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Phenotypic clusters and survival analyses in interstitial pneumonia with myositis-specific autoantibodies

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Abstract. Background: Idiopathic inflammatory myopathy (IIM) is highly combined with interstitial pneumonia (IP), often as the initial or solo presentation with positive myositis-specific autoantibodies (MSAs) but does not fulfill the diagnostic criteria. **Objectives:** We aimed to explore the phenotypic clusters and prognosis of the patients with IP and positive MSA, which is called MSA-IP in the present study. **Methods:** A total of 178 patients with MSA-IP were prospectively enrolled for analysis. Serum MSAs were detected using Western blotting. Radiological patterns of IP were determined according to the classification of idiopathic IPs. Clusters of patients with MSA-IP were identified using cluster analysis. Predictors for acute/subacute onset, therapeutic response, IP progression and survival were also analyzed. **Results:** Patients with MSA-IP were classified into four distinct clusters. Cluster 1 were the elderly with chronic onset, nearly normal oxygenation and good survival. Cluster 2 had dyspnea on exertion and nonspecific IP pattern, with moderate survival. Patients in cluster 3 had chronic onset and were prone to IP progression (OR 2.885). Cluster 4 had multi-systemic involvements, positive anti-melanoma differentiation associated gene 5 antibody, and were prone to acute/subacute onset (OR 3.538) and IP progression (OR 5.472), with poor survival. Corticosteroids combined immunosuppressants showed therapeutic response in MSA-IP (OR 4.303) and had a protective effect on IP progression (OR 0.136). **Conclusions:** Four clusters of the patients with MSA-IP suggested the distinct clinical, radiological and prognostic features.

Key words: myositis, autoantibody, cluster analysis, interstitial pneumonia, prognosis

Introduction

Interstitial lung disease (ILD) is one of the well-acknowledged manifestations of connective tissue diseases (CTDs), and it is referred to as CTD-associated ILD (CTD-ILD) when occurring within the context of CTDs (1,2). A study summarized the incidence of CTDs combined with ILD, including systemic sclerosis, rheumatoid arthritis, Sjoegren's syndrome, mixed CTD, idiopathic inflammatory myopathy (IIM) and systemic lupus erythema and etc. The estimated incidence of CTD-ILDs is approximately 15% (3). Interstitial pneumonia (IP) can be the primary or sole manifestation of CTDs (4), leading to difficulties in obtaining an accurate diagnosis at the first clinical visit. When patients with IP have clinical, serological, and/or morphological features likely stemming from underlying autoimmune conditions but do not satisfy the diagnostic criteria for any CTD, they may be diagnosed with IP with autoimmune features (IPAF) (2) or not fulfill any of the above diagnostic criteria.

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IIMs are the unusual subtypes of CTDs. IIMs are characterized by skeletal muscle inflammation and include polymyositis, dermatomyositis, amyopathic dermatomyositis (ADM) etc. (5,6). IP is one of the most common extra-muscular manifestations of IIMs (7). The prevalence of IIM-associated ILDs (IIM-ILDs) is ranged from 19.9% to 86% (8-14). The autoantibodies of IIMs consist of myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs). MSAs are highly specific, including anti-aminoacyl-tRNA-synthetase antibodies (anti-ARS) and non-anti-ARS MSAs, whereas MAAs are less specific and can be detected in other CTDs (15). A cohort study showed that 26.7% (44/165) of patients with IP at the initial diagnosis were positive for myositis autoantibodies (16). MSAs are essential for assessing the clinical characteristics, diagnosis and prognosis of patients (17). MSAs may indicate unique IPAF phenotypes featured by clinical characteristics and survival that were similar to patients with IIM-ILD (18).

Previous studies have mainly focused on patients with IIM-ILDs, but the clinical characteristics and prognosis of IP with positive MSA (MSA-IP) are vague (19). For the patients with MSA-IP, the selective diagnosis of CTD-ILD or IPAF may lead to the different therapeutic timing and regimens (20). Cluster analysis is an effective method for identifying homogeneous phenotypes among patients with heterogeneous disorders (21,22). The purpose of this study was to explore the clinical characteristics, potential predictors for acute/subacute onset, therapeutic response, IP progression, and survival of the patients with MSA-IP by cluster analysis.

Methods

Study cohort

A total of 2,115 patients with IP from Clinical Center for Interstitial Lung Diseases of Beijing Chao-Yang Hospital were sequentially and prospectively included from November 2018 to December 2020. IP was diagnosed according to the 2013 American Thoracic Society (ATS) and European Respiratory Society (ERS) Consensus Classification of idiopathic interstitial pneumonias (IIPs) (23).

Among enrolled patients, 42 patients were diagnosed with polymyositis, 43 with dermatomyositis, 23 with ADM and 70 with IPAF. IIM and the sub-classification were diagnosed according to the European League Aginst Rheumatism (EULAR)/American College of Rheumatology (ACR) criteria (24). IPAF was diagnosed using the ERS/ATS research statement (2). Of the 2,115 patients with IP, 42 underwent pathological examinations of the lungs, and no patients received a lung transplant.

Data collection and definitions

At the first clinical visit, the patients' medical records were reviewed to uniformly extract clinical data (online supplementary e-Appendix). Acute/subacute onset was defined as less than three months from symptoms onset to the first clinical visit, and chronic onset was defined as a duration of more than three months.

Serological markers and MSAs (online supplementary e-Appendix) were obtained from patients. IP was diagnosed by high-resolution computed tomography (HRCT) (23). All patients underwent HRCT, pulmonary function tests (25) and echocardiography (26). HRCT patterns, test items and the definition of pulmonary hypertension were provided in the online supplementary e-Appendix (Supplementary file).

Treatment regimens included corticosteroids, corticosteroids combined immunosuppressants, triple therapy which means corticosteroids, immunosuppressants combined antifibrotic agents, and others.

Follow-up and endpoint of the study

The follow-up interval was 3 or 6 months, and the follow-up ended in October 2020. Therapeutic response was defined as no reduction in the median annual rate of decline in absolute forced vital capacity (FVC) or FVC% predicted from the beginning of treatment to end of the follow-up (27). The outcome of this study was IP progression within the follow-up period. Full definitions were provided in the online supplementary e-Appendix (28). Survival time was calculated from the onset of IP-related symptoms to the outcome or end of the follow-up.

Statistical analysis

Quantitative data were reported as the mean ± standard deviations or median (interquartile ranges), and categorical data are reported as numbers and percentages. The cluster analysis was performed through the Two Step Cluster algorithm. The detailed analysis were provided in the online supplementary e-Appendix. Analysis of variance and the non-parametric Mann–Whitney U test were used for comparisons of quantitative data. The chi-square test was used for comparisons of categorical data. Multivariable logistic regression was applied to determine predictors for acute/subacute onset, therapeutic response and IP progression. Survival curves were obtained using Kaplan–Meier method and a multivariable Cox proportional hazards model was constructed to identify prognostic factors for patients with MSA-IP. Statistical analysis was performed using SPSS software (version 23.0, IBM), and $P<0.05$ was statistically significant.

