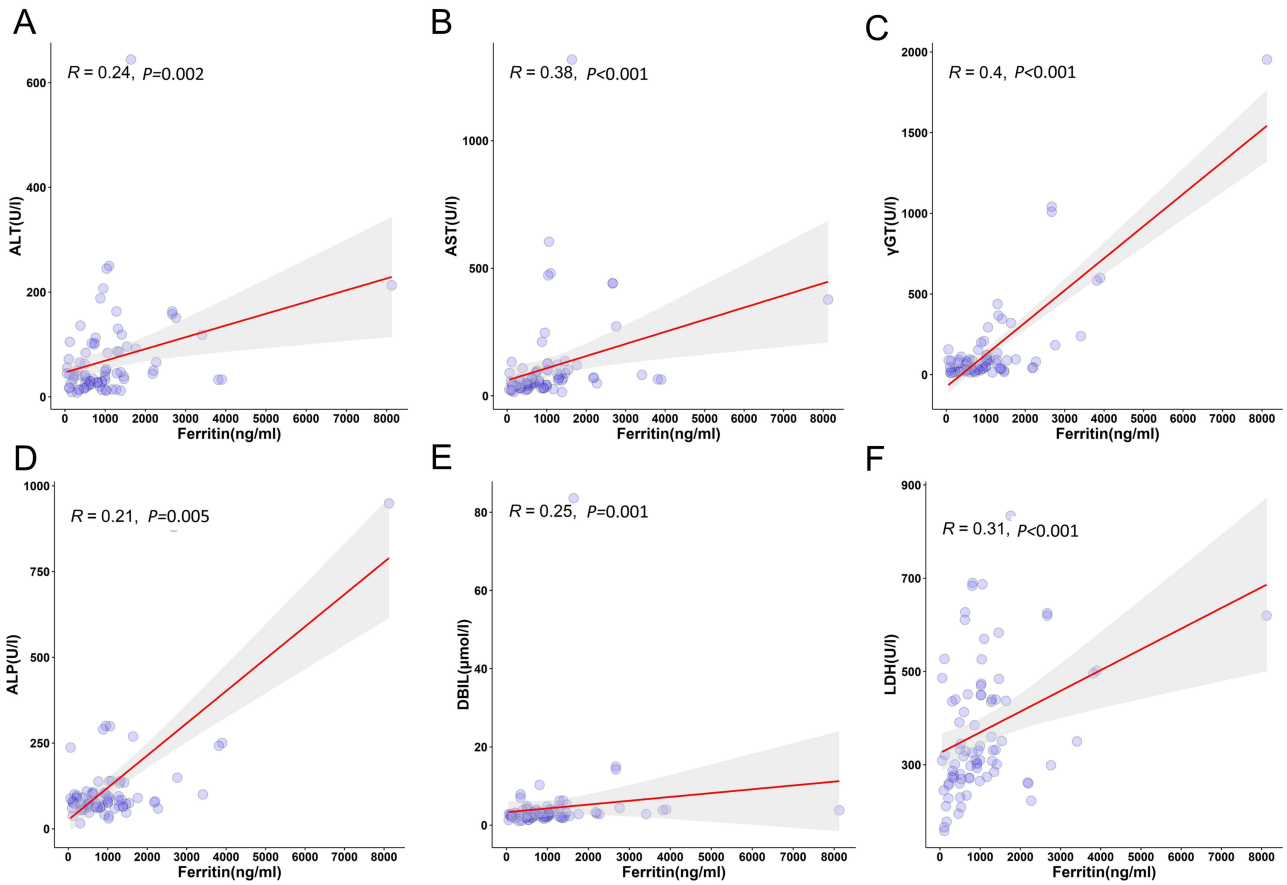

Figure 1 Correlations between serum ferritin and liver enzymes. **(A)** Serum ferritin and ALT; **(B)** Serum ferritin and AST; **(C)** Serum ferritin and γGT; **(D)** Serum ferritin and ALP; **(E)** Serum ferritin and DBIL; **(F)** Serum ferritin and LDH.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, γ-glutamyl transferase; ALP, alkaline phosphatase; DBIL, direct bilirubin; LDH, lactate dehydrogenase.

