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Clinical significance of myositis-specific autoantibody profiles in Japanese patients with polymyositis/dermatomyositis

Jumpei Temmoku, MD[a], Shuzo Sato, MD[a], Yuya Fujita, MD[a], Tomoyuki Asano, MD[a], Eiji Suzuki, MD[b], Takashi Kanno, MD[b], Makiko Yashiro Furuya, MD[a], Naoki Matsuoka, MD[a], Hiroko Kobayashi, MD[a], Hiroshi Watanabe, MD[a], Tomohiro Koga, MD[c], Toshimasa Shimizu, MD[c], Atsushi Kawakami, MD[c], Kiyoshi Migita, MD[a],*

Abstract

Myositis-specific autoantibodies, such as anti-melanoma differentiation associated gene 5 (MDA5) and anti-anti-amino acyl-tRNA synthetases (ARS) antibodies, are associated with interstitial lung diseases (ILD), which determine the prognosis of polymyositis/dermatomyositis (PM/DM) patients. However, there is a paucity of data on the clinical correlation between anti-Sjögren syndrome-related antigen A (anti-SSA)/Ro52 antibodies in PM/DM. We investigated the prevalence of myositis-specific autoantibodies including anti-SSA/Ro52 antibody and assessed the clinical significance of these antibodies in patients with PM/DM.

We retrospectively reviewed demographic data and clinical outcomes in patients with PM/DM. The study population comprised 24 patients with PM and 60 patients with DM. The presence of anti-myositis-specific antibodies (MDA5, ARS, Jo-1, SSA/Ro52) was determined by immunosorbent assay (ELISA).

Anti-MDA5 antibody was detected in 18 patients with DM (n = 60). Anti-ARS/anti-SSA/Ro52 antibodies were detected in 31 and 39 patients with PM/DM (n = 84). Rapidly progressive ILD patients were mainly found in the anti-MDA5 antibody-positive DM group. During the follow-up period, 9 patients died. Kaplan–Meier analysis demonstrated that survival rates seem to be lower in DM patients with anti-MDA5 antibodies compared with those without anti-MDA5 antibodies. Furthermore, dual positivity for anti-SSA/Ro52 and anti-MDA5 antibodies was significantly higher in nonsurviving DM patients compared with survivors.

Although the presence of anti-ARS or anti-MDA5 antibodies is a prognostic marker in patients with PM/DM, combined presence of anti-SSA/Ro52 and anti-MDA5 antibodies represent another marker for clinical outcome in DM patients. Our results suggest that anti-SSA/Ro52 antibody positivity in DM patients with anti-MDA5 antibody reveals a subgroup of DM patients with poor prognosis.

Abbreviations: Ab = antibody, ARS = anti-amino acyl-tRNA synthetases, CADM = clinically amyopathic dermatomyositis, CK = creatine kinase, DM = dermatomyositis, ILD = interstitial lung disease, MDA-5 = melanoma differentiation associated gene 5, MSA = myositis-specific autoantibody, PM = polymyositis, RP-ILD = rapid progressive interstitial lung disease.

Keywords: anti-MDA5 antibody, anti-SSA/Ro52 antibody, dermatomyositis, myositis-specific autoantibody, polymyositis

1. Introduction

Dermatomyositis (DM) and polymyositis (PM) are systemic autoimmune diseases characterized by muscle inflammation and skin lesions.[1] Interstitial lung disease (ILD) is the most frequent pulmonary complication and determines the prognosis of patients with DM/PM.[2] The clinical features of ILD complicated with PM/DM vary widely; however, rapidly progressive interstitial lung disease (RP-ILD) is a serious complication of DM, especially clinical amyopathic DM (CADM).[3] Serum myositis-specific autoantibodies (MSAs) are useful markers for the diagnosis of PM/DM and are associated with distinct clinical phenotypes of the disease.[4] Anti-melanoma differentiation-associated protein 5 (MDA5) antibody (Ab) has been shown to be associated with RP-ILD in patients with DM and CADM, and its presence results in unfavorable prognosis.[5] Studies of Japanese patients have shown that anti-MDA5 Ab is detected exclusively in patients with DM and ADM, with 35% positivity in DM and 73% in ADM.[6] It was reported that 79% of DM patients positive for anti-MDA5 Ab developed RP-ILD and 50% of these died of respiratory failure.[7] In RP-ILD with anti-MDA5 Ab, immunological mechanisms including macrophage activation resulting in increased levels of type 1 interferon and inflammatory

cytokine production might play roles in the development of RP-ILD.[8] Anti-aminoacyl-tRNA synthetases (anti-ARS) Abs are a further group of autoantibodies associated with ILD in PM/DM.[9] Approximately 50% of PM/DM patients with ILD complication are positive for 1 of the anti-ARS Ab.[10] Patients with anti-ARS Abs show similar clinical features (ILD, myositis, mechanical hands), and this condition is termed anti-ARS syndrome.[9] In contrast to MDA5 Ab, anti-ARS Ab-positive ILD generally develops gradually and responds well to steroids.[11] Therefore, patients positive for anti-ARS Abs are characterized by chronic progression, with good response to steroids and a fair prognosis.[12] The clinical significance of anti-MDA5 and anti-ARS Abs has been investigated in PM/DM[13,14]; however, there are still heterogeneities of clinical phenotypes associated with these autoantibodies. Anti-SSA/Ro52 Abs have been frequently identified in rheumatic diseases without Sjögren syndrome,[15] making it a common autoantibody in PM/DM patients. Given its high prevalence and the few data available on its significance in PM/DM, we investigated whether anti-Sjögren syndrome-related antigen A (anti-SSA)/Ro52 Abs are associated with the clinical phenotype of PM/DM. The objective of this study was to analyze the phenotype and clinical outcomes of a series of patients with PM/DM regarding the association of anti-MDA5 or anti-ARS Abs, and also to assess the value of anti-SSA/Ro52 Ab as a prognostic marker.