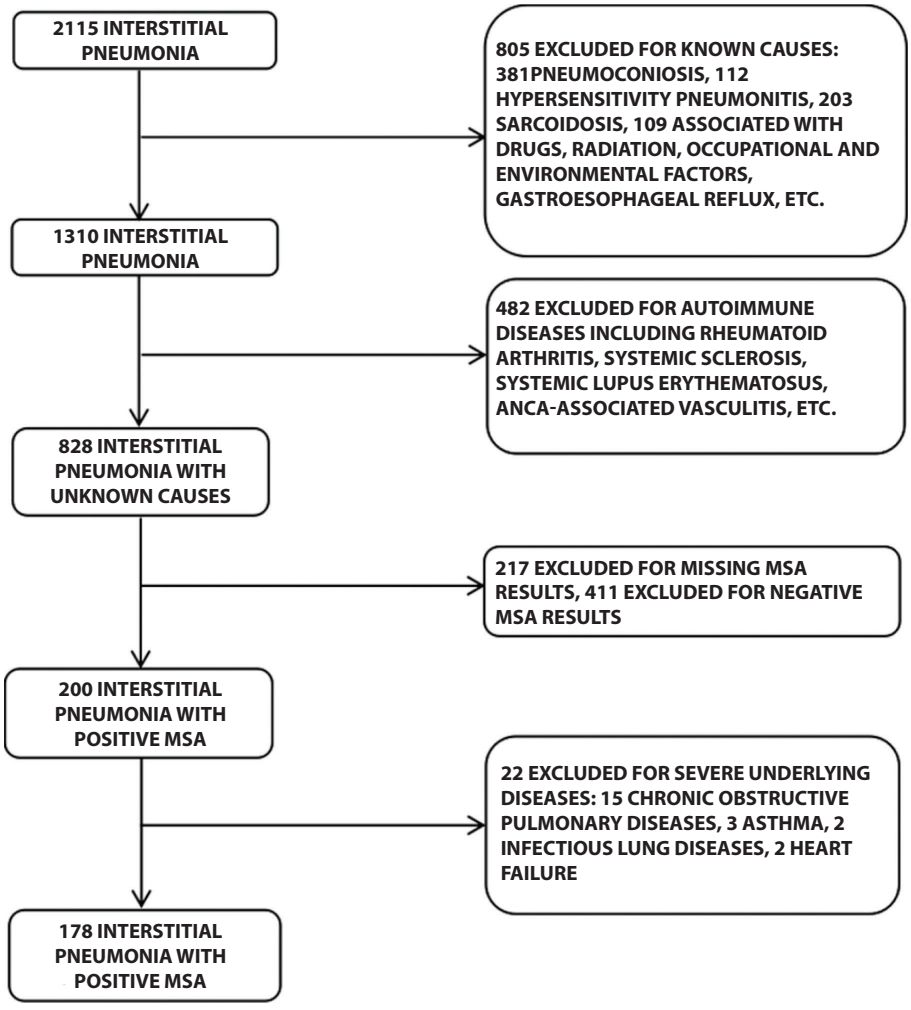

Figure 1. Flow chart showing the enrolment of patients with MSA-IP

A total of two thousand one hundred and fifteen patients with interstitial pneumonia were initially included in this study. One hundred and seventy-eight patients with MSA-IP were enrolled in the final analysis after evaluation.

Results

Demographics

A total of 178 patients with MSA-IP were enrolled in cluster analysis after evaluation (Figure 1) and were categorized into four clusters. As shown in Table 1, cluster 1 had 38 (21.3%) patients with a mean age of 60.2±10.7 years, and 60.5% had chronic onset. Cluster 2 was the largest (58/178, 32.6%). Cluster 3 comprised 41 patients, and chronic onset was common (70.7%, *P*=0.006). Cluster 4 had the largest proportion of females (68.3%) and a high proportion of patients with acute/subacute onset (63.4%, *P*=0.006).

Clinical characteristics

In the study population, dyspnea was frequently observed in all clusters except cluster 1 (online supplementary e-Table 1). Patients in cluster 1 tended to have proximal muscle weakness, nearly normal PaO_2/FiO_2. Gottron was frequently seen in cluster 2 (online supplementary e-Table 2). In cluster 4, low PaO_2/FiO_2, skin rash (31.7%, *P*=0.035), oral ulcer (9.8%, *P*=0.031) and joint involvement were present.

MSA subtypes and laboratory features

As shown in e-Table 3, of all patients, 66.9%(119/178) had positive anti-ARS, which were diagnosed as antisynthetase syndrome. 33.1%(59/178) patients were patients with anti-non-ARS positive antibodies. Among which, 38 of 119 patients with positive anti-histidyl-tRNA synthetase (Jo-1) antibody were the most frequent subtype of antisynthetase syndrome. 25 of 59 patients with anti-melanoma differentiation associated gene (MDA) 5 antibody were the most frequent subtype of anti-non-ARS MSAs patients in present cohort (online supplementary e-Table 3). Patients in cluster 2 often had positive anti-glycy1-tRNA synthetase (EJ) antibodies (24.1%). In cluster 3, anti-threonyl-tRNA synthetase (PL-7) (24.4%, *P*=0.012) and anti-isoleucy1-tRNA synthetase (OJ) (12.2%, *P*=0.010) antibodies could be detected (online supplementary e-Table 4). The distinct MSA subtypes of cluster 4 were anti-MDA5 (34.1%, $P < 0.001$) and anti-EJ (24.4%, *P*=0.019) antibodies.

HRCT patterns

Among all patients, the most frequent HRCT pattern was nonspecific interstitial pneumonia (NSIP) (39.9%) followed by organic pneumonia (OP) (21.3%) (online supplementary e-Table 5). Cluster 1 patients had NSIP or usual interstitial pneumonia (UIP) (42.1%

Table 1. Demographics of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	T/U/X^2	P^* value
N	178	38	58	41	41		
Age, yrs	57.6 ± 11.2	60.2 ± 10.7	58.0 ± 12.1	56.6 ± 11.7	55.7 ± 9.3	1.243	0.296
Female, n (%)	111 (62.4)	22 (57.9)	37 (63.8)	24 (58.5)	28 (68.3)	1.244	0.743
Smoking status						4.507	0.608
Current smokers, n (%)	21 (11.8)	5 (13.2)	7 (12.1)	5 (12.2)	4 (9.8)		
Ex-smokers, n (%)	25 (14.0)	6 (15.8)	7 (12.1)	9 (22.0)	3 (7.3)		
Non-smokers, n (%)	132 (74.2)	27 (71.1)	44 (75.9)	27 (65.9)	34 (82.9)		
Onset forms						12.544	0.006
Acute/subacute, n (%)	72 (40.4)	15 (39.5)	19 (32.8)	12 (29.3)	26 (63.4)		
Chronic, n (%)	106 (59.6)	23 (60.5)	39 (67.2)	29 (70.7)	15 (36.6)		

Values were given as mean (standard deviation) or n (%).
*The *P* value represents comparison among four clusters.

