JD Original Article

Clinical manifestations of skin, lung, and muscle diseases in dermatomyositis positive for anti-aminoacyl tRNA synthetase antibodies

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Abbreviations: aldolase (ALD), anti-aminoacyl tRNA synthetase (ARS), antisynthetase syndrome (ASS), bronchoalveolar lavage (BAL), cellular non-specific interstitial pneumonia (cNSIP), clinically amyopathic dermatomyositis (CADM), creatinine phosphokinase (CK), fibrotic non-specific interstitial pneumonia (fNSIP), interstitial lung disease (ILD), melanoma differentiation-associated gene 5 (MDA5), organizing pneumonia/eosinophilic pneumonia (OP/EP), transcriptional intermediary factor (TIF), usual interstitial pneumonia (UIP)

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Key messages

Patients with anti-ARS antibody form a distinct subtype of dermatomyositis. In addition to ILD, the patients may have so-called mechanic's hands with a psoriasiform tissue reaction, and fasciitis-dominant myopathy. The patients' relatives may have collagen diseases in high incidence.

Key words: dermatomyositis, aminoacyl tRNA synthetase, mechanic's hand, interstitial lung disease, myositis/fasciitis, familial occurrence, aldolase

Abstract

Patients with dermatomyositis positive for anti-aminoacyl tRNA synthetase (ARS) antibodies, also known as anti-synthetase syndrome (ASS), frequently present with mechanic's hand and interstitial lung disease (ILD). We first screened the antibody profiles of 59 patients with dermatomyositis, and then examined the cutaneous, muscular and pulmonary manifestations characteristic for patients with ASS. The anti-ARS antibodies Jo-1, PL-7, PL-12, EJ and KS, along with antibodies to TIF1-γ, MDA5 and Mi-2 were examined. Among the 59 patients, 20, 21, 15 and 3 patients were classified into the ASS, non-ASS, myositis-specific antibody-negative, and unknown groups, respectively. Five of 16 patients (31%) with ASS had 6 relatives with a history of collagen diseases, within the second-degree of relationship, including 2 cases of dermatomyositis (vs. the non-ASS group, P=0.018). Patients with ASS more frequently presented with fever and arthralgia, and had elevated levels of C-reactive protein. Nine of the 11 finger lesions (82%) clinically-diagnosed as mechanic's hands showed a psoriasiform tissue reaction. ILD was observed in 19 of 20 patients (95%) with ASS, and 8 of 21 patients (38%) in the non-ASS group, in which 6 patients possessed anti-MDA5 antibody. Patients with ASS showed higher serum levels of muscle enzymes, and 4 of 12 patients (33%) had fasciitis-dominant myopathy, while only 1 of 11 patients (9%) in the non-ASS group had fasciitis-dominant myopathy. Patients with ASS often present with a psoriasiform tissue reaction in the hand lesions and fasciitis-dominant

myopathy, and the relatives of those with ASS are at high risk for collagen diseases.

Introduction

Recent studies have revealed that some myositis-specific autoantibodies are related to the clinical subtypes of poly- and dermatomyositis. For instance, the presence of anti-melanoma differentiation-associated gene 5 (MDA5) antibody or anti-aminoacyl-tRNA synthetase (ARS) antibodies is closely related to the occurrence of interstitial lung disease (ILD).[1,2] Similarly, positive status for the anti-transcriptional intermediary factor (TIF) 1-γ antibody in patients with adult dermatomyositis is strongly associated with internal malignancies.[3,4] Anti-MDA5 antibody is often detected in patients with clinically amyopathic dermatomyositis (CADM), a disorder characterized by a high risk of rapidly progressive, treatment-resistant ILD.[5-7] Patients with anti-Mi-2 antibody may present with typical cutaneous symptoms of dermatomyositis and a significantly higher level of serum CK (mean above 5000 IU/L).[8, 9]

So far, eight anti-ARS antibodies (Jo-1, PL-7, PL-12, EJ, OJ, KS, Zo, and Ha) have been found, and patients who are positive for an anti-ARS antibody are diagnosed with antisynthetase syndrome (ASS),[10] since they share common clinical manifestations that include ILD, mechanic’s hands, fever, polyarthritis, Raynaud's phenomenon, and various degrees of myositis.[11,12] The first aim of the present study was to examine the autoantibody profiles of patients with dermatomyositis, and compare the results with clinical manifestations and serum levels of muscular enzymes including creatinine

phosphokinase (CK) , myoglobin (Mb) and aldolase (ALD) in patients of both the ASS and non-ASS groups. The second aim was to examine differences in the ILD pattern by computed tomography (CT), and differences in myopathy by magnetic resonance imaging (MRI).

