Comparison of Clinical Features and Prognostic Factors of Polymyositis/Dermatomyositis Associated Interstitial Lung Disease According to Autoantibodies: Anti-Aminoacyl tRNA Synthetase Antibodies Versus Anti-Melanoma Differentiation-Associated Gene 5 Antibody.

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Abstract

Objective: The serum myositis-specific autoantibodies has been considered to be relatively specific and be useful for diagnosis of polymyositis/dermatomyositis associated interstitial lung disease (PM/DM-ILD). The goal of our retrospective study was to identify clinical features and prognostic factors for PM/DM-ILD based on serological phenotypes.

Methods: PM/DM-ILD patients were diagnosed in the Department of Respiratory Medicine, Nanjing Drum Tower Hospital. MSAs were measured by anti-myositis antibody profile IgG detection kit. Based on the results of MSAs, the patients were divided into three groups: anti-MDA5 group, anti-Jo-1 group and other anti-ARS group. Kaplan-Meier, log rank, Kruskal-Wallis test and chi-square tests were used for analysis.

Results: We identified 30 patients (22.0%) with positive anti-MDA5, 42 patients (31.0%) with positive anti-Jo-1 and 64 patients(47.0%) with other anti-ARS. acute disease onset was more frequently observed in the anti-MDA5 group ($P = 0.005$). The highest mortality rate was in the anti-MDA5 group (66.7%, $P < 0.001$). The overall survival of patients with anti-Jo-1 was significantly better than that of patients with anti-MDA5 ($P < 0.001$) and similar to that of patients with other anti-ARS. The acute disease onset ($P = 0.002$), DAD pattern for HRCT imaging ($P < 0.001$), current smokers ($P = 0.02$), presence of Anti-MDA5 ($P < 0.001$), fever ($P < 0.001$) were significantly associated with the mortality of the study population. Interestingly, in the subgroup survival analysis for anti-MDA5 group, age was a risk factor for death of patients with anti-MDA5 ($P = 0.02$), and the treatment with PSL pulse and IVIG were markedly correlated with high mortality ($P < 0.001$ and $P = 0.001$, respectively).

Conclusion: Anti–MDA5 antibody is significantly associated with associated with worse prognosis in PM/DM-ILD patients. The application of PSL pulse and IVIG are not necessarily an effective treatment for positive anti-MDA5 patients.

Introduction

The diagnosis of interstitial lung disease needs meticulous evaluation for the environmental exposures, medications, and especially connective tissue diseases (CTDs), including rheumatoid arthritis (RA), polymyositis/dermatomyositis (PM/DM), systemic sclerosis (SSc) and so on, which is important to therapy and prognosis[1]. PM/DM associated ILD (PM/DM-ILD) is a leading cause of connective tissue diseases associated ILD (CTD-ILD) death, with an estimated mortality rate of 40% and poor response to corticosteroids therapy[2, 3]. The extrapulmonary findings including mechanic hands, Gottron’s papules and serological data are particularly important for the classification and diagnosis[2, 4, 5]. In clinical practice, the patient's clinical performance is consistent with PM/DM-ILD but is not completely diagnosed with Bohan and Peter's criteria for PM/DM. Current guidelines recommend that both clinical manifestation and measurement of autoantibodies (AAbs) are evaluated for the patients with ILD underlying CTD[6, 7]. Recently, the serum myositis-specific autoantibodies (MSAs) has been defined as autoantibody specificities that are considered relatively specific for PM/DM[8–10]. Prior reports for the

patients with anti-aminoacyl-tRNA synthetase(ARS) antibodies revealed different manifestations in patients that were positive and negative[11–13]. Several studies have identified anti-melanoma differentiation associated protein 5 (MDA5) antibody as a prognostic factor for PM/DM-ILD[10, 14, 15]. The available data on the characteristics of long-term follow-up in patients with PM/DM-ILD based on classification of serological MSAs are limited. It is important to identify the prognostic factors based on serological phenotypes and optimize disease management.

In this study, the clinical data, therapies and clinical outcomes in patients with PM/DM were retrospectively assessed to identify prognostic factors for PM/DM-ILD patients who grouped by autoantibodies.

Patients And Methods

Patients

We retrospectively studied all PM/DM-ILD patients diagnosed between 2016 and 2018 in Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital Affiliated to Medical School of Nanjing University, measured the presence of MSAs by anti-myositis antibody profile IgG detection kit (EUROLINE) (from EUROMIMMUN Medizinische Labordiagnostika AG, Germany) with the serum samples collected at the time of initial ILD diagnosis. 136 PM/DM-ILD patients with positive MSAs were included in the study.

The diagnosis of definite or probable PM/DM was based on the Bohan and Peter criteria[16].CADM was diagnosed as a distinct subgroup of DM according to Sontheimer criteria when the patient had typical rash but with little or no evidence of myositis during the hospitalization[17]. The interstitial lung disease was evaluated by chest High Resolution Computed Tomography (HRCT). None of the patients had advanced malignancies at the time of initial diagnosis. The clinical data including gender, age, smoking history, clinical presentations, physical findings, pulmonary function tests and laboratory findings were obtained from medical records. Disease onset was classified as acute (< 3 month from the onset of respiratory symptoms to the admission) or chronic (> 3 months from the onset of respiratory symptoms to the admission). We also analyzed the major therapy modality (corticosteroids, immunosuppressant and intravenous immunoglobulin) and clinical outcomes during the study period.