2. Patients and methods

2.1. Patients

We conducted a retrospective study of patients with PM/DM who achieved disease stabilization during treatment at Fukushima Medical University Hospital and Ohta Nishinouchi Hospital, from September, 2006 to August, 2018. The study population consisted of 24 patients (17 females and 7 males) with PM plus 60 patients (43 females and 17 males) with DM. The diagnosis of classical DM was based on Bohan and Peter criteria.[16,17] The diagnosis of clinically amyopathic DM (CADM) was based on Sontheimer criteria.[3] Nine patients did not fulfill Bohan and Peter criteria for DM,[16,17] but fulfilled Sontheimer criteria of CADM,[3] because of the absence of clinical skeletal muscle symptoms and the presence of persistent clinical DM skin features.

All of the subjects underwent routine examination of internal malignancies. This study was approved by the Ethics Committee of the Fukushima Medical University (No. 2940).

2.2. Review of the clinical data

Clinical manifestations, laboratory data, radiographic data, and the presence of internal malignancies were obtained by a retrospective review of medical records. Patients seen from March, 2004 to May, 2018 are enrolled in this retrospective cohort study. The following data were collected at baseline: age, sex, time to diagnosis, underlying comorbidities, clinical symptoms, laboratory data, histological data, and treatment status. Clinical findings included the presence or absence of inflammatory myopathy, ILD, arthritis, Raynaud phenomenon, mechanic's hands, fever, skin rash, heart involvement, and other relevant clinical features. The patients were diagnosed with ILD according to the results of chest x-ray and high-resolution chest CT, reported by Japanese board-certified radiologists. A subset of patients with RP-ILD was defined

as those presenting with progressive dyspnea and progressive hypoxemia, and a worsening of interstitial change on chest radiography within 1 month from the onset of respiratory symptoms, as described previously.[7]

2.3. Serological analysis

Sera samples were obtained from all patients with PM/DM who were undergoing medical treatment at the first visit, and were stored at −20°C until use. Most of the sera samples were obtained at the first visit, so the interval from initiation of therapy was minimal. Laboratory data before the initial treatments included creatine kinase (CK), C-reactive protein (CRP), ferritin, and sialylated carbohydrate antigen (KL-6). Anti-SS-A/Ro and anti-Jo-1 Abs were measured using ELISA kit (MESACUP-2/ Medical and Biological Laboratories, MBL, Nagoya, Japan). Anti-MDA5 Abs were measured by ELISA using the MESACUP anti-MDA5 test (MBL). Anti-ARS Abs were measured using ELISA kit (MESACUP anti-ARS test, MBL) that can detect 5 anti-ARS Abs (Jo-1, PL-7, PL-12, EJ, and KS). We analyzed the clinical and serological findings, including autoantibodies, at the time of diagnosis. Owing to the retrospective nature of the study, serological analysis was carried out on stocked samples (sera) at the diagnosis in some patients.

2.4. Statistical analysis

Clinical characteristics were presented as the mean ± standard deviation (SD) of the number of patients. All categorical variables were reported as frequency (percentages). Qualitative variables were compared using the chi-square test (or Fisher exact test when appropriate). Continuous variables were compared using Student t test or the Mann–Whitney U test depending on data distributions. Survival, related to follow-up time, was analyzed using the Kaplan–Meier method and compared using the log-rank test. Two-sided P values less than .05 were considered statistically significant. All calculations were performed using PASW Statistics version 20 (SPSS Japan Inc., Tokyo, Japan).

2.5. Ethical approval and consent to participate

The study was approved by the Ethics Committee of Fukushima Medical University (No. 2940).

3. Results

3.1. Clinical features of the enrolled patients with PM/DM

Demographic data of the enrolled 84 patients are presented in Table 1. The study population comprised 24 patients with PM and 60 patients with DM. Demographic data were comparable between PM and DM patients, except for elderly onset in PM patients. Myopathic symptoms, such as muscle weakness, were detected in similar proportions in those with PM and DM, whereas serum levels of CK were significantly higher in PM patients compared with those with DM. Skin lesions, such as Gottron sign, were exclusively observed in patients with DM. The presence of anti-MSAs (MDA5, ARS, Jo-1, SSA/Ro52) was determined by ELISA. Of 84 patients, 31 (36.9%) were positive for anti-ARS Abs, 18 patients were positive for anti-MDA5 Abs, and 39 patients (41.4%) were positive for anti-SSA/Ro52 Abs, with some patients demonstrating positivity for multiple Ab. Anti-MDA5 Abs were exclusively detected in patients with DM.

Table 1

Baseline characteristics of enrolled patients with PM and DM.

Characteristics	PM (n=24)	DM (n=60)	P
Age at onset, y	60.25 (16.1)	52.1 (14.8)	.0296
Female, n (%)	17 (70.8)	43 (71.7)	.9391
Clinical diagnosis			
CADM, n (%)	0 (0)	9 (15.0)	.0446
Skeletal muscle and skin features			
Muscle weakness, n (%)	22 (91.7)	46 (76.7)	.1137
Gottron sign, n (%)	2 (8.3)	53 (88.3)	<.001*
Skin ulcer, n (%)	0 (0)	5 (8.3)	.1448
Heliotrope, n (%)	0 (0)	26 (43.3)	<.001*
Palmar papules, n (%)	0 (0)	21 (35.0)	<.001*
Periungual erythema, n (%)	1 (4.2)	28 (46.7)	<.001*
Pulmonary involvement and malignancy			
Interstitial lung disease, n (%)	18 (75.0)	47 (78.3)	.7415
RP-ILD, n (%)	0 (0)	5 (8.3)	.0600
Malignancies, n (%)	5 (20.8)	16 (26.7)	.5770
Laboratory data			
CK (IU/l), mean (SD)	3293.6 (1970.0)	1711.1 (3343.5)	<.001*
Ferritin (ng/mL), mean (SD)	315.5 (279.7)	604.3 (896.2)	.4867
KL-6 (U/mL), mean (SD)	923.5 (1157.2)	942.7 (919.5)	.4445
IgG (mg/dL), mean (SD)	1795.8 (738.7)	1578.0 (563.8)	.2108
MSA profile			
Anti-MDA5, n (%)	0 (0)	18 (30.0)	.0026*
Anti-ARS, n (%)	13 (54.2)	18 (30.0)	.0344
Anti-Jo-1, n (%)	8 (33.3)	9 (15.0)	.0589
Anti-SSA/Ro52, n (%)	13 (54.2)	26 (43.3)	.3636
Follow up periods, mos (SD)	41.7 (49.1)	48.0 (57.3)	.9311

ARS = anti-amino acyl-tRNA synthetases antibody, CADM = clinically amyopathic dermatomyositis, CK = creatine kinase, DM = dermatomyositis, IgG = Immunoglobulin G, KL-6 = sialylated carbohydrate antigen KL-6, MDA5 = melanoma differentiation associated gene 5, MSA = myositis-specific autoantibody, PM = polymyositis, RP-ILD = rapid progressive interstitial pneumonia.
* P < 0.05.