Table 2. Multivariable Logistic regression model for acute/subacute onset

	OR	95% CI	*P* value
Age	1.038	1.006-1.070	0.020
Female	0.770	0.314-1.892	0.570
Smoking status			0.594
Non-smokers*	ref.		
Current smokers	1.934	0.579-6.461	0.284
Ex-smokers	1.184	0.374-3.752	0.774
Clusters			0.003
Cluster 1+	ref.		
Cluster 2	0.809	0.336-1.947	0.636
Cluster 3	0.692	0.261-1.832	0.458
Cluster 4	3.538	1.357-9.224	0.010

*take non-smokers as a reference; +take cluster 1 as a reference.
Abbreviations: OR, odds ratio; CI, confidence interval.

Table 3. Multivariable Logistic regression model for therapeutic response

	OR	95% CI	*P* value
Age	0.982	0.933-1.034	0.497
Female	0.435	0.076-2.504	0.351
Smoking status			0.271
Non-smokers*	ref.		
Current smokers	0.304	0.041-2.270	0.246
Ex-smokers	1.854	0.161-21.356	0.621
Clusters			0.521
Cluster 1+	ref.		
Cluster 2	0.481	0.118-1.971	0.309
Cluster 3	0.867	0.160-4.708	0.868
Cluster 4	1.477	0.229-9.537	0.682
Treatment regimens			0.172
Others‡	ref.		
Corticosteroids	3.828	0.646-22.675	0.139
Corticosteroids combined immunosuppressants	4.303	1.132-16.361	0.032
Triple therapy§	2.301	0.370-14.286	0.371

*take non-smokers as a reference; +take cluster 1 as a reference; ‡take others as a reference;
§means corticosteroids, immunosuppressants combined antifibrotic agents.
Abbreviations: OR, odds ratio; CI, confidence interval.

or 21.1%, respectively). NSIP was frequent in cluster 2 (94.8%, *P*<0.001). Patients in cluster 3 often had UIP (41.5%, *P*<0.001). In cluster 4, diffuse ground glass opacities (GGOs) were representative (22.0%, *P*<0.001).

Predictors for acute/subacute onset, therapeutic response or IP progression

Multivariable Logistic regression analysis showed that patients who were older (OR 1.038, 95% CI 1.006–1.070, *P*=0.020) and in cluster 4 (OR 3.538, 95% CI 1.357–9.224, *P*=0.010) were at higher risks of acute/subacute onset (Table 2). Half of the patients were treated with corticosteroids combined immunosuppressants (50.6%) (online supplementary e-Table 6). After adjusting for age, sex, smoking status and

Table 4. Multivariable Logistic regression model for IP progression

	OR	95% CI	*P* value
Age	1.081	0.996-1.173	0.062
Female	0.699	0.098-4.962	0.720
Smoking status			0.473
Non-smokers*	ref.		
Current smokers	0.159	0.008-3.061	0.221
Ex-smokers	1.021	0.067-12.215	0.998
Clusters			0.340
Cluster 1+	ref.		
Cluster 2	4.383	0.603-31.872	0.144
Cluster 3	0.692	0.261-1.832	0.978
Cluster 4	3.538	1.357-9.224	0.960
Treatment regimens			0.134
Others‡	ref.		
Corticosteroids	0.154	0.013-1.832	0.138
Corticosteroids combined immunosuppressants	0.136	0.021-0.875	0.036
Triple therapy§	0.116	0.008-1.603	0.108

*take non-smokers as a reference; +take cluster 1 as a reference; ‡take others as a reference;
§means corticosteroids, immunosuppressants combined antifibrotic agents.
Abbreviations: IP, interstitial pneumonia; OR, odds ratio; CI, confidence interval.

clusters, multivariable Logistic regression analysis showed that corticosteroids combined immunosuppressants predicted good response of the treatment (OR 4.303, 95% CI 1.132–16.361, *P*=0.032) (Table 3) and were protective for IP progression (OR 0.136, 95% CI 0.021–0.875, *P*=0.036) (Table 4).

Survival

The outcome and median survival time of the four clusters were shown in online supplementary e-Table 7. A total of 71 patients developed IP progression. The Kaplan–Meier curves showed that the prognosis of patients in cluster 4 was the worst (χ^2=15.874, log rank *P*=0.001) (shown in Figure 2). The median survival time of cluster 4 was also the shortest (median 29.0m, *P*=0.001). After adjusting for age, sex, smoking status and treatment regimens, a multivariable Cox proportional hazards model indicated that patients in cluster 3 (HR 2.885, 95% CI 1.116–7.453, *P*=0.029) and cluster 4 (HR 5.472, 95% CI 2.073–14.442, *P*=0.001) were prone to IP progression (Table 5), which was in line with the Kaplan–Meier curves.

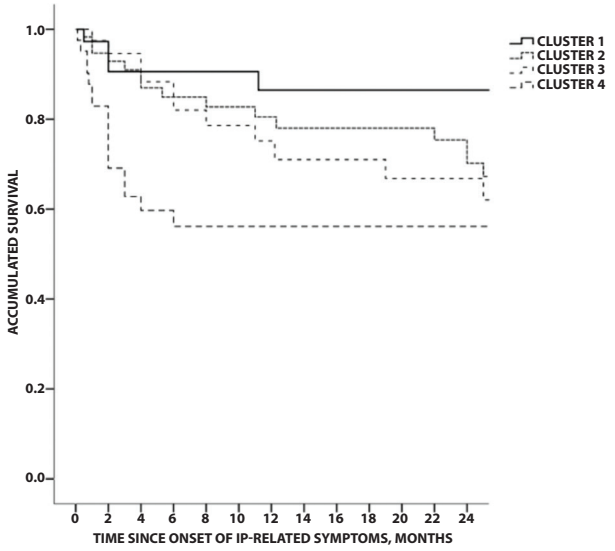

Figure 2. Kaplan–Meier curves of patients with MSA-IP in four (cluster 1, solid line; cluster 2, dotted line; cluster 3, short dashed line; cluster 4, long dashed line). Survival time was calculated from the onset of IP-related symptoms to the outcome or end of the follow-up. Median survival time of all patients was 48.1 months. Median survival time of cluster 4 was 29.0 months, which was the shortest. The prognosis of cluster 4 patients was the worst among all other clusters (χ^2=15.874, log rank *P*=0.001).clusters