Patients and methods

The present study was performed with the approval of the IRB of Okayama University Hospital (#1622-012). Fifty-nine patients with dermatomyositis were enrolled: 55 who visited the Okayama University Hospital Dermatology Department from 2008 to 2016, and 4 patients who were referred to us from the Dermatology Department of the Okayama Japanese Red Cross Hospital from 2015 to 2016. Fifty-two patients met the criteria for the classification tree model by the International Myositis Classification Criteria Project (https://www.niehs.nih.gov/research/resources/imacs/). Among the 7 excluded patients, there were 4 “possible” cases of Bohan and Peter’s criteria, 1 “probable” case, and 2 patients with amyopathic dermatomyositis having typical skin findings and ILD.

Enzyme-linked immunosorbent assay (ELISA) for dermatomyositis-related antibodies

We screened serum samples obtained from 48 of the 59 dermatomyositis patients and

20 control serum samples from patients with dermatological diseases other than collagen disease, using an ELISA kit (MESACUP anti-ARS test). The ELISA plate was coated with 5 different ARS antigens: Jo-1, PL-7, PL-12, EJ, and KS. In a preliminary study, we screened 20 control serum samples from patients with inflammatory skin diseases unrelated to collagen diseases, using a MESACUP anti-ARS test. All 20 samples were negative for any anti-ARS antibody.

To identify the targeted antigen, all the positive sera were further examined using ELISA plates coated with one of the 5 ARSs. All 48 serum samples were also examined for antibodies to TIF1-γ, MDA5 and Mi-2. The ELISA kits used in the present study were provided by Medical & Biological Laboratories (MBL; Nagoya, Japan). The other 11 serum samples were examined for anti-ARS antibodies by immunoblotting (EUROLINE) and a fluorescence enzyme immunoassay kit, or by a double immunodiffusion kit in the case of Jo-1.

In addition to the myositis-specific antibodies, we examined the presence of autoantibodies by a commercially available indirect immunofluorescence (IIF) study using Hep-2 cells in 43 patients.

Evaluation of cutaneous and systemic manifestations

Dermatologists checked the presence and absence of dermatomyositis-related cutaneous manifestations: heliotrope, Gottron’s papules, erythema on the extensor surface of the

extremities, mechanic's hands, Shawl-sign/V-sign, Raynaud's phenomenon, periungual erythema, poikiloderma and scratch dermatitis. Histopathologic findings of the finger lesions clinically-diagnosed as mechanic's hands were examined in the ASS group (n=11). Systemic symptoms such as fever and arthralgia, and related blood test results including serum C-reactive protein (CRP) were evaluated.

Evaluation of pulmonary disease

In order to determine the radiological pattern of the ILD, a radiologist (S.N.) evaluated the CT findings in a blind fashion. Bronchoalveolar lavage (BAL) examinations were performed in 13 patients, or 9 and 4 from the ASS and non-ASS groups, respectively. Since the therapeutic regimens differed from case to case, the initial serum KL-6 levels on diagnosis of ILD are described in Table 1.

Evaluation of myopathy

We performed routine blood tests including complete blood counts, and blood chemistry tests including CK, Mb, and ALD. Magnetic resonance imaging (MRI) examination was carried out in 31 patients—i.e., 13 patients with ASS, and 18 patients in the non-ASS group. The MRI patterns were evaluated in a blind fashion, and classified into 5 patterns: the fasciitis-only, the fasciitis-dominant, the comparable, the myositis-dominant, and the myositis-only patterns. The results were analyzed in relation

to the serum levels of CK, Mb, and ALD.

Statistical analysis

We analyzed the results using Fisher's exact test and Brunner-Munzel test. All statistical analyses were performed with EZR[13] software (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Differences were considered significant at $P<0.05$.

Results

Antibody profiles

Of the 59 patients, 20 patients (33.9%) had one of the anti-ARS antibodies: anti-Jo-1 (n=9), anti-PL-7 (n=4), anti-PL-12 (n=2), anti-EJ (n=2), and anti-KS (n=1) antibodies (**Table 1, Fig. 1**). In 2 cases (cases 19 and 20), the anti-ARS antibodies could be detected by screening ELISA test, but no further serological examination was performed. This group of 20 patients was designated as the ASS group in the present study. When compared with the results of IIF test using Hep-2 cells, the interpretation of the results was sometimes difficult because of the coexisting autoantibodies unrelated to dermatomyositis. For instance, 13 of 20 patients (65%) with ASS, and 5 of 14 patients (36%) in the non-ASS group had anti-SS-A antibodies as well. These results support the observation in a previous study that ASS patients often had anti-SS-A antibodies.[14]

Of 21 patients positive for myositis-specific antibody, 12 patients were positive for

anti-TIF1-γ antibody, 7 for anti-MDA5 antibody, and 2 for anti-Mi-2 antibody. Antibody profiles of all patients except for one (case 41 in **Table 1**) showed a mutually exclusive pattern: each patient possessed only one kind of ARS antibody, if any. The exceptional case (case 41) had a high titer of anti-Mi-2 antibody, which is known to cross-react with TIF1-γ,[9] and as a result, showed a weak false-positive reaction against TIF1-γ. This group of 21 patients was referred to as the non-ASS group in the present study.

