summing the scores of 3 HAMD subscales for insomnia (initial, middle, and delayed insomnia, each value: 0–2). Daily-living activity was evaluated using Performance Status according to the Eastern Cooperative Oncology Group. Patients also completed a questionnaire regarding pain and fatigue using visual analog scales (VAS) for pain and fatigue (0–100 mm, 100 mm being most severe).

Assessment of disease activity and neurological/immunological markers. Patient records were reviewed by an experienced rheumatologist (MH). Disease duration was defined as the time between onset of SLE-attributable symptoms and assessment. Global SLE disease activity was evaluated using the SLE Disease Activity Index 2000 (SLEDAI-2K)[32] at the same time as the neurocognitive assessment or within the preceding 10 days. Anti-dsDNA antibodies and serum complement CH50 were measured as markers of disease activity. Based on published reports regarding NPSLE including NCI[8], the following potentially relevant laboratory and neurologic variables were selected: aPL, cerebrospinal fluid (CSF) tests [immunoglobulin G index, interleukin 6 (IL-6), IL-8, and interferon-α], magnetic resonance imaging (MRI) of the brain, and electroencephalography (EEG). Of the 43 patients, CSF tests were performed in 37, brain MRI in 41, and EEG in 41. These neuropsychological, laboratory, and neurological tests were completed within 1 week after admission and before corticosteroid or other immunosuppressive therapies were administered. Control participants completed the neuropsychological tests, but did not have MRI of the brain, EEG, or CSF tests.

Statistical analyses. For the univariate analyses, a non-parametric Mann-Whitney U test was used to identify differences between groups for continuous variables, and Fisher's exact test was used for categorical variables. To identify (1) neuropsychological variables that best discriminated between the patients with SLE and the healthy controls; and (2) independent risk factors for NCI in patients with SLE, multiple logistic regression analysis was performed, with forward stepwise variable selection. Variables from the univariate analyses with $p < 0.25$ were entered into a forward logistic regression model. Regression coefficients were used to calculate the OR and 95% CI of the OR. In all statistical analyses, p values < 0.05 were considered statistically significant. We performed all analyses using the SPSS Statistics 17.0 (SPSS Inc.).

RESULTS

Prevalence and profile of NCI. Although MMSE scores were significantly lower in the SLE group than in the control group ($p = 0.017$), both groups had a median score of 30 (ranges, 26–30 and 26–30; interquartile range, 29–30 and 30–30, respectively; Table 1). However, while 40% (17/42) of the patients scored below 30 on the MMSE, only 17% (5/30) of healthy controls did.

NCI was identified in significantly more patients with SLE (12, 27.9%) than normal controls (2, 6.7%; $p = 0.033$). Univariate analysis of the group comparison using the Mann-Whitney U test indicated that patients with SLE showed significantly worse scores for the following tests than control subjects: RAVLT Trials 1 to 5 ($p = 0.022$), reflecting immediate recall; Trail Making Test, Part B ($p = 0.001$), reflecting complex attention/executive function; Trail Making Test, Part A ($p = 0.001$); and Digit Symbol Substitution ($p < 0.001$), both reflecting psychomotor speed (Table 3).

The multiple logistic regression analysis showed that Digit Symbol Substitution was the only variable that could be used to differentiate patients with SLE from controls. The OR for Digit Symbol Substitution was 0.924 (95% CI, 0.881–0.969, $p = 0.001$).

Risk factors for NCI in patients with SLE. Patients were divided into 2 groups according to the presence or absence of NCI, and demographic, psychological/health characteristics, and neurological/immunological markers were compared between the 2 groups (Table 4). Among these items, only SLEDAI-2K score was significantly higher in patients with NCI than in those without ($p = 0.022$). No significant differences between the 2 groups were found in age, sex, education, disease duration, time since SLE diagnosis, performance status, VAS pain/fatigue, mood state (HAMD-17, HAMA, POMS scores), or sleep disturbance. Neither were significant differences between the 2 groups found in any of the neurological/immunological markers.

The multiple logistic regression analysis showed that SLEDAI-2K was the only independent risk factor for NCI in SLE patients. The OR for SLEDAI-2K was 1.141 (95% CI, 1.001–1.300, $p = 0.048$).

