Clinical characteristics of Japanese patients with anti-PL-7 (anti-threonyl-tRNA synthetase) autoantibodies

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Abstract

Objective

The clinical and laboratory features of seven Japanese patients with anti-aminoacyl-tRNA synthetase (ARS) autoantibodies against PL-7 (anti-threonyl-tRNA synthetase) were analyzed and compared with previously published findings.

Methods

Serum samples from 1,135 Japanese patients with various autoimmune diseases were screened for anti-PL-7 antibodies using RNA and protein immunoprecipitation assays. The patients whose sera contained anti-PL-7 antibodies were assessed regarding clinical symptoms and clinical course.

Results

Sera from seven patients were found to have anti-PL-7 antibodies. These autoantibodies were associated with polymyositis/dermatomyositis (PM/DM) and/or interstitial lung disease (ILD). The clinical diagnoses of these seven patients were PM - systemic sclerosis (SSc) overlap (5 patients), DM (1 patient) and idiopathic pulmonary fibrosis (IPF) (1 patient). All patients had ILD with a chronic course and six also had arthritis (85%) and five sclerodactyly (71%).

Conclusions

These results indicate that anti-PL-7 autoantibodies are closely associated with PM-SSc overlap as well as ILD, arthritis and sclerodactyly in our series of Japanese patients.

Key words

Polymyositis/dermatomyositis (PM/DM), interstitial lung disease (ILD), anti-aminoacyl-tRNAsynthetases (ARS) antibodies.

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Introduction

The aminoacyl-tRNA synthetases are a set of cellular enzymes, each of which catalyzes the formation of aminoacyl-tRNA from a specific amino acid and its cognate tRNA. Autoantibodies to six anti-aminoacyl-tRNA synthetases (anti-ARS) have been identified in patients with inflammatory myopathies, as follows: anti-histidyl (anti-Jo-1), anti-threonyl (anti-PL-7), anti-alanyl (anti-PL-12), anti-glycyl (anti-EJ), anti-isoleucyl (anti-OJ), and anti-asparaginyl (anti-KS) tRNA synthetases (1-10). Among these anti-ARS antibodies, the most common, anti-Jo-1, are found in approximately 20-30% of polymyositis/dermatomyositis (PM/ DM) patients (8, 10-11).

Each of these anti-ARS antibodies has been reported to be associated with a similar syndrome. This syndrome is characterized by myositis with a high frequency of interstitial lung disease (ILD) (50-80%) and arthritis (50-90%), as well as an increase (compared with the overall myositis population) of Raynaud's phenomenon (60%), fever with exacerbations (80%), and the skin lesions of the fingers referred to as "mechanic's hands" (70%) (1,7). Although the similarity of clinical features in patients with different anti-ARS antibodies is striking, further observation and analysis has shown that there are certain differences in clinical symptoms associated with each of the anti-ARS antibodies.

Hirakata *et al.* examined clinical features of anti-synthetase syndromes in detail and reported that anti-Jo-1 antibodies are common in patients with myositis, but anti-PL-12 and anti-KS antibodies are found in patients with ILD without any signs of myositis (10). The latter are more likely to have ILD and/or arthritis without clinical evidence of myositis (10,12-13).

Anti-PL-7 antibodies are the first non-Jo-1 anti-ARS, found in patients with PM/DM accompanied by ILD, the frequency of which is low (2). In previous studies, this antibody was found in only 3-4% of all patients with PM/DM (2,6, 8,14). Targoff *et al*. reported that patients with anti-PL-7 antibodies had a high incidence of arthritis and ILD (15). However, the presence of anti-PL-7 antibodies and their clinical significance has not been reported in Japanese patients so far.

In the present study, we describe the clinical and laboratory features of Japanese patients with antibodies against anti-PL-7 and review previously published reports from elsewhere.

Patients, materials and methods

Patients and sera

Serum samples were obtained from 1,135 Japanese patients suspected of having connective tissue diseases seen at the current or previous collaborating centers of the authors between 1990 and 2000. These included 120 with PM/DM, 400 with systemic lupus erythematosus (SLE), 192 with systemic sclerosis (SSc), 58 with rheumatoid arthritis (RA), 101 with mixed connective tissue disease (MCTD)/overlap syndrome, 114 with idiopathic pulmonary fibrosis (IPF), and finally, 150 patients who had arthritis or erythema but did not meet the criteria for other connective tissue diseases.