The OS rate was remarkably higher in anti-MDA5 positive DM patients treated with tacrolimus than in those treated without tacrolimus (p<0.001, Supplementary Figure 2A). We also analyzed the efficacy of tofacitinib and found that patients treated with tofacitinib had a substantially better prognosis than those not treated with it (p<0.001, Supplementary Figure 2B).

To further validate whether the aforementioned differentially expressed markers are prognostic risk factors, the "surv_cutpoint" function of the R package "survminer" was used to evaluate the optimal cut-off values of ALT, AST, γGT, ALP, LDH, ALB, ferritin and DBIL at diagnosis for the risk of mortality. Patients were stratified into high-level and low-level groups according to the optimal cut-off value of the markers above. As shown Supplementary Figure 3A-F, Kaplan–Meier survival analysis showed that serum ALT >103.0U/l (p<0.001), AST >49.0U/l (p<0.001), γGT >82.0U/l (p<0.001), ALP >133.0U/l (p<0.001), DBIL >2.80 μmol/l (p=0.002) and ALB <35.7 g/l (p<0.001) predicted poor OS. Moreover, patients with ferritin >1030.0 ng/mL (p<0.001) and LDH >474.0U/l (p<0.001) had lower OS (Supplementary Figure 3G and H).

Due to the relatively small sample size of deceased patient (n=29), 2 multivariable Cox proportional hazards models (model 1: adjusting for age and ferritin levels; model 2: adjusting for age, ferritin levels and treatment with/without tacrolimus) were performed to examine the association of ALT levels and mortality. Multivariate Cox regression analysis revealed that both high ALT level (Model 1: HR 3.9, 95% CI 1.6, 9.5, p<0.001; Model 2: HR 3.7, 95% CI 1.4, 9.5, p=0.008) and high serum ferritin (Model 1: HR 6.0, 95% CI 2.3, 16.2, p<0.001; Model 2: HR 3.3, 95% CI 1.2, 9.5, p=0.024) were independent risk factors for mortality of the anti-MDA5 positive DM patients (Table 4).

Table 4 Initial Parameters Associated with Death Using Cox Regression Model

	Univariate Analysis			Multivariate Analysis Model 1			Multivariate Analysis Model 2		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Age >38 years	12.0	1.6–89.0	0.014	11.5	1.5–88.5	0.019	18.4	2.3–147.9	0.006
Gender, female	0.5	0.3–1.1	0.098						
ALT >103.0U/l	5.7	2.6–13.0	<0.001	3.9	1.6–9.5	<0.001	3.7	1.4–9.5	0.008
AST >49.0U/l	13.0	3.0–54.0	<0.001	-	-	-			
γGT>82.0U/l	3.6	1.6–8.0	0.002	-	-	-			
ALP>133.0U/l	4.1	1.8–9.2	<0.001	-	-	-			
ALB< 35.7 g/l	5.8	1.7–19.6	0.004						
DBIL >2.80 μmol/l	3.9	1.5–9.8	0.004						
LDH >474.0U/l	4.3	1.9–9.7	<0.001	-	-	-			
CK >199.0 U/l	5.2	2.2–12.0	<0.001						
Ferritin>1030.0ng/mL	8.7	3.4–22.0	<0.001	6.0	2.3–16.2	<0.001	3.3	1.2–9.5	0.024
Treatment without tofacitinib	4.8	1.8–13.0	0.002						
Treatment without Tacrolimus	6.3	2.4–17.0	<0.001				6.2	1.9–20.2	0.002

Notes: Statistical significance: $p < 0.05$.
Abbreviations: LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyl transferase; ALP, alkaline phosphatase; ALB, albumin; DBIL, direct bilirubin; CK, creatine kinase.