HRCT findings and pulmonary function tests

The HRCT images were taken with 16-row or 64-row spiral CT scan produced by GE, composed of 1-2.5 mm collimation sections at 10 mm intervals, reconstructed by a high spatial frequency algorithm and were exhibited for viewing the lung parenchyma (window level, -600 Hu; window width, 1500 Hu) and the mediastinum (window level, 35–45 Hu; window width, 400–450 Hu). HRCT findings were reviewed independently by one expert pulmonologists and one well-trained chest radiologist. HRCT patterns were

classified as organizing pneumonia (OP), nonspecific interstitial pneumonia (NSIP), NSIP with organizing pneumonia (OP) and diffuse alveolar damage (DAD) according to the guidelines for interstitial pneumonias[18]. The disagreements regarding HRCT patterns between doctors were resolved by consensus agreement.

We also reviewed lung function tests including forced vital capacity [FVC (% pred.)], forced expired volume in 1st second [FEV1 (% pred.)] and single-breath diffusion capacity of CO corrected for hemoglobin [DLCO SB (% pred.)].

Detection of myositis-specific autoantibodies

The serum samples were collected at the time of initial ILD diagnosis in Nanjing Drum Tower Hospital for all 136 PM/DM-ILD patients, measured the presence of MSAs by anti-myositis antibody profile IgG detection kit (EUROLINE) (from EUROMIMMUN Medizinische Labordiagnostika AG, Germany). The MSAs included anti-MDA5, anti-signal recognition particle(SRP), anti-Mi-2, anti-PM-scl and autoantibodies against aminoacyl-tRNA synthetase (ARS) such as anti-Jo-1, EJ, PL-7, PL-12, OJ, KS which have been found to be highly specific for myositis. Based on the results of MSAs, we further divided the patients into three groups: anti-MDA5 group (n = 30), anti-Jo-1 group (n = 42) and other anti-ARS group (n = 64).

Statistical analysis

Binary data were expressed as number (percentage) and continuous data were expressed as median (range). Either the chi-square test or Fisher's exact test was used as appropriate for comparing proportions. Comparisons of continuous data were performed using the Kruskal-Wallis

test followed by the Mann-Whitney U test. The survival time was calculated from the date of first admission to our hospital for ILD to the last visit or death. Overall survival was evaluated by the Kaplan-Meier method using the log-rank test. Univariate and multivariate logistic regression analysis were employed to determine factors that could predict prognosis. Variables that were significant in the univariate analysis were included in the multivariate analysis. A p-value of 0.05 was considered statistically significant. All statistical analyses were performed with SPSS software, version 22.0 (SPSS, Inc., Chicago, IL, USA). All tests were two-sided and performed at a significance level of 0.05.

Results

The clinical characteristics of patients

Table 1 showed the clinical differences among the PM/DM-ILD patients based on the autoantibodies status. We identified 30 patients (22.0%) with positive anti-MDA5, 42 patients (31.0%) with positive anti-Jo-1 and 64 patients (47.0%) with other anti-ARS. There were no significant differences in age, sex ratio, smoking status among the three groups. Skin lesion, fever and arthralgia were seen more frequently in anti MDA-5 group (P = 0.002, 0.003 and 0.02, respectively). However, other respiratory symptoms (cough, sputum and dyspnea) and other muscle symptoms did not differ significantly among the three groups. The crackle was present in almost all patients, but the clubbing did not. In addition, acute disease onset was more frequently observed in the anti-MDA5-positive group (P = 0.005). MDA5 antibodies are only found in patients with DM and CADM and do not be present in patients with PM (P = 0.01), which is consistent with previous report. In contrast, anti-Jo-1 antibodies and other ARS antibodies can exist in PM, DM and CADM. In the anti-Jo-1 group, 4 (9.5%) of 42 patients died and 3 (4.7%) of 64 patients died in the other anti-ARS group. The highest mortality rate was in the anti-MDA5 group (66.7%, 20 of 30, P< 0.001).

Table 1
Characteristics of 136 PM/DM-ILD patients

	Anti-MDA5 N = 30(22.0%)	Anti-Jo-1 N = 42(31.0%)	Other anti-ARS N = 64(47.0%)	*P* value
Age, yrs, median (range)	53.5(35–77)	57.5(33–76)	55.0(31–84)	0.16
Gender				0.50
Female	17 (56.7)	26 (61.9)	44 (68.8)	
Male	13 (43.3)	16 (38.1)	20 (31.3)	
Smoking status				0.60
Never smokers	21 (70.0)	34 (81.0)	52 (81.3)	
Former smokers	4 (13.3)	5 (11.9)	5 (7.8)	
Current smokers	5 (16.7)	3 (7.1)	7 (10.9)	
Skin lesion	25 (83.3)	20 (47.6)	30 (46.9)	**0.002**
Muscle symptoms	9 (30.0)	11 (26.2)	8 (12.5)	0.08
Arthralgia	12 (40.0)	16 (38.1)	11 (17.2)	**0.02**
Dysphagia	1 (3.3)	0 (0)	1 (1.6)	0.49
Raynaud	1 (3.3)	2 (4.8)	6 (9.4)	0.49
Fever	17 (56.7)	12 (28.6)	14 (21.9)	**0.003**
Cough	26 (86.7)	39 (92.9)	61 (95.3)	0.33
Sputum	18 (60.0)	25 (59.5)	43 (67.2)	0.67
Dyspnea	26 (86.7)	39 (92.9)	62 (96.9)	0.15
Crackle	28 (93.3)	39 (92.9)	57 (89.1)	0.79
Clubbing	0 (0)	1 (2.4)	2 (3.1)	1.00
Disease onset				**0.005**
Acute	23(76.7)	23(54.8)	26(40.6)	
Chronic	7(23.3)	19(45.2)	38(59.4)	
Myositis diagnosis				**0.01**
PM	0 (0)	10 (23.8)	10 (15.6)	

Data are presented as the medians (ranges) or as n (%).