Table 2

Comparison of anti-ARS antibody-positive and negative patients with PM/DM.

| Characteristics | Anti-ARS (n=84) | | |
	Positive (n=31)	Negative (n=53)	P
Age at onset, y	51.2 (15.9)	56.3 (15.2)	.0980
Female, n (%)	24 (77.4)	36 (64.2)	.3526
Clinical diagnosis			
PM, n (%)	13 (41.9)	11 (20.8)	.0381
DM, n (%)	18 (58.1)	32 (60.4)	.8349
CADM, n (%)	0 (0)	10 (18.9)	.0100*
Skeletal muscle and skin features			
Muscle weakness, n (%)	16 (51.6)	45 (84.9)	<.001*
Gottron sign, n (%)	18 (58.1)	37 (69.8)	.2745
Skin ulcer, n (%)	0 (0)	5 (9.4)	.0778
Heliotrope, n (%)	5 (16.1)	21 (39.6)	.0246
Palmar papules, n (%)	6 (19.4)	15 (28.3)	.3608
Periungual erythema, n (%)	10 (32.3)	19 (35.8)	.7384
Pulmonary involvement and malignancy			
Interstitial lung disease, n (%)	28 (90.3)	37 (69.8)	.0301
RP-ILD, n (%)	1 (3.2)	4 (7.5)	.4192
Malignancies, n (%)	7 (22.6)	14 (26.4)	.6953
Laboratory data			
CK (IU/l), mean (SD)	2571.1 (3466.7)	1928.8 (2841.6)	.1923
Ferritin (ng/mL), mean (SD)	491.9 (846.9)	554.8 (771.0)	.8761
KL-6 (U/mL), mean (SD)	1336.6 (1377.8)	689.6 (509.1)	.0508
IgG (mg/dL), mean (SD)	1712.8 (679.2)	1598.9 (590.1)	.3979
MSA profile			
Anti-MDA5, n (%)	0 (0)	18 (34.0)	.0105
Anti-Jo-1, n (%)	17 (54.8)	0 (0)	<.001*
Anti-SSA/Ro52, n (%)	22 (71.0)	17 (32.1)	<.001*
Follow-up periods, mos (SD)	65.0 (57.8)	36.2 (51.2)	.0093

ARS = anti-amino acyl-tRNA synthetases antibody, CADM = clinically amyopathic dermatomyositis, CK = creatine kinase, DM = dermatomyositis, IgG = immunoglobulin G, KL-6 = sialylated carbohydrate antigen KL-6, MDA5 = melanoma differentiation associated gene 5, MSA = myositis-specific autoantibody, PM = polymyositis, RP-ILD = rapid progressive interstitial pneumonia.
* P < 0.05.

3.2. Differences of clinical characteristics between anti-ARS or MDA5 Ab-positive and negative patients

Clinical characteristics of anti-ARS or anti-MDA5 Ab-positive PM/DM patients were analyzed. The clinical characteristics of anti-ARS Ab-positive or negative groups are summarized in Table 2. There were no differences in clinical parameters except for disease status (PM) and the association rates of ILD, which were significantly higher in the anti-ARS Ab-positive group. The anti-ARS-Ab group was shown to be less frequently associated with muscle weakness and heliotrope rash compared with those negative for anti-ARS Ab. Anti-MDA5 Abs were exclusively detected in patients with DM; therefore, we compared the clinical characteristics of anti-MDA5 Ab-positive or negative DM patients (Table 3). The anti-MDA5 Ab-positive group was shown to be more frequently associated with RP-ILD and skin lesions, such as heliotrope rash or palmar papules, compared with patients negative for anti-MDA5 Ab. These characteristic clinical phenotypes of anti-ARS or MDA5 Ab-positive patients were consistent with previous findings.[9,13]

3.3. Clinical course and autoantibodies

Of the total 84 patients, 9 patients died during the study period with a mean follow-up time of 38.2 months. The most common causes of death were ILD progression or respiratory failure (n=6) and infections including pneumonia (n=3). To further determine the prognostic factors associated with mortality in patients with PM/DM, we compared the baseline clinical features between survivors and nonsurvivors (Table 4). There were no significant differences in clinical features between survivors and non-survivors, except for the association rates of RP-ILD. Serologically, the rate of anti-MDA5 Ab-seropositivity was significantly higher in nonsurvivors compared with survivors (55.6% vs 17.3%; P=0.0083). Although the rate of double-positivity of anti-SSA/Ro52 and anti-ARS Abs was not different between survivors and nonsurvivors, individuals with double-positivity for both anti-MDA5 and anti-SSA/Ro52 Abs were significantly higher in nonsurvivors compared with survivors (44.4% vs 5.3%; P < 0.001).

3.4. Clinical outcomes and autoantibody profiles in patients DM

Anti-SSA/Ro52 Ab-positive patients (n=39, 46.4%) were included in a total of 84 patients with PM/DM. Clinical features were compared between PM/DM patients with and without anti-SSA/Ro52 antibodies. As shown in Table 5, anti-SSA/Ro52 Ab-positive patients were more frequently associated with female sex and higher levels of serum IgG compared with anti-SSA/Ro52 Ab-negative patients. However, there were no significant differences in other clinical parameters (Table 5). We compared the prognosis of DM patients stratified by the seropositivity for anti-MDA5 or anti-SSA/Ro52 Abs using Kaplan–Meier survival curves. In Kaplan–Meier survival curves stratified by the presence

Table 3

Comparison of anti-MDA5 antibody-positive and negative patients with DM.