Discussion

To the best of our knowledge, the current study is the first report to use cluster analysis to classify patients with MSA-IP into four distinct clusters. Patients in cluster 1 were mainly the elderly without dyspnea, with chronic onset, nearly normal PaO_2/FiO_2 and good survival. Patients in cluster 2 all had dyspnea, and mostly presented NSIP and moderate survival. Patients in cluster 3 mainly had positive anti-PL-7 antibodies, UIP and chronic onset, and were prone to IP progression. Patients in cluster 4 mostly had multi-system involvements, positive anti-MDA5 antibodies, OP and

Table 5. Multivariable Cox proportional hazards model for IP progression

Variables	HR	95% CI	*P* value
Age	1.013	0.990-1.038	0.271
Female	0.732	0.337-1.590	0.430
Smoking status			0.523
Non-smokers*	ref.		
Current smokers	0.818	0.281-2.386	0.713
Ex-smokers	0.587	0.228-1.507	0.268
Clusters			0.004
Cluster 1+	ref.		
Cluster 2	2.163	0.891-5.251	0.088
Cluster 3	2.885	1.116-7.453	0.029
Cluster 4	5.472	2.073-14.442	0.001
Treatment regimens			0.505
Others‡	ref.		
Corticosteroids	1.456	0.602-3.520	0.404
Corticosteroids combined immunosuppressants	1.006	0.444-2.280	0.989
Triple therapy§	0.775	0.267-2.137	0.597

*take non-smokers as a reference; +take cluster 1 as a reference; ‡take others as a reference; §means corticosteroids, immunosuppressants combined antifibrotic agents.
Abbreviations: IP, interstitial pneumonia; HR, hazard ratio; CI, confidence interval.

diffuse GGO, and were prone to acute/subacute onset and IP progression with poor survival. Corticosteroids combined immunosuppressants showed therapeutic response in patients with MSA-IP, and had a protective effect for IP progression.

Previous studies have indicated that the evaluation of MSAs was valuable for the recognition and management of patients with MSA-IP, even more important than the diagnosis of IIMs (6,29,30). However, when the patients with MSA-IP are classified only by MSAs, the clinical features, therapeutic regimens and survival are still unclear. A cohort study indicated that the overall survival rate of patients with IP and anti-ARS antibodies was higher than that of patients with idiopathic pulmonary fibrosis (IPF), and was similar regardless of whether IIM was diagnosed, but the clinical course among patients with different individual anti-ARS antibodies were unknown (19). According to EULAR/ACR criteria, some of the patients with MSA-IP could be diagnosed with IIMs or major subgroups, such as polymyositis, dermatomyositis, or ADM (24). And according to the ERS/ATS statement, some of the patients with MSA-IP who did not meet the criteria of IIMs can be diagnosed with IPAF (2). The uncertain regimens to MSA-IP patients with various diagnoses may lead to different prognosis (20). Compared with MSA-IP, the diagnosis of IPAF was more heterogeneous and may lead to the delayed clinical interventions (20). Thus, we used cluster analysis to classify patients with MSA-IP into four distinct clusters, based on clinical features, autoantibodies and HRCT patterns. The results may assist clinicians in identifying the characteristics and assessing the risk of IP progression in patients with MSA-IP.

The majority of patients in cluster 1 presented NSIP and had positive anti-ARS antibodies with nearly normal PaO_2/FiO_2 and the longest median survival time, without dyspnea. Almost all patients in cluster 2 presented NSIP and had dyspnea and positive anti-ARS antibodies with moderate survival. Patients with anti-ARS antibodies often present myositis, IP, arthritis and mechanic's hands, Raynaud's phenomenon and fever, known as anti-synthetase syndrome (ASS) (31,32). The incidence of IP is higher in patients with ASS than in patients with other IIM subtypes (33). A previous study showed that NSIP was the main HRCT patterns in patients with ASS-associated ILD (72.5%), followed by OP (22.5%), but patients with various anti-ARS antibodies were not able to be distinguished by HRCT patterns of IPs (34).

The majority of patients in cluster 3 had UIP and positive anti-PL-7 antibodies with chronic onset. Patients in cluster 3 were prone to IP progression. A previous study explored the clinical, radiological and histopathological features of UIP, which had been confirmed by surgical lung biopsies (35). The results showed that various causes may lead to ILDs with UIP. The most common diseases were IPF, rheumatic ILD and chronic hypersensitivity pneumonitis (CHP). The histopathological features of these diseases were different. Spatial and temporal heterogeneity, fibroblastic foci, a peripheral lobular distribution, and microscopic honeycomb were observed in IPF; airway-centred fibrosis, NSIP-like alveolar septal fibrosis, follicular bronchiolitis, and pleural fibrosis were observed in rheumatic ILD. Finally, patchy fibrosis along the bronchovascular bundle with rare fibroblast foci, honeycomb cysts in the upper and lower lobes, extensive peribronchiolar metaplasia, and bridging fibrosis across lobules in CHP (35). A cohort study compared the prognosis of 203 patients with IPF and UIP versus 36 patients with collagen vascular disease (CVD) and UIP. The results showed that mean survival time of patients with CVD and UIP was longer than that of patients with IPF and UIP (125.5±16.0 vs 66.9±6.5, P=0.001) (36). Different histopathological features of UIP might lead to differences in prognosis to some extent. In the current study, the median survival time of the patients in cluster 3 was 41.0 months, which was even shorter than of the patients with IPF/UIP, possibly due to the IP progression. These results indicated that the patients with UIP with possible MSA-IP, such as the patients with cluster 3, should be considered for differentiation of IPF.

Most patients in cluster 4 had anti-MDA5 antibodies, diffuse GGOs, low PaO_2/FiO_2 and multi-system symptoms, including dyspnea, cough, skin rash, arthralgia, morning stiffness and xerostomia. Acute/subacute onset, susceptibility to IP progression and the poor survival were characteristics of patients in cluster 4. Among dermatomyositis patients in the U.S. and Japan, 13.1% to 37.3% were positive for anti-MDA5 antibodies (37;38). Anti-MDA5 antibodies

were found to be associated with progressive ILD and poor survival with a mortality rate as high as 71.4% (37). The results of our study were consistent with previous studies. These data indicated that when the patients present acute and progressive dyspnea, new diffuse pulmonary infiltrates on HRCT and poor oxygenation, MSA, especially anti-MDA5 antibodies should be checked in patients to increase diagnostic sensitivity (20). Furthermore, clinicians should administer appropriate and timely treatment to improve the survival of patients with progressive IP.