DISCUSSION

Although results from our present study did not directly establish whether SLE-associated NCI was related to corticosteroid treatment, there were 3 major findings. First, the prevalence of NCI in corticosteroid-naive patients with SLE without overt NP symptoms was much higher than that of healthy control subjects. Second, the NCI was associated with general SLE disease activity as assessed by the SLEDAI-2K but not with other relevant laboratory and neurologic variables. Third, multivariate analysis showed that the dominant pattern of NCI in this population was a decrease in psychomotor speed.

The prevalence rate of NCI observed here (28%) was lower than that of studies[33,34,35,36,37] using similar neuropsychological test batteries[1]. We speculate that this reflects nonuse of corticosteroids and shorter disease durations, which were characteristic of the patients in our study. Regular treatment with prednisone has been reported to be associated with decreased cognitive functioning in patients with SLE[21]. Although a metaanalysis using a random-effects model demonstrated that the prevalence rate for NCI was estimated to be 19.7% (95% CI 10.7%–36%), NCI prevalence was interpreted as underestimated because the analysis included studies that did not involve formal neuropsychological testing[38]. When proper neuropsychological testing was conducted in patients with SLE, the prevalence of cognitive dysfunction was much higher (23–60% in most series)[33,34,35,36,37].

Here, NCI was associated with general SLE disease activity as assessed by the SLEDAI-2K. Past studies regarding the association between disease activity and NCI in patients with SLE have drawn conflicting conclusions. While some studies[6,39,40] are consistent with our results and show that higher disease activity is an independent predictor of NCI in patients with SLE, other studies[5,19,41] have not. Unlike other studies, most patients here had overall disease

Table 3. Neuropsychological performances of corticosteroid-naive patients with SLE versus healthy controls: univariate analysis. Data are median (interquartile range).

Neurocognitive Tests	Patients with SLE, n = 43	Controls, n = 30	p
Digit Span, Forward[a]	8 (7–9)	8 (6.8–9.3)	0.814
Trail Making Test, Part B[b]	75 (65–95)	60.5 (54.8–78.3)	0.001[c]
Digit Span, Backward[a]	7 (6–8)	7 (6–8.3)	0.977
KWCST			
Categories achieved	5 (1–6)	5 (3.8–6)	0.148
Total errors[b]	13 (9.8–20.3)	11 (10–13.3)	0.126
Perseverative errors[b]	0 (0–2)	0 (0–1)	0.190
Difficulty of maintaining set[b]	1 (0–3)	0 (0–2)	0.144
RAVLT			
Trial I to V, immediate recall	55 (48–61)	60 (53.8–64)	0.022[c]
Trial VII, delayed recall	13 (11–14)	13 (12–14)	0.256
Recognition	14 (13–15)	14 (14–15)	0.722
Block Design[a]	45 (42–49)	47 (42.8–51.5)	0.090
Word Fluency Test (Animal Naming)	17 (16–20)	18.5 (17–22.3)	0.099
Similarities[a]	18 (16–21)	20 (17.5–22.3)	0.066
Trail Making Test, Part A[b]	65 (54–78)	51.5 (43.8–59.8)	0.001[c]
Digit Symbol Substitution[a]	65 (61–73)	79 (68.8–85.3)	< 0.001[c]

P values were determined by Mann-Whitney U test. [a] Subsets of the Wechsler Adult Intelligence Scale-Revised. [b] Higher score signifies worse function. [c] Significant variables. KWCST: Wisconsin Card Sorting Test, Keio version; RAVLT: Rey Auditory-Verbal Learning Test.

activity (SLEDAI-2K ≥ 6 in 88.4%) that was moderate or high and free from the potential influence of medication. Thus we assert that a positive association between NCI and disease activity was clearly shown.

Additionally, because the disease duration of our patients was not long and because they had no history of corticosteroid therapy, most patients did not have disease-related or treatment-related damage, which have been reported to be the main factors affecting severity of cognitive impairment in SLE[37]. Although uncomfortable disease-related physical and emotional symptoms derived from high disease activity might have affected cognitive performance, we found no association between NCI and pain, fatigue, mood state, or sleep disturbance.