Characteristics	Anti-MDA5 (n = 60)		
	Positive (n = 18)	Negative (n = 42)	P
Age at onset, y	50.1 (13.7)	52.9 (15.4)	.6271
Female, n (%)	12 (66.7)	31 (66.0)	.5737
Skeletal muscle and skin features			
Muscle weakness, n (%)	12 (66.7)	34 (81.0)	.2306
Gottron's sign, n (%)	17 (94.4)	36 (85.7)	.3344
Skin ulcer, n (%)	3 (16.7)	2 (4.8)	.1263
Heliotrope, n (%)	13 (72.2)	13 (31.0)	.0031*
Palmar papules, n (%)	11 (61.1)	10 (23.8)	.0055
Periungual erythema, n (%)	11 (61.1)	17 (40.4)	.1420
Pulmonary involvement and malignancy			
Interstitial lung disease, n (%)	18 (100.0)	29 (69.0)	.0077
RP-ILD, n (%)	4 (22.2)	1 (2.4)	.0108
Malignancies, n (%)	1 (5.6)	15 (35.7)	.0155
Laboratory data			
CK (IU/l), mean (SD)	465.1 (820.1)	2258.1 (3862.4)	.0024*
Ferritin (ng/mL), mean (SD)	931.5 (1079.6)	434.7 (751.9)	.2482
KL-6 (U/mL), mean (SD)	961.4 (551.5)	934.2 (1050.2)	.1391
IgG (mg/dL), mean (SD)	1518.9 (392.6)	1601.0 (620.7)	.8800
MSA profile			
Anti-ARS, n (%)	0 (0)	18 (42.9)	<.001*
Anti-Jo-1, n (%)	0 (0)	9 (21.4)	.0332
Anti-SSA/Ro52, n (%)	6 (33.3)	20 (47.6)	.3062
Follow-up periods, mos (SD)	47.0 (63.0)	48.5 (55.1)	.7058

ARS = anti-amino acyl-tRNA synthetases antibody, CK = creatine kinase, DM = dermatomyositis, IgG = immunoglobulin G, KL-6 = sialylated carbohydrate antigen KL-6, MDA-5 = melanoma differentiation associated gene 5, MSA = myositis-specific autoantibody, RP-ILD = rapid progressive interstitial pneumonia.
* P < 0.05.

Table 4

Comparisons of clinical characteristics between survivors and nonsurvivors with PM/DM.

Characteristics	Dead (n = 9)	Alive (n = 75)	P
Age at onset, y	63.9 (6.9)	53.4 (15.9)	.0509
Female, n (%)	5 (55.6)	55 (72.4)	.2646
Clinical diagnosis			
PM, n (%)	0 (0)	24 (32.0)	.0446*
DM, n (%)	8 (88.9)	42 (56.0)	.2368
CADM, n (%)	1 (11.1)	9 (12.0)	.9380
Skeletal muscle and skin features			
Muscle weakness, n (%)	7 (77.8)	61 (81.3)	.7974
Gottron sign, n (%)	9 (100)	46 (61.3)	.0211*
Skin ulcer, n (%)	1 (11.1)	4 (0.05)	.4888
Heliotrope, n (%)	5 (55.6)	21 (28.0)	.0911
Palmar papules, n (%)	4 (44.4)	17 (22.7)	.1540
Periungual erythema, n (%)	4 (44.4)	25 (33.3)	.5077
Pulmonary involvement and malignancy			
Interstitial lung disease, n (%)	7 (77.8)	58 (77.3)	.9760
RP-ILD, n (%)	3 (33.3)	2 (2.7)	<.001*
Laboratory data			
CK (IU/l), mean (SD)	1111.1 (1111.4)	2297.3 (3224.9)	.5422
Ferritin (ng/mL), mean (SD)	1303.9 (1063.1)	418.1 (691.0)	.0074
KL-6 (U/mL), mean (SD)	1097.1 (1117.7)	917.2 (974.3)	.6767
IgG (mg/dL), mean (SD)	1485.9 (276.0)	1662.1 (653.2)	.9936
MSA profile			
Anti-MDA5, n (%)	5 (55.6)	13 (17.3)	.0083
Anti-ARS, n (%)	1 (11.1)	30 (40.0)	.0897
Anti-SSA/Ro52, n (%)	5 (55.6)	33 (44.0)	.5105
Anti-MDA5/anti-SSA double positive, n (%)	4 (44.4)	4 (5.3)	<.001*
Anti-ARS/anti-SSA double positive, n (%)	1 (11.1)	21 (28.0)	.2762
Therapy			
Initial PSL dose (mg) (SD)	54.4 (11.3)	69.7 (148.4)	.1027
mPSL pulse, n (%)	7 (77.8)	40 (53.3)	.1628
Immunosuppressant, n (%)	5 (55.6)	61 (81.3)	.0749
Follow-up periods, mos (SD)	6.8 (12.0)	51.7 (56.3)	.0014*

ARS = anti-amino acyl-tRNA synthetases antibody, CADM = clinically amyopathic dermatomyositis, CK = creatine kinase, DM = dermatomyositis, IgG = immunoglobulin G, KL-6 = sialylated carbohydrate antigen KL-6, MDA5 = melanoma differentiation associated gene 5, mPSL = methylprednisolone, MSA = myositis-specific autoantibody, PM = polymyositis, PSL = prednisolone, RP-ILD = rapid progressive interstitial pneumonia.
* P < 0.05.

of anti-MDA5 Ab, DM patients with anti-MDA5 Abs had worse survival compared with anti-MDA5 Ab-negative DM patients (Fig. 1). Additionally, anti-MDA5 Ab-positive DM patients with anti-SSA/Ro52 Abs showed worse survival than those without anti-SSA/Ro52 Ab (Fig. 2), although this was not statistically significant. We compared the prognosis according to the seropositivity for anti-SSA/Ro52 Ab in MDA5 Ab-negative DM/PM patients. In Kaplan–Meier analysis, there was no difference in survival curves between MDA5 Ab-negative DM/PM patients with and without anti-SSA/Ro52 Abs (Fig. 3). Whereas the seropositivity of anti-MDA5 Ab is associated with the significant worth survival compared with those without anti-MDA5 Ab in anti-SSA/Ro52 Ab-positive patients (Fig. 4). These findings suggest that patients with dual positivity for anti-SSA/Ro and anti-MDA5 Abs exhibit poor prognosis.

