Clinical features and prognosis of idiopathic inflammatory myopathies with coexistent multiple myositis-specific antibodies

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Abstract

Objective

This study aimed to evaluate the clinical significance of the coexistence of 2 or more myositis-specific antibodies (multiple MSAs) in adult patients with idiopathic inflammatory myopathies (IIM).

Methods

We assessed a cohort of 202 consecutive patients with IIM. Clinical features and survival rates were compared between patients with and without multiple MSAs.

Results

Of those 202 patients, 44 (21.8%) were found to have multiple MSAs. 63.6% of the 44 patients tested positive for anti-aminoacyl-tRNA synthetase antibodies (anti-ARS+) and 52.3% positive for anti-melanoma differentiation-associated protein-5 antibody (anti-MDA5+). The presence of multiple MSAs was associated with less rapidly progressive interstitial lung disease (RP-ILD), fever, rash, periungual erythema, more muscle involvement and dysphagia, higher albumin level, and higher positive rate of ANA antibody in anti-MDA5+ population. In anti-ARS+ population with multiple MSAs, there were more V-neck sign, skin ulcers, dysphagia and peripheral edema. No differences in survival rates were observed between patients with or without multiple MSAs in the overall and anti-ARS+ populations. However, the survival rate in anti-MDA5+ population with multiple MSAs was significantly higher than those without multiple MSAs (p=0.003). Moreover, multiple MSAs remained an independent protective factor against mortality in multivariable Cox regression analysis of anti-MDA5+ population [HR 0.108 (95% CI 0.013, 0.908), p=0.041].

Conclusion

Multiple MSAs coexist in some IIM patients and their existence indicates mixed features from concomitant MSAs in anti-MDA5+ population and anti-ARS+ population. Identifying multiple MSAs could help to discover a more favourable disease phenotype with decreased mortality in anti-MDA5+ population.

Key words

myositis-specific antibodies, idiopathic inflammatory myopathy, anti-melanoma differentiation-associated protein-5 antibody, anti-aminoacyl-tRNA synthetase antibodies, prognosis

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Introduction

Idiopathic inflammatory myopathies (IIM) encompass a wide range of autoimmune disorders that impact skeletal muscle and various organs, with interstitial lung disease (ILD) being the leading cause of morbidity and mortality, particularly rapidly progressive ILD (RP-ILD) with a high mortality rate (1, 2). An array of autoantibodies have been detected in individuals with IIM and are categorised into two groups: myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) (3). MSAs are crucial for predicting distinct clinical phenotypes and prognosis of IIM (2). Among all MSAs, the anti-melanoma differentiation-associated protein-5 (anti-MDA5) and anti-aminoacyl-tRNA synthetase (anti-ARS) antibodies are particularly noteworthy as they have a robust correlation with ILD and can represent distinct entities of IIM (2, 4, 5).

MSAs are typically considered to be mutually exclusive before (3, 6). However, some studies found that 2 or more MSAs (multiple MSAs) coexisted, particularly in cases where individuals exhibited simultaneous positivity for anti-MDA5 and anti-ARS antibodies (7-16). When 2 or more MSAs coexist, clinicians encounter challenges in clinical interpretation owing to the significant clinical heterogeneity of various MSAs. The coexistence of multiple MSAs may impact clinical features and disease prognosis of IIM patients, which is important to consider. However, due to the limited number of reported cases regarding this topic, the clinical characteristics and prognosis of IIM patients with multiple MSAs remain unknown.

Here we assessed the clinical features and prognosis associated with the coexistence of multiple MSAs in IIM patients, including those who were positive for anti-MDA5 antibody (anti-MDA5+ population) or anti-ARS antibodies (anti-ARS+ population).

Materials and methods

Study population and design

We retrospectively reviewed the medical records of 202 patients with IIM treated at the Nanfang Hospital between December 2015 and June 2022. Patients were enrolled on a consecutive basis without selection. The diagnosis of IIM was determined using either the Bohan and Peter criteria or the EULAR/ACR 2017 classification criteria (17, 18). The inclusion criterion was age ≥18 years. Those with tumours or other connective tissue diseases were excluded. Baseline characteristics of patients on admission, including demographics, clinical manifestations, laboratory data, and treatment regimens were acquired from the medical records. Diagnosis of ILD was established through the radiological evaluation of HRCT imaging. Within 3 months of the original diagnosis of ILD, patients who developed acute and progressive exacerbation of dyspnoea due to ILD were considered to have RP-ILD (19). Following guidelines from the American Thoracic Society/European Respiratory Society, the HRCT pictures were categorised into distinct ILD patterns (20): non-specific interstitial pneumonia (NSIP), organising pneumonia (OP), and NSIP combined with OP. Lower lung zone consolidation was characterised by a uniform elevation in opacity of the pulmonary parenchyma, resulting in the obscuration of vascular and airway wall boundaries and the lesions distributed below the inferior pulmonary vein (21). HRCT imaging score was evaluated based on the classification by Ichikado *et al*. (22, 23). Follow-up data were collected until January 2023. The cumulative survival rates were assessed. The study complies with the Helsinki Declaration and was approved by the Ethics Committee Board of Nanfang Hospital, Southern Medical University (NFEC2022378).