In patients with SLE, notable deficits appear in attention, information processing, learning and memory, and executive function[8]. Here, although univariate analysis demonstrated deficits in verbal memory, complex attention/executive function, and psychomotor speed in the corticosteroid-naive patients, multivariate analysis demonstrated a deficit only in psychomotor speed as assessed by the Digit Symbol Substitution Test. A lower score on this test has been reported in patients with SLE[4,20,35,42] and NPSLE[4,35] and is currently considered to reflect difficulty in visually guided active and speedy psychomotor coordination. Impairment seen here in Trail Making Tests A and B may reflect similar problems with speedy visuomotor ability, despite not being statistically significant. In fact, Glanz, *et al*[42] and Kozora, *et al*[35] found that patients with SLE performed worse than controls on the Digit Symbol Substitution Test and on Trail Making Tests A and B. Lower psychomotor speed may result from reduction in corpus callosum volume or other white matter abnormalities[43]. Indeed, injury to white matter myelin may underlie the earliest cognitive dysfunction observed in SLE[8].

Regarding memory, the analysis demonstrated that patients with SLE were impaired in serial verbal learning, but were comparable to control subjects in their delayed recall and recognition. Accordingly, we speculate that their ability to memorize or learn new facts *per se* would be well preserved. Impaired serial learning may reflect problems with time constraints and high-load cognitive activities similar to the Trail Making Tests and Digit Symbol Substitution Test. These findings are consistent with those in the literature suggesting deficits in declarative (verbal) memory after acute (high-dose) and chronic (relatively low-dose) corticosteroid use[12]. Therefore, similar memory deficits that have been reported in patients with SLE might also have their roots in corticosteroid therapy.

Results from our present study suggest the need to consider the presence of NCI in early SLE when determining treatment. Because autoantibodies appear long before clinical symptoms and have been associated with inflammation in SLE[44], they may also play a relevant role in the development of NCI in patients with early SLE. Although persistent elevation of aPL has been consistently reported as a significant risk factor for SLE-associated NCI[21,39,45,46,47], we found no association between NCI and aPL. Other relevant autoantibodies associated with NPSLE such as anti-NR2 or antiribosomal P protein were not

Table 4. Health/psychological and clinical characteristics of patients with SLE who have neurocognitive impairment versus those who do not. Data are no./no. assessed (%) or median (interquartile range).

Variables	Impaired, n = 12	Not Impaired, n = 31	p
Demographics			
Age, years	34.5 (28–38.8)	26 (21–33)	0.086
Sex, female/male	12/0	30/1	> 0.999
Education, years	12 (11.3–15.5)	14 (12–15)	0.328
Health/psychological characteristics			
Disease duration, mos	9 (5–38)	14 (5–35)	0.841
Time since SLE diagnosis, mos	0 (0–0)	0 (0–0)	0.542
SLEDAI-2K	15.5 (9.5–21.3)	9.0 (8.0–14.0)	0.022[a]
Performance Status[b]	1 (0–1)	0 (0–1)	0.063
Pain, VAS, mm	51.5 (7.5–66.0)	23.0 (9.0–40.0)	0.174
Fatigue, VAS, mm	39.0 (2.0–65.0)	25.0 (13.0–57.0)	0.841
HAMD-17, total	5.0 (1.3–9.5)	2.0 (1.0–5.0)	0.126
HAMA, total	3.5 (2.3–10.0)	3.0 (1.0–5.0)	0.246
POMS			
Tension-Anxiety	15.0 (6.0–21.8)	14.0 (9.0–19.0)	0.989
Depression-Dejection	12.0 (5.3–22.3)	13.0 (6.0–22.0)	0.924
Anger-Hostility	5.0 (1.0–13.3)	5.0 (3.0–15.0)	0.495
Vigor	5.5 (0.3–14.5)	9.0 (5.0–11.0)	0.212
Fatigue	12.0 (7.0–20.5)	10.0 (6.0–14.0)	0.260
Confusion	9.5 (6.5–12.5)	8.0 (6.0–14.0)	0.871
Total mood disturbance	47.5 (19.0–75.8)	44.0 (22.0–68.0)	0.862
Sleep disturbance[c]	0 (0–2)	0 (0–1)	0.655
Clinical characteristics			
Anti-DNA antibody, IU/ml	36 (13–196)	39 (9–97.3)	0.691
CH50, U/ml	12.6 (10.0–33.1)	16.2 (10.0–31.4)	0.964
Antiphospholipid antibody, positive[d]	1/12 (8.3)	13/31 (41.9)	0.067
Cerebrospinal fluid tests			
IgG index, positive (normal < 0.70)	0/10 (0)	6/27 (22.2)	0.162
Interleukin 6, pg/ml	2.9 (1.2–9.7)	3.3 (1.4–7.1)	0.973
Interleukin 8, pg/ml	62.1 (27.5–133.8)	42.0 (22.4–125.6)	0.638
Interferon-α, IU/l	0 (0–5.7)	0 (0–14.8)	0.349
Brain MRI, abnormal	2/12 (16.7)	4/29 (13.8)	> 0.999
Electroencephalogram, abnormal	5/12 (41.7)	13/29 (44.8)	> 0.999