Progressive fibrosing ILDs (PF-ILDs) refer to fibrotic ILDs that present progressive phenotypes with multiple causes and are characterized by worsening dyspnea, deterioration of lung function, limited response to immunomodulatory therapies and even death (39). The clinical, radiological and pathological features of PF-ILDs overlap with those of IPF (39). There was no evidence-based treatment for patients with PF-ILDs. The patients often receive corticosteroids combined immunosuppressants with various responses (40). Given the similarities in pathogenesis of fibrosis, the results of a clinical trial showed that Nintedanib can reduce the annual rate of decline in FVC in patients with PF-ILDs (41). It is thought that antifibrotic therapy could be beneficial in the progressive fibrosis of IP (40). The results of our study indicated that after adjusting for gender, age, smoking status and clusters, corticosteroids combined immunosuppressants was independent predictors of therapeutic response and IP progression in patients with MSA-IP.

Several limitations of this study should be considered. Firstly, selection bias might exist because the enrolled patients did not fully represent the diversity of organ involvements in MSA-IP as they were derived from a single medical center. Secondly, due to the limited patients who recieved antifibrotic drugs (20, 11.2%), the present study did not have the power to show the potential effect of the triple therapy. Thirdly, the follow-up was limited for observing IP progression.

We applied cluster analysis to MSA-IP for the first time, resulting in the categorization of four clusters. The clusters may be helpful in evaluating the prognosis and select treatment in the patients with MSA-IP when the symptoms are atypical and before the diagnosis of IIM. However, the clusters are needed to be verified. Further studies are warranted to explore the correlation of clinical characteristics with underlying genetic mechanisms of corresponding MSA subtypes.

Abbreviations: ACR, American College of Rheumatology; ADM, amyopathic dermatomyositis; anti-ARS, anti-aminoacyl-tRNA-synthetase antibodies; ASS, anti-synthetase syndrome; ATS, American Thoracic Society; CHP, chronic hypersensitivity pneumonitis; CTDs, connective tissue diseases; CTD-ILD, CTD-associated ILD; CVD, collagen vascular disease; EJ, glycy1-tRNA synthetase; ERS, European Respiratory Society; EULAR, European League Against Rheumatism; FVC, forced vital capacity; GGO, ground glass opacity; HRCT, high-resolution computed tomography; IIM, idiopathic inflammatory myopathy; IIM-ILDs, IIM-associated ILDs; ILDs, Interstitial lung diseases; IP, interstitial pneumonia; IPAF, interstitial pneumonia with autoimmune features; IPF, idiopathic pulmonary fibrosis; Jo-1, histidyl-tRNA synthetase; MAA, myositis-associated autoantibody; MDA, melanoma differentiation associated gene; MSA-IP, IP and MSA; NSIP, nonspecific interstitial pneumonia; OJ, isoleucy1-tRNA synthetase; OP, organic penumonia; PF-ILDs, progressive fibrosing ILDs; PL-7, threonyl-tRNA synthetase; UIP, usual interstitial pneumonia.

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Conflicts of interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material: The data set used in the current study is available from the corresponding author upon reasonable request.

Code availability: SPSS software: version 23.0, IBM.

Authors' contributions: Y Li was responsible for completing the analysis of data and writing. Y Fan and Y Wang performed all data collection. S Yang and X Du were responsible for recruiting the patients and collecting plasma samples. Q Ye contributed as primary investigator and was responsible for designing the study, recruiting the patients and writing the manuscript. All authors have read and approved the final manuscript.

Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University (No. 2018-KE-289).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

References

1. Vij R, Strek ME. Diagnosis and treatment of connective tissue disease-associated interstitial lung disease. Chest 2013; 143(3):814-824.
2. Fischer A, Antoniou KM, Brown KK, et al. An official European Respiratory Society/American Thoracic Society research statement: Interstitial pneumonia with autoimmune features. Eur Respir J 2015; 46(4):976-987.
3. Koo SM, Uh ST. Treatment of connective tissue disease -associated interstitial lung disease: the pulmonologist's point of view. Korean J Intern Med 2017; 32(4): 600-610.
4. Fischer A, West SG, Swigris JJ, Brown KK, du Bois RM. Connective tissue disease-associated interstitial lung disease: a call for clarification. Chest 2010; 138(2):251-256.
5. Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975; 292(8):403-407.
6. Cavagna L, Trallero-Araguβs E, Meloni F, et al. Influence of antisynthetase antibodies specificities on antisynthetase syndrome clinical spectrum time course. J Clin Med 2019; 8(11):2013.
7. Morisset J, Johnson C, Rich E, Collard HR, Lee JS. Management of Myositis-Related interstitial lung disease. Chest 2016; 150(5):1118-1128.
8. Labirua A, Lundberg IE. Interstitial lung disease and idiopathic inflammatory myopathies: progress and pitfalls. Curr Opin Rheumatol 2010; 22(6):633-638.
9. Mimori T, Nakashima R, Hosono Y. Interstitial lung disease in myositis: clinical subsets, biomarkers, and treatment. Curr Rheumatol Rep 2012; 14(3):264-274.
10. Fathi M, Lundberg IE. Interstitial lung disease in polymyositis and dermatomyositis. Curr Opin Rheumatol 2005; 17(6):701-706.
11. Fathi M, Vikgren J, Boijsen M, et al. Interstitial lung disease in polymyositis and dermatomyositis: longitudinal evaluation by pulmonary function and radiology. Arthritis Rheum 2008; 59(5):677-685.
12. Marie I, Hachulla E, Cherin P, et al. Interstitial lung disease in polymyositis and dermatomyositis. Arthritis Rheum 2002; 47(6):614-622.
13. Chen IJ, Jan WY, Lin CW, et al. Interstitial lung disease in polymyositis and dermatomyositis. Clin Rheumatol 2009; 28(6):639-646.
14. Richards TJ, Eggebeen A, Gibson K, et al. Characterization and peripheral blood biomarker assessment of anti-Jo-1 antibody-positive interstitial lung disease. Arthritis Rheum 2009; 60(7):2183-2192.
15. Zanframundo G, Tripoli A, Cometi L, et al. One year in review 2020: idiopathic inflammatory myopathies. Clin Exp Rheumatol 2021;39(1):1-12.
16. Fidler L, Doubelt I, Kandel S, et al. Screening for myositis antibodies in idiopathic interstitial lung disease. Lung 2019; 197(3):277-284.
17. Ghirardello A, Zampieri S, Tarricone E, et al. Clinical implications of autoantibody screening in patients with autoimmune myositis. Autoimmunity 2006; 39(3):217-221.
18. Graham J, Bauer VI, Newton CA, et al. Myositis-specific antibodies identify a distinct interstitial pneumonia with autoimmune features phenotype. Eur Respir J 2020; Jul 16:2001205. doi: 10.1183/13993003.01205-2020. Epub ahead of print.
19. Tanizawa K, Handa T, Nakashima R, et al. The long-term outcome of interstitial lung disease with anti-aminoacyl-tRNA synthetase antibodies. Respir Med 2017; 127:57-64.
20. Scirè CA, Gonzalez-Gay MA, Selva-O'Callaghan A, Cavagna L. Clinical spectrum time course of interstitial pneumonia with autoimmune features in patients positive for antisynthetase antibodies. Respir Med 2017,132:265-266.
21. van de Velden M, D'Enza A I, Palumbo F. Cluster correspondence analysis. Psychometrika 2017; 82(1):158-185.
22. Szodoray P, Hajas A, Kardos L, et al. Distinct phenotypes in mixed connective tissue disease: Subgroups and survival. Lupus 2012; 21(13):1412-1422.
23. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013; 188(6):733-748.
24. Lundberg I E, Tjarnlund A, Bottai M, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis 2017; 76(12):1955-1964.
25. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. Eur Respir J 2005; 26(3):511-522.
26. Galiè N, Humbert M ,Vachiery JL, et al. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension: The joint task force for the diagnosis and treatment of pulmonary hypertension of the european society of cardiology (ESC) and the european respiratory society (ERS): Endorsed by: Association for european paediatric and congenital cardiology (AEPC), international society for heart and lung transplantation (ISHLT). Eur Heart J 2016; 37(1):67-119.
27. Eaden JA, Barber CM, Renshaw SA, Chaudhuri N, Bianchi SM. Real world experience of response to pirfenidone in patients with idiopathic pulmonary fibrosis: a two centre retrospective study. Sarcoidosis Vasc Diffuse Lung Dis 2020; 37(2):218-224.