Detection of autoantibodies

A total of 16 autoantigens were detected in immunoblot testing (EUROIMMUN, Lübeck, Germany) based on the manufacturer's instructions. The antibody band's semiquantitative results were obtained by scanning its grey-scale value. Grey-scale values of 0 to 5 units/L were defined accordingly: <10 units/L as -, 11 to 25 units/L as +, 26 to 50 units/L as ++, and >50 units/L as +++. All serum samples were obtained at hospital admission and the results of

MSAs were based on the first examination. There were no other examination kits used for testing MSAs in our cohort. ANA was determined by the Nova Lite Hep-2 ANA kit (Inova Diagnostics, San Diego, CA, USA).

Statistical analysis

Chi-squared or Fisher's exact test was used to compare categorical variables between groups. For continuous data, we employed either one-way ANOVA or Kruskal-Wallis test, depending on the data distribution. The Kaplan-Meier (log-rank) test was used to assess differences in survival. We conducted a univariate Cox regression analysis to assess the relationship between variables and survival, and all variables with $p<0.5$ in the univariate analysis were subsequently served as candidate predictors. Then, with the use of a stepwise selection method based on the Akaike information criterion (AIC) collaborated with the least absolute shrinkage and selection operator (LASSO) technique, the final multivariate Cox proportional hazards model was selected. Statistical analysis was performed using the SPSS software package (v. 26.0; IBM Corp, Armonk, NY) and the R statistical package (v. 4.2.3; R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org). *p*-values <0.05 were considered statistically significant.

Results

Initial clinical features

The clinical features of the 202 enrolled patients with IIM are summarised in Supplementary Table S1. The mean age of these patients at diagnosis was 48.9 years (S.D. 13.6), and 58.9% (119/202) were female. 39 patients (19.3%) developed RP-ILD during follow-up. Among these participants, 12 (5.9%) had negative MSAs, 146 (72.3%) had single MSAs and 44 (21.8%) had multiple MSAs. The prevalence of different MSAs in the multiple MSAs (+) group of the overall population is shown in Table I. Of the 44 patients with multiple MSAs, anti-MDA5 antibody and anti-ARS antibodies were the most common MSAs, which was corresponding to previous studies (7-16), accounting for 23 (52.3%) and 28 (63.6%) respectively. Meanwhile, these 2 types of antibodies are the most studied MSAs at present, representing entirely different disease phenotypes, making it easy to compare them. Furthermore, both of them are well-known MSAs associated with ILD in IIM, which was the significant domain in our study necessitating to explore. Therefore, we extracted patients with anti-MDA5 antibody or anti-ARS antibodies as 2 separate entities as the anti-MDA5+ population and anti-ARS+ population, respectively.

Table I. The prevalence of different MSAs in the multiple MSAs (+) group of the overall population.

	Overall population (n=202)	Multiple MSAs (+) (n=44)	Single MSAs (+) (n=146)	*p*-value
Myositis-specific antibodies, n (%)				
Anti-MDA5	74 (36.6)	23 (52.3)	51 (34.9)	**0.039**
Anti-ARS	76 (37.6)	28 (63.6)	48 (31.5)	**0.000**
Anti-Jo-1	36 (17.8)	12 (27.3)	24 (16.4)	0.108
Anti-OJ	7 (3.5)	2 (4.6)	2 (1.4)	**0.008**
Anti-PL7	18 (8.4)	7 (15.9)	11 (7.5)	0.138
Anti-PL12	11 (5.4)	9 (20.5)	2 (1.4)	**0.000**
Anti-EJ	10 (5.0)	1 (2.3)	9 (6.2)	0.458
Anti-SAE	2 (1.0)	2 (4.6)	0 (0.0)	0.053
Anti-HMGCR	15 (7.4)	8 (18.2)	7 (4.8)	**0.008**
Anti-Mi-2	19 (9.4)	12 (27.3)	8 (5.5)	**0.001**
Anti-NXP2	20 (9.9)	8 (18.2)	12 (8.2)	0.088
Anti-TIF-γ	12 (5.9)	4 (9.1)	8 (5.5)	0.478
Anti-SRP	25 (12.4)	13 (29.6)	12 (8.2)	**0.000**

Bold indicates statistical significance.
MSAs: myositis-specific autoantibodies; MDA5: melanoma differentiation-associated gene 5; TIF1-γ: transcriptional intermediary factor 1 gamma; SAE: small ubiquitin-like modifier activating enzyme; NXP2: nuclear matrix protein 2; ARS: aminoacyl-tRNA synthetase; Jo-1: histidyl-tRNA-synthetase; PL-12: alanyl-tRNA synthetase; PL-7: threonyl-tRNA synthetase; EJ: glycyl-tRNA synthetase; OJ: isoleucyl-tRNA synthetase; HMGCR:3-hydroxy-3-methylglutaryl-coenzyme A reductase; SRP: signal recognition particle.

Among the anti-ARS+ population and anti-MDA5+ population, 36.8% (28/76) and 31.1% (23/74) had multiple MSAs, respectively. In some cases, more than one anti-ARS antibodies were tested positive simultaneously, which was why 28 patients with anti-ARS antibodies were discovered to have 31 positive anti-ARS antibodies. This kind of phenomenon could also be observed in other tables. The distribution of various MSAs in the anti-ARS+ population and anti-MDA5+ population with multiple MSAs are presented in Supplementary Tables S2 and S3, respectively. The most frequent antibodies found in the anti-ARS+ population and anti-MDA5+ population with multiple MSAs were anti-MDA5 [42.9%, (12/28)] and anti-ARS [52.2%, (12/23)] antibodies, respectively. During the follow-up, RP-ILD was developed by 8 patients (10.5%) in the anti-ARS+ population and by 33 patients (44.6%) in the anti-MDA5+ population (Tables II and III).