In the current study, the incidence of RP-ILD was notably higher in anti MDA5 positive DM patients with liver involvement than in patients without liver involvement. We investigated whether initial liver injury markers could predict RP-ILD in anti MDA5 positive DM patients. Univariate regression analysis revealed that ALT >103.0 U/l (HR 6.8, CI 2.9, 16, p<0.001), AST >49.0 U/l (HR 3.0, CI 1.3–6.9, p=0.008), γGT >82.0U/l (HR 2.1, CI 1–4.5, p=0.045), ALP >133.0 U/l (HR 4, CI 1.8–9, p<0.001), LDH >474.0U/l (HR 2.2, CI 0.9–4.9, p=0.007), ALB <35.7g/l (HR 6.4, CI 2.2–19, p<0.001) and ferritin >1030.0 ng/mL (HR 3.8, CI 1.7–8.5, p=0.001) predicted RP-ILD (Table 5). Levels of serum ferritin, ALT and ALB were then included for multivariate regression analysis. As expected, multivariate COX regression analysis for risk factors of RP-ILD also showed a marked impact of elevated ALT level on RP-ILD (HR 5.9, 95% CI 2.2, 15.9, p<0.001).

Table 5 Initial Parameters Associated with RP-ILD Using Cox Regression Model

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age >38, years	2.2	0.9–5.8	0.100			
Gender, female	1.4	0.7–2.9	0.390			
ALT >103.0U/l	6.8	2.9–16	<0.001	5.9	2.2–15.9	<0.001
AST >49.0U/l	3.0	1.3–6.9	0.008	-	-	-
γGT>82.0U/l	2.1	1–4.5.0	0.045	-	-	-
ALP>133.0U/l	4.0	1.8–9.0	<0.001	-	-	-
LDH >474.0U/l	2.2	0.9–4.9	0.007	-	-	-
ALB< 35.7 g/l	6.4	2.2–19.0	<0.001	9.5	2.1–43.2	0.004
DBIL >2.80 μmol/l	1.0	1.0–1.1	0.007			
CK >199.0 U/l	2.4	1.0–5.8	0.041			
Ferritin>1030.0 ng/mL	3.8	1.7–8.5	0.001	1.0	0.4–2.6	0.967

Notes: Statistical significance: $p < 0.05$.
Abbreviations: RP-ILD, rapidly progressive interstitial lung disease; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyl transferase; ALP, alkaline phosphatase; ALB, albumin; DBIL, direct bilirubin; CK, creatine kinase.

Discussion

In the present study, we included 159 patients diagnosed with anti-MDA5 positive DM or ASyS. We comprehensively compared the clinical characteristics and liver injury markers between the two groups. We found that the levels of ALT, AST, γGT and ALP in anti-MDA5 positive DM patients were all notably higher than those in ASyS group. Liver involvement was more common in anti-MDA5 positive DM patients than ASyS patients (34.4% vs 12.3%). Consistent with previous studies, the ferritin level was strikingly elevated and significantly higher in anti-MDA5 positive DM group than that in ASyS group. Moreover, in patients with anti-MDA5 positive DM, levels of ALT and AST were significantly higher in non-survivors compared with survivors. Survival analysis identified high levels of ALT, AST, γGT and ALP as poor prognostic factors in anti-MDA5 positive DM group. Additionally, increased ALT level was also defined as an independent risk factor for RP-ILD in anti-MDA5 positive DM patients.

Liver involvement was previously reported as an extra-muscular manifestation in anti-MDA5 positive DM.[10,11] Surprisingly, in Nagashima et al's study, patients with liver dysfunction were all positive for the anti- MDA5 antibody. Liver damage may be associated with autoimmune-mediated inflammation or other causes, including viral hepatitis, fatty liver and drug-induced hepatitis. In the current anti-MDA5 positive DM cohort, no patients were complicated with HBV infection. Only one patient was complicated with chronic HCV infection. Twenty-five percent patients were complicated with fatty liver on admission; however, there was a similar percentage of patients complicated with fatty liver in anti-MDA5 positive DM patients with and without liver involvement (32.1% vs 21.4%). Additionally, as high as 77.4% patients were treatment-naive. Therefore, we can largely exclude the effect of viral hepatitis, fatty liver and medication on the elevation of liver injury markers. ALT and AST levels can be elevated in patients with muscle involvement. However, in our study, there was no difference between liver involvement group and non-liver involvement group regarding the presence of myogenic pattern at EMG, MRI testing indicative of myositis and serum CK level. Therefore, the effect of muscle involvement on ALT and AST levels can be largely ruled out. We proposed that the elevation of the liver injury markers was mainly due to anti-MDA5 positive DM itself.