28. George PM, Spagnolo P, Kreuter M, et al. Progressive fibrosing interstitial lung disease: clinical uncertainties, consensus recommendations, and research priorities. Lancet Respir Med 2020; 8(9):925-934.
29. Hervier B, Devilliers H, Stanciu R, et al. Hierarchical cluster and survival analyses of antisynthetase syndrome: phenotype and outcome are correlated with anti-tRNA synthetase antibody specificity. Autoimmun Rev 2012; 12(2):210-217.
30. Mariampillai K, Granger B, Amelin D, et al. Development of a new classification system for idiopathic inflammatory myopathies based on clinical manifestations and Myositis-Specific autoantibodies. JAMA Neurol 2018; 75(12):1528-1537.
31. Hamaguchi Y, Fujimoto M, Matsushita T, et al. Common and distinct clinical features in adult patients with anti -aminoacyl-tRNA synthetase antibodies: Heterogeneity within the syndrome. Plos One 2013; 8(4):e60442.
32. Watanabe K, Handa T, Tanizawa K, et al. Detection of antisynthetase syndrome in patients with idiopathic interstitial pneumonias. Respir Med 2011; 105(8):1238-1247.
33. Andersson H, Aal kken TM, Günther A, et al. Pulmonary involvement in the antisynthetase syndrome: a comparative cross-sectional study. J Rheumatol 2016; 43(6):1107-1113.
34. Bartoloni E, Gonzalez-Gay MA, Scirè C, et al. Clinical follow-up predictors of disease pattern change in anti-Jo1 positive anti-synthetase syndrome: results from a multicenter, international and retrospective study. Autoimmun Rev 2017;16(3):253-257.
35. Smith M, Dalurzo M, Panse P, Parish J, Leslie K. Usual interstitial pneumonia-pattern fibrosis in surgical lung biopsies. Clinical, radiological and histopathological clues to aetiology. J Clin Pathol 2013; 66(10):896-903.
36. Park J H, Kim DS, Park IN, et al. Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes. Am J Respir Crit Care Med 2007; 175(7):705-711.
37. Moghadam-Kia S, Oddis CV, Sato S, Kuwana M, Aggarwal R. Anti-Melanoma Differentiation-Associated gene 5 is associated with rapidly progressive lung disease and poor survival in US patients with amyopathic and myopathic dermatomyositis. Arthritis Care Res (Hoboken) 2016; 68(5):689-694.
38. Motegi SI, Sekiguchi A,Toki S, et al. Clinical features and poor prognostic factors of anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis with rapid progressive interstitial lung disease. Eur J Dermatol 2019; 29(5):511-517.
39. Cottin V, Hirani NA, Hotchkin DL, et al. Presentation, diagnosis and clinical course of the spectrum of progressive-fibrosing interstitial lung diseases. Eur Respir Rev 2018; 27(150): 180076.
40. Cottin V. Treatment of progressive fibrosing interstitial lung diseases: a milestone in the management of interstitial lung diseases. Eur Respir Rev 2019; 28(153): 190109.
41. Flaherty KR, Wells AU, Cottin V, et al; INBUILD Trial Investigators. Nintedanib in progressive fibrosing interstitial lung diseases. N Engl J Med 2019;381(18):1718-1727.

Supplementary material for:

Phenotypic clusters and survival analyses in interstitial pneumonia with myositis-specific autoantibodies

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Clinical data extracted from medical records

At the first clinical visit, the patients' medical records were reviewed to uniformly extract clinical data, including demographics (age, sex, and smoking status), patient-reported information (date of IP-related symptoms onset, including cough and dyspnea), clinical characteristics, laboratory features, radiological patterns and treatment regimens. Smoking status was categorized into non-smokers, ex-smokers (quit smoking ≥12 months previously) and current smokers (currently smoking or quit smoking <12 months previously).

Serological markers and MSAs

Serological markers were obtained within one month of presentation to the clinic including C-reactive protein, erythrocyte sedimentation rate, fibrinogen, immunoglobulin (Ig) A, IgG, IgM and autoantibodies.

MSAs, including anti-ARS [anti- histidyl-tRNA synthetase (Jo-1), anti- histidyl-tRNA synthetase (PL-7), anti- threonyl-tRNA synthetase (PL-12), anti-alany1-tRNA synthetase (OJ), and anti-isoleucy1-tRNA synthetase (EJ)], anti-signal recognition particle (SRP), anti-nucleosomes reshape the deacetylase complex (Mi2) α, anti-Mi2β, anti-transcriptional intermediary factor (TIF) 1γ, anti-melanoma differentiation associated gene (MDA) 5, anti-nuclear matrix protein (NXP) 2 and anti-small ubiquitin—like modifier activating enzyme (SAE) 1 antibodies were detected by Western blotting (Yahuilong Biological Technology Company, Shenzhen, China).

HRCT patterns, pulmonary function test items and the definition of pulmonary hypertension

All enrolled patients underwent chest high-resolution computed tomography (HRCT) with a 1-s scan time, 0.625-mm sections, and 10-mm intervals from the lung apex to the base including both lungs in the field of view. Each HRCT scan was reviewed independently by two experienced thoracic radiologists blinded to the clinical data. HRCT patterns were classified as usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), organic pneumonia (OP) or diffuse ground-glass opacity (GGO) according to the classification of IIP. The interobserver correlation was good. The kappa value was 0.83.

A pulmonary function test was performed for each patient. The test items included forced vital capacity (FVC) and the diffusing capacity of the lung for carbon monoxide (DLCO) using the single-breath method.