Comparison of clinical features between different groups in the overall population, anti-ARS+ population and anti-MDA5+ population

No significant differences were found in clinical features between the MSAs (-), single MSAs (+) and multiple MSAs (+) groups in the overall population (Suppl. Table S1).

In the anti-ARS+ population, those with multiple MSAs were more likely to exhibit certain symptoms than those without. These included V-neck sign ($p=0.002$), skin ulcers ($p=0.007$), dysphagia ($p=0.026$), and peripheral oedema ($p=0.005$). Additionally, this group was more likely to have been exposed to high-dose glucocorticoid ($p=0.043$) and had lower HRCT scores ($p=0.049$), as well as higher ESR ($p=0.043$) than the multiple MSAs (-) (single-positive anti-ARS antibody) group (Table II). We then compared the clinical features between the anti-ARS+ population with

Table II. Comparison of clinical features between multiple MSAs (+) and multiple MSAs (–) groups in the anti-ARS+ population.

Characteristics	Total (n=76)	Multiple MSAs (-) (n=48)	Multiple MSAs (+) (n=28)	*p*-value
Demographics				
Follow-up, months, (median [IQR])	27.50 [17.00, 52.25]	34.00 [17.00, 56.75]	24.50 [16.00, 42.00]	0.202
Age, years, mean (S.D.)	51.84 (11.69)	52.92 (11.24)	50.00 (12.40)	0.297
Female gender, n (%)	44 (57.9)	31 (64.6)	13 (46.4)	0.152
Smoking, n (%)	17 (22.4)	8 (16.7)	9 (32.1)	0.156
Clinical manifestations				
Fever at presentation, n (%)	23 (30.3)	15 (31.2)	8 (28.6)	1.000
Rash, n (%)	47 (61.8)	26 (54.2)	21 (75.0)	0.089
Heliotrope rash, n (%)	15 (19.7)	7 (14.6)	8 (28.6)	0.231
Gottron papule/sign, n (%)	27 (35.5)	15 (31.2)	12 (42.9)	0.331
V-neck sign, n (%)	17 (22.4)	5 (10.4)	12 (42.9)	**0.002**
Periungual erythema, n (%)	3 (3.9)	2 (4.2)	1 (3.6)	1.000
Skin ulcers, n (%)	12 (15.8)	3 (6.2)	9 (32.1)	**0.007**
Mechanic's hands, n (%)	30 (39.5)	21 (43.8)	9 (32.1)	0.343
Raynaud phenomenon, n (%)	15 (19.7)	10 (20.8)	5 (17.9)	1.000
Dysphagia, n (%)	12 (15.8)	4 (8.3)	8 (28.6)	**0.026**
Hoarseness, n (%)	4 (5.3)	2 (4.2)	2 (7.1)	0.623
Peripheral oedema, n (%)	14 (18.4)	4 (8.3)	10 (35.7)	**0.005**
Articular symptom, n (%)	48 (63.2)	31 (64.6)	17 (60.7)	0.807
Cardiovascular involved, n (%)	9 (11.8)	6 (12.5)	3 (10.7)	1.000
Serous effusion, n (%)	33 (43.4)	23 (47.9)	10 (35.7)	0.344
Muscle involvement, n (%)	56 (73.7)	35 (72.9)	21 (75.0)	1.000
Infection at presentation, n (%)	10 (13.2)	4 (8.3)	6 (21.4)	0.158
ILD domain				
ILD, n (%)	70 (92.1)	46 (95.8)	24 (85.7)	0.185
HRCT score, (median [IQR])	125.75 [109.03, 161.52]	129.32 [113.22, 163.44]	117.33 [104.53, 133.63]	**0.049**
RP-ILD, n (%)	8 (10.5)	5 (10.4)	3 (10.7)	1.000
Lower lung zone consolidation, n (%)	29 (38.2)	18 (37.5)	11 (39.3)	1.000
HRCT pattern, n (%)				0.298
NSIP	18 (25.7)	12 (26.1)	6 (25.0)	
OP	27 (38.6)	15 (32.6)	12 (50.0)	
NSIP + OP	25 (35.7)	19 (41.3)	6 (25.0)	
Laboratory features				
WBC, ×109 /l, mean (S.D.)	8.38 (4.43)	9.08 (3.96)	7.19 (5.00)	0.073
HB, g/L, mean (S.D.)	127.28 (16.73)	128.98 (15.74)	124.36 (18.23)	0.248
LY%, mean (S.D.)	21.23 (11.38)	20.08 (9.86)	23.20 (13.57)	0.252
NLR, (median [IQR])	3.68 [2.37, 5.93]	3.84 [2.44, 6.02]	3.16 [2.34, 5.54]	0.426
CK level, U/L, (median [IQR])	214.50 [70.75, 1688.11]	297.00 [72.75, 2164.25]	117.50 [63.50, 619.75]	0.226
LDH, U/L, (median [IQR])	331.00 [228.50, 489.75]	303.50 [228.50, 483.00]	358.00 [229.75, 571.25]	0.404
ALT, U/L, (median [IQR])	42.50 [18.25, 92.87]	30.03 [14.75, 81.25]	47.50 [21.00, 104.12]	0.368
AST, U/L, (median [IQR])	37.00 [21.62, 104.75]	30.35 [19.00, 95.50]	50.00 [28.00, 121.00]	0.139
ESR, mm/h, (median [IQR])	26.50 [12.00, 44.75]	22.00 [11.75, 34.25]	34.31 [16.75, 54.25]	**0.043**
CRP, mg/L, (median [IQR])	4.74 [2.04, 16.87]	4.72 [2.13, 16.10]	5.62 [1.93, 20.03]	0.834
Albumin, g/L, mean (S.D.)	35.19 (5.57)	35.34 (5.10)	34.92 (6.39)	0.754
ANA (≥1:80), n (%)	55 (72.4)	35 (72.9)	20 (71.4)	0.889
Anti-PM-SCL75, n (%)	5 (6.6)	3 (6.3)	2 (7.1)	1.000
Anti-SSA, n (%)	25 (32.9)	17 (35.4)	8 (28.6)	0.540
Anti-Ro52, n (%)	53 (69.7)	36 (75.0)	17 (60.7)	0.197
Therapies				
Exposure to high-dose glucocorticoid (≥80 mg), n (%)	24 (31.6)	11 (22.9)	13 (46.4)	**0.043**
No. of immunosuppressants, on top of steroid, n (%)				0.658
0	15 (19.7)	11 (22.9)	4 (14.3)	
1	42 (55.3)	26 (54.2)	16 (57.1)	
≥2	19 (25.0)	11 (22.9)	8 (28.6)	
IVIg, n (%)	25 (32.9)	13 (27.1)	12 (42.9)	0.207
Exposure to pirfenidone, n (%)	18 (23.7)	12 (25.0)	6 (21.4)	0.786