** P value calculated by using the Kruskal-Wallis test.*

	Anti-MDA5 N = 30(22.0%)	Anti-Jo-1 N = 42(31.0%)	Other anti-ARS N = 64(47.0%)	*P* value
DM	14 (46.7)	20 (47.6)	22 (34.4)	
CADM	16 (53.3)	12 (28.6)	32 (50.0)	
Mortality	20 (66.7)	4 (9.5)	3 (4.7)	**< 0.001**
Data are presented as the medians (ranges) or as n (%).				
** P value calculated by using the Kruskal-Wallis test.*				

The laboratory data, pulmonary function tests and HRCT findings.

The laboratory data, pulmonary function tests and HRCT findings in three groups are presented in Table 2. Among the laboratory data, serum LDH, CEA and NSE were significantly higher in the anti-MDA5 group compared to the other two groups($P < 0.001$), while CD4 + T cells, NK cells and lymphocyte counts were markedly lower than the anti-Jo-1 group and the other ARS group($P = 0.01$, $P < 0.001$ and $P < 0.001$, respectively). In addition, the median PaO_2 level and oxygenation index were relatively low in anti-MDA5 group and significantly differed among the three groups (both $P < 0.001$). Likewise, patients in the anti-MDA5 group were more likely to have lower $PaCO_2$ level ($P = 0.01$).

The pulmonary function tests including %FVC, %FEV1 and %DLCO were tend to elevate in anti-MDA5 group although no significant difference was observed. Chest HRCT images were available for all 136 patients. NSIP pattern was more likely to be observed in 25(59.5%) patients of the anti-Jo-1 group and 40(62.5%) patients of the other anti-ARS group ($P < 0.001$). Conversely, OP + NSIP and DAD patterns were more commonly found in patients with anti-MDA5, especially DAD pattern. 7 (23.3%) cases of the anti-MDA5 group were interpreted as exhibiting DAD pattern compared with 2(4.8%) patients in the anti-Jo-1 group and there was no DAD pattern found in the other anti-ARS group($P < 0.001$)).

Table 2
The differences of laboratory data, pulmonary function tests and HRCT findings in three groups

	Anti-MDA5 N = 30(22.0%)	Anti-Jo-1 N = 42(31.0%)	Other anti-ARS N = 64(47.0%)	*P* value
PaO2, torr,mmHg (n = 115)	60.0 (50.0–85.0) (n = 29)	72.0 (51.1–117.0) (n = 36)	81.5 (52.0-164.0) (n = 50)	< 0.001
PaCO2, torr,mmHg (n = 115)	33.7 (24.7–43.7) (n = 29)	36.9 (24.9–48.9) (n = 36)	38.2 (23.0-45.7) (n = 50)	0.001
OI (n = 115)	201.0 (56–404) (n = 29)	333.0 (98–557) (n = 36)	371.0 (80–565) (n = 50)	< 0.001
CK, U/L (n = 130)	37.0 (11–377) (n = 29)	58.0 (15-4796) (n = 40)	50.0 (16-1442) (n = 61)	0.41
LDH, U/L (n = 135)	408.0(214-15743) (n = 29)	295.5 (162–2150) (n = 42)	263.5 (166–596) (n = 64)	< 0.001
CRP, mg/dL (n = 135)	16.9 (2.2–79.1) (n = 29)	4.9 (1.4–91.2) (n = 42)	6.4 (1.2-151.6) (n = 64)	0.17
ESR, mm/h(n = 130)	33.0 (7–81) (n = 27)	25.0 (2–94) (n = 41)	27.5 (4-139) (n = 62)	0.08
CD4 + T cells, $\times 10^9$/L(n = 115)	0.235 (0.037–1.166) (n = 27)	0.372 (0.140–1.462) (n = 36)	0.414(0.087–1.009) (n = 52)	0.01
NK cells, $\times 10^9$/L (n = 115)	0.067 (0.013–0.401) (n = 27)	0.267(0.029–1.087) (n = 36)	0.170(0.035–0.533) (n = 52)	< 0.001
IgG, g/L (n = 130)	12.5 (7.4–25.7) (n = 28)	11.1 (7.2–25.2) (n = 41)	13.7 (8.2–21.9) (n = 61)	0.07
CEA,ng/ml (n = 131)	5.32 (0.34-47.0) (n = 30)	1.72 (0.50–18.40) (n = 40)	1.29 (0.50-11.73) (n = 61)	< 0.001

Data are presented as the medians (ranges) or as n (%). P value calculated by using the Kruskal-Wallis test.*

OI: Oxygenation Index, CK: Creatine kinase, LDH: Lactate dehydrogenase, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, CEA: Carcinoembryonic antigen, NSE: Neuron-specific enolase, CyFRA21-1: Cytokeratin 19 fragment, PLT: Platelet, WBC: White blood cell.