P values were determined by Fisher's exact test or Mann-Whitney U test. [a] Significant variable. [b] Defined by Eastern Cooperative Oncology Group criteria. [c] Total scores of sleep-related 3 items in HAMD-17. [d]Antiphospholipid antibodies include anticardiolipin-β_2-glycoprotein-I complex and SLE anticoagulant. SLE: systemic lupus erythematosus; SLEDAI-2K: SLE Disease Activity Index 2000; VAS: visual analog scale; HAMD-17: Hamilton Depression Rating Scale–17 item; HAMA: Hamilton Anxiety Rating Scale; POMS: Profile of Mood States; MRI: magnetic resonance imaging; aPL: antiphospholipid antibodies.

evaluated in our present study. There have been both negative[36,48] and positive[49] findings for the association between anti-NR2 and NCI, while no association has been reported between antiribosomal P protein antibodies and NCI[8,50]. Thus, we think that monitoring levels of these autoantibodies may be crucial for clarifying the pathogenesis of NCI in early SLE. Further studies are certainly needed.

The strengths of our study are the early-stage, corticosteroid-naive SLE population, an appropriate neuropsychological test battery, and the use of multivariate methods to identify specific patterns and predictive factors for NCI in patients with SLE. However, our study has several limitations. First, because the published normative data for the psychological tests were limited in Japanese representation, we used our data from control subjects as a reference for the normal population. The number of control subjects was relatively small, and may not be the best estimate of population data. Second, because this study used a cross-sectional design, it lacked longitudinal analysis that could have been helpful for defining predictors of neurocognitive deficits. Third, the power in this study was relatively low for identifying the specific patterns and detecting predicting factors for NCI in patients with SLE. Fourth, because the study subjects were limited to Japanese people who spoke Japanese, our findings may not be applicable to other ethnicities and languages. Fifth, because results from our study were limited to relatively young patients with SLE, the findings may not be applicable for all patients with SLE, in particular those with longer disease duration.

The dominant NCI in a corticosteroid-naive SLE population was decreased psychomotor speed that was associated with higher general SLE disease activity. Verbal-memory deficits that have been reported in patients with SLE were not evident. Results from our study suggest that impaired psychomotor speed may be added to the symptoms of early SLE. Further followup studies using larger sample sizes are needed.

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Recent Treatment of Interstitial Lung Disease with Idiopathic Inflammatory Myopathies

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Supplementary Issue: Current Developments in Interstitial Lung Disease

ABSTRACT: Interstitial lung disease (ILD) is a prognostic factor for poor outcome in polymyositis (PM)/dermatomyositis (DM). The appropriate management of ILD is very important to improve the prognosis of patients with PM/DM. ILD activity and severity depend on the disease subtype. Therefore, clinicians should determine therapeutic strategies according to the disease subtype in each patient with PM/DM. Anti–melanoma differentiation-associated gene 5 antibody and hyperferritinemia predict the development and severity of rapidly progressive (RP) ILD, particularly in East Asian patients. Combination therapy with corticosteroids, intravenous cyclophosphamide pulse, and calcineurin inhibitors should be administered in RP-ILD. In contrast, patients with anti–aminoacyl-tRNA synthetase (ARS) show better responses to corticosteroids alone. However, ILDs with anti-ARS often display disease recurrence or become refractory to corticosteroid monotherapy. Recent studies have demonstrated that the administration of tacrolimus or rituximab in addition to corticosteroids may be considered in ILD patients with anti-ARS. Large-scale, multicenter randomized clinical trials should be conducted in the future to confirm that the aforementioned agents exhibit efficacy in ILD patients with PM/DM. The pathophysiology of ILD with PM/DM should also be elucidated in greater detail to develop effective therapeutic strategies for patients with ILD in PM/DM.