4. Discussion

This study was conducted to determine the significance of the MSAs, anti-ARS, anti-MDA5, and anti-SSA/Ro52 Abs in the clinical course of PM/DM. We identified 31 (36.9%) patients who were positive for anti-ARS Abs, 15 for anti-MDA5 Abs, and 39 (41.4%) for anti-SSA/Ro52 Abs. Several prognostic factors for PM/DM have been identified. Previous studies demonstrated that the presence of anti-MDA5 antibodies is associated with RP-ILD and CADM without classic DM symptoms.[18] A recent meta-analysis also demonstrated that anti-MDA5 Ab have good sensitivity and specificity for identifying the risk of RP-ILD in patients with DM.[19] In agreement with these reports,[18,19] our

data showed that anti-MDA5 Abs were observed more frequently in DM patients with RP-ILD. Consistent with the previous studies,[9,10] our data also indicated that anti-ARS Ab-positive PM/DM patients had better prognosis, whereas the presence of anti-MDA5 Ab was associated with poor prognosis. Kaplan–Meier analysis demonstrated that of anti-MDA5 antibody was associated with poor prognosis. These findings suggest that the clinical phenotypes and prognosis of PM/DM patients could be influenced by the MSA profile.

Antibodies to SSA antigen (Ro52) have been histologically described as a marker for Sjögren syndrome; however, clinical investigations have documented an association of anti-SSA/Ro52 antibodies with ILD.[20] Additionally, an association between anti-SSA/Ro52 antibodies and aggressive anti-ARS syndrome has been reported.[21,22] However, there have been few studies addressing the frequency of anti-SSA/Ro52 Ab and its significance in PM/DM patients. In this study, we thus evaluated the role of anti-SSA/Ro52 Ab as a prognostic factor in PM/DM patients. The data presented in this study demonstrate that the prevalence of anti-SSA/Ro52 Ab in PM/DM patients was similar

**Comparison of anti-SSA/Ro antibody-positive and negative patients with PM/DM.**

Characteristics	Anti-SSA/Ro (n=84)		
	Positive (n=39)	Negative (n=45)	P
Age at onset, y	52.3 (14.8)	56.2 (16.1)	.2021
Female, n (%)	34 (87.2)	26 (57.8)	.0029*
Clinical diagnosis			
PM, n (%)	13 (33.3)	11 (24.4)	.3684
DM, n (%)	22 (56.4)	28 (62.2)	.5884
CADM, n (%)	4 (10.3)	6 (13.3)	.6641
Skeletal muscle and skin features			
Muscle weakness, n (%)	29 (74.4)	39 (86.7)	.1520
Gottron sign, n (%)	26 (66.7)	29 (64.4)	.8308
Skin ulcer, n (%)	2 (5.1)	3 (6.7)	.7663
Heliotrope, n (%)	8 (20.5)	18 (40.0)	.0540
Palmar papules, n (%)	10 (25.6)	11 (24.4)	.8995
Periungual erythema, n (%)	15 (38.5)	14 (31.1)	.4798
Pulmonary involvement and malignancy			
Interstitial lung disease, n (%)	33 (84.6)	32 (71.1)	.1401
RP-ILD, n (%)	4 (10.3)	1 (2.2)	.1206
Malignancies, n (%)	7 (17.9)	14 (31.1)	.1647
Laboratory data			
CK (IU/l), mean (SD)	2201.5 (3337.8)	2139.6 (2881.1)	.9056
Ferritin (ng/mL), mean (SD)	655.2 (954.1)	419.3 (612.1)	.3451
KL-6 (U/mL), mean (SD)	1010.1 (1084.3)	869.5 (891.4)	.4029
IgG (mg/dL), mean (SD)	1936.0 (697.9)	1383.2 (407.3)	<.001*
MSA profile			
Anti-MDA5, n (%)	8 (20.5)	8 (17.8)	.7658
Anti-ARS, n (%)	22 (56.4)	9 (20.0)	.0010*
Anti-Jo-1, n (%)	11 (28.2)	6 (13.3)	.0907
Follow-up periods, mos (SD)	46.9 (56.3)	45.8 (54.6)	.9536

ARS=anti-amino acyl-tRNA synthetases antibody, CADM=clinically amyopathic dermatomyositis, CK=creatine kinase, DM=dermatomyositis, IgG=immunoglobulin G, KL-6=sialylated carbohydrate antigen KL-6, MDA5=melanoma differentiation associated gene 5, MSA=myositis-specific autoantibody, RP-ILD=rapid progressive interstitial pneumonia, SSA, PM=polymyositis.
* $P < 0.05$.