No myositis-specific antibody was detected in serum samples from 15 patients by ELISA for anti-ARS, TIF-1γ, MDA5 or Mi-2 antibodies, and these 15 patients were classified into the antibody-negative group. However, we could not exclude the possibility that this group contained some patients with positivity for anti-ARS antibodies to OJ, Zo, and Ha, because those ARS antigens were not contained in the ELISA plates used for the screening test.

In the remaining 3 patients, referred to as the unknown group, we confirmed the negative results for anti-ARS antibodies by immunoblotting (EUROLINE) or ELISA kit, but did not have an opportunity to examine the serum samples by ELISA for TIF-1γ, MDA5 or Mi-2 antibodies.

In sum, the patients were classified into four groups according to their antibody profiles: 1) patients positive for anti-ARS antibodies (the ASS group) (n=20); 2) patients with one of the other myositis-specific antibodies, i.e., the anti-TIF-1γ, MDA5 or Mi-2 antibodies (the non-ASS group) (n=21); 3) patients with no ELISA-detected antibodies

(the antibody-negative group) (n=15); and 4) patients only examined for ARS antibodies by ELISA and immunoblotting (the unknown group) (n=3).

The age of onset, family history and cutaneous manifestations related to the ASS group

The onset age of patients in the ASS group was slightly younger than that of the non-ASS group in our series, but the difference was not significant (mean: 51.7 vs 59.0; *P*=0.09). Medical interview on familial occurrence of collagen diseases in relatives within the second degree of relationship revealed that 5 of 16 patients (31%) in the ASS group had 6 relatives with a history of collagen diseases—i.e., dermatomyositis (n=2: siblings), microscopic polyangiitis (n=1: mother), Sjögren syndrome (n=1: mother), rheumatoid arthritis (RA) (n=1: mother), RA with vasculitis (n=1: father)—while none of 17 patients in the non-ASS group had collagen disease, except for 1 relative who had a history of autoimmune thyroiditis/Hashimoto disease (case 29 **in Table 1, Table 2**). Therefore, the ASS patients' relatives were susceptible to collagen diseases (*P*=0.018).

Mechanic's hands were frequently observed in the ASS group, but there was no significant difference when compared with the incidence in the non-ASS group: 14 of 18 patients (78%) in the ASS group had mechanic's hands, vs. 8 of 16 patients (50%) in the non-ASS group (*P*=0.15) (**Table 2**). Gottron's papules were observed in 12 of 19 patients (63%) with ASS, and all 21 patients (100%) in the non-ASS group (*P*=0.0027).

The occurrence of Shawl-sign and/or V-sign was lower in the ASS group: 3 of 14 patients (21%) in the ASS group exhibited one of these signs vs. 13 of 18 patients (72%) in the non-ASS group (P=0.011). Heliotrope rash was observed in 5 of 20 patients (25%) in the ASS group and 15 of 19 patients (79%) in the non-ASS group (P=0.0012). There were no significant differences in other cutaneous manifestations, including erythema on the extensor surfaces of the extremities, Raynaud's phenomenon, periungual erythema, poikiloderma, or scratch dermatitis between the ASS and non-ASS groups.

We also examined the histopathological findings of finger lesions that had been clinically-diagnosed as mechanic's hands in 11 patients with ASS, and classified them into three categories: 1) liquefaction degeneration, defined as vacuolizing necrosis and saw-tooth appearance of the epidermis, 2) psoriasiform tissue reaction with elongation of the rete ridges and hyperkeratosis, and 3) a miscellaneous group. Skin biopsy specimens obtained form different cutaneous lesions of the same patient (case 18) showed distinct histopathological findings: liquefaction degeneration in the Gottron's papules on the metacarpophalangeal (MP) joint surface, while a psoriasiform tissue reaction in the mechanic's hands on the second finger (**Fig 2a-d**). Of the 11 samples from the clinically-diagnosed mechanical hands, a psoriasiform tissue reaction was observed in 9 samples (82%), and liquefaction degeneration in 2 samples (18%). These results suggest that mechanic's hands associated with ASS often shows a psoriasiform

tissue reaction rather than liquefaction degeneration.

Systemic manifestations related to the ASS group

Patients with ASS frequently presented with systemic symptoms such as fever of >38.0ºC (OR 8.87, 95%CI 1.36 to 103.87; *P*=0.0095), arthralgia/arthritis (OR 4.15, 95%CI 0.91 to 21.53; *P*=0.05), and elevated serum CRP levels (OR 12.29, 95%CI 2.35 to 91.16; *P*=0.00054) (**Table 2**).

Coincident malignancy (within 6 years, except for case16) was found in 4 of 20 patients (20%) with ASS, 8 of 12 patients (67%) with anti-TIF1-γ antibody in the non-ASS group, and 5 of 15 patients (33%) in the antibody-negative group (**Tables 1, 2**). None of the patients with antibodies to MDA5 and Mi-2 had malignancies. No specific association was found between the antibody profile and the type of concomitant neoplasms (**Table 1**).