The mechanism of liver injury in anti-MDA5 positive DM patients is still unclear. In Nagashima et al's study, liver biopsies from anti-MDA5 positive DM patients showed hepatocyte ballooning, pigmented macrophages, and glycogenated nuclei and mild steatosis.[10] Our study revealed significant positive correlations between serum liver enzymes and ferritin. High serum ferritin level, a key marker of systemic macrophage activation, has been reported as a hallmark inflammatory marker in anti-MDA5 positive DM patients. Accumulating evidence suggests that hyperferritinemia may play a pathogenetic role in increasing the inflammatory process.[20] Thus, we hypothesized that macrophage activation may be essential in the pathogenesis of liver injury in anti-MDA5 positive DM patients. In the autopsy of an anti-MDA5 positive DM patient with RP-ILD and hyperferritinemia, systemic ferritin producing macrophages were detected in the alveoli, bone marrow, liver, and spleen.[21] Moreover, soluble CD206, a marker of activated macrophages, was shown to significantly increase in fatal cases of anti-MDA5 positive DM-ILD patients. In that study, dense accumulation of CD206+ macrophages into the airspace of a fatal DM-ILD case was also demonstrated.[22] Levels of other macrophage activation markers, including serum chitotriosidase, neopterin and serum-soluble CD163, were significantly elevated in anti-MDA5 positive DM patients.[23–25] Peng et al showed that serum levels of neopterin were significantly associated with RP-ILD and poor prognosis in DM patients.[24] Immunohistochemical analysis of the lung specimens showed patients with RP-ILD had significantly higher amounts of CD163-positive macrophages at the alveolar spaces when compared with the patients with chronic ILD.[26]

In a recent study by Zhao et al, high HScore was identified as a risk factor for poor survival among anti-MDA5-positive DM-ILD patients, suggesting the involvement of macrophage activation in the pathogenesis.[27] In Zhao et al's study, serum AST concentration was also notably higher in survivors than in non-survivors.

MDA5 recognizes intracellular viral nucleic acids and triggers type I interferon (IFN) production to suppress the replication of viruses.[28] Integrated miRNA-mRNA association analysis using circulating monocytes from patients with anti-MDA5 positive DM-ILD patients has shown an anti-viral inflammatory response.[29] Great similarities of clinical and pathogenic features have been reported between Coronavirus disease 2019 (COVID-19) and anti-MDA5 positive DM. They presented with similar radiologic appearance on chest CT and hyperferritinemia.[4,30] Besides, anti-MDA5 antibodies

have been identified in the serum of 48.2% Chinese patients with COVID-19.[31] Cytokines and chemokines have been evaluated in anti-MDA5 positive DM patients by many studies. Increased levels of Interferon-a (INF-a), INF-β, IFNλ3, macrophagecolony stimulating factor (M-CSF), interleukin-2 (IL-2), IL-6, IL-8, IL-10, IL15, IL-18, C-C motif ligand 2 (CCL2), C-X-C motif ligand 10 (CXCL10), CXCL11 and tumor necrosis factor α were reported recently, indicating that anti-MDA5 positive DM is characterized by "cytokine storm" conditions.[32–36] Taken together, we may hypothesize that viral infection may induce the activation of macrophage and further triggers the "cytokine storm", leading to injuries of different target organs in anti-MDA5 positive DM patients. In our study, the occurrence of RP-ILD and mortality was significantly higher in liver involvement than in non-liver involvement group. Multivariate COX regression analysis confirmed that higher ALT level was independently associated with higher risk of RP-ILD and mortality. Thus, we speculate that elevated liver enzymes may indicate uncontrolled hyperinflammation and macrophage activation, leading to increased risk of RP-ILD and mortality.

