Clinical and Pathological Findings of Interstitial Lung Disease Patients with Anti-Aminoacyl-tRNA Synthetase Autoantibodies

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Abstract

Objective The aim of this study was to investigate the clinicopathological characteristics of interstitial lung disease (ILD) patients with anti-aminoacyl-tRNA synthetase (anti-ARS) autoantibodies.

Patients and Methods We examined 14 ILD patients with anti-ARS autoantibodies between 2004 and 2007 and retrospectively investigated their clinical, radiographic, and pathological findings.

Results Anti-Jo-1 antibodies were the most common (10 of 14), followed by anti-OJ, anti-KS, and anti-EJ (1 each for 3 patients); 1 patient with polymyositis had both anti-Jo-1 and anti-PL-12 antibodies. Ten patients had a chronic clinical course, whereas 4 presented with subacute deterioration. Of 8 patients with myositis, 1 (12.5%) had myositis-preceding ILD, 3 (37.5%) had ILD-preceding myositis, and 4 (50%) had simultaneous onset. Chest high-resolution computed tomography frequently showed lung-base predominant ground glass opacities (GGO) with volume loss. The results of surgical lung biopsies indicated that 4 patients had nonspecific interstitial pneumonia (NSIP) and/or organizing pneumonia (OP) patterns. All but 1 received corticosteroid therapy, and 6 patients were also given cyclosporin. The mean duration of follow-up was 22 months (range, 5-47 months). ILD improved in 9 patients and stabilized in 3; however, in 1 patient, it initially improved during 6 months, then progressively worsened despite treatment, and finally resulted in death.

Conclusion These results indicate that ILD patients with anti-ARS antibodies usually have a chronic clinical course, lung-base predominant GGO with volume loss, NSIP and/or OP patterns, and a good response to corticosteroid treatment; however, some have a rapidly worsening course and recurrence, despite therapy.

Key words: anti-aminoacyl-tRNA synthetase autoantibodies, interstitial lung disease, myositis

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Introduction

Aminoacyl-tRNA synthetase (ARS) is a family of cytoplasmic enzymes that catalyze formation of aminoacyl-tRNA from a specific amino acid and its cognate tRNA and play a crucial role in protein synthesis. Eight anti-ARS autoantibodies have been identified until date. These include anti-histidyl (anti-Jo-1), anti-threonyl (anti-PL-7), anti-alanyl (anti-PL-12), anti-isoleucyl (anti-OJ), anti-glycyl (anti-EJ), anti-asparaginyl (anti-KS), anti-phenylalanyl (anti-Zo), and anti-tyrosyl-tRNA synthetases (1-3). Anti-ARS autoantibodies are associated with myositis, arthritis, and interstitial lung disease (ILD), clinically referred to as “anti-synthetase syndrome” (2).

There have been few reports that showed the clinical, radiographic, and pathological findings of ILD patients with anti-ARS autoantibodies. The aim of this study was to investigate the clinicopathological characteristics and the response to treatments in 14 ILD patients with anti-ARS...
autoantibodies. These results indicate that they generally have a chronic clinical course, lung-base predominant ground glass opacities (GGO) with volume loss, nonspecific interstitial pneumonia (NSIP) patterns, and a good response to corticosteroid treatment.

### Patients and Methods

**Patients**

We evaluated 14 clinical diagnosed ILD patients with anti-ARS autoantibodies who were admitted to Kagoshima University Hospital between 2004 and 2007; we retrospectively investigated their clinical, radiographic, and pathological findings. Anti-Jo-1 antibody was measured by double immunodiffusion, whereas other anti-synthetases were tested by immunoprecipitation. Patients with polymyositis (PM) and dermatomyositis (DM) was diagnosed according to the criteria described by Bohan and Peter (4). Bronchoalveolar lavage was undertaken with an infusion of 40 mL of sterile 0.9% saline for 3 times at the same site where a bronchoscope was wedged at the orifice of the objective subsegment of a lobe.