Evaluation of ILD

Of 20 patients with ASS, 19 patients (95%) had ILD by CT imaging (**Tables 1, 2, Fig. 3a**). Of 21 patients in the non-ASS group, 8 patients (38%) had ILD. The incidence of ILD was significantly higher in the ASS group than in the non-ASS group (OR 23.80, 95%CI 2.70 to 1172.96; *P*=0.00043) (**Table 2**). Among the patients of the non-ASS group, there was a high prevalence of ILD in the group of patients positive for the

anti-MDA5 antibody: 6 of 7 patients (86%), including 2 patients who showed rapidly progressive ILD. By contrast, patients with anti-TIF1-γ antibody showed the lowest incidence of ILD: 1 of 12 patients (8%).

In the ASS group, CT findings showed the fibrotic non-specific interstitial pneumonia (fNSIP) pattern in 9 patients (47%), the cellular NSIP pattern in 4 patients (21%), and the organizing pneumonia/eosinophilic pneumonia (OP/EP) pattern in 5 patients (26%) (**Table 1, Fig. 3b**). On the other hand, in 7 patients with anti-MDA5 antibody, the OP/EP pattern was observed in 4 patients (57%), and the fNSIP pattern in 2 patients (29%). In our series, patients with anti-MDA5 antibody had no CT findings suggestive of diffuse alveolar ILD at the time of diagnosis, but 2 patients (cases 37 and 39) who showed the OP/EP pattern died of ILD at 4 months and 2 months, respectively (**Table 1**).

There was no significant difference in serum KL-6 levels between patients with ILD in the ASS group and patients with ILD positive for anti-MDA5 antibody in the non-ASS group: the serum KL-6 levels ranged from 132 to 3439 (mean: 965.1; median: 562) in the former group and from 177 to 940 (mean: 546.7; median: 539) in the latter. Although the treatment protocols varied from case to case, the ILD associated with ASS responded well to the initial treatments: the response rates were 70%. Over the more than one-year observation period, additional treatments were required for flare of ILD in 4 of 10 patients (40%) with ASS, and one patient (case 15) with anti-PL12 antibody

died of ILD with the OP/EP pattern.

Evaluation of myopathy

Twenty-one of 59 patients (36%) showed no or only slight abnormalities in serum levels of muscle enzymes, and were diagnosed with clinically amyopathic dermatomyositis (CADM): 7 of the 20 patients (35%) with ASS, 10 of 21 patients (48%) in the non-ASS group, and 4 of 15 patients (27%) in the antibody-negative group. Thirty-one patients with overt myositis who met the following criteria were enrolled in the comparative study: patients with elevated serum levels of CK, Mb, and/or ALD that were at least 1.5-fold greater than the upper limit of normal, i.e., 229.5 (IU/L) for CK, 105 (ng/mL) for Mb, and 11.3 (IU/L) for ALD, or patients with the presence of myopathy by MRI.

The measurement of muscle enzymes in the sera of the 31 patients with myopathy revealed higher mean levels of CK (P=0.0026), ALD (P=0.0000095), and Mb (P=0.0094) in the ASS group (n=13) than the non-ASS group (n=18): for CK, 1617.8 vs. 895.3 IU/L; for ALD, 40.6 vs. 14.4 IU/L; and for Mb, 1922.7 vs. 747.2 ng/mL, respectively **(Table 3, Fig. 4a)**. In general, the increase of serum CK levels was correlated to that of Mb and ALD levels both in the ASS and non-ASS groups (data not shown).

MRI was performed in 13 patients with ASS, and 12 patients showed significant MRI findings. The fasciitis-only pattern was apparent in 2 (cases 14 and 16, **Table 3**),

the fasciitis-dominant pattern in 2 (cases 5 and 10), the comparable pattern in 1 (case 7), the myositis-dominant pattern in 2 (cases 8 and 9), and the myositis-only pattern in 5 patients (cases 6, 13, 15, 17, and 18) (**Fig. 4b**). In the non-ASS group, 11 of 18 patients were found to have significant MRI findings: the fasciitis-dominant pattern in 1 (case 31), the comparable pattern in 1 (case 28), the myositis-dominant pattern in 2 (cases 36 and 41), and the myositis-only pattern in 7 patients (cases 25, 26, 27, 32, 33, 35, and 37). Therefore, MRI-proven overt fasciitis, including the fasciitis-only and the fasciitis-dominant patterns, was observed in 4 of 12 patients (33%) in the ASS group, and 1 of 11 (9%) in the non-ASS group, although there was no significant difference (**Fig 4c**). Regarding the serum levels of muscle enzymes, no statistically significant difference was found between the MRI patterns and each of the serum CK, ALD and Mb levels.

Discussion

We confirmed the previous observations that patients with ASS possessed a single anti-ARS antibody in a mutually exclusive fashion, and showed high incidences of systemic inflammation and ILD (**Table 2**).[8,10] In addition to these findings, we found that a psoriasiform tissue reaction was frequently observed in the mechanic's hands or Gottron's papules of ASS, and approximately one-third of patients with ASS had

MRI-proven fasciitis. Moreover, the relatives of patients with ASS had a high risk for the occurrence of collagen diseases.