Bold indicates statistical significance.

MSAs: myositis-specific autoantibodies; ARS: aminoacyl-tRNA synthetase; ILD: interstitial lung disease; RP-ILD: rapidly progressive interstitial lung disease; HRCT: high-resolution computed tomography. NSIP: nonspecific interstitial pneumonia; OP: organising pneumonia; WBC: white blood cell count; HB: haemoglobin; LY%: percentage of lymphocyte; NLR: neutrophil/lymphocyte ratio; CK: creatine kinase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ANA: anti-nuclear antibody.

coexisted anti-MDA5 antibody and those without multiple MSAs (Suppl. Table S4). In the anti-ARS+ population with coexisted anti-MDA5 antibody, there was a higher incidence of heliotrope rash (p=0.015), V-neck sign (p=0.005), and skin ulcers (p=0.006) compared to those without multiple MSAs. These patients also exhibited lower level of WBC (p=0.005) and HRCT scores (p=0.025), and were more likely to have been exposed to

Table III. Comparison of clinical features between multiple MSAs (+) and multiple MSAs (–) groups in the anti-MDA5+ population.

Characteristics	Total (n=74)	Multiple MSAs (-) (n=51)	Multiple MSAs (+) (n=23)	*p*-value
Demographics				
Follow-up, months, (median [IQR])	14.50 [2.00, 26.75]	7.60 [1.00, 24.00]	22.00 [11.50, 40.00]	**0.004**
Age, years, mean (S.D.)	47.20 (13.44)	47.20 (13.79)	47.22 (12.94)	0.995
Female gender, n (%)	43 (58.1)	30 (58.8)	13 (56.5)	1.000
Smoking, n (%)	15 (20.3)	10 (19.6)	5 (21.7)	1.000
Clinical manifestations				
Fever at presentation, n (%)	32 (43.2)	27 (52.9)	5 (21.7)	**0.021**
Rash, n (%)	69 (93.2)	50 (98.0)	19 (82.6)	**0.030**
Heliotrope rash, n (%)	49 (66.2)	35 (68.6)	14 (60.9)	0.598
Gottron papule/sign, n (%)	59 (79.7)	44 (86.3)	15 (65.2)	0.059
V-neck sign, n (%)	42 (56.8)	30 (58.8)	12 (52.2)	0.621
Periungual erythema, n (%)	23 (31.1)	20 (39.2)	3 (13.0)	**0.031**
Skin ulcers, n (%)	39 (52.7)	28 (54.9)	11 (47.8)	0.621
Mechanic's hands, n (%)	27 (36.5)	17 (33.3)	10 (43.5)	0.442
Raynaud phenomenon, n (%)	10 (13.5)	5 (9.8)	5 (21.7)	0.268
Dysphagia, n (%)	14 (18.9)	6 (11.8)	8 (34.8)	**0.027**
Hoarseness, n (%)	11 (14.9)	8 (15.7)	3 (13.0)	1.000
Peripheral oedema, n (%)	43 (21.3)	31 (19.6)	12 (27.3)	0.300
Articular symptom, n (%)	46 (62.2)	30 (58.8)	16 (69.6)	0.444
Cardiovascular involved, n (%)	10 (13.5)	9 (17.6)	1 (4.3)	0.158
Serous effusion, n (%)	30 (40.5)	24 (47.1)	6 (26.1)	0.125
Muscle involvement, n (%)	44 (59.5)	26 (51.0)	18 (78.3)	**0.040**
Infection at presentation, n (%)	20 (27.0)	17 (33.3)	3 (13.0)	0.092
ILD domain				
ILD, n (%)	73 (98.6)	50 (98.0)	23 (100.0)	1.000
HRCT score, (median [IQR])	125.06 [104.72, 149.52]	127.70 [105.94, 158.64]	111.23 [104.45, 136.16]	0.140
RP-ILD, *n* (%)	33 (44.6)	28 (54.9)	5 (21.7)	**0.011**