So far, the optimal treatment therapy for anti-MDA5 positive DM has not been established.[8] It is generally agreed that GCs should remain the first-line drug for initial treatment. High-dose GCs in combination with immunosuppressive agents have been commonly used for the treatment of anti-MDA5 positive DM-ILD.[37,38] A recent prospective study supported the benefit of triple combination therapy (GCs, tacrolimus and cyclophosphamide) in the management of anti-MDA5 positive DM-ILD patients.[39] Tacrolimus has been increasingly used in the treatment of DM-ILD and may lead to a better prognosis.[40] A retrospective study from Japan demonstrated that the addition of tacrolimus significantly improves the event-free and disease-free survival of patients with PM-/DM-related ILD.[41] In a recent prospective study, the 52-week survival rate in the prospective group initially treated with tacrolimus and GCs was 88.0%, suggesting that combination treatment with tacrolimus and GCs may improve the short-term mortality of PM/DM-ILD patients.[42] In our cohort, the combination of tacrolimus with conventional treatment (GCs alone or GCs plus other immunosuppressants except tacrolimus) significantly improved OS of patients with anti-MDA5 positive DM.

Tofacitinib has also been used as a promising immunosuppressant in treating patients with anti-MDA5 positive DM.[43] In a single-center, open-label clinical study, early-stage anti-MDA5 positive DM-ILD patients treated with tofacitinib exhibited significantly higher OS compared with historical controls.[44] As for the treatment of refractory anti-MDA5 positive DM-ILD, Kurasawa et al reported patients who had poor prognostic factors and who were predicted to have a poor prognosis and failed to respond to triple therapy received a combination therapy with tofacitinib. The survival rate of the patients treated with the combination therapy was significantly better than that of the historical controls before tofacitinib.[45] Consistent with previous studies, the efficacy of tofacitinib was also proved in our study.

It should be noted that our study had several limitations. First, this study was a single-center, retrospective study. Biases (selection bias and information bias) were inevitable with such studies. Numerous baseline data on admission were missing due to the retrospective design. Second, the relatively small sample size forbad comprehensive multivariate regression analysis to remove all the confounding effects. Thirdly, cytokine data, liver biopsies and disease activity measurements were not performed in the present study. Prospective randomized controlled trial studies are needed to validate our findings.

In conclusion, the current study indicated that liver involvement is common in Chinese patients with anti-MDA5 positive DM. Elevated serum ALT level is an independent risk factor for RP-ILD and mortality of patients with anti-MDA5 positive DM.

Conclusion

The current study indicated that liver involvement is common in Chinese patients with anti-MDA5 positive DM. Elevated serum ALT level is an independent risk factor for RP-ILD and mortality of patients with anti-MDA5 positive DM.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the medical ethics committee of The Second Affiliated Hospital of Xi'an Jiaotong University (ID: 2023304).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors have read and approved the final submitted manuscript.