Echocardiography was performed for all of the enrolled patients. The probability of pulmonary hypertension based on tricuspid regurgitation velocity at rest as high (＞3.4 m/s), intermediate (2.9–3.4 m/s) or low (≤2.8 m/s or not measurable), and on the presence of additional echocardiographic variables suggested pulmonary hypertension.

Full definitions of IP progression

IP progression was defined by the presence of at least one of the following (within 24 months): a relative decline in FVC% predicted of ≥10%; a relative decline in FVC% predicted of ≥5% and a relative decline in DLCO% predicted of ≥15%; a relative decline in FVC% predicted of ≥5% and increased extent of fibrosis on HRCT; a relative decline in FVC% predicted of ≥5% and worsening of respiratory symptoms; worsening of respiratory symptoms and increased extent of IP on HRCT.

Steps of TwoStep Cluster algorithm

With the TwoStep Cluster algorithm, the clustering criterion was the Bayesian Information Criterion, the distance measurement form was logarithmic likelihood, the number of clusters was automatically determined by the algorithm, and the maximum value was set as 15 clusters. The variables included in the cluster analysis were all categorical variables related to the patients' clinical characteristics, myositis autoantibodies and imaging findings. The variables included dyspnea, proximal muscle weakness, MSA subtypes (anti-Jo-1, anti-PL-7, anti-PL-12, anti-OJ, anti-EJ, anti-SRP, anti-Mi2α, anti-Mi2β, anti-TIF1γ, anti-MDA5, anti-NXP2, and anti-SAE) and HRCT patterns (UIP, NSIP, OP, diffuse GGO, unclassifiable patterns). These variables were available for all participants.

e-Table 1 Respiratory characteristics of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	X^2	P^* value
N	178	38	58	41	41		
Fever, n (%)	55(30.9)	6 (15.8)	19 (32.8)	12 (29.3)	18 (43.9)	7.455	0.059
Cough, n (%)	107 (60.1)	18 (47.4)	32 (55.2)	30 (73.2)	27 (65.9)	6.644	0.084
Dyspnea, n (%)	135 (75.8)	0	58 (100)	36 (87.8)	41 (100)	154.038	<0.001
Pulmonary hypertension, n (%)	9 (5.1)	2 (5.3)	2 (3.4)	3 (7.3)	2 (4.9)	0.742	0.863
PaO_2 / FiO_2, mmHg (room air, at rest)	354.9 (316.5, 433.7)	397.1 (356.3, 440.3)	378.4 (323.0, 435.1)	338.5 (320.3, 347.3)	302.7 (172.4, 342.4)	5.963	0.113
CPI	38.9 ± 16.6	33.7 ± 19.8	43.1 ± 13.9	40.8 ± 17.7	35.2 ± 13.0	2.501	0.063

Values were given as n (%), median (interquartile range) or mean (standard deviation).

* The *P* value represents comparison among four clusters.

Abbreviations: CPI, composite physiologic index.

e-Table 2 Multisystem involvements of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	X^2	P^* value
N	178	38	58	41	41		
Proximal muscle weakness, n (%)	11 (6.2)	4 (10.5)	5 (8.6)	1 (2.4)	1 (2.4)	4.106	0.282
Dysphagia, n (%)	12 (6.7)	1 (2.6)	8 (13.8)	1 (2.4)	2 (4.9)	6.726	0.081
Skin rash, n (%)	33 (18.5)	8 (21.1)	9 (15.5)	3 (7.3)	13 (31.7)	8.636	0.035
Gottron, n (%)	11 (6.2)	2 (5.3)	7 (12.1)	1 (2.4)	1 (2.4)	5.353	0.148
Mechanic hands, n (%)	6 (3.4)	1 (2.6)	2 (3.4)	2 (4.9)	1 (2.4)	0.443	0.931
Photaesthesia, n (%)	10 (5.6)	2 (5.3)	5 (8.6)	1 (2.4)	2 (4.9)	1.890	0.596
Sclerodactyly, n (%)	4 (2.2)	1 (2.6)	2 (3.4)	0	1 (2.4)	2.223	0.527
Arthralgia, n (%)	34 (19.1)	6 (15.8)	11 (19.0)	6 (14.6)	11 (26.8)	2.384	0.497
Joint swelling, n (%)	14 (7.9)	5 (13.2)	`5 (8.6)	2 (4.9)	2 (4.9)	2.441	0.486
Morning stiffness, n (%)	25 (14.0)	6 (15.8)	10 (17.2)	1 (2.4)	8 (19.5)	6.176	0.103
Raynard phenomenon, n (%)	6 (3.4)	3 (7.9)	1 (1.7)	0	2 (4.9)	5.399	0.145
Fingertip vasculitis, n (%)	6 (3.4)	2 (5.3)	4 (6.9)	0	0	7.694	0.053
Xerophthalmia, n (%)	26 (14.6)	3 (7.9)	12 (20.7)	6 (14.6)	5 (12.2)	3.284	0.350
Xerostomia, n (%)	49 (27.5)	8 (21.1)	19 (32.8)	10 (24.4)	12 (29.3)	1.859	0.602
Rampant teeth, n (%)	25 (14)	5 (13.2)	10 (17.2)	4 (9.8)	6 (14.6)	1.152	0.765
Alopecia, n (%)	11 (6.2)	1 (2.6)	5 (8.6)	1 (2.4)	4 (9.8)	3.619	0.306
Oral ulcer, n (%)	6 (3.4)	0	2 (3.4%)	0	4 (9.8)	8.862	0.031
Gastroesophageal reflux, n (%)	12 (6.7)	2 (5.3)	4 (6.9)	4 (9.8)	2 (4.9)	0.919	0.821

Values were given as n (%).

* The *P* value represents comparison among four clusters.

e-Table 3 MSA subtypes of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	T/U/χ^2	P^* value
N	178	38	58	41	41		
Anti-ARS, n (%)	119 (66.9)	24 (63.2)	41 (70.7)	32 (78)	22 (53.7)	6.160	0.104
Anti-non-ARS MSA, n (%)	59 (33.1)	14 (36.8)	17 (29.3)	9 (22.0)	19 (46.3)	6.160	0.104
Anti-Jo-1, n (%)	38 (21.3)	10 (26.3)	11 (19.0)	7 (17.1)	10 (24.4)	1.427	0.699
Anti-PL-7, n (%)	29 (16.3)	8 (21.1)	11 (19.0)	10 (24.4)	0	10.887	0.012
Anti-PL-12, n (%)	15 (8.4)	4 (10.5)	3 (5.2)	6 (14.6)	2 (4.9)	3.604	0.307
Anti-OJ, n (%)	8 (4.5)	0	3 (5.2)	5 (12.2)	0	11.254	0.010
Anti-EJ, n (%)	30 (16.9)	2 (5.3)	14 (24.1)	4 (9.8)	10 (24.4)	9.920	0.019
Anti-SRP, n (%)	14 (7.9)	6 (15.8)	3 (5.2)	3 (7.3)	2 (4.9)	3.857	0.277
Anti-Mi-2α, n (%)	4 (2.2)	1 (2.6)	2 (3.4)	1 (2.4)	0	2.223	0.527
Anti-Mi-2β, n (%)	16 (9.0)	3 (7.9)	3 (5.2)	6 (14.6)	4 (9.8)	2.223	0.527
Anti-TIF1γ, n (%)	12 (6.7)	0	6 (10.3)	4 (9.8)	2 (4.9)	7.119	0.068
Anti-MDA5, n (%)	25 (14.0)	3 (7.9)	8 (13.8)	0	14 (34.1)	21.616	<0.001
Anti-NXP2, n (%)	8 (4.5)	4 (10.5)	3 (5.2)	1 (2.4)	0	6.683	0.083
Anti-SAE1, n (%)	4 (2.2)	1 (2.6)	1 (1.7)	1 (2.4)	1 (2.4)	0.116	0.990