Lower lung zone consolidation, n (%)	35 (47.3)	25 (49.0)	10 (43.5)	0.802
HRCT pattern, n (%)				0.290
NSIP	8 (11.0)	4 (8.0)	4 (17.4)	
OP	39 (53.4)	26 (52.0)	13 (56.5)	
NSIP + OP	26 (35.6)	20 (40.0)	6 (26.1)	
Laboratory features				
WBC, ×109 /l, mean (S.D.)	5.81 (2.76)	5.59 (1.93)	6.31 (4.04)	0.302
HB, g/L, mean (S.D.)	117.88 (19.70)	115.25 (20.73)	123.70 (16.14)	0.088
LY%, mean (S.D.)	16.96 (9.24)	15.90 (6.22)	19.32 (13.66)	0.142
NLR, (median [IQR])	4.43 [2.92, 6.84]	4.64 [3.17, 6.87]	3.60 [2.66, 6.74]	0.265
CK level, U/L, (median [IQR])	93.50 [48.25, 366.50]	80.00 [46.50, 200.50]	183.00 [62.00, 570.00]	0.129
LDH, U/L, (median [IQR])	371.00 [291.78, 565.75]	369.00 [295.56, 581.50]	373.00 [279.50, 454.50]	0.645
ALT, U/L, (median [IQR])	45.00 [23.25, 83.25]	46.00 [23.50, 72.00]	42.00 [25.00, 146.00]	0.820
AST, U/L, (median [IQR])	56.50 [33.25, 95.00]	61.00 [43.00, 94.00]	48.00 [28.00, 90.50]	0.362
ESR, mm/h, (median [IQR])	34.00 [21.00, 60.00]	34.00 [22.00, 57.00]	34.00 [18.50, 64.21]	0.829
CRP, mg/L, (median [IQR])	7.03 [3.84, 16.18]	8.66 [4.37, 20.04]	4.97 [2.94, 10.75]	0.187
Albumin, g/L, mean (S.D.)	32.44 (5.57)	31.58 (5.58)	34.37 (5.16)	**0.046**
ANA (≥1:80), n (%)	28 (38.8)	13 (25.5)	15 (65.2)	**0.005**
Anti-PM-SCL75, n (%)	2 (2.7)	2 (3.9)	0 (0.0)	1.000
Anti-SSA, n (%)	12 (16.2)	8 (15.7)	4 (17.4)	1.000
Anti-Ro52, n (%)	55 (74.3)	39 (76.5)	16 (69.6)	0.529
Therapies				
Exposure to high-dose glucocorticoid (≥80 mg), n (%)	39 (52.7)	30 (58.8)	9 (39.1)	0.137
No. of immunosuppressants, on top of steroid, n (%)				0.404
0	16 (21.6)	12 (23.5)	4 (17.4)	
1	30 (40.5)	18 (35.3)	12 (52.2)	
≥2	28 (37.8)	21 (41.2)	7 (30.4)	
IVIg, n (%)	27 (36.5)	21 (41.2)	6 (26.1)	0.298
Exposure to pirfenidone, n (%)	9 (12.2)	5 (9.8)	4 (17.4)	0.446

Bold indicates statistical significance.

MSAs: myositis-specific autoantibodies; MDA5: melanoma differentiation-associated gene 5; ILD: interstitial lung disease; RP-ILD: rapidly progressive interstitial lung disease; HRCT: high-resolution computed tomography. NSIP: nonspecific interstitial pneumonia; OP: organising pneumonia; WBC: white blood cell count; HB: haemoglobin; LY%: percentage of lymphocyte; NLR: neutrophil/lymphocyte ratio; CK: creatine kinase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ANA: anti-nuclear antibody.

high-dose glucocorticoid (p=0.031).

It was discovered that the multiple MSAs (+) group in the anti-MDA5+ population had a higher prevalence of muscle involvement (p=0.040), as well as dysphagia (p=0.027), but a lower incidence of RP-ILD (p=0.011), fever (p=0.021), rash (p=0.030), and periungual erythema (p=0.031) than multiple MSAs (-) (single-positive anti-MDA5 antibody) group. In addition, they had higher albumin level (p=0.046) and