Funding

This work was supported by Key R&D Project in Shaanxi Province (NO.2019SF-050).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Lundberg IE, Fujimoto M, Vencovsky J, et al. Idiopathic inflammatory myopathies. *Nat Rev Dis Primers*. 2021;7(1):86. doi:10.1038/s41572-021-00321-x
2. Tanboon J, Uruha A, Stenzel W, Nishino I. Where are we moving in the classification of idiopathic inflammatory myopathies? *Curr Opinion Neurol*. 2020;33(5):590–603. doi:10.1097/WCO.0000000000000855
3. Sato S, Hoshino K, Satoh 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(7):2193–2200. doi:10.1002/art.24621
4. Nombel A, Fabien N, Coutant F. Dermatomyositis With Anti-MDA5 Antibodies: bioclinical Features, Pathogenesis and Emerging Therapies. *Front Immunol*. 2021;12:773352. doi:10.3389/fimmu.2021.773352
5. Narang NS, Casciola-Rosen L, Li S, Chung L, Fiorentino DF. Cutaneous Ulceration in Dermatomyositis: association With Anti-Melanoma Differentiation-Associated Gene 5 Antibodies and Interstitial Lung Disease: analysis of Skin Ulcers in Dermatomyositis. *Arthritis Care Res*. 2015;67(5):667–672. doi:10.1002/acr.22498
6. Lu X, Peng Q, Wang G. Anti-MDA5 antibody-positive dermatomyositis: pathogenesis and clinical progress. *Nat Rev Rheumatol*. 2024;20(1):48–62. doi:10.1038/s41584-023-01054-9
7. Wu W, Guo L, Fu Y, et al. Interstitial Lung Disease in Anti-MDA5 Positive Dermatomyositis. *Clin Rev Allergy Immunol*. 2021;60(2):293–304. doi:10.1007/s12016-020-08822-5
8. McPherson M, Economidou S, Liampas A, Zis P, Parperis K. Management of MDA-5 antibody positive clinically amyopathic dermatomyositis associated interstitial lung disease: a systematic review. *Semin Arthritis Rheumatism*. 2022;53:151959. doi:10.1016/j.semarthrit.2022.151959
9. Jiang Y, Liu Y, Zhao Y, et al. Mitochondrial morphology and MAVS-IFN1 signaling pathway in muscles of anti-MDA5 dermatomyositis. *Ann Clin Transl Neurol*. 2021;8(3):677–686. doi:10.1002/acn3.51311
10. Nagashima T, Kamata Y, Iwamoto M, Okazaki H, Fukushima N, Minota S. Liver dysfunction in anti-melanoma differentiation-associated gene 5 antibody-positive patients with dermatomyositis. *Rheumatol Int*. 2019;39(5):901–909. doi:10.1007/s00296-019-04255-2
11. Gono T, Kawaguchi Y, Satoh T, et al. Clinical manifestation and prognostic factor in anti-melanoma differentiation-associated gene 5 antibody-associated interstitial lung disease as a complication of dermatomyositis. *Rheumatology*. 2010;49(9):1713–1719. doi:10.1093/rheumatology/keq149
12. Opinc AH, Makowska JS. Antisynthetase syndrome – much more than just a myopathy. *Semin Arthritis Rheumatism*. 2021;51(1):72–83. doi:10.1016/j.semarthrit.2020.09.020
13. Solomon J, Swigris JJ, Brown KK. Myositis-related interstitial lung disease and antisynthetase syndrome. *J Bras Pneumol*. 2011;37(1):100–109. doi:10.1590/S1806-37132011000100015
14. Bohan A, Peter JB. Polymyositis and Dermatomyositis: (Second of Two Parts). *N Engl J Med*. 1975;292(8):403–407. doi:10.1056/NEJM197502202920807
15. Bohan A, Peter JB. Polymyositis and Dermatomyositis: (First of Two Parts). *N Engl J Med*. 1975;292(7):344–347. doi:10.1056/NEJM197502132920706
16. Mammen AL, Allenbach Y, Stenzel W, et al. 239th ENMC International Workshop: classification of dermatomyositis, Amsterdam, the Netherlands, 14–16 December 2018. *Neuromuscul Disord*. 2020;30(1):70–92. doi:10.1016/j.nmd.2019.10.005