to the previously described 37.0% in tested sera.[23] Seropositivity of anti-SSA/Ro52 Ab itself did not affect the clinical outcome of PM/DM patients. However, when anti-SSA/Ro52 Ab was detected in PM/DM patients with anti-MDA5 Ab, anti-SSA/Ro52 Ab seropositivity was associated with lower survival. Our results show that the detection of anti-SSA/Ro52 Ab in conjunction with anti-MDA5 Ab predicts a unique subgroup of PM/DM patients and facilitates the characterization of PM/DM-related ILD and its clinical course. Anti-SSA/Ro52 antibody is prevalent throughout the rheumatic diseases suggesting that this antibody is linked with the phenotype or clinical manifestations of connective tissue diseases. Previous study demonstrated an association between anti-SAA/Ro-52 Ab with aggressive anti-ARS syndrome.[24] More recently, Tatebe et al[25] reported that anti-SAA/Ro-52 Ab was associated with relapse in patients with PM/DM. In our data, anti-SSA/Ro-52 Ab was also detected in a significant number of PM/DM patients with other MSAs. Assessment of anti-ARS and anti-MDA5 Abs is useful for predicting the clinical course, prognosis, and treatment response in patients with PM/DM. Our results suggest that in combination with anti-MDA5 Ab, anti-SSA/Ro52 Ab seropositivity affects the clinical course of patients with PM/DM. The mechanisms by which anti-SSA/Ro52 Ab may contribute to poor prognosis in anti-MDA5 Ab-positive DM patients are as yet unknown. Recent epitope mapping studies demonstrated that anti-SSA/Ro52 Abs that bound to a peptide in the coil domain of Ro52 correlated with morbidity and severity of ILD in rheumatic diseases.[26] Yamaguchi et al[27] reported that anti-MDA5-positive CADM patients with myositis-associated autoantibodies (MAAs) had a better prognosis than those without MAAs. In this study, CADM patients had various autoantibodies, such as anti-SSA/Ro52 Ab (5/9) Abs and anti-CCP Ab (5/9) Abs in addition to MAAs. These findings are contrasting to our data demonstrating the worth survival in MDA5+ DM patients with anti-SSA/Ro52 Ab. The differential clinical phenotype of the subjected patients may
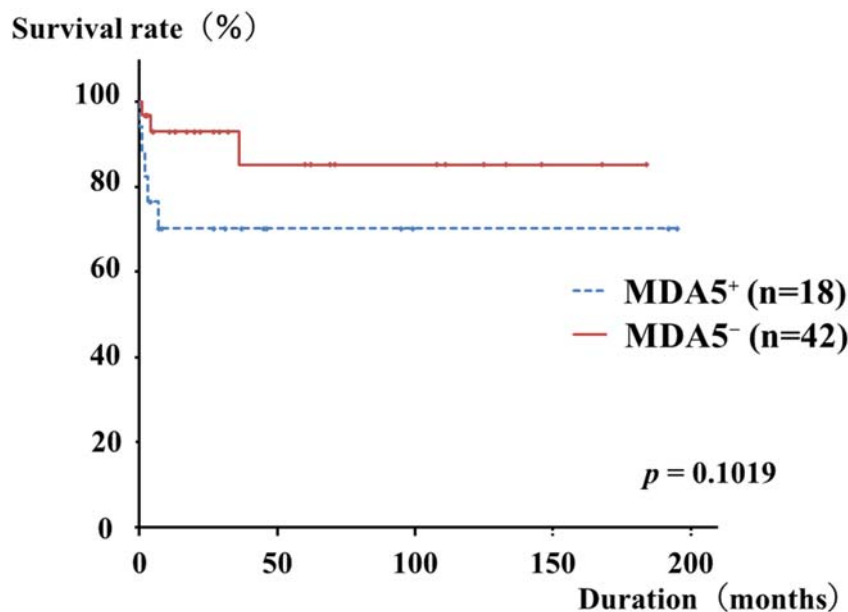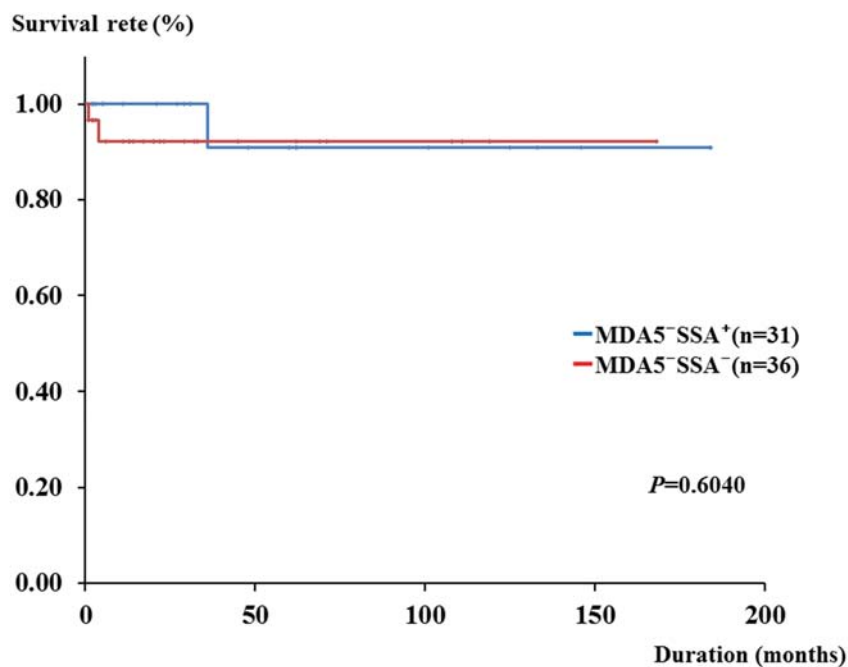

Figure 1. Kaplan–Meier curves of survival of DM patients with or without anti-MDA5 antibodies. DM patients are stratified by the presence or absence of anti-MDA5 Ab. DM patients with anti-MDA5 Ab seemed to be associated with worse survival compared with those without anti-MDA5 Ab, whereas statistically significant difference was not observed (P=.102, log-rank test).
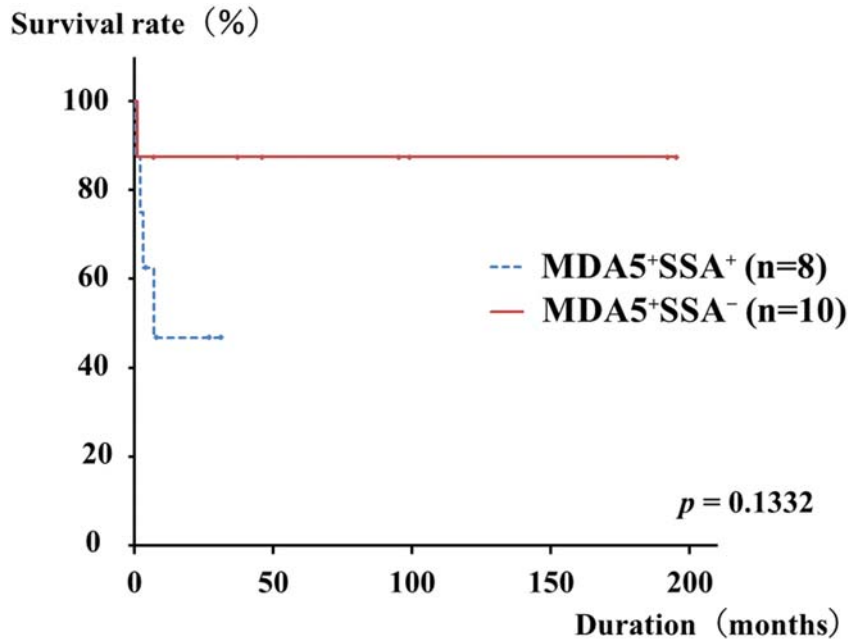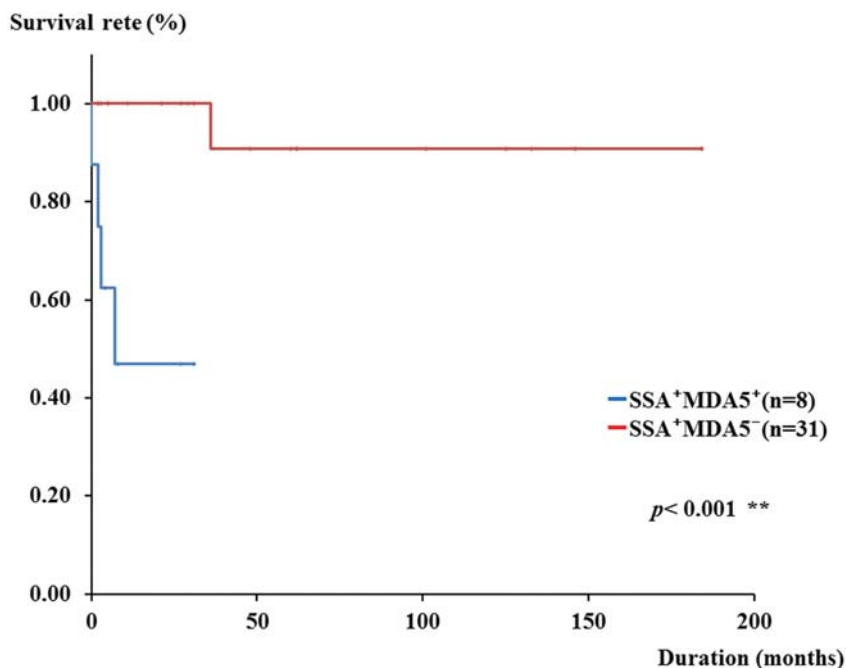
Figure 2. Kaplan–Meier curves of survival of anti-MDA5 Ab-positive DM patients with or without anti-SSA/Ro52 antibodies. Anti-MDA5 Ab-positive DM patients are stratified by the presence or absence of anti-SSA/Ro52 Ab. Anti-MDA5 Ab-positive DM patients with anti-SSA/Ro52 Ab seemed to be associated with worse survival compared with those without anti-SSA/Ro52 Ab, whereas statistically significant difference was not observed ($P = .133$, log-rank test).