PM/DM was diagnosed based on the criteria of Bohan and Peter (16). The assessment of muscle weakness was performed using a manual muscle test (17). The diagnosis of SSc was based on the criteria for the classification of SSc defined by the American College of Rheumatology in 1980 (18). ILD was considered to be present if an interstitial change was observed on both chest radiography and computed tomography (CT) or a restrictive pattern found on pulmonary function testing in patients with IPF or PM/DM.

Detection of anti-PL-7 antibodies

The immunoprecipitation (IPP) from HeLa cell extracts was performed as previously described (1, 6). For analysis of RNAs, 10 μl of patient sera was mixed with 2 mg of protein A-Sepharose CL-4B (Pharmacia Biotech AB, Uppsala, Sweden) in 500 μl of IPP buffer (10 mM Tris-HCl, pH 8.0, 500 mM NaCl, 0.1% Nonidet P-40) and incubated with end-over-end rotation (Labquake shaker; Lab Industries, Berkeley, CA) for 2 h at 4°C. The IgG-coated Sepharose was washed 4 times

in 500 µl of IPPbuffer using 10-second spins in a microfuge and was resuspended in 400 µl of NET-2 buffer (50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.05% Nonidet P-40). This suspension was incubated with 100 µl of extracts, derived from $6x10^6$ cells, on the rotator for 2 h at 4°C. The antigen-bound Sepharose beads were then collected by centrifugation for 10 s in the microfuge, washed 4 times with NET-2 buffer, and resuspended in 300 µl of NET-2 buffer. To extract bound RNAs, 30 µl of 3.0 M sodium acetate, 30 µl of 10% SDS, 2 µl of carrier yeast tRNA (10 mg/ml; Sigma, St. Louis, MO) and 300 µl of phenol/chloroform/isoamyl alcohol (50: 50: 1, containing 0.1% 8-hydroxyquinoline) were added to the Sepharose beads. After agitation in a Vortex mixer and spinning for 1 min, RNAs were recovered in the aqueous phase after ethanol precipitation and dissolved in 20 µl of electrophoresis sample buffer composed of 10 M urea, 0.025% bromophenol blue, and 0.025% xylene cyanol-FF in TBE buffer (90 mM Tris-HCl, pH 8.6, 90 mM boric acid, and 1 mM EDTA). The RNA samples were denatured at 65°C for 5 min and then resolved in 7 M urea-10% polyacrylamide gels, which were then silver-stained (Bio-Rad Laboratories, Hercules, CA).

For the protein studies, antibody-coated Sepharose beads were mixed with 400 µl of [^{35}S] methionine-labeled HeLa extracts derived from $2x10^5$ cells, and rotated at 4°C for 2 h. After four washes with IPP buffer, the Sepharose beads were resuspended in SDS - sample buffer (2% SDS, 10% glycerol, 62.5 mM Tris-HCl, pH 6.8, 0.005% bromophenol blue). After heating (90° C for 5 min), the proteins were fractionated by 10% SDS-PAGE, enhanced with 0.5 M sodium salicylate, and dried. Radiolabeled protein components were analyzed by autoradiography.

With these assays, anti-ARS, anti-signal recognition particle, anti-Mi-2, anti-SSA, anti-SSB, anti-U1-RNP, anti-Scl-70, anti-PM-Scl and anti-Ku autoantibodies are detectable in comparison with corresponding standard sera (1). We also examined anticentromere antibody by ELISA (Medical & Biological Laboratories Co., Ltd. Nagoya, Japan).

Clinical features

Clinical information was retrospectively assessed in all PM/DM patients as well as non-PM/DM patients positive for anti-PL-7 antibodies. Clinical findings included clinical symptoms, serum creatine kinase (CK) level, electromyogram (EMG), muscle biopsy, chest radiograph and chest CT. The resolution of the myositis symptoms was defined as having both improvement of muscle weakness on a manual muscle test and the normalization of serum CK level. The two groups of PM/DM patients with or without anti-PL-7 antibodies were compared. Moreover, our cases were compared with those previously reported in the literature.

Statistical analysis

All comparisons between the two patient groups were performed using the χ^2 test. Significance level was set at 5%.