17. Patel J, Ravishankar A, Maddukuri S, Vazquez T, Grinnell M, Werth VP. Identification of Similarities Between Skin Lesions in Patients With Antisynthetase Syndrome and Skin Lesions in Patients With Dermatomyositis by Highly Multiplexed Imaging Mass Cytometry. *Arthritis Rheumatol*. 2022;74(5):882–891. doi:10.1002/art.42050
18. Allenbach Y, Uzunhan Y, Toquet S, et al. Different phenotypes in dermatomyositis associated with anti-MDA5 antibody: study of 121 cases. *Neurology*. 2020;95(1):e70–e78. doi:10.1212/WNL.0000000000009727
19. Leise MD, Poterucha JJ, Talwalkar JA. Drug-Induced Liver Injury. *Mayo Clin Proc*. 2014;89(1):95–106. doi:10.1016/j.mayocp.2013.09.016
20. Ruscitti P, Di Cola I, Di Muzio C, et al. Expanding the spectrum of the hyperferritinemic syndrome, from pathogenic mechanisms to clinical observations, and therapeutic implications. *Autoimmunity Rev*. 2022;21(7):103114. doi:10.1016/j.autrev.2022.103114
21. Gono T, Miyake K, Kawaguchi Y, Kaneko H, Shinozaki M, Yamanaka H. Hyperferritinaemia and macrophage activation in a patient with interstitial lung disease with clinically amyopathic DM. *Rheumatology*. 2012;51(7):1336–1338. doi:10.1093/rheumatology/kes012
22. Horiike Y, Suzuki Y, Fujisawa T, et al. Successful classification of macrophage-mannose receptor CD206 in severity of anti-MDA5 antibody positive dermatomyositis associated ILD. *Rheumatology*. 2019;58(12):2143–2152. doi:10.1093/rheumatology/kez185
23. Kawasumi H, Katsumata Y, Nishino A, et al. Association of Serum Soluble CD163 with Polymyositis and Dermatomyositis, Especially in Anti-MDA5 Antibody–positive Cases. *J Rheumatol*. 2018;45(7):947–955. doi:10.3899/jrheum.170997
24. Peng QL, Zhang YM, Liang L, et al. A high level of serum neopterin is associated with rapidly progressive interstitial lung disease and reduced survival in dermatomyositis. *Clin Exp Immunol*. 2020;199(3):314–325. doi:10.1111/cei.13404
25. Fujisawa T, Hozumi H, Yasui H, et al. Clinical Significance of Serum Chitotriosidase Level in Anti-MDA5 Antibody–positive Dermatomyositis-associated Interstitial Lung Disease. *J Rheumatol*. 2019;46(8):935–942. doi:10.3899/jrheum.180825
26. Zuo Y, Ye L, Liu M, et al. Clinical significance of radiological patterns of HRCT and their association with macrophage activation in dermatomyositis. *Rheumatology*. 2020;59(10):2829–2837. doi:10.1093/rheumatology/keaa034
27. Zhao S, Ma X, Zhang X, et al. Clinical significance of HScore and MS score comparison in the prognostic evaluation of anti-MDA5-positive patients with dermatomyositis and interstitial lung disease. *Modern Rheumatol*. 2022;32(2):373–379. doi:10.1093/mr/roab017
28. Kurtzman DJB, Vleugels RA. Anti-melanoma differentiation–associated gene 5 (MDA5) dermatomyositis: a concise review with an emphasis on distinctive clinical features. *J Am Acad Dermatol*. 2018;78(4):776–785. doi:10.1016/j.jaad.2017.12.010
29. Gono T, Okazaki Y, Kuwana M. Antiviral proinflammatory phenotype of monocytes in anti-MDA5 antibody-associated interstitial lung disease. *Rheumatology*. 2021;keab371. doi:10.1093/rheumatology/keab371
30. De Lorenzis E, Natalello G, Gigante L, Verardi L, Bosello SL, Gremese E. What can we learn from rapidly progressive interstitial lung disease related to anti-MDA5 dermatomyositis in the management of COVID-19? *Autoimmunity Rev*. 2020;19(11):102666. doi:10.1016/j.autrev.2020.102666