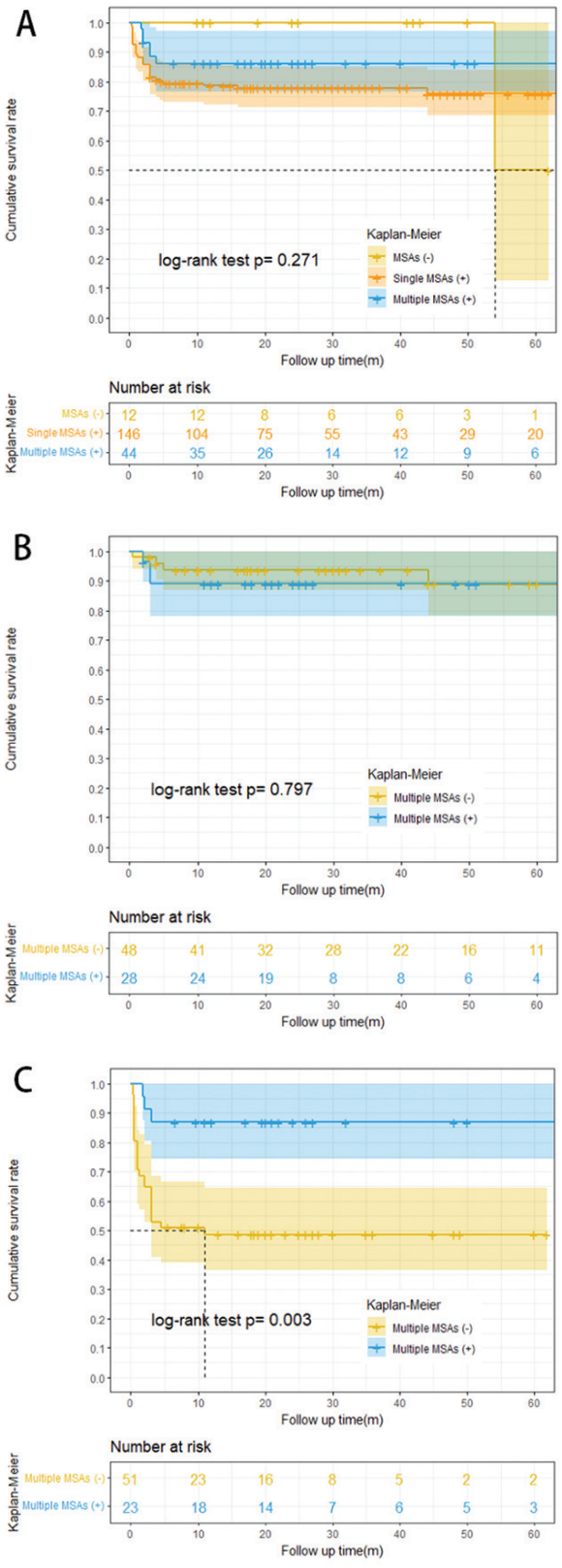

Fig. 1. Kaplan-Meier survival curves for patients with and without multiple MSAs.
A: The cumulative survival rate between patients without MSAs, with single MSAs and with multiple MSAs in the overall population was similar (91.7% *vs*. 76.7% *vs*. 84.1%; p=0.271).
B: The cumulative survival rate between patients with and without multiple MSAs in the anti-ARS+ population was similar (85.7% *vs*. 89.6%; p=0.797).
C: The cumulative survival rate was significantly higher in the anti-MDA5+ population with multiple MSAs than those without multiple MSAs (87.0% *vs*. 49.0%; p=0.003).
MSAs: myositis-specific autoantibodies; ARS: aminoacyl-tRNA synthetase; MDA5: melanoma differentiation-associated gene 5.

had a higher positive rate of ANA antibody (p=0.005) (Table III). Following that, we compared the clinical features of the anti-MDA5+ population with coexisted anti-ARS antibodies to those without multiple MSAs (Suppl. Table S5). When the anti-MDA5+ population coexisted with anti-ARS antibodies, the incidence of Gottron papule/sign (p=0.012), periungual erythema (p=0.048), and RP-ILD (p=0.024) were lower than the multiple MSAs (-) group. These patients also exhibited elevated levels of LY% (p=0.046) and CRP (p=0.039) and had a higher ANA antibody positive rate (p=0.002).

Prognosis among different groups in the overall population, anti-ARS+ population and anti-MDA5+ population

The survival rates for the overall population, those with anti-ARS antibodies, and those with anti-MDA5 antibody were 79.2%, 88.2%, and 60.8%, respectively. RP-ILD was the primary cause of death, with most of these fatalities occurring within the first three months following diagnosis. We analysed the survival rates of patients between the different groups using Kaplan-Meier curves. This analysis was done for the overall population, the anti-MDA5+ population and anti-ARS+ population. There were no significant differences in survival rates observed between the MSAs (-), single MSAs (+) and multiple MSAs (+) groups in the overall population (91.7% *vs*. 76.7% *vs*. 84.1%; p=0.271), as well as the multiple MSAs (+) and multiple MSAs (–) groups in anti-ARS+ population (85.7% *vs*. 89.6%; p=0.797) (Fig. 1A and 1B, respectively). However, in the anti-MDA5+ population, consistent with a lower frequency of RP-ILD (21.7% *vs*. 54.9%; p=0.011), the multiple MSAs (+) group had a significantly better prognosis than multiple MSAs (-) group (87.0% *vs*. 49.0%; p=0.003 (Fig. 1C). Further comparison showed that the survival rate of the anti-ARS+ population with coexisted anti-MDA5 antibody was similar to those without multiple MSAs (91.7% *vs*. 89.6%; p=0.909; Suppl. Fig. S1A). Nevertheless, the anti-MDA5+ population with coexisted anti-ARS antibodies had a significantly higher survival rate than those without multiple MSAs (91.7% *vs*. 49.0%; p=0.011; Suppl. Fig. S1B).

The prognostic significance of multiple MSAs in anti-MDA5+ population

Because we observed a significantly higher survival rate in the anti-MDA5+

Table IV. Results of multivariable Cox regression analysis for mortality in anti-MDA5+ population.