Results

Identification of anti-PL-7 antibodies

Of the 1,135 sera tested, samples from seven patients were found to immunoprecipitate a characteristic identical pattern of tRNAs. Representative examples are shown in Figure 1. This pattern of tRNAs was clearly distinguishable from those precipitated by the five other described anti-synthetases and identical in mobility and appearance to anti-PL-7 standard serum (Fig. 1a). The same sera also immunoprecipitated a protein band from [^{35}S] methionine-labeled HeLa cell extracts migrating at 80 kDa. This was clearly different from those immunoprecipitated by sera reactive with the other described anti-synthetases (Fig. 1b). Thus, it is concluded that they contained anti-PL-7 antibodies.

Clinical features of patients with anti-PL-7 antibodies

Clinical features of the 7 patients with anti-PL-7 antibodies are summarized in Table I. Five patients were clinically diagnosed as having PM-SSc overlap syndrome and the other two as DM and IPF. Six (86%) had muscle weakness and arthritis. Four (57%) had Raynaud's phenomenon. It was of note that 5 patients had scleroderma: the extent of skin thickness was diffuse scleroderma in 2 (29%), proximal scleroderma in one (14%) and sclerodactyly alone in 2 (29%). Although two (29%) had mechanic's hands, sclerodactyly of these patients was clearly distinguished from mechanic's hands. All 7 patients were diagnosed as having ILD from the results of chest radiography and chest CT or pulmonary function testing. One patient had anti-SS-A antibodies and another had anticentromere antibodies.

Characteristics of myositis in patients with anti PL-7 antibodies

The characteristics of 6 patients suffering from myositis are summarized in Table II. Only one patient manifested a DM rash and was accordingly diagnosed as having DM. The maximum level of CK (IU/l) was relatively low throughout the clinical course (maximum CK was 2,830 IU/l, seen in patient #1). EMG was performed in all 6 myositis patients and all showed a myogenic pattern: low-amplitude polyphasic units of short duration and resting fibrillation, complex repetitive discharges and positive sharp waves in needle EMG. The muscle biopsy revealed atrophic fibers, active necrosis with regeneration and infiltration of lymphocytes in all 3 patients tested. The administration of prednisolone (PSL) alone without other immunosuppressant in 5 resulted in an improvement of both muscle strength and serum CK value in all. One patient had no PSL medication due to concomitant tuberculosis infection. PSLwas tapered gradually and 3 patients maintained inactive myositis by continuing on a low dose of PSL. Two patients (#1 and #3) died of cardiac failure and respiratory failure due to bacterial infection. The duration of the disease was 159 months and 44 months in these latter patients. All 7 PM/DM patients had ILD, classified as chronic course. The symptoms of ILD preceded muscle involvement in 5 patients.