**Pathological diagnosis**

We performed lung biopsy under video-assisted thoracoscopic surgery in 4 of 14 patients. Tissues were fixed with formalin solution and embedded in paraffin. The cut sections were stained with hematoxylin and eosin (HE) or elastica-Masson-Goldner. Specimens were diagnosed pathologically according to histopathological patterns described in the American Thoracic Society/European Respiratory Society (ATS/ERS) international consensus classification of idiopathic interstitial pneumonias (IIP) (5). Furthermore, specimens diagnosed as NSIP were divided into 3 groups (G1-3) according to Katzenstein’s classification (6).

**Response to treatment**

Response to ILD treatment was determined according to the criteria of the response to idiopathic pulmonary fibrosis treatment in the international consensus statement of the ATS and ERS (7).

### Results

**Clinical, laboratory, and radiographic findings**

The 14 ILD patients comprised 7 men and 7 women, and their mean age was 58.4 years (range, 41-78 years; Table 1). Seven were nonsmokers, 3 current smokers, and 4 ex-smokers. All 14 ILD patients had anti-ARS autoantibodies; anti-Jo-1 antibodies were the most common (10 of 14, 71%), followed by anti-OJ, anti-EJ, and anti-KS antibodies (1 of 14 each, 7%). One patient (patient 4) had both anti-Jo-1 and anti-PL-12 antibodies. In patient 6 with ILD-preceding DM, anti-Jo-1 antibodies changed to positive during follow-up. In patient 10 with DM-preceding ILD, anti-Jo-1 changed to positive with the onset of ILD. Regarding PM/DM patients, 7 of 8 had anti-Jo-1, and for patients without PM/DM, 4 of 6 had anti-Jo-1.

Eight patients had coexistent myositis, including PM in 4 and DM in 4. With regard to the onset of obvious symptoms of myositis or ILD, 1 (12.5%) of 8 patients had myositis-preceding ILD, 3 (37.5%) had ILD-preceding myositis, and 4 (50%) had simultaneous onset of both conditions. Three patients were complicated with other collagen vascular diseases (CVD), including mixed connective tissue disease (MCTD), PM-rheumatoid arthritis (RA)-systemic sclerosis (SSc) overlap, and secondary Sjögren’s syndrome (SJS) with PM. One patient had a postoperative ovarian low malignant potential tumor, and another had squamous cell lung carcinoma.

Ten (71%) patients had a chronic clinical course, whereas 4 (29%) presented with subacute deterioration. Cough and arthritis (4 of 14 each) were most frequent as initial symptoms, followed by muscle weakness (2 of 14). Three asymptomatic patients were noted with an abnormal shadow in the chest. In the total clinical course, the most frequent clinical manifestation was cough (8 of 14, 57%), followed by dyspnea, arthritis, and weight loss (7 of 14, 50% each). Muscle weakness (6 patients), fever (5 patients, including low grade in 4 and transient in 1), sclerodactyly (5 patients), morning stiffness (4 patients), Raynaud’s phenomenon (3 patients), and facial flushing (3 patients) were also observed.

Six of 8 patients with myositis had elevated serum creatine kinase levels, and all patients had myogenic patterns on electromyography. Seven underwent muscle biopsy and received a pathological diagnosis. Thirteen of the 14 patients had elevated KL-6 levels, 8 of 9 patients measured had elevated SP-A levels, and 7 of 12 patients measured had elevated SP-D levels. Four patients had a positive indirect immunofluorescence test for anti-nuclear antibody (ANA) using HEp-2 cells as the tissue substrate, and 5 patients had cytoplasmic staining on the indirect immunofluorescence test for ANA. Four patients had positive rheumatoid factor activity. Anti-Ro/SS-A, anti-agonalactosyl IgG, anti-centromere (2 patients each), anti-La/SS-B, anti-RNP, anti-Scl-70, PR3-ANCA, and anti-cyclic citrullinated peptides (1 patient each) were also observed. Based on the findings of arterial blood gas analysis, none of the patients had less than 60 Torr of PaO2. A pulmonary function test revealed that 7 patients (50%) had a restrictive pattern with reduction (<80% predicted) in vital capacity (VC), and all of them presented volume loss on chest radiographs. All 12 patients examined for carbon monoxide diffusing capacity (DLco) showed diffusion disturbance of less than 80%.