Variables	Hazard ratio	95% Confidence interval	*p*-value
WBC	1.159	1.000 -1.343	0.051
Multiple MSAs	0.108	0.013-0.908	**0.041**
RP-ILD	13.827	4.409-43.356	**<0.001**
LY%	0.927	0.867-0.991	**0.025**
Cardiovascular involvement	3.656	1.256-10.642	**0.017**
Albumin	0.855	0.764-0.957	**0.007**

Bold indicates statistical significance.
MSAs: myositis-specific autoantibodies; MDA5: melanoma differentiation-associated gene 5; WBC: white blood cell count; RP-ILD: rapidly progressive interstitial lung disease; LY%: percentage of lymphocyte.

population with multiple MSAs than those without multiple MSAs, we aimed to verify if multiple MSAs was an independent predictor of prognosis within the anti-MDA5+ population. Clinical features of anti-MDA5+ population with non-survivors and predictors of mortality are summarised in Supplementary Table S6. Based on the results of the univariate Cox regression analysis, higher age (p=0.015), higher WBC (p=0.027), higher ESR (p=0.001), higher CRP (p=0.011), higher HRCT score (p=0.001), fever (p=0.001), cardiovascular involvement (p=0.006), serous effusion (p<0.001), infection (p<0.001) and RP-ILD (p<0.001) were discovered to be potential predictors of mortality. Meanwhile, multiple MSAs (p=0.007), higher LY% (p=0.014), higher albumin level (p<0.001), the use of 1 immunosuppressant (p=0.007), and the use of 2 or more immunosuppressants (p<0.001) were found to be protective factors against mortality.

15 candidate predictors with p<0.5 in the univariate analysis were reduced to 10 most valuable variables using LASSO Cox regression in Supplementary Fig. S2. Then, in the backward stepwise selection algorithm, we identified the optimal multivariable Cox regression model with the lowest AIC value, which included 6 variables, as shown in Table IV. Final multivariable Cox regression model indicated: multiple MSAs was the independent protective factor against mortality [HR 0.108 (95% CI 0.013, 0.908), p=0.041] after adjusting for other covariates. RP-ILD (p<0.001) and cardiovascular involvement (p=0.017) were independent predictors of higher mortality. Higher LY% (p=0.025) and higher level of albumin (p=0.007) were independent protective factors for reduced mortality.

Discussion

In the present study, we reported that the coexistence of multiple MSAs may aid in identifying a distinct subtype of the anti-ARS+ population and anti-MDA5+ population. For the anti-MDA5+ population with multiple MSAs, this subgroup was less likely to develop fever and RP-ILD and had a higher albumin level compared to the multiple MSAs (-) group, all of which could predict lower mortality. Finally, the anti-MDA5+ population with multiple MSAs had a better prognosis than those without multiple MSAs.

The occurrence of multiple MSAs in patients with IIM was relatively unclear at present, because the exclusivity of MSAs was widely accepted and the presence of multiple MSAs was absent of attention before. Most of the available studies on this topic were limited case reports or small case series, providing details on the clinical characteristics and outcomes of IIM patients with multiple MSAs. The others were cross-sectional but only reported the frequency of cases (0.2% to 16.7%) with detected multiple MSAs (6, 24). In our cohort, we found that up to 21.8% of patients with IIM had multiple MSAs, mainly concentrated on those with anti-MDA5 or anti-ARS antibodies. It is currently unclear whether IIM patients with multiple MSAs have mixed clinical features. A study found 4 IIM patients with both anti-HMGCR and anti-MDA5 antibodies exhibited characteristic rash and ILD indicative of anti-MDA5-associated dermatomyositis (DM), but without myasthenia and elevated serum CK levels which imply anti-HMGCR-related immune-mediated necrotising myopathy (IMNM) (8). Similarly, Huang *et al*. reported that in 8 cases of IIM patients with double-positive MSAs, these patients showed similar clinical phenotypes to those with single-positive MSAs, and their phenotypes skewed to one of the co-existed MSAs (14). However, Chen *et al*. reported 6 cases of DM with double positivity for anti-MDA5 and anti-ARS antibodies (anti-MDA5+/ARS+), and anti-MDA5+/ARS+ DM showed clinical characteristics that combined the features of anti-MDA5+ DM and anti-ARS+ DM (11). In our cohort, what is noteworthy is that the anti-MDA5+ population with multiple MSAs had a higher occurrence of muscle involvement and dysphagia than those without multiple MSAs. It is well-known that the anti-MDA5 antibody is a DM-associated antibody, which is typically associated with the presence of DM skin rashes and polyarthralgia and ILD, especially with a high frequency of RP-ILD, whereas the clinical signs of myositis are often not present (25-27). This might suggest that there are combined characteristics from concurrent MSAs presented in the anti-MDA5+ population with multiple MSAs. For instance, a higher occurrence of dysphagia in the anti-MDA5+ population with multiple MSAs may be attributed to the high frequency of dysphagia-associated MSAs such as anti-SRP (21.7%) and NXP2 antibodies (17.4%) (8, 28, 29). Previous studies have suggested that the presence of ANA antibody in IIM patients reflects the presence of overlapping features of two or more autoimmune diseases (30). Thus, we assumed that the higher positive rate of ANA antibody in the anti-MDA5+ population with multiple MSAs was likely associated with overlapping features. Lower incidence of rash and periungual erythema further indicates that these patients are more mixed than pure anti-MDA5-associated DM. This phenomenon could also be observed in the anti-ARS+ population. Antisynthetase syndrome (ASS) is a well-described clinical syndrome with