Frequencies of several clinical mani-

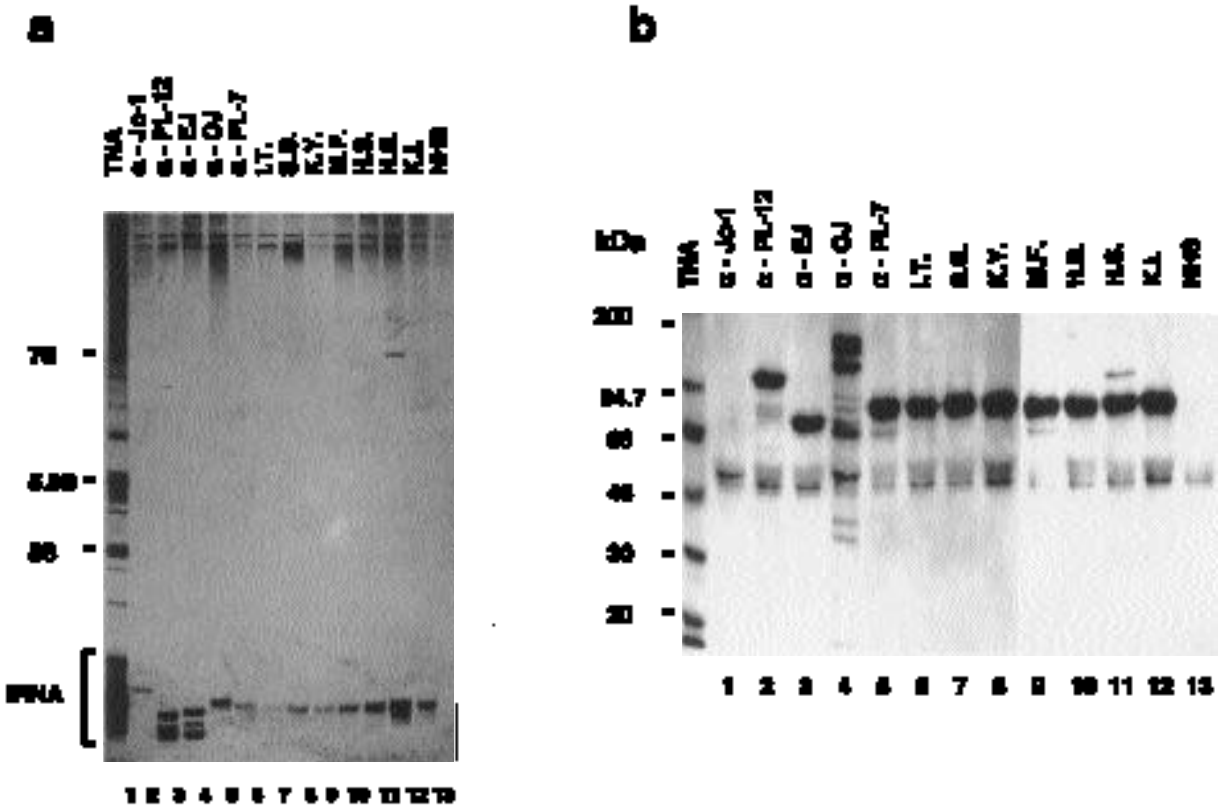

Fig. 1. (**a**) Immunoprecipitation (IPP) of nucleic acids with anti-PL-7 sera and controls. Urea (7 M) and 10% PAGE of phenol-extracted immunoprecipitates from HeLa cell extracts were developed with silver stain. TNA, total nucleic acids, with the 5.8 and 5.0 S small ribosomal RNAs and the tRNAregion indicated. Sera used for IPPinclude: lanes 1-5, the anti-synthetase sera indicated, with antibodies to Jo-1 (histidyl-tRNAsynthetase), PL-12 (alanyl-tRNAsynthetase), EJ (glycyl-tRNAsynthetase), OJ (isoleucyl-tRNAsynthetase), PL-7 (threonyl-tRNAsynthetase); lanes 6-12, anti-PL-7 sera as indicated; and lane 13, control serum (NHS, normal human serum). The tRNApattern with anti-PL-7 sera is easily distinguishable from that of the other anti-synthetases.
(**b**) IPP of proteins with anti-PL-7 sera and controls. Autoradiogram of 10% SDS-PAGE of immunoprecipitates from [^{35}S] methionine-labeled HeLa cell extracts. Mr, molecular weight markers of the sizes indicated to the left (kDa). The sera used for IPPare the same as those in (**a**). The same characteristic pattern of 80 kDa protein bands was seen with each of the seven anti-PL-7 sera. The pattern was clearly different from the bands immunoprecipitated by sera against the other anti-synthetases.

Table I. Clinical features of patients with anti-PL-7 antibodies.

Clinical findings	#1	#2	#3	#4	#5	#6	#7
Age/ gender	51/ male	59/ female	32/ female	53/ female	51/ female	64/ female	57/ female
Diagnosis	DM	PM-SSc	PM-SSc	PM-SSc	PM-SSc	IPF	PM-SSc
Fever	(-)	(-)	(+)	(+)	(+)	(+)	(-)
Arthritis	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Muscle weakness	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Raynaud's phenomenon	(-)	(+)	(+)	(+)	(-)	(-)	(+)
Extent of scleroderma	None	Proximal scleroderma	Sclerodactyly alone	Diffuse scleroderma	Diffuse scleroderma	None	Sclerodactyly alone
Digital pitting scar	(-)	(+)	(+)	(+)	(-)	(-)	(+)
Mechanic's hands	(-)	(-)	(-)	(+)	(+)	(-)	(-)
ILD	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Hypergammagloblinemia	(+)	(+)	(-)	(+)	(+)	(-)	(-)
Sjögren's syndrome	(+)	(+)	(+)	(+)	(-)	(-)	(-)
Other autoantibodies	(-)	(-)	Anti-SSA	(-)	(-)	(-)	Anti centromere

PM: polymyositis; DM: dermatomyositis; SSc: systemic sclerosis; ILD: interstitial lung disease.

Table II. Characteristics of myositis in patients with anti-PL-7 antibodies.