Bronchoalveolar lavage was performed in 11 patients, and 6 had an increased total cell count. There was an increase (>15%) in lymphocytes in 7, neutrophils (>5%) in 4, and eosinophils (>1%) in 5. A decreased CD4/CD8 ratio (<1) was demonstrated in 8 patients.

Chest high-resolution computed tomography (HRCT) on
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<th>Clinical Features of Patients with Anti-Synthetase Autoantibodies and Interstitial Lung Disease</th>
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*Anti-Jo-1 antibodies changed to positive during follow-up; # low grade fever.

Table 1.
admission revealed GGO with peribronchovascular interstitial thickening and traction broncho-bronchiolocystasis in 10 patients, and 5 also had consolidation (Table 2). One only had remarkable honeycombing, and 3 had mild honeycombing. Volume loss of both lower lobes was observed in 8 patients. Twelve patients presented predominance of lung base, including the middle lobes and the lingula close to the diaphragm, rather than lower lobe predominance (Figs. 1A and 1C). Furthermore, 7 of these 12 patients also presented with peribronchovascular predominance.

Pathological findings

Histopathological evaluation using surgical lung biopsy was performed in 4 patients (Table 1). Results of histopathological findings showed a predominantly organizing pneumonia (OP) pattern with a focal NSIP pattern in patient 1 (Figs. 2A and 2B) and an NSIP pattern in patients 2, 4 (Figs. 2C and 2D), and 11 (Figs. 2E and 2F). Fibrotic lesions in the lobules were comparatively predominant in the centrilobular area in 3 of 4 patients. Infiltrating inflammatory cells in the interstitium, consisting of lymphocytes and plasma cells, were found in all patients. Mild infiltration of lymphocytes in the vessel walls was shown in 1 patient and that in the pleura was shown in 2 patients.

Response to treatment

All but 1 patient received therapy with oral prednisolone (PSL), including 7 who were treated by high-dose intravenous methylprednisolone treatment, administered daily as 1 g methylprednisolone for 3 consecutive days (Table 3). In 13 patients receiving therapy, the initial PSL dose was 0.5 mg/kg in 2 and 1.0 mg/kg in 9, except for 2 patients in whom PSL was maintained because of stable mild ILD. Seven patients were also given oral immunosuppressive drugs, including oral cyclosporin (CyA) microemulsion in 6 patients. CyA was started at 3 mg/kg/day, and the dose was adjusted to target the trough level in the range of 100-200 ng/mL. In patient 11, polymyxin-B immobilized fiber-direct hemoperfusion (PMX-DHP), sivelestat, and cyclophosphamide pulse were also administered for the acute phase. The mean duration of follow-up was 22 months, and the range was 5-47 months. ILD improved in 9 patients (Figs. 1B and 1D) and stabilized in 3, whereas in 1 patient (patient 14), it initially improved in the first 6 months, but then progressively worsened despite treatment, and resulted in death 18 months after treatment.

Discussion

Among anti-ARS autoantibodies, anti-Jo-1 was the most commonly found both in our ILD patients with PM/DM (7 of 8) and without PM/DM (4 of 6). Compared with our patients without PM/DM or anti-Jo-1, in the patients with PM/DM or anti-Jo-1 no difference in clinical, radiography and pathologic findings was recognized. Anti-Jo-1 is found most frequently in 20%-30% of such patients, whereas other anti-ARS autoantibodies are less common and are found in less than 4% of patients (1). Anti-ARS autoantibodies are found in approximately 25%-35% of patients with PM/DM (8). Among our patients without PM/DM, two patients had the anti-OJ or anti-KS. Both anti-OJ (9) and anti-KS (10) have been reported to be more closely associated with ILD than with myositis. Here, one patient had both anti-Jo-1 and anti-PL-12 antibodies. It is extremely rare for a patient to have more than one anti-synthetase (8). To our knowledge, 2 patients have been reported to have both anti-Jo-1 and another anti-ARS antibody (11, 12), the same as our patient with PM and ILD.