	Anti-MDA5 N = 30(22.0%)	Anti-Jo-1 N = 42(31.0%)	Other anti-ARS N = 64(47.0%)	*P* value
NSE, ng/ml(n = 131)	20.61 (12.64–67.93) (n = 30)	16.865 (7.84–64.41) (n = 40)	14.77 (8.74–31.24) (n = 61)	< 0.001
CyFRA21-1, ng/ml(n = 131)	6.525 (1.74–48.59) (n = 30)	4.525 (1.36–18.72) (n = 40)	4.06 (1.13–36.05) (n = 61)	0.09
Lymphocyte, $\times 10^9$/L (n = 136)	0.7 (0.2–3.4) (n = 30)	1.4 (0.2–13.4) (n = 42)	15. (0.6–4.9) (n = 64)	< 0.001
PLT, $\times 10^9$/L (n = 136)	196.5 (97–347) (n = 30)	241.0 (125–422) (n = 42)	223.5 (5-346) (n = 64)	0.10
WBC, $\times 10^9$/L (n = 136)	7.1 (1.9–16.9) (n = 30)	7.5 (4.4–23.3) (n = 42)	7.2 (3.4–16.1) (n = 64)	0.42
HRCT findings				< 0.001
OP	4(13.3)	3(7.1)	7(10.9)	
NSIP	7(23.3)	25(59.5)	40(62.5)	
OP + NSIP	12(40.0)	12(28.6)	17(26.6)	
DAD	7(23.3)	2(4.8)	0(0)	
FVC, % predicted median (range) (n = 96)	67.1 (36.1–86.2) (n = 10)	57.8 (18.7-101.6) (n = 32)	59.0 (28.5–97.9) (n = 54)	0.79
FEV1, % predicted median (range) (n = 96)	70.8 (44.1–95.2) (n = 10)	62.6 (20.3-103.3) (n = 32)	63.15 (32.2-100.6) (n = 54)	0.83
DLCO, % predicted median (range) (n = 85)	68.6 (43.2–81.7) (n = 8)	50.4 (10.1-136.2) (n = 25)	47.0 (5.4–86.4) (n = 52)	0.04

Data are presented as the medians (ranges) or as n (%). P value calculated by using the Kruskal-Wallis test.*

OI: Oxygenation Index, CK: Creatine kinase, LDH: Lactate dehydrogenase, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, CEA: Carcinoembryonic antigen, NSE: Neuron-specific enolase, CyFRA21-1: Cytokeratin 19 fragment, PLT: Platelet, WBC: White blood cell.

The survival analysis for the patients with PM/DM-ILD.

Figure 1 shows the Kaplan-Meier survival curves for the entire cohort. The overall survival of patients with anti-Jo-1-ILD was significantly better than that of patients with anti-MDA5-ILD (log-rank, P<0.001) and similar to that of patients with other anti-ARS-ILD.

Univariate Cox hazard analysis showed that acute disease onset [hazard ratio (HR) 4.51; 95%CI: 1.70–11.90, P = 0.002), DAD pattern for HRCT imaging (HR 24.25; 95%CI: 8.76–67.14, P < 0.001), current smokers (HR 3.00; 95%CI: 1.19–7.57, P = 0.02), presence of Anti-MDA5(HR 23.01; 95%CI: 6.79–78.02, P < 0.001), fever(HR5.21; 95%CI: 6.79–78.02, P < 0.001) were significantly associated with the CTD-ILD death of the study population (Table 3). Among laboratory data,lower PaO2(HR 0.92; 95%CI: 0.89–0.96, P < 0.001), decreased PaCO2(HR 0.89; 95%CI: 0.83–0.96, P = 0.001), LDH level ≥ 300U/L(HR 15.39; 95%CI: 3.64–65.17, P < 0.001), elevated ESR, CEA, NSE and CyFRA21-1(HR 1.03, 1.140, 1.064, 1.098, respectively, all P < 0.001) were found to be positively correlated with mortality. The patients with higher CD4 + T cells counts (HR 0.996; 95%CI: 0.994–0.999, P = 0.002) and NK cells counts (HR 0.993; 95%CI: 0.989–0.997, P = 0.002) associated with lower mortality. Interestingly, increasing in platelet count was found associated with lower mortality risk (HR 0.992; 95%CI: 0.987–0.998, P = 0.004). (HR 2.47; 95%CI: 1.13–5.42, P = 0.02).

It is worth mentioning that the administration of high-dose prednisolone (PSL) pulse and intravenous immunoglobulin (IVIG) were positively correlated with mortality (HR 23.04; 95%CI: 10.30-51.53; HR 12.35; 95%CI: 5.60-27.24, respectively, both P < 0.001). Corticosteroids and immunosuppressive agents combination therapy can reduce the risk of death than using corticosteroid alone during hospitalization (HR 0.36, 95%CI: 0.14–0.95, P = 0.04).