Clinical and laboratory findings	#1	#2	#3	#4	#5	#7
DM rash	(+)	(-)	(-)	(-)	(-)	(-)
Maximun CK level (IU/l)	2,830	748	930	1,682	1,663	1,005
EMG findings	Myogenic*	Myogenic	Myogenic	Myogenic	Myogenic	Myogenic
Muscle biopsy	Myopathy	n.d.	n.d.	n.d.	Myopathy	Myopathy
Atrophy	(+)				(+)	(+)
Necrosis with regeneration	(+)				(+)	(+)
Infiltration of lymphocytes	(+)				(+)	(+)
Initial dose of PSL(mg/ day)	60	(-)	40	40	50	30
Duration of treatment (mos.)	159	(-)	44	24	110	93
Efficacy of PSLfor myositis	(+)	n.d	(+)	(+)	(+)	(+)
Present status	Death	Alive	Death	Alive	Alive	Alive

*Low amplitude, resting fibrillation, positive sharpe wave (denervatrion potencials) were present. DM: dermatomyositis, CK: creatine kinase, EMG: electromyogram, PSL: prednisolone.

festations were compared between anti-PL-7-positive and negative PM/DM patients (Table III). The frequencies of ILD and sclerodactyly were found to be significantly higher in antibody-positive patients.

Comparison of the clinical features of patients with anti-PL-7 antibodies in the present study and those in the literature

The clinical features of patients with anti-PL-7 antibodies reported in the English-language literature were reviewed (2, 13, 15, 16, 19, 20). A summary of clinical data including our study is shown in Table IV. The frequencies of arthritis, myositis, and Raynaud's phenomenon in our series is similar to those of previously reported patients with anti-PL-7 antibodies. On the other hand, the occurrence of sclerodactyly in our series is greater compared with previous reports from North America and the United Kingdom.

Case 1 (patient #5)

This 51-year-old woman noticed dyspnea on exertion in 1995, after which symptoms progressively worsened. Her general practitioner identified an abnormal lung shadow in the chest radiogram. She was admitted to the Keio University Hospital in October 1995. She had dyspnea on exertion, and muscle weakness predominantly in the proximal muscle. She also had diffuse scleroderma and Raynaud's phenomenon. The CK level was elevated (1,663 IU/l). Myopathic changes detected by EMG mainly in proximal muscles and active necrosis with regeneration seen in a muscle biopsy specimen suggested the presence of myopathy. %VC was 59% and %DLco was 43% on lung function testing, indicating restricted respiratory impairment. A chest radiograph showed bilateral reticular shadow and infiltration. The chest CT revealed interstitial fibrosis and infiltration accompanied by air-bronchogram. A diagnosis of PM/SSc overlap syndrome was established based on proximal muscle weakness, elevated muscle enzymes, typical EMG and muscle biopsy findings and diffuse scleroderma. Treatment with 50 mg/day of PSL was started, resulting in improvement of clinical symptoms including muscle weakness, and dyspnea on exertion, and decrease in CK levels. However, dyspnea worsened again when the dose of PSL was tapered to 11 mg/day. In October 1997, she was re-admitted to our hospital and the dose of PSL was increased to 40 mg/day. %VC improved from the baseline (60%) to the level after treatment (74%). PSL was gradually tapered and she is now taking 10 mg/day of PSL. Although moderate dyspnea on exertion has persisted, she has no muscle weakness and serum CK level is within the normal range.

Case 2 (patient #7)

A 57-year-old woman developed dyspnea on exertion and had a non-productive cough in 1994. She was admitted to the Keio University Hospital in November 1994 due to worsening dyspnea. Chest radiography revealed a reticular shadow in both lower lung fields. A chest CT also showed bibasilar interstitial fibrosis. The pulmonary function test showed a decreased %VC (59%) and decreased $\%DL_{CO}$ (35%). A diagnosis of ILD was made, and PSL 40 mg/day was initiated, resulting in improvement of respiratory symptoms. The dose of PSL was tapered and discontinued in November 1995.

In August 1997, she gradually devel-

Table III. Comparison of clinical features in anti-PL-7-positive versus negative PM/DM.

Clinical and laboratory findings	Anti-PL-7(+) (n = 6)	Anti-PL-7(-) (n = 119)	Pvalue
Male / female	1/ 5	36/83	NS
Fever (%)	3 (50)	59 (50)	NS
Arthritis (%)	6 (100)	73 (61)	NS
ILD (%)	6 (100)	52 (44)	P< 0.05
Raynaud's phenomenon (%)	4 (67)	35 (29)	NS
Sclerodactyly (%)	5 (83)	17 (14)	P< 0.005

* PM/DM: polymyositis/dermatomyositis, ILD: interstitial lung disease.