contribute to the differential clinical outcomes of anti-MA5+ CADM or DM patients between this previous study and our study. More recently, the coexistence of anti-SSA/Ro52 Ab was associated with the severity of ILD in anti-MDA5-positive CADM patients.[28] In accord to this report, our data suggest that anti-SSA/Ro52 antibodies could be associated with anti-MDA5 Ab, which may result in the poor prognosis of DM patients. Further large-scale prospective studies will be required to determine the pathogenic role of anti-SAA/Ro52 Ab in patients with DM.

Figure 3. Kaplan–Meier curves of survival of anti-MDA5 Ab-negative DM/PM patients with or without anti-SSA/Ro52 antibodies. Anti-MDA5 Ab-negative patients are stratified by the presence or absence of anti-SSA/Ro52 Ab. There was no significant difference in survival between anti-MDA5 Ab-negative DM/PM patients with or without anti-SAA/Ro52 Ab ($P = .604$, log-rank test).

Figure 4. Kaplan–Meier curves of survival of anti-SAA/Ro52 Ab-positive patients with or without anti-MDA5 antibodies. Anti-SAA/Ro52 Ab-positive patients are stratified by the presence or absence of anti-MDA5 Ab. Anti-SAA/Ro52 Ab-positive patients with anti-MDA5 Ab are associated with significantly worse survival compared with those without anti-MDA5 Ab ($P < .001$, log-rank test).

This study had several limitations. First, given its retrospective design, the study was subjected to several possible biases. Second, the sample size was small because of the relative rarity of PM/DM. Thus, we could not identify independent risk factors by multivariate analysis. Third, patients who were positive for anti-ARS Abs were analyzed collectively as the anti-ARS-positive group and detailed analysis of MSAs, such as anti-PL-7, anti-TIFI-γ, anti-Mi-2, and anti-SRA antibodies, were not performed. Owing to the retrospective nature of the study, pulmonary function tests or follow-up testing with computed tomography were not available. Our study focused on patients with anti-ARS, anti-MDA5, and anti-SSA/Ro-52 Abs, making our results less generalized than PM/DM patients with other autoantibodies. Among total patients, 5 patients (PM 3, DM 2) were diagnosed as having Sjögren syndrome according to the 2016 American College of Rheumatology/European League Against Rheumatism diagnostic criteria.[29] Therefore, it is possible that the coexistence of Sjögren syndrome affects the clinical outcome of anti-MDA5-positive DM patients. These 5 patients with Sjögren syndrome did not have anti-MDA5 Ab. Whereas we cannot rule out the coexistence of Sjögren syndrome in rest of the patients completely, because we performed the minor salivary gland biopsy in a part of the patients. Our results may be affected by indication bias because the treatments were determined at the discretion of the participating physicians, and clinical information was obtained from patient's medical record. Finally, during the study period, the treatment regimen against PM/DM or PM/DM-ILD was changed, which may have affected the clinical course of these diseases.

5. Conclusions

In conclusion, we observed high frequencies of anti-SAA/Ro-52 Ab in patients with PM/DM. Anti-ARS or anti-MDA5 Abs were correlated with the phenotype of ILD and its prognosis in DM patients. Additionally, we found the presence of anti-SSA/Ro-52 Ab to be associated with poor prognosis in a subset of anti-MDA5 Ab-positive PM/DM patients. Further large-scale prospective studies are needed to clarify whether anti-Ro-52 antibody is associated with the clinical phenotype of PM/DM, particularly in patients stratified by anti-MDA5 Ab seropositivity.

Author contributions

Conceptualization: Kiyoshi Migita, Jumpei Temmoku, Shuzo Sato, Atsushi Kawakami.

Data curation: Shuzo Sato, Yuya Fujita, Tomoyuki Asano, Eiji Suzuki, Takashi Kanno, Makiko Yashiro Furuya, Naoki Matsuoka, Hiroko Kobayashi, Hiroshi Watanabe, Toshimasa Shimizu.

Investigation: Yuya Fujita, Tomoyuki Asano, Tomohiro Koga.

Methodology: Makiko Yashiro Furuya, Tomohiro Koga.

Project administration: Tomohiro Koga, Toshimasa Shimizu.

Resources: Eiji Suzuki, Atsushi Kawakami.