Values were given as n (%).

* The *P* value represents comparison among four clusters.

Abbreviations: MSA, myositis specific antibodies; ARS, aminoacyl-tRNA synthetase; Jo-1, histidyl-tRNA synthetase; PL-7, threonyl-tRNA synthetase; PL-12, alany1-tRNA synthetase; OJ, isoleucy1-tRNA synthetase; EJ, glycy1-tRNA synthetase; SRP, signal recognition particle; Mi-2, nucleosomes reshape the deacetylase complex; TIF1, anscripltional intermediary factor-1; MDA, melanoma differentiation associated gene; NXP, nuclear matrix protein; SAE, small ubiquitin—like modifier activating enzyme.

e-Table 4 Laboratory features of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	T/U/X^2	P^* value
N	178	38	58	41	41		
Elevated CRP, n (%) (n=136)	62 (45.6)	10 (38.5)	27 (58.7)	13 (39.4)	12 (38.7)	4.820	0.185
ESR, mm/h (n=126)	16.0 (9.0, 28.0)	13.0 (4.5, 21.0)	18.5 (11.0, 30.8)	16.5 (8.8, 29.0)	18.0 (8.0, 28.0)	4.353	0.226
Fibrinogen, mg/dl (n=128)	324.0 (258.1, 426.2)	317.0 (257.4, 393.5)	341.1 (242.8, 458.6)	337.8 (279.1, 429.0)	293.4 (250.1, 424.3)	1.783	0.619
Elevated IgG, n (%) (n=149)	43 (28.9)	7 (24.1)	18 (36.0)	13 (34.2)	5 (15.6)	5.106	0.164
IgA, mg/dl (n=139)	277.0 (193.0, 341.0)	264.0 (189.0, 318.0)	284.0 (228.0, 364.0)	296.0 (236.0, 380.0)	192.5 (168.3, 293.0)	10.519	0.015
IgM, mg/dl (n=139)	104.0 (70.9, 159.0)	78.2 (65.5,107.0)	143.0 (73.3, 191.0)	111.0 (62.4, 136.0)	104.9 (83.9, 172.0)	10.390	0.016
Positive ANA, n (%) (n=157)	57 (36.5)	13 (41.9)	17 (31.5)	18 (46.2)	9 (27.3)	3.768	0.288
Elevated pANCA, n (%) (n=115)	4 (2.9)	2 (7.7)	1 (2.1)	0	1 (3.3)	3.661	0.300
Elevated cANCA, n (%) (n=115)	3 (2.2)	0	1 (2.2)	1 (2.8)	1 (3.3)	1.363	0.714
RF positive, n (%) (n=110)	14 (12.2)	2 (8.3)	5 (13.5)	3 (9.4)	4 (18.2)	1.337	0.720
Elevated CCP, n (%) (n=117)	10 (8.5)	1 (4.3)	5 (13.9)	1 (3.3)	3 (10.7)	3.236	0.357

Values are given as n (%) or median (interquartile range).

* The *P* value represents comparison among four clusters.

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; ANA, antinuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; RF, rheumatoid factor; CCP, cyclic citrullinated peptide.

e-Table 5 HRCT patterns of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	X^2	P^* value
N	178	38	58	41	41		
UIP, n (%)	26 (14.6)	8 (21.1)	0	17 (41.5)	1 (2.4)	39.762	<0.001
NSIP, n (%)	71 (39.9)	16 (42.1)	55 (94.8)	0	0	127.503	<0.001
OP, n (%)	38 (21.3)	7 (18.4)	0	3 (7.3)	28 (68.3)	74.556	<0.001
Diffusing GGO, n (%)	11 (6.2)	1 (2.6)	0	1 (2.4)	9 (22.0)	20.744	<0.001
Unclassifiable IP, n (%)	32 (18.0)	6 (15.8)	3 (5.2)	20 (48.8)	3 (7.3)	14.612	0.004

Values were given as n (%).

* The *P* value represents comparison among four clusters.

Abbreviations: HRCT, high resolution computed tomography; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; OP, organic pneumonia; GGO, ground glass opacity.

e-Table 6 Treatment regimens of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	X^2	P^* value
N	178	38	58	41	41		
Corticosteroids, n (%)	35 (19.7)	4 (10.5)	8 (13.8)	12 (29.3)	11 (26.8)	7.007	0.072
Corticosteroids combined immunosuppressants, n (%)	90 (50.6)	15 (39.5)	33 (56.9)	21 (51.2)	21 (51.2)	2.814	0.419
Triple therapy[#], n (%)	20 (11.2)	5 (13.2)	9 (15.5)	2 (4.9)	4 (9.8)	3.253	0.354
Others, n (%)	33 (18.5)	14 (36.8)	8 (13.8)	6 (14.6)	5 (12.2)	5.042	0.145
Responds to treatment regimens, n (%)	161 (90.4)	34 (89.5)	50 (86.2)	38 (92.7)	39 (95.1)	2.615	0.455

Values were given as n (%).

[*] The *P* value represents comparison among four clusters.

[#] Triple therapy means corticosteroids, immunosuppressants combined antifibrotic agents.

e-Table 7 IP progression and survival time of the four clusters

	All	Cluster 1	Cluster 2	Cluster 3	Cluster 4	X^2	P^* value
N	178	38	58	41	41		
IP progression, n (%)	71 (39.9)	8 (21.1)	26 (44.8)	19 (46.3)	18 (43.9)	7.200	0.066
Median survival time, months	48.1	360.0	60.0	41.0	29.0	15.874	0.001
SE	10.381	0	14.612	6.866	24.779		
95% CI	27.754-68.446	NA	31.361-88.639	27.542-54.458	0-77.567		

Values were given as n (%).

* The *P* value represents comparison among four clusters.

Abbreviations: IP, interstitial pneumonia; SE, standard error; CI, confidence interval. NA, not available.