Table 3
Univariate Cox hazards analysis for mortality of PM/DM-ILD patients

	HR	95% CI	*P* value
Age, yrs	1.02	0.99–1.06	0.23
≤55	Ref		
>55	1.79	0.83–3.86	0.14
Gender			
Male	Ref		
Female	0.63	0.29–1.34	0.22
Disease onset			
Chronic	Ref		
Acute	4.51	1.70–11.90	0.002
HRCT findings			
NSIP	Ref		
OP	1.34	0.28–6.31	0.71
OP + NSIP	2.11	0.81–5.46	0.12
DAD	24.25	8.76–67.14	< 0.001
Smoking status			
Never smokers	Ref		
Former smokers	1.33	0.39–4.50	0.65
Current smokers	3.00	1.19–7.57	0.02
Myositis diagnosis			
PM	Ref		
DM	2.22	0.50–9.91	0.30
CADM	2.23	0.50–9.88	0.29
Myositis-specific Abs			
Other anti-ARS	Ref		
Anti-Jo-1	2.00	0.45–8.95	0.36
Anti-MDA5	23.01	6.79–78.02	< 0.001

	HR	95% CI	*P* value
Skin lesion			
No	Ref		
Yes	2.00	0.88–4.58	0.10
Muscle symptoms			
No	Ref		
Yes	1.58	0.69–3.62	0.28
Arthralgia			
No	Ref		
Yes	1.24	0.56–2.76	0.60
Dysphagia			
No	Ref		
Yes	2.93	0.40-21.67	0.29
Fever			
No	Ref		
Yes	5.21	2.34–11.61	< 0.001
Cough			
No	Ref		
Yes	2.26	0.31–16.63	0.42
Sputum			
No	Ref		
Yes	1.87	0.79–4.44	0.15
Dyspnea			
No	Ref		
Yes	2.01	0.27–14.79	0.49
Crackle			
No	Ref		
Yes	0.85	0.26–2.82	0.79
PaO2, torr,mmHg (n = 115)	0.92	0.89–0.96	< 0.001

	HR	95% CI	*P* value
PaCO2, torr,mmHg (n = 115)	0.89	0.83–0.96	0.001
OI (n = 115)	0.98	0.98–0.99	< 0.001
CK,U/L (n = 130)	0.999	0.996–1.001	0.263
LDH, U/L (n = 135)	1.0003	1.0001–1.0004	< 0.001
<300	Ref		
≥300	15.39	3.64–65.17	< 0.001
CRP, mg/dL (n = 135)	1.03	1.01–1.04	< 0.001
ESR, mm/h(n = 130)	1.01	1.00-1.03	0.14
CD4 + T cells, $\times 10^9$/L (n = 115)	0.996	0.994–0.999	0.002
NKcells, $\times 10^9$/L,(n = 115)	0.993	0.989–0.997	0.002
IgG,g/L (n = 130)	0.929	0.833–1.036	0.186
CEA, ng/ml (n = 131)	1.140	1.095–1.186	< 0.001
NSE, ng/ml (n = 131)	1.064	1.041–1.087	< 0.001
CyFRA21-1, ng/ml (n = 131)	1.098	1.064–1.133	< 0.001
Lymphocyte, $\times 10^9$/L (n = 136)			
≥1.5%	Ref		
<1.5%	5.64	1.34–23.83	0.02
PLT, $\times 10^9$/L (n = 136)	0.992	0.987–0.998	0.004
WBC, $\times 10^9$/L (n = 136)	1.000	0.889–1.126	0.994
Treatment during Hospitalization (n = 134)			
CS alone	Ref		
CS + IM	0.36	0.14–0.95	0.04
PSL pulse			
No	Ref		
Yes	23.04	10.30-51.53	< 0.001
IVIG			
No	Ref		

	HR	95% CI	*P* value
Yes	12.35	5.60-27.24	< 0.001

Table 4 showed the results of multivariate Cox proportional hazard analyses for myositis-specific Abs including anti-MDA5, anti-Jo-1 and other anti-ARS. After adjusting for disease onset, HRCT findings, smoking status, treatment before admission, treatment after admission,fever、PaO_2、$PaCO_2$、LDH、CRP、$CD4^+$T cells、NK cells、CEA、NSE、CyFRA21-1、lymphocyte and PLT, the patients with anti-MDA5 antibody had a 17.61-fold (95%CI: 2.28-135.95, $P = 0.006$) higher risk for the mortality than those in other anti-ARS groups (model 1, Table 4). As shown in model 2, anti-MDA5 still predicted a worse outcome (HR, 8.44; 95% CI, 1.09–65.03; $P = 0.04$) after adjusting for all the risk factors showed significance in univariate Cox regression analysis including PSL pulse and IVIG.

Table 4
Multivariate Cox hazards analysis for myositis-specific Abs

	Model 1			Model 2		
	HR	95% CI	P value	HR	95% CI	P value
Other anti-ARS	Ref			Ref		
Anti-Jo-1	2.45	0.30–19.70	0.40	1.46	0.16–12.95	0.73
Anti-MDA5	17.61	2.28-135.95	**0.006**	8.44	1.09–65.03	0.04

The Cox hazard analysis for mortality in anti-MDA5 subgroup.