Mechanic's hands, a characteristic cutaneous sign for ASS, were observed in 14 of 18 patients (78%) in the ASS group, but no significant difference was noted against the incidence in the non-ASS group (8 of 16 patients (50%): P=0.15). This discrepancy was probably due to the difficulty in differential diagnosis of mechanic's hands from the extended Gottorn's papules on the fingers. Otherwise, we should consider the limitation of our study with a small size of case series. The incidence of Shawl sign and/or V-sign was significantly lower in the ASS group than the non-ASS group (P=0.011). In contrast to the previous reports,[11,12] there was no difference between the ASS and non-ASS groups in the incidence of Raynaud's phenomenon, which is known to be more frequently associated with patients with ASS.

Regarding the histopathologic findings of mechanic's hands or Gottron's papules of ASS, the liquefaction degeneration, a histopathologic hallmark for dermatomyositis, was observed in only 2 of the 11 finger lesions (18%), while the remaining 9 lesions (82%) showed a psoriasiform tissue reaction. This finding suggests that inflammatory processes in the mechanic's hands of ASS may differ from those in the liquefaction degeneration.

We confirmed that both patients with ASS and patients positive for anti-MDA5 antibody had high incidences of ILD: 19 of 20 (95%), and 6 of 7 (86%), respectively. A

meta-analysis by Lega et al. reported that ILD was diagnosed in 66% of patients positive for the anti-Jo-1 antibody, and 84% of patients positive for other ARS antibodies.[15] Rapidly progressive ILD with a fatal outcome frequently occurs in patients with anti-MDA5 antibody, but this type of ILD is less common in patients with ASS.[16] In our present study, however, 1 patient (case 15) with ASS positive for anti-PL12 antibody died of progressive ILD. As reported previously,[17] more serious ILD or acute pulmonary arterial hypertension might occur in patients with anti-PL/7PL12 antibody.

At initial diagnosis of ILD, there was no difference in the serum levels of KL-6 between the ASS group and the anti-MDA5-positive group. Our comparative studies of the CT image showed that the fNSIP pattern was predominant in the ASS group (47%), and the OP/EP pattern was most frequent in patients with anti-MDA5 antibody (57%). One patient with ASS and 2 patients with anti-MDA5 antibody who showed the OP/EP pattern died of progressive ILD. These observations suggest the possibility that in addition to the type of myositis-specific antibodies, the CT image might be a prognostic indicator for ILD. Because of the variety of initial doses of corticosteroids and therapeutic regimens, we could not accurately evaluate the response rate to the treatments in the ASS group.

MRI revealed that 4 of 12 patients (33%) with ASS, and 1 of 11 patients (9%) in the non-ASS group had overt fasciitis-dominant myopathy. Previous case reports have described that MRI-proven fasciitis without myositis was observed in some patients

with CADM.[18] Our case series study indicates that fasciitis is not rare in patients with ASS, and not limited to cases of CADM. Yoshida et al. reported that fasciitis was more predominant in patients with dermatomyositis evaluated by MRI earlier than 2 months after the onset of muscle symptoms.[19] In the present study, however, there was no clear correlation between the presence of fasciitis and the time after the onset of muscle symptoms.

Alternatively, the rather high incidence of fasciitis in patient with ASS may account for the different inflammatory processes in the subtypes of dermatomyositis: type1 interferon-mediated myositis, and perifascicular necrosis rather characteristic for ASS.[20,21] It has been reported that patients with myopathy with “perimysial pathology” showed a selective increase of ALD, and the clinical symptoms of such patients are similar to ASS.[22] Furthermore, the serum ALD level is thought to be a useful indicator of disease activity in eosinophilic fasciitis.[23] Thus, we compared the MRI findings with the serum levels of ALD in the ASS and non-ASS groups. The results showed that the serum ALD levels, together with CK and Mb, were higher in the ASS group, and no selective increase of ALD was observed in the ASS group, or in the fasciitis-dominant group.

Patients with dermatomyositis share genetic features with other patients with autoimmune diseases, and some disease-predisposing genes have been found by means of genome scan technology.[24] The present study disclosed that relatives within the

second-degree of relationship in the ASS group showed higher risk for collagen diseases than those in the non-ASS group. Therefore, some genetic risk factors might be more closely associated with patients with ASS, although little is known about them.

In conclusion, the present study confirmed that patients with anti-ARS antibody form a distinct clinical subtype of dermatomyositis characterized by the presence of ILD, and systemic symptoms such as fever and arthralgia. Although there is the limitation of our study because of a small size of case series, we newly found that patients with ASS often present with mechanic's hands with a psoriasiform tissue reaction, and fasciitis-dominant myopathy. The relatives of patients with ASS might be susceptible to collagen diseases.