Software: Tomoyuki Asano.

Supervision: Kiyoshi Migita, Hiroko Kobayashi, Hiroshi Watanabe.

Writing – original draft: Jumpei Temmoku, Atsushi Kawakami.

Writing – review & editing: Atsushi Kawakami.

References

[1] Cavazzana I, Fredi M, Selmi C, et al. The clinical and histological spectrum of idiopathic inflammatory myopathies. Clin Rev Allergy Immunol 2017;52:88–98.

[2] Hirakata M, Nagai S. Interstitial lung disease in polymyositis and dermatomyositis. Curr Opin Rheumatol 2000;12:501–8.

[3] Gerami P, Schope JM, McDonald L, et al. A systematic review of adult-onset clinically amyopathic dermatomyositis (dermatomyositis siné myositis): a missing link within the spectrum of the idiopathic inflammatory myopathies. J Am Acad Dermatol 2006;54:597–613.

[4] Satoh M, Tanaka S, Ceribelli A, et al. A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. Clin Rev Allergy Immunol 2017;52:1–9.

[5] Sato S, Hoshino K, Satoh T, et al. RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: association with rapidly progressive interstitial lung disease. Arthritis Rheum 2009;60:2193–200.

[6] Nakashima R, Imura Y, Kobayashi S, et al. The RIG-I-like receptor IFIH1/MDA5 is a dermatomyositis-specific autoantigen identified by the anti-CADM-140 antibody. Rheumatology (Oxford) 2010;49:433–40.

[7] Koga T, Fujikawa K, Horai Y, et al. The diagnostic utility of anti-melanoma differentiation-associated gene 5 antibody testing for predicting the prognosis of Japanese patients with DM. Rheumatology 2012;51:1278–84.

[8] Horai Y, Koga T, Fujikawa K, et al. Serum interferon-alpha is a useful biomarker in patients with anti-melanoma differentiation-associated gene 5 (MDA5) antibody-positive dermatomyositis. Mod Rheumatol 2015;25:85–9.

[9] Levine SM, Rosen A, Casciola-Rosen LA. Anti-aminoacyl tRNA synthetase immune responses: insights into the pathogenesis of the idiopathic inflammatory myopathies. Curr Opin Rheumatol 2003;15:708–13.

[10] Hozumi H, Enomoto N, Kono M, et al. Prognostic significance of anti-aminoacyl-tRNA synthetase antibodies in polymyositis/dermatomyositis-associated interstitial lung disease: a retrospective case control study. PLoS One 2015;10:e0120313.

[11] Nakashima R, Imura Y, Hosono Y, et al. The multicenter study of a new assay for simultaneous detection of multiple anti-aminoacyl-tRNA synthetases in myositis and interstitial pneumonia. PLoS One 2014;9:e85062.

[12] 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.

[13] Gono T, Kaneko H, Kawaguchi Y, et al. Cytokine profiles in polymyositis and dermatomyositis complicated by rapidly progressive or chronic interstitial lung disease. Rheumatology 2014;53:2196–203.

[14] Isoda K, Kotani T, Takeuchi T, et al. Comparison of long-term prognosis and relapse of dermatomyositis complicated with interstitial pneumonia according to autoantibodies: anti-aminoacyl tRNA synthetase antibodies versus anti-melanoma differentiation-associated gene 5 antibody. Rheumatol Int 2017;37:1335–40.

[15] Defendenti C, Atzeni F, Spina MF, et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. Autoimmun Rev 2011;10:150–4.

[16] Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292:403–7.

[17] Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344–7.

[18] Hall JC, Casciola-Rosen L, Samedy LA, et al. Anti-melanoma differentiation-associated protein 5-associated dermatomyositis: expanding the clinical spectrum. Arthritis Care Res (Hoboken) 2013;65:1307–15.

[19] Chen Z, Cao M, Plana MN, et al. Utility of anti-melanoma differentiation-associated gene 5 antibody measurement in identifying patients with dermatomyositis and a high risk for developing rapidly progressive interstitial lung disease: a review of the literature and a meta-analysis. Arthritis Care Res 2013;65:1316–24.

[20] Boitiaux JF, Debray MP, Nicaise-Roland P, et al. Idiopathic interstitial lung disease with anti-SSA antibody. Rheumatology 2011;50:2245–50.

[21] La Corte R, Lo Mo Naco A, Locaputo A, et al. In patients with antisynthetase syndrome the occurrence of anti-Ro/SSA antibodies causes a more severe interstitial lung disease. Autoimmunity 2006;39:249–53.

[22] Schulte-Pelkum J, Fritzler M, Mahler M. Latest update on the Ro/SS-A autoantibody system. Autoimmun Rev 2009;8:632–7.

[23] Ghillani P, André C, Toly C, et al. Clinical significance of anti-Ro52 (TRIM21) antibodies non-associated with anti-SSA 60kDa antibodies: results of a multicentric study. Autoimmun Rev 2011;10:509–13.

[24] Yamasaki Y, Satoh M, Mizushima M, et al. Clinical subsets associated with different anti-aminoacyl transfer RNA synthetase antibodies and their association with coexisting anti-Ro52. Mod Rheumatol 2016;26:403–9.

[25] Tatebe N, Sada KE, Asano Y, et al. Anti-SS-A/Ro antibody positivity as a risk factor for relapse in patients with polymyositis/dermatomyositis. Mod Rheumatol 2018;28:141–6.

[26] Okumura M, Ozawa T, Hamana H, et al. Autoantibodies reactive to PEP08 are clinically related with morbidity and severity of interstitial lung disease in connective tissue diseases. Eur J Immunol 2018;48:1717–27.

[27] Yamaguchi K, Yamaguchi A, Kashiwagi C, et al. Differential clinical features of patients with clinically amyopathic dermatomyositis who have circulating anti-MDA5 autoantibodies with or without myositis-associated autoantibodies. Respir Med 2018;140:1–5.

[28] Huang W, Ren F, Wang Q, et al. Clinical features of thirty-two patients with anti-melanoma differentiation-associated gene 5 antibodies. Clin Exp Rheumatol 2019;[Epub ahead of print].

[29] Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European League against rheumatism classification criteria for primary Sjögren syndrome: a consensus and data-driven methodology involving three international patient cohorts. Arthritis Rheumatol 2017;69:35–45.