the following characteristics: arthritis, Raynaud phenomenon, fever, ILD, 'mechanic's hands' and myositis accompanied by one of the anti-ARS antibodies, whereas lacking typical DM rashes, and the frequency of RP-ILD is lower than anti-MDA5+ DM (5, 7, 31). Our findings showed that anti-ARS+ individuals with multiple MSAs had an increased frequency of skin ulcers and V-neck sign, both of which are common DM rashes. This may indicate the presence of "mixed phenotypes" which could be associated with accompanied DM-related MSAs such as anti-MDA5 antibody, which has a frequency of 42.9%. The anti-ARS+ population with multiple MSAs also had a higher incidence of dysphagia and peripheral oedema, which may corresponded to the high prevalence of anti-SRP (17.9%) and anti-NXP2 (17.9%) antibodies, the latter antibody was also distinguished by a notable prevalence of peripheral oedema (29). A more specific comparison showed that the anti-ARS+ population with coexisted anti-MDA5 antibody had a higher incidence of typical DM rashes like heliotrope rash and V-neck sign than those with only anti-ARS antibodies. Meanwhile, the anti-MDA5 population with coexisted anti-ARS antibodies were less likely to develop RP-ILD and experience DM rashes like Gottron papule/sign, and had a higher ANA antibody positive rate than those with single-positive anti-MDA5 antibody. Above all, the presence of multiple MSAs indicates "mixed phenotypes" from concomitant MSAs in the anti-MDA5+ population and anti-ARS+ population, and we suggest that at least some IIM patients could have real coexisting MSAs as evidenced by such phenomenon.

The prognosis of IIM patients with multiple MSAs is undetermined, mainly due to the limited case reports available on this topic. In the case series from Huang's team, patients with double-positive MSAs had similar severity of clinical course as those with single-positive MSAs (14). However, Chen *et al*. showed that anti-MDA5+/ARS+ DM tended to have a similar clinical course to anti-ARS+ DM and a higher survival rate than anti-MDA5+/ARS- DM (11). In our study, when the anti-MDA5+ population coexisted with anti-ARS antibodies, a significantly higher survival rate than those with pure anti-MDA5 antibody was achieved. More importantly, we also observed that the anti-MDA5+ population with multiple MSAs had a significantly lower mortality rate than those without, and multiple MSAs was the independent protective factor against mortality. Most anti-MDA5+ patients with multiple MSAs achieved remission in our study, which was in line with previous case reports. Previously, 19 cases of anti-MDA5+ patients with multiple MSAs were published. Of these, most of them (78.9%) recovered, and 21.1% died of respiratory failure (7-16). Our study also found that the anti-MDA5+ population with multiple MSAs had a lower incidence of fever and RP-ILD, and a higher level of albumin compared to those without. Additionally, fever, RP-ILD, and reduced albumin level were associated with increased mortality in the anti-MDA5+ population. These findings together suggest that coexistence of multiple MSAs could predict a more favourable disease phenotype with better prognosis in the anti-MDA5+ population. In recent years, patients with anti-MDA5 antibody have gained significant attention owing to their exceedingly poor prognosis, leading to an increasing number of studies seeking prognostic markers to predict the clinical outcome (5, 32, 33). Our study suggests that multiple MSAs could serve as a potential indicator for better prognosis in the anti-MDA5+ population. We postulate that in the anti-MDA5+ population with multiple MSAs, the prognosis is affected not only by the anti-MDA5 antibody but also by other coexisted antibodies, which results in a better prognosis than those with pure anti-MDA5 antibody. The mixed clinical features seen in these patients are behind this hypothesis. To our best knowledge, this is the first study that has assessed multiple MSAs as a predictor of clinically significant outcomes in a retrospective cohort of patients with IIM.

This study presents noteworthy information regarding multiple MSAs. However, we do acknowledge that there are certain limitations. First, this study was conducted retrospectively at a single institution, which had several inevitable limitations, including selection bias, reporting bias, and information bias. Second, our study relied on a semi-quantitative analysis of MSAs levels using a commercial line blot assay. Line blot assay may suffer from low specificity with a high false positivity rate, which may lead to misclassification, such as the relatively high prevalence of patients with anti-MDA5+ in our study, which could be on account of possible false positives of the detection method and selection bias of this retrospective study. However, several studies have shown that the commercial line blot assay could be a reliable confirmatory test for IIM against in-house assays (34, 35). The gold standard immunoprecipitation assay is powerful, but the inevitable problem of this in-house assay is technically complex, time-consuming, and cannot be applied at scale. Actually, immunoprecipitation assay is not accessible to clinicians for routine clinical diagnosis of IIM; a simple/ rapid test as a confirmatory serological test in the suspected IIM is a realistic choice. Notably, the commercial line blot assay is the most extensively used assay worldwide, which greatly promotes early diagnosis of IIM (36). And the diagnostic accuracy of the commercial line blot assay is clinically validated, representing a reliable alternative to more complex procedures. According to Fabricio's study, the overall concordance rate between the above two assays was 78% (35). Above all, probably line blot assay isn't the "gold standard" method in detecting MSAs, but it is a realistic and credible choice for early diagnosis of IIM in clinical practice. Third, the assessment of dysphagia is based on the medical records in this retrospective cohort and majority of patients lack endoscopic evidence. More objective evaluation methods of dysphagia such as fibreoptic endoscopic evaluation of swallowing (FEES) (37) are needed in future research. Finally, another major shortcoming was the lack of pulmonary function testing and monitoring of alterations in levels of myositis-specific autoantibodies.

In conclusion, this is the first study to systematically illustrate the clinical features related to multiple MSAs and assess multiple MSAs as a predictor of prognosis in a retrospective cohort of patients with IIM. Multiple MSAs coexist in some IIM patients and the presence of which indicates a combination of features from concurrent MSAs in the anti-MDA5+ population and anti-ARS+ population. Identifying multiple MSAs could help recognise a more favourable disease phenotype with decreased mortality in the anti-MDA5+ population.

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