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Table 1. Detection of Myositis-Specific Autoantibodies, Incidences of ILD and Malignancy, and Outcomes of Our Patients

No.	ARS (<25)	TIF1-γ (<32)	MDA5 (<32)	Mi-2 (<53)	IIF	Autoantibodies	ILD pattern	KL-6 (<500U/mL)	malignancy	observation period (months) / outcome
1	150.2	8.5	1.1	0.3	＜x40	SS-A	fNSIP	3439	—	42
2	165.1	0.8	0	0.9	＜x40	SS-A	fNSIP	492	—	32
3	168.4	7.4	0.8	1	ND	-	fNSIP	876	—	96
4	118.3	0.2	0.5	0.7	x40, Granular	SS-A	OP/EP	2272	colon ca.	32
5	155.4	11.5	7.1	12.8	x40, Homo	SS-A	cNSIP	529	—	9
6	103.1	1.8	0.8	8.8	x40, Homo	-	cNSIP	1241	—	49
7	192.5	1.6	0.7	1.4	＜x40	-	cNSIP	296	melanoma	45
8*	157.1 (Ouchterlony:Jo-1)	ND	ND	ND	x160, Sp	SS-A, CCP	fNSIP	ND	—	ND
9*	185.4 (IB/FEIA:Jo-1)	ND	ND	ND	＜x40	SS-A	—	132	—	1
10	169.9	0.7	0	0.4	x640, Sp	SS-A, RF	cNSIP	373	—	50
11	150.5	1.9	1.6	1.4	＜x40	SS-A	OP/EP	2358	colon ca.	8
12	174.2	0.9	0.8	4.1	ND	-	OP/EP	ND	—	125
13	126.6	1	0.8	4.9	ND	SS-A	fNSIP	315	—	21
14	168.1	0.9	0.7	0.5	＜x40	SS-A, SRP	fNSIP	296	—	45
15*	(IB: PL12)	ND	ND	ND	＜x40	SS-A	OP/EP	562	—	3/death of ILD
16	185.5	1.2	0	1.6	x80, Granular	SS-A	fNSIP	511	—	32
17*	(IB: EJ)	ND	ND	ND	x40, Sp	SS-A	unclassified	785	renal ca.	1
18	162.1	0.7	0	0.9	ND	ss-DNA	fNSIP	696	—	89
19*	129.7	ND	ND	ND	＜x40	-	OP/EP	1233	—	ND
20*	154.3	ND	ND	ND	＜x40	-	fNSIP	ND	—	ND
21	9.5	104.3	0	0.6	ND	-	—	144	gastric ca.	14/death of ca.
22	3.2	138.6	0	0.6	x320, Centro	CENP-B, RF (SS-A;ND)	—	ND	—	69
23	2.7	47.4	0	1.6	＜x40	SS-A	—	194	breast ca.	55
24	2.5	36.5	3	0.6	ND	SS-A, TGAB	—	102	—	81
25	2.3	48	0.5	0.1	ND	-	—	361	gastric ca.	16/dath of ca.
26	4.6	141.1	1.9	2.6	320, Homo		-		lung ca.	12
26	4.6	141.1	1.9	2.6	x320, Homo	CENP-B	—	147	—	12
27	1.4	127.2	0	3.5	＜x40	-	—	200	breast ca.	10
28	2.1	104.4	1.1	1.2	ND	-	—	197	breast ca.	8
29	10.9	42	1.7	1.4	x640, Homo	SS-A, CENP-B	OP/EP	971	—	75
30	17.8	139	1.7	1.9	ND	SS-A;ND	—	186	bladder ca.	3
31	1.4	174.1	0.6	2.6	x160, Centro	SS-A, CENP-B	—	209	colon ca.	14
32	6.6	155.9	0.5	1.5	＜x40	-	—	226	rectal ca.	5
33	1.4	0.5	72	1.3	＜x40	-	OP/EP	685	—	36
34	2.2	1.7	186.9	0.5	ND	-	fNSIP	467	—	27
35	1.1	1.4	32.3	1	x80, Sp	ds-DNA (SS-A; ND)	OP/EP	539	—	100
36	2.4	4.5	36.4	0.8	＜x40	ds-DNA	—	177	—	36
37	1.1	0.7	222.2	0.2	＜x40	SS-A	OP/EP	616	—	4/death of ILD
38	2.1	0.6	194.8	0.8	＜x40	-	fNSIP	940	—	9
39*	5.8	＜5	150＜	＜5	＜x40	(SS-A;ND)	OP/EP	403	—	2/death of ILD
40	1.2	14.8	0.7	123.6	ND	(SS-A;ND)	—	358	—	81
41*	5	43	＜5	150≦	x320, Homo Sp	(SS-A;ND)	cNSIP	241	—	4
42	0.7	0	0	0.3	＜x40	-	—	279	—	37
43	7.5	0.2	0.5	0.3	＜x40	-	fNSIP	2386	—	40
44	1.3	0.2	8.7	0.3	＜x40	(SS-A;ND)	OP/EP	467	—	83
45	2.7	4.5	0.1	0.7	x40, Homo Sp	(SS-A;ND)	—	518	abdominal leiomyoma	49/death of ca.
46	3.6	0.8	0	1.7	x40, Sp	(SS-A;ND)	unknown	630	—	ND
47	1.8	5.2	0	0.5	x40, Sp	(SS-A;ND)	—	142	breast ca.	40
48	4.7	23.8	0.9	1	ND	(SS-A;ND)	—	178	lung ca.	49
49	4.9	28.3	2.3	4.5	＜x40	SS-A, Th/To	—	429	—	26
50	3.5	4.3	1.2	2.8	x40, Homo	(SS-A;ND)	—	205	—	10
51	4.3	2.8	1.1	9.0	x160, Granular	(SS-A;ND)	—	435	—	3
52	3.1	2.6	7.4	2.9	ND	-	fNSIP	335	lung ca.	10
53	14.4	1.6	3	7.4	ND	CENP-B	cNSIP	2209	—	5
54	1.5	31.7	1.6	1.5	x160, Granular	-	—	182	—	18
55	1.7	4.2	2.4	2.4	x40, Centro	SS-A	cNSIP	1166	—	34
56	2.8	18.6	1	4.6	ND	-	UIP	605	lung ca.	21/death of ca.
57*	(IB:anti-ARS Ab-)	ND	ND	ND	ND	SS-A, RF	OP/EP	618	—	6
58*	(IB: SRP)	ND	ND	ND	＜x40	-	—	ND	—	2
59*	＜5.0	ND	ND	＜5	＜x40 (fluorescence in cytoplasm)	SS-A, RF, CCP	OP/EP	992	—	4