In 2 patients of our series, anti-Jo-1 antibodies changed to positive during follow-up after the onset of both ILD and myositis. In a patient who was considered to have IIP, it has been reported that anti-Jo-1 became with positive before the onset of myositis (13). It suggests that changing to positive for anti-ARS autoantibodies and the onset of ILD or myositis may vary in anti-synthetase syndrome. Even if anti-ARS antibodies are initially negative in patients with ILD or myositis, it is useful to reexamine the autoantibodies during follow-up.

With regard to the onset time of myositis and ILD in anti-synthetase syndrome, myositis-preceding type constitutes 7%-33%, ILD-preceding type is 29%-50%, and the simultaneous type is 38%-60% (12, 14, 15). Our patients with myositis and ILD had a similar composition. This finding suggests that the detection of anti-ARS autoantibodies in patients with either myositis or ILD is useful for predicting the coincidence of another condition.

Here, 3 of 14 patients (21%) had a complication of a CVD other than PM/DM, such as MCTD, PM-RA-SSc overlap, and secondary SjS. It was reported that 18%-25% of patients with anti-ARS autoantibodies presented with overlap syndrome of PM/DM and other CVD (16, 17). They included RA, lupus, and SSc, as well as sicca syndrome in 59% of patients with anti-synthetase syndrome (18). In our study, 2 (14%) of the 14 patients had anti-Ro/S- AA antibodies. In patients with anti-ARS autoantibodies, 24%-25% had anti-Ro/S- AA antibodies (15, 18, 19), 10% had anti-La/SS-B antibodies (18, 19), and 10% had anti-RNP antibodies (19). Moreover, anti-Ro/S- AA antibodies were detected most frequently in PM/DM patients with anti-ARS autoantibodies (20).

In our study, 1 patient had a medical history of a postoperative ovarian low malignant potential tumor, and 1 patient had squamous cell lung carcinoma as a complication. Patients with anti-synthetase syndrome do not often have malignancies (18, 19); however, they may present with cancer-associated myositis, and the coincidence of malignancies requires attention.

Most patients with anti-ARS autoantibodies have chronic and mild ILD, but some patients presenting with acute ILD suffer severe, rapidly progressive courses (21, 22). None of our patients had severe ILD with less than 60 Torr of PaO₂, and most patients had a chronic clinical course. However,
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Table 2. Findings of Chest High-Resolution Computed Tomography
Figure 1. Chest high-resolution computed tomography (HRCT) findings of patient 1 (A, B) and 11 (C, D). A: Chest HRCT findings of patient 1 on admission shows ground glass opacities (GGO) and consolidation with volume loss that presented in the lung base and was peribronchovascular predominant. B: In patient 1, GGO and volume loss improved 2 months after starting therapy. C: Chest HRCT findings of patient 11 on admission shows GGO and consolidations with volume loss presented in the lower lobe and peribronchovascular predominant. D: In patient 11, GGO and volume loss improved 3.5 months after starting therapy.

some patients (29%) presented with subacute deterioration, thus suggesting that attention should be paid to acute deterioration during follow-up. Most ILD patients with anti-ARS autoantibodies present with either an abnormal chest radiograph without symptoms or slowly progressive dyspnea (22). In our 14 patients, 3 asymptomatic patients had abnormal shadows in the chest, thus indicating that there are many asymptomatic ILD patients with anti-ARS autoantibodies. Most of the present patients had elevated levels of KL-6, SP-A, and SP-D. A similar finding was also reported in previous studies (23, 24).