Univariate Cox hazard analysis for mortality in anti-MDA5 subgroup showed that DAD pattern for HRCT imagings (HR 5.36; 95%CI: 1.44–20.01, $P = 0.01$), current smokers (HR 5.83; 95%CI: 1.90-17.87, $P = 0.002$), elevated CEA, NSE and CyFRA21-1(HR 1.088, 1.106, 1.163, respectively, all $P < 0.001$) were still significantly associated with the death of the patients with anti-MDA5 (Table 5). (HR 1.03, 1.140, 1.064 and 1.098, respectively, all $P < 0.001$). Increased CD4 + T cells (HR 0.996; 95%CI: 0.993–0.999, $P = 0.011$) and lymphocyte counts (HR 0.71; 95%CI: 0.59–0.86, $P < 0.001$) can reduce mortality. Higher PaO_2 (HR 0.95; 95%CI: 0.91–0.99, $P = 0.02$) and $PaCO_2$(HR 0.89; 95%CI: 0.81–0.98, $P = 0.01$), were also found to be found to be beneficial to survival. Age was a risk factor for death of patients with anti-MDA5 (HR 1.05; 95%CI: 1.01–1.09, $P = 0.02$). Those older than 55 years old showed higher mortality risk compared to those younger than 55 years old (HR 2.61; 95%CI: 1.06–6.39, $P = 0.04$). In addition, the presence of sputum (HR 3.18; 95%CI: 1.14–8.84, $P = 0.03$) and high level of CRP (HR 1.026; 95%CI: 1.004–1.048, $P = 0.019$) showed associated with higher mortality. Interestingly, in the subgroup analysis for anti-MDA5 group, the treatment with PSL pulse and IVIG were still markedly correlated with high mortality (HR 21.78, 95%CI: 4.71-100.73, $P < 0.001$; HR 5.65; 95%CI: 2.06–15.48, $P = 0.001$, respectively)

Table 5
Univariate Cox hazards analysis for mortality in anti-MDA5 group

	HR	95% CI	P value
Age, yrs	1.05	1.01–1.09	0.02
≤55	Ref		
>55	2.61	1.06–6.39	0.04
Gender			
Male	Ref		
Female	0.67	0.28–1.62	0.37
Disease onset			
Chronic	Ref		
Acute	3.61	0.83–15.68	0.09
HRCT findings			
NSIP	Ref		
OP	0.72	0.13–3.95	0.71
OP + NSIP	0.74	0.22–2.53	0.63
DAD	5.36	1.44–20.01	0.01
Smoking status			
Never smokers	Ref		
Former smokers	1.77	0.49–6.35	0.38
Current smokers	5.83	1.90-17.87	0.002
Myositis diagnosis			
DM	Ref		
CADM	0.83	0.34–1.99	0.67
Skin lesion			
No	Ref		
Yes	0.60	0.20–1.81	0.37
Muscle symptoms			
No	Ref		

	HR	95% CI	P value
Yes	0.55	0.20–1.53	0.25
Arthralgia			
No	Ref		
Yes	0.87	0.35–2.12	0.75
Dysphagia			
No	Ref		
Yes	1.39	0.18–10.58	0.75
Fever			
No	Ref		
Yes	2.51	0.95–6.61	0.06
Cough			
No	Ref		
Yes	4.64	0.62–34.82	0.14
Sputum			
No	Ref		
Yes	3.18	1.14–8.84	0.03
Dyspnea			
No	Ref		
Yes	5.04	0.67–37.92	0.12
Crackle			
No	Ref		
Yes	1.72	0.23–12.85	0.60
PaO2, torr, mmHg (n = 115)	0.95	0.91–0.99	0.02
PaCO2, torr, mmHg (n = 115)	0.89	0.81–0.98	0.01
OI (n = 115)	0.992	0.986–0.997	0.004
CK,U/L (n = 130)	1.0004	0.9952–1.0056	0.8888
LDH, U/L (n = 135)	1.0002	1.00004–1.0004	0.015
<300	Ref		

	HR	95% CI	P value
≥300	6.18	0.82–46.56	0.08
CRP, mg/dL (n = 135)	1.026	1.004–1.048	0.019
ESR, mm/h(n = 130)	0.997	0.977–1.017	0.764
CD4 + T cells, $\times 10^9$/L (n = 115)	0.996	0.993–0.999	0.011
NKcells, $\times 10^9$/L,(n = 115)	0.994	0.986–1.003	0.184
IgG,g/L (n = 130)	0.901	0.781–1.039	0.150
CEA, ng/ml (n = 131)	1.088	1.038–1.139	< 0.001
NSE, ng/ml (n = 131)	1.106	1.046–1.169	< 0.001
CyFRA21-1, ng/ml (n = 131)	1.163	1.076–1.258	< 0.001
Lymphocyte,%, 0.1 increase (n = 30)	0.71	0.59–0.86	< 0.001
PLT, $\times 10^9$/L (n = 30)	0.994	0.986–1.001	0.090
WBC, $\times 10^9$/L (n = 30)	1.048	0.902–1.217	0.539
Treatment before admission			
None	Ref		
CS alone	1.35	0.54–3.37	0.52
CS + IM	0.74	0.09–5.93	0.78
Treatment during Hospitalization			
CS alone	Ref		
CS + IM	0.41	0.12–1.41	0.16
PSL pulse			
No	Ref		
Yes	21.78	4.71-100.73	< 0.001
IVIG			
No	Ref		
Yes	5.65	2.06–15.48	0.001

Discussion

In the last 10 years, MSAs as useful biomarkers have been shown to be highly specific for the diagnosis of PM/DM, and several new MSAs with important clinical significance have been identified[10]. There have been a few single-center studies focusing on the effects of different types of MSAs on the prognosis of different form of PM/DM/CADM-ILD patients[11, 12, 14]. Our study is a retrospective study of the Chinese population, trying to explore the prognostic factors of PM-ILD based on serological myositis antibody phenotypes. The patients with PM, DM, and CADM were divided into anti-MDA5 group, anti-Jo-1 group and other anti-ARS group according to the results of myositis spectrum. We reviewed the medical records to determine their differences in clinical features and impact on prognosis.