KEYWORDS: interstitial lung disease, idiopathic inflammatory myopathies, dermatomyositis, polymyositis, treatment

SUPPLEMENT: Current Developments in Interstitial Lung Disease

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Introduction

Polymyositis (PM)/dermatomyositis (DM) are idiopathic inflammatory myopathies (IIM) that are occasionally complicated with extramuscular lesions, such as interstitial lung disease (ILD), cardiomyopathy, and malignancy. These complications are poor prognostic factors in PM/DM patients. ILD is found in approximately 50% of patients with PM/DM.[1] ILD associated with DM is more refractory to treatment and more strongly associated with a poorer prognosis than ILD associated with PM.[2] Recent research has revealed that myositis-specific autoantibodies (MSAs) are closely linked to clinical phenotypes in PM/DM. ILD activity and severity are dependent on the subtype of PM/DM. Therefore, physicians should evaluate MSAs and determine the therapeutic strategies for ILD according to the subtype of PM/DM in each patient.

Classification of ILD with PM/DM

ILD is classified by clinical course or pathohistological findings. The clinical course of ILD is divided into two subtypes: acute/subacute interstitial pneumonia (A/SIP), also called rapidly progressive ILD (RP-ILD), and chronic IP (CIP). The pathohistological classification includes usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), organizing pneumonia (OP), diffuse alveolar damage (DAD), desquamative interstitial pneumonia, and lymphoid interstitial pneumonia.[3] NSIP is a typical form of ILD in PM/DM patients. UIP, OP, and DAD are also complicated in patients with PM/DM.[4,5] DAD is typically characterized by RP-ILD, and pulmonary function deteriorates daily or weekly in RP-ILD with PM/DM patients. NSIP is usually recognized as CIP. ILD develops gradually (monthly) in CIP but develops acutely/subacutely in some cases. Some patients with NSIP or UIP with PM/DM do not exhibit respiratory symptoms, although ILD is demonstrated by radiological imaging, such as X-ray radiography and/or high-resolution computed tomography (HRCT).

Clinical Characteristics of ILD with PM/DM

The measurement of MSAs is useful for predicting clinical course, clinical characteristics, response to treatment, and prognosis in PM/DM patients. The MSAs that are strongly associated with ILD include the anti–melanoma differentiation-associated gene 5 (MDA5) and anti–aminoacyl-tRNA synthetase (ARS) antibodies (Table 1).[6,7]

The presence of anti-MDA5 is associated with clinically amyopathic DM (CADM),[8,9] especially cutaneous ulcers.

Table 1. Differences in clinical characteristics between anti-MDA5 and anti-ARS antibodies in PM/DM.

	ANTI-MDA5	ANTI-ARS
Type of PM/DM	CADM	PM/DM/CADM
Clinical course of ILD	RP*	Chronic progressive
Response to corticosteroids in ILD	Poor*	Favorable
Recurrence of ILD	Rare*	Frequent

Note: *These findings are frequently found in East Asia. ILD in Caucasians is generally not RP. Clinical manifestations may depend on race.