Table IV. Clinical features of patients with anti-PL-7 antibodies in literature and our study.

	Previous reports from North America and the United Kingdom							Present study	Pvalue
Year/ Author	1984 Mathews	1988 Targoff	1990 Marguerie	1994 Satoh	1995 Mchugh	1999 Wasko	Total	Sato	
No.	5	4	4	1	1	1	16	7	
Male: female	1:4	2:2	1:3	0:1	0:1	1:0	5:11	1:6	n.s.
Arthritis no. (%)	3 (60%)	4 (100%)	4 (100%)	1 (100%)	0	1 (100%)	13 (81)	6 (86)	n.s.
Myositis no. (%)	4 (80%)	4 (100%)	3 (75%)	1 (100%)	0	1 (100%)	13 (81)	6 (86)	n.s.
ILD no. (%)	1 (20%)	3 (75%)	3 (75%)	0	1 (100%)	0	8 (50)	7 (100)	n.s.
RPno. (%)	2 (40%)	1 (25%)	4 (100%)	0	1 (100%)	1 (100%)	9 (56)	4 (57)	n.s.
Sclerodactyly no. (%)	0	0	2 (50%)	0	1 (100%)	0	3 (19)	5 (71)	P< 0.05

ILD: interstitial lung disease, RP: Raynaud's phenomenon

oped muscle weakness and polyarthralgia. In January 1998, the patient was re-admitted. She had sclerodactyly and digital pitting scar as well as muscle weakness and polyarthralgia. Blood tests revealed an elevated CK level (1005 IU/l). The EMG showed myopathic changes. A muscle biopsy revealed chronic inflammatory cell infiltrates in the endomysium, indicating myopathy. The diagnosis of PM-SSc overlap syndrome was made and administration of PSL 30 mg/day was reinstated. The muscle weakness and arthralgia were improved markedly and the CK level normalized in 1998.

Discussion

In the present study, we found 7 patients who had anti-PL-7 autoantibodies among 1,135 patients suspected to have CTD. With regard to clinical symptoms, the features of these patients with anti-PL-7 appeared to reside within the spectrum of the "anti-synthetase syndrome" that has been noted in other patients with anti-ARS antibodies (13). However, it should be noted that the frequency of sclerodactyly in our series was significantly higher than in our PM/DM patients without anti-PL-7 antibodies or anti-PL-7 antibody-positive patients previously reported in the English-language literature. In addition, 2 patients had diffuse scleroderma and one had proximal scleroderma. In fact, 5 of 7 (71%) patients were diagnosed as having PM-SSc overlap syndrome. Anti-PL-7 antibodies are likely to be associated with PM-SSc overlap syndrome in Japanese patients. It is thought that there could be certain racial difference in frequencies of autoantibodies. For instance, anti-PM-Scl antibodies known to be associated with PM-SSc overlap were detected in Caucasian SSc patients but not in Japanese SSc patients (21). Because the number of patients with anti-PL-7 is limited, further studies are required to confirm our hypothesis.

Refractory myositis with anti-ARS antibodies has been reported (22). However the degree of myositis of our cases was relatively mild. Treatment with corticosteroid alone resulted in the resolution of muscle weakness and the normalization of serum CK level successfully in all patients although 2 died from complications unrelated to myositis.

Arthritis and chronic ILD are characteristics of anti-ARS seropositive patients (7, 8) and these features were frequently detected in our series of patients with anti-PL-7 antibodies. It is known that certain patients with PM/DM have ILD preceding the appearance of muscle symptoms (1,8,23). Although patient #6 was diagnosed with IPF at this point, the possibility remains that muscle symptoms may arise in the future. Therefore, continuous careful follow-up observation will be necessary to monitor future muscle involvement.

In conclusion, clinical features detected in 7 Japanese patients with anti-PL-7 antibodies are essentially consistent with anti-ARS syndrome previously reported, such as high frequencies of arthritis, chronic ILD and relatively mild PM/DM for which corticosteroid therapy is effective. An additional clinical manifestation unique to anti-PL-7-positive patients is concomitant scleroderma, and anti-PL-7 are likely to be associated with PM-SSc overlap syndrome in Japanese patients. The detection of anti-PL-7 antibodies may be useful in the diagnosis and disease classification of patients with connective tissue diseases.

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