Anti–MDA5 has been reported to be significantly associated with ILD, rapidly progressive ILD and poor survival in DM patients[15]. Consistent with previous studies, the patients with anti-MDA5 frequently have acute progressive ILD and their diagnosis of myositis will not be PM. Skin lesion and fever are more likely to be observed in anti-MDA5 group, and these patients are more prone to hypoxemia with lower $PaCO_2$ level. The predicted factors associated with mortality in our cohort were acute disease onset, anti-MDA5 antibody, lower PaO_2 and $PaCO_2$ levels, elevated serum LDH and CRP. Moreover,,it has been shown that serum CEA and CA 19 – 9 were elevated and can be predictors of mortality in CTD-ILD patients[19], which also was confirmed in our study on patients with PM/DM-ILD. In addition, the data suggest that the most common radiologic pattern found in anti-Jo-1 group and other anti-ARS group was NSIP(59.5% and 62.5%, respectively), a finding similarly described in the literature in association with other AS syndrome and the inflammatory myopathies[20–22]. The predominance of a radiological DAD is considered a major reason for higher mortality in anti-MDA5 group. Infections and acute interstitial pneumonia are the most common causes of DAD[23], which probably represents evidence of more severe lung injury. The pulmonary function tests including %FVC, %FEV1 and %DLCO were prone to elevate in anti-MDA5 group, because some critically ill patients in the anti-MDA5 group whose lung functions were very poor could not complete the pulmonary function tests, thereby their lung function data missed.

Unlike the previous studies, the current study has some new findings. First of all, in current study, NK cells and T lymphocytes counts, especially CD4 + T cells, are significantly reduced in peripheral blood of patients with positive anti-MDA5, which are significantly associated with mortality. Little is known about the relationship between immune cells and the prognosis of CTD-ILD. Patrizia F et.al[24] showed that CD8 + T cells and NK cells are decreased in SSc associated ILD patients, and there was a correlation between the reduced number of NK cells and increased inflammation. In addition, decreased percentage of CD3 + T cells and CD8 + T cells were indicated to be present in peripheral blood of active dermatomyositis but not in the inactive form of the disease[25]. However, whether the reduced lymphocytes are related to prognosis is still unknown. In this study, we found that patients with anti-MDA5 had lower CD4 + T cells levels and had a worse prognosis, while the CT performance of DAD was more common, suggesting that a part of patients developed acute interstitial pneumonia, and another patients may be combined infections including cytomegalovirus and Pneumocystis carinii infection. This theory can explain the results of the univariate Cox hazard analysis on PSL pulse therapy. The Cox hazard analysis for mortality in all patients showed the administration of PSL pulse and IVIG were significantly correlated with high

mortality. The reason might be MDA5-positive patients were more inclined to take PSL and IVIG therapy compared to MDA5-negative patients. Interestingly, the subsequent Cox hazard analysis for anti-MDA5 subgroup indicated that the administration of PSL pulse and IVIG were still risk factors for mortality, which revealed the conventional high-dose corticosteroid pulse therapy for PM/DM-ILD patients with anti-MDA5 antibody may be ineffective,using immunosuppressive agents combined with corticosteroids is more important to reduce mortality.

In addition, current smoking status was found to be positively correlated with mortality. To our knowledge, smoking has been shown to be associated with autoantibody production as well as the development and course of many rheumatic disorders[26–28]. Smokers had a higher frequency of myositis-associated autoantibodies[28, 29]. A possible dose-response relationship existed between cigarette smoking and increasing risk of ILD in Caucasian PM/DM patients[28]. Our study provides evidence for the relationship of smoking status and risk of death. There was no statistically significant relationship between former smoking status and mortality, which suggested that smoking cessation may be one of the effective treatments.

Our study also has several limitations. Since this study was performed in a specialized single-center for the respiratory and critical diseases, the research was subject to some possible biases. First, many patients have been treated with corticosteroids for more than a week before admission so that the serum CK levels have returned to normal at the initial visit, which leading to a selection bias for the diagnosis of PM/DM. Second, several patients with positive anti-MDA5 were too serious to cooperate with pulmonary function tests, resulting in the average levels of FVC% and DLCO% in the anti-MDA5 group did not differ from the other two groups. At last, due to the retrospective nature of our study, the previously reported prognostic factors for PM/DM-ILD including the serum ferritin, KL-6 and SP-D was not available in all patients. However, our study is the largest descriptive report for patients with PM/DM-ILD in the Chinese population, and all patients had myositis spectrum measurement results. The application of PSL pulse and IVIG are not effective treatment for positive anti-MDA5 patients,and new strategies for the treatment need to be explored.