31. Wang G, Wang Q, Wang Y, et al. Presence of Anti-MDA5 Antibody and Its Value for the Clinical Assessment in Patients With COVID-19: a Retrospective Cohort Study. *Front Immunol*. 2021;12:791348. doi:10.3389/fimmu.2021.791348
32. Fukada A, Fujisawa T, Hozumi H, et al. Prognostic Role of Interferon-λ3 in Anti–Melanoma Differentiation–Associated Gene 5–Positive DERMATOMYOSITIS-ASSOCIATED Interstitial Lung Disease. *Arthritis Rheumatol*. 2024:art.42785. doi:10.1002/art.42785
33. Matsuda S, Kotani T, Ishida T, et al. Exploration of pathomechanism using comprehensive analysis of serum cytokines in polymyositis/dermatomyositis-interstitial lung disease. *Rheumatology*. 2020;59(2):310–318. doi:10.1093/rheumatology/kez301
34. Gono T, Kaneko H, Kawaguchi Y, et al. Cytokine profiles in polymyositis and dermatomyositis complicated by rapidly progressive or chronic interstitial lung disease. *Rheumatology*. 2014;53(12):2196–2203. doi:10.1093/rheumatology/keu258
35. Shimizu T, Koga T, Furukawa K, et al. IL-15 is a biomarker involved in the development of rapidly progressive interstitial lung disease complicated with polymyositis/dermatomyositis. *J Intern Med*. 2021;289(2):206–220. doi:10.1111/joim.13154
36. Zhou J, Zhao L, Xiao Y, et al. The Expression of Cytokine Profiles and Related Receptors in Idiopathic Inflammatory Myopathies. *Front Pharmacol*. 2022;13:852055. doi:10.3389/fphar.2022.852055
37. Oddis CV, Aggarwal R. Treatment in myositis. *Nat Rev Rheumatol*. 2018;14(5):279–289. doi:10.1038/nrrheum.2018.42
38. Kondoh Y, Makino S, Ogura T, et al. 2020 guide for the diagnosis and treatment of interstitial lung disease associated with connective tissue disease. *Respiratory Investigation*. 2021;59(6):709–740. doi:10.1016/j.resinv.2021.04.011
39. Tsuji H, Nakashima R, Hosono Y, et al. Multicenter Prospective Study of the Efficacy and Safety of Combined Immunosuppressive Therapy With High-Dose Glucocorticoid, Tacrolimus, and Cyclophosphamide in Interstitial Lung Diseases Accompanied by Anti–Melanoma Differentiation–Associated Gene 5–Positive Dermatomyositis. *Arthritis Rheumatol*. 2020;72(3):488–498. doi:10.1002/art.41105
40. Fujisawa T, Hozumi H, Kamiya Y, et al. Prednisolone and tacrolimus versus prednisolone and cyclosporin A to treat polymyositis/dermatomyositis-associated ILD: a randomized, open-label trial. *Respirology*. 2021;26(4):370–377. doi:10.1111/resp.13978
41. Kurita T, Yasuda S, Oba K, et al. The efficacy of tacrolimus in patients with interstitial lung diseases complicated with polymyositis or dermatomyositis. *Rheumatology*. 2015;54(1):39–44. doi:10.1093/rheumatology/keu166
42. Takada K, Katada Y, Ito S, et al. Impact of adding tacrolimus to initial treatment of interstitial pneumonitis in polymyositis/dermatomyositis: a single-arm clinical trial. *Rheumatology*. 2020;59(5):1084–1093. doi:10.1093/rheumatology/kez394
43. Wang CR, Lin WC, Wong TW. Extended-Release Tofacitinib Therapy for a MDA5 Antibody-Positive Amyopathic Dermatomyositis Patient with Early-Stage Interstitial Lung Disease. *ITT*. 2023;12:187–192. doi:10.2147/ITT.S445971
44. Chen Z, Wang X, Ye S. Tofacitinib in Amyopathic Dermatomyositis–Associated Interstitial Lung Disease. *N Engl J Med*. 2019;381(3):291–293. doi:10.1056/NEJMc1900045
45. Kurasawa K, Arai S, Namiki Y, et al. Tofacitinib for refractory interstitial lung diseases in anti-melanoma differentiation-associated 5 gene antibody-positive dermatomyositis. *Rheumatology*. 2018;57(12):2114–2119. doi:10.1093/rheumatology/key188