All of our 12 patients examined for diffusing capacity showed reduced DLco, whereas only 7 (50%) of 14 had reduction in VC. The decrease in DLco and the development of a restrictive pattern are reported as the earliest abnormality in patients with anti-synthetase syndrome (25). Marguerie et al reported that in 17 patients with anti-ARS autoantibodies, 12 (71%) had reduction (<80% predicted) in VC and 16 (94%) had reduced DLco (<80% predicted) (18). ILD patients with anti-Jo-1 antibodies have predominant CD8 lymphocytosis in bronchoalveolar lavage fluids (26). Similarly, we frequently found increased percentages of lymphocytes and decreased CD4/CD8 ratios in bronchoalveolar lavage fluid.

Chest HRCT of ILD patients with PM/DM revealed frequent reticular opacities, GGO, and consolidation; however, honeycombing is rarely found (27). It was reported that in ILD patients with anti-Jo-1, the predominantly inferior location was frequent, whereas honeycombing was rare (26). In our study, lung-base predominant GGO with or without con-
Figure 2. Histopathological findings of patient 1 (A, B), 4 (C, D) and 11 (E, F). A: Frequent bud-type intraalveolar fibrosis in alveolar spaces, and the alveolar structure is minimally remodeled. This specimen was diagnosed as the organizing pneumonia (OP) pattern [Hematoxylin and Eosin staining, ×40]. B: Alveolar walls are partially fused and associated with obliterative-type intraalveolar fibrosis. Diagnosis was the nonspecific interstitial pneumonia (NSIP) pattern, group 2 (Elastica-Masson-Goldner stain, ×100). C: The alveolar structure is slightly remodeled in the left segment 5 and diagnosed as the NSIP pattern, group 2. Mural incorporation-type intraalveolar fibrosis is shown and the distribution is comparatively predominant in the centrolobular area (Hematoxylin and Eosin staining, ×40). D: The alveolar structure is remodeled in the left segment 8 and diagnosed as the NSIP pattern, group 3. Fibrotic lesions have mural incorporation- and obliterative-type intraalveolar fibrosis (Hematoxylin and Eosin staining, ×40). E: The alveolar structure is moderately remodeled in the left segment 8, and fibrotic lesions with mural incorporation-type intraalveolar fibrosis are diffusely distributed (Hematoxylin and Eosin staining, ×200). F: Moderate infiltration of lymphocytes is shown in the interstitium, forming an incomplete lymph follicle (Hematoxylin and Eosin staining, ×200).

Solidation was frequently identified (Figs. 1A and 1C). Furthermore, peribronchovascular predominance and volume loss were frequently present, and remarkable honeycombing was rarely observed.

In the present study 4 patients underwent surgical lung biopsy and showed NSIP and OP patterns, which could explain the good response to treatment. Histopathological reports have indicated that in ILD patients with anti-Jo-1 antibodies, the usual interstitial pneumonia (UIP) pattern is the most common, but NSIP and OP patterns are also observed (22). Another study reported that ILD patients with anti-ARS autoantibodies display NSIP or OP patterns, but not UIP or diffuse alveolar damage patterns (21).

Here, 13 of the patients received therapy with corticoster-
oids, including 6 who were also given CyA. ILD was improved or stabilized in all but 1, thus suggesting a good response to treatment. Treatment with corticosteroids and CyA is reported to be effective for ILD patients with anti-ARS autoantibodies (28), but the disease frequently recurs (12, 19). Therefore, attention should be paid to corticosteroid tapering, and corticosteroid treatment combined with CyA may be more useful for patients with deteriorated ILD. It was suggested that the reason why our one patient worsened despite treatment and resulted in death was the pathologic condition; the aggravation of fibrosis was more notable than in the other patients and there was marked honeycombing which was not detected in the other patients.

Identifying anti-ARS autoantibodies in patients with ILD or myositis is useful for predicting the onset of symptoms, clinical course, good response to corticosteroid treatment, and prognosis. Furthermore, we must remain alert to the fact that undiscovered anti-ARS autoantibodies may be present. In conclusion, these results indicate that ILD patients with anti-ARS antibodies usually have a chronic clinical course, lung-base predominant GGO with volume loss, NSIP and/or OP patterns, and a good response to corticosteroid treatment, but some have a rapidly worsening course and recurrence, despite therapy.

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**References**