In conclusion, reduced NK cells, T lymphocytes counts, especially CD4 + T cells and anti–MDA5 antibody are significantly associated with associated with worse prognosis in PM/DM-ILD patients. The application of PSL pulse and IVIG are not necessarily an effective treatment for positive anti-MDA5 patients.

Abbreviations

MSAs: myositis-specific autoantibodies;

PM/DM-ILD: polymyositis/dermatomyositis associated interstitial lung disease;

CTDs: connective tissue diseases,

RA: rheumatoid arthritis,

SSc: systemic sclerosis,

CTD-ILD: connective tissue diseases associated ILD,

AAbs: autoantibodies,

ARS: aminoacyl-tRNA synthetase,

MDA5: melanoma differentiation associated protein 5,

HRCT: High Resolution Computed Tomography,

OP: organizing pneumonia,

NSIP: nonspecific interstitial pneumonia,

DAD: diffuse alveolar damage,

OI: Oxygenation Index,

CK: Creatine kinase,

LDH: Lactate dehydrogenase,

CRP: C-reactive protein,

ESR: Erythrocyte sedimentation rate,

CEA: Carcinoembryonic antigen,

NSE: Neuron-specific enolase,

PLT: Platelet,

WBC: White blood cell,

PSL: prednisolone,

IVIG: intravenous immunoglobulin.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Nanjing Drum Tower Hospital of Medical School of Nanjing University (No.31/93, 84/93, 29/01). Written informed consent was obtained from all subjects in the study protocol.

Consent for publication

The consent for publication has been obtained from the patients.

Availability of data and material

All data generated or analysed during this study are included in this manuscript. All data will be available by personal communication with corresponding author.

Competing interests

All authors declare no competing interests.

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Authors' contributions

Gao Yujuan: Data curation, Writing-Original draft preparation. **Yan Xin**: Methodology, Validation, Investigation. **Li Yan**: Data curation, Formal analysis, Validation. **Xie Miaomiao**: Provision of study materials, reagents and patients, Validation. **Li Hui**: Data Curation. **Huang Mei, Ding Jingjing and Cao Min**: Provision of study patients. **Dai Jinghong**: Provision of study patients, Supervision. **Cai Hourong**: Conceptualization, Writing- Reviewing and Editing.
All authors read and approved the final manuscript.

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Figures

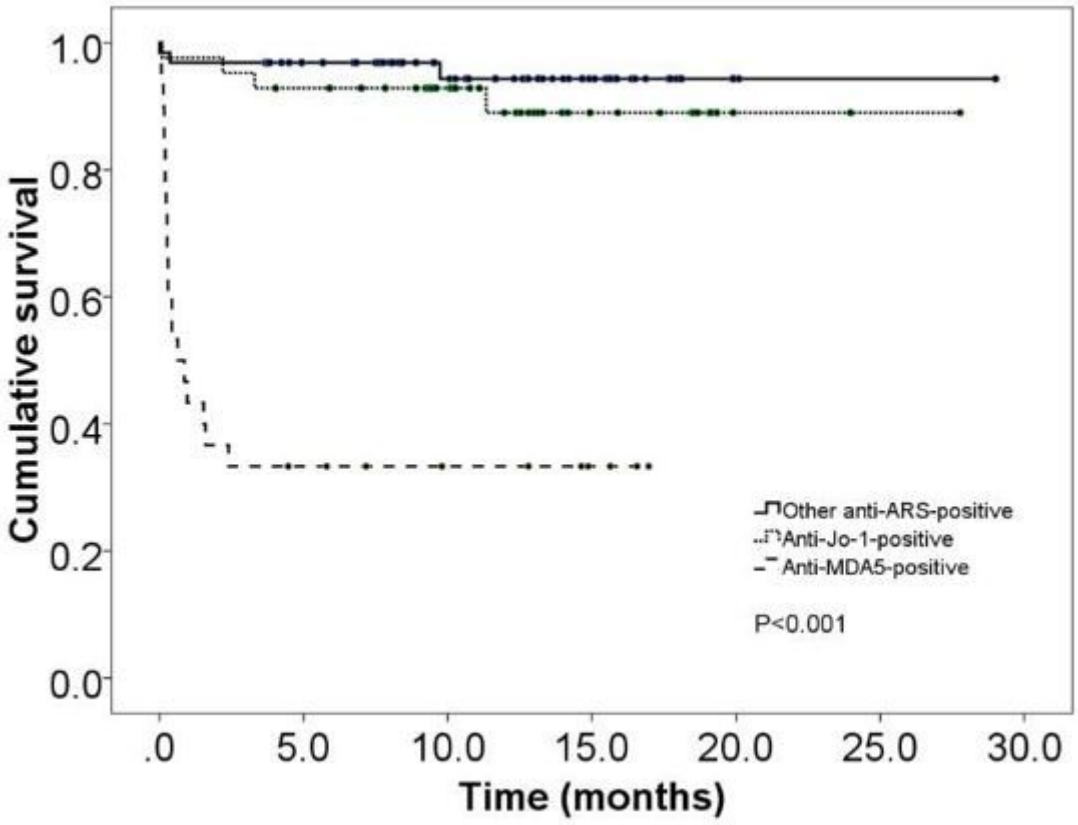

Figure 1

Kaplan–Meier survival curve for the patients with PM/DM-ILD