CADM involves the typical skin lesions that are revealed in DM with amyopathy or hypomyopathy. Therefore, patients with CADM do not typically present with muscle symptoms, such as myalgia and muscle weakness.[10] The risk of ILD development is increased in patients with anti-MDA5 in East Asian and Western countries.[8,9,11–13] However, the clinical course and severity of ILD are different between Asians and Westerners. ILD in Japan and China is generally RP and frequently causes fatal outcomes in patients with anti-MDA5. RP-ILD is not frequently found in the United States and Europe. The clinical characteristics of ILD may depend on race in patients with anti-MDA5. Approximately half of patients with anti-MDA5 present with RP-ILD in East Asia.[8,14,15] RP-ILD is generally complicated with CADM, although it is not found in all patients with CADM. Anti-MDA5 is also not found in all patients with CADM. Therefore, anti-MDA5 is a useful predictor for the complication of RP-ILD in patients with CADM, especially in East Asia. Ferritin is the other useful predictive marker for RP-ILD, and serum ferritin levels predict the development and severity of RP-ILD in PM/DM patients. Hyperferritinemia is frequently found in RP-ILD with anti-MDA5, and serum ferritin levels correlate with the disease activity of ILD with anti-MDA5. Gono et al demonstrated that serum ferritin levels were higher in PM/DM patients with A/SIP than in patients with CIP.[16]

Anti-ARS is associated with clinical manifestations, including arthritis, mechanic's hand, Raynaud's phenomenon, myositis, and ILD, which are known as antisynthetase syndromes. This type of ILD generally manifests as NSIP or UIP and typically exhibits chronic progressive pulmonary dysfunction, which may acutely/subacutely deteriorate. ILD with anti-ARS exhibits a better response to corticosteroid therapy and better prognosis than ILD without anti-ARS in PM/DM.[17] However, corticosteroid therapy alone often causes a recurrence of myositis and/or ILD in patients with anti-ARS.[18] Therefore, combination therapy of corticosteroids and immunosuppressive agents should be considered.

Anti-ARS includes anti–Jo-1, anti-EJ, anti-OJ, anti–PL-7, anti–PL-12, anti-KS, anti-Zo, and anti-Ha. Hamaguchi et al demonstrated the similarities and differences of clinical manifestations in Japanese patients with individual anti-ARSs.[19] DM-specific rashes, such as heliotrope and Gottron's sign, have been frequently observed in patients with anti–Jo-1, anti-EJ, anti–PL-7, and anti–PL–12. ILD alone has been frequently found in patients with anti-OJ and anti-KS, and myositis is not complicated with ILD in some patients with anti-ARS.

Prognosis

The overall 5-year survival rate is 77%–95% in PM/DM,[20–22] and cardiac dysfunction, malignancy, respiratory failure, and infection are the major causes of fatal outcome. Previous studies reported that cardiac involvement and respiratory muscle involvement are significant prognostic factors for death in patients with PM/DM, except in patients with cancer.[22] Yamasaki et al retrospectively investigated 197 patients with PM, DM, and CADM.[1] Survival in the entire group at 1, 5, and 10 years was 85%, 75%, and 67%, respectively, and the mortality in CADM and DM was 61% and 77% at 5 years, respectively. DM patients exhibited significantly lower survival compared to PM (91% at 5 years), and ILD was the major cause of death in CADM (71%) and DM (60%) patients. Most of these patients died within the first few months.

The overall 5-year mortality rate of ILD with PM/DM patients is 50%.[23] Dankó et al reported survival curves of patients with PM/DM patients with and without ILD.[22] The cumulative survival rate was significantly worse in DM with ILD patients than DM without ILD patients, although there was no difference in survival rate between PM with and without ILD patients. One Japanese study revealed that acute/subacute ILD, forced vital capacity (FVC), age, neutrophils in bronchoalveolar lavage fluid, and CADM were significantly associated with poor outcome.[24] The overall 6-month survival rate of ILD with anti-MDA5 is 50%–60% in Japan.[14,15] Serum ferritin levels also predict clinical outcome in ILD with PM/DM. The survival rate in patients with serum ferritin levels higher than 1500 ng/mL on admission is lower than patients with levels less than 1500 ng/mL. The 10-year survival rate in another study was significantly higher in ILD patients with anti-ARS than in ILD patients without anti-ARS (91.6% vs. 58.7%). Therefore, prognosis is dependent upon clinical phenotype, serum ferritin levels, and MSAs.[17]

Efficacy of Each Drug in ILD with PM/DM

Corticosteroids and immunosuppressive agents are considered a first-line therapy in ILD with PM/DM patients. Some specialists recommend the use of immunosuppressive agents with corticosteroids for myositis