Table 1. Footnotes

Familial occurrence of autoimmune diseases includes rheumatoid arthritis (RA) in case 1 (mother), RA with vasculitis and microscopic polyangiitis in case 4 (father and mother, respectively), Sjögren syndrome in case 9 (mother), dermatomyositis in cases 10 and 12 (both sisters) in the ASS group, and autoimmune thyroiditis/Hashimoto disease in case 29 (mother) in the non-ASS group.

Abbreviations: ILD, interstitial lung disease; IB, immunoblot; ND, no data ; ca., carcinoma. Homo, Homogeneous; Sp, Speckled; Centro, Centromere

Table 2. Comparison of Clinical Symptoms and Signs of the ASS and non-ASS groups

Clinical symptoms and signs	ASS	non-ASS	OR,	95%CI;	*P* value
Age of onset, y	51.7±14.4	59.0±13.4		*0.47-0.84;	.091
Sex ratio, M : F	7:13	7:14	**1.08,	0.24-4.74;	1.00
Heliotrope rash	5/20 (25%)	15/19 (79%)	**0.096,	0.015-0.48;	.0012
Gottron's papule	12/19 (63%)	21/21 (100%)	**0,	0.00-0.49;	.0027
Erythema on the extensor surface of extremities	15/18 (83%)	16/18 (89%)	**0.63,	0.047-6.35;	1.00
Mechanic's hand	14/18(78%)	8/16 (50%)	**3.37,	0.65-20.64;	.15
Shawl-sign/V-sign	3/14 (21%)	13/18 (72%)	**0.11,	0.014-0.67;	.011
Raynaud phenomenon	3/13 (23%)	1/11 (9%)	**2.87,	0.19-172.77;	.60
Periungal erythema	11/16 (69%)	16/18 (89%)	**0.29,	0.023-2.14;	.21
Poikiloderma	0/9 (0%)	5/14 (36%)	**0,	0.00-1.50;	0.00
Scratch dermatitis	3/10 (30%)	6/13 (46%)	**0.52,	0.058-3.73;	.67
Proximal muscle weakness	10/18 (56%)	14/21(67%)	**0.63,	0.14-2.76;	.53
Muscle grasping and spontaneous pain	10/19 (53%)	11/21 (52%)	**1.01,	0.25-4.16;	1.00
Elevated serum ALD level	12/12(100%)	5/13 (38%)			*.0000095
Arthralgia/arthritis	13/19 (68%)	6/18 (33%)	**4.15,	0.91-21.53;	.05
Elevated serum CRP level	16/19(84%)	6/21 (29%)	**12.29,	2.35-91.16;	.00054
Fever of over 38°C	8/16 (50%)	2/21(10%)	**8.87,	1.36-103.87;	.0095
Incidence of interstitial lung disease	19/20 (95%)	8/21 (38%)	**23.80,	2.70-1172.96;	.00043
Incidence of malignancy	4/20 (20%)	8/21 (38%)	**0.42,	0.074-1.99;	.31
History of autoimmune disease in relatives	5/16 (31%)	0/17 (0%)	**Inf,	1.13-Inf;	.018

Table 2 Footnotes

* Brunner-Munzel test, ** Fisher’s exact test

Table3. MRI Findings and Serum Levels of CK and ALD in 31 Cases

	No.	myositis specific autoantigens	Age, sex	MRI findings	Period from onset of myositis to MRI imaging		CK (41-153 IU/L)	ALD (2.7-7.5 IU/L)	Mb(18-70 ng/ml)
ASS (n=13)	2	Jo-1	34, F	not done	no muscle symptoms		525	ND	277
	5	Jo-1	51, F	fasciitis dominance	11 months		1114	26.9	585
	6	Jo-1	40, F	myositis only	4 months		274	11.6	155
	7	Jo-1	60, M	comprable	2 months		5654	97.4	7330
	8	Jo-1	45, M	myositis dominance	no muscle symptoms		711	21.3	ND
	9	Jo-1	29, F	myositis dominance	2-3 weeks		3350	74.4	2646
	10	PL-7	59, F	fasciitis dominance	no muscle symptoms		461	18.5	748
	13	PL-7	75, F	myositis only	no muscle symptoms		584	29.4	686
	14	PL-12	56, F	fasciitis only	1.5 months		2522	39.1	1930
	15	PL-12	52, F	myositis only	1 month		292	12.2	170
	16	EJ	55, F	fasciitis only	within 1 month		1917	77.9	2200
	17	EJ	72, M	myositis only	unknown		3048	52.2	4423
	18	KS	57, M	myositis only	2.5 months		580	26.0	ND
						mean	1617.8	40.6	1922.7
						median	711	28.2	748.0
non-ASS (n=18)	21	TIF1-γ	63, M	no significant findings	1-2 weeks		171	10.9	231
	22	TIF1-γ	79, F	no significant findings	no muscle symptoms		163	ND	183
	24	TIF1-γ	39, F	no significant findings	unknown		370	5.5	174
	25	TIF1-γ	71, M	myositis only	2-3 weeks		4006	18.1	4350
	26	TIF1-γ	31, F	myositis only	1 month		143	11.4	106
	27	TIF1-γ	61, F	myositis only	1 month		1181	ND	854
	28	TIF1-γ	57, F	comprable	1.5 months		67	3.4	46
	30	TIF1-γ	65, M	not done	unknown		64	ND	125
	31	TIF1-γ	67, F	fasciitis dominance	within 1 month		213	7.0	218
	32	TIF1-γ	55, F	myositis only	2 weeks		439	6.7	382
	33	MDA5	53, F	myositis only	within 1 month		339	6.9	146
	34	MDA5	55, F	no significant findings	no muscle symptoms		72	18.6	48
	35	MDA5	66, F	myositis only	within 1 month		394	12.0	682
	36	MDA5	26, F	myositis dominance	1 month		18	10.4	50
	37	MDA5	73, F	myositis only	no muscle symptoms		26	ND	ND
	38	MDA5	69, M	not done	no muscle symptoms		29	7.8	108
	40	Mi-2	58, M	no significant findings	within 1 month		1187	ND	1085
	41	Mi-2	75, M	myositis dominance	1 month		7233	68.9	3914
						mean	895.3	14.4	747.2
						median	192	10.4	183.0

Table 3. Footnotes

Of 20 patients with ASS, 12 patients had MRI findings of myositis and/or fasciitis associated with elevated serum levels of CK, ALD or Mb over the 1.5 times of reference value. In the non-ASS group, 11 of 18 patients showed MRI findings consistent with myositis and/or fasciitis, and 15 patients showed elevated serum levels of CK or ALD.

Figure legends

Fig 1. Detection Rates of Myositis-Specific Autoantibodies

Twenty patients with ASS had one of the ARS-related antigens. Twenty-one patients with non-ASS included 12 patients positive for anti-TIF1-γ, 7 for anti-MDA5, and 2 for anti-Mi-2 antibody.

Fig 2. Representative Cutaneous Manifestations and Histopathologic Findings in ASS

Basal cell damage suggestive of liquefaction degeneration in Gottron's papules **(a, c)**, and a psoriasiform tissue reaction in mechanic's hands **(b, d)** in the same patient (case 18).

Fig 3. CT Patterns of ILD and the Relative Incidence

(a) Representative CT patterns of ILD**:** CT images are classified into four patterns: fNSIP pattern (upper left, case 16), cNSIP pattern (upper right, case 10), OP/EP pattern (lower left, case 37), and UIP pattern (lower right, case 56). **(b)**The fNSIP pattern is the most common finding in the ASS group (47%), followed by the OP/EP pattern (26%), and the cNSIP pattern (21%). There is no statistical significance in the incidence of the CT patterns between the ASS and the non-ASS groups.
fNSIP: fibrotic non-specific interstitial pneumonia, cNSIP: cellular non-specific interstitial pneumonia, OP/EP: organizing pneumonia/eosinophilic pneumonia, UIP: usual interstitial pneumonia.

Fig 4. Serum Levels of Muscle Enzymes and MRI Findings

(a) Serum CK, ALD and Mb levels in patients with ASS and non-ASS with overt myositis: each serum level is significantly higher in the ASS group than the non-ASS group. **(b)** MRI findings (T2 STIR) of representative cases indicated by symbols in Fig 4(a). **(c)** Relative incidence of MRI-proven fasciitis: MRI findings of fasciitis are frequently observed in the ASS group, but there is no significant difference in the incidence when compared with that in the non-ASS group. There is no correlation in the presence of fasciitis and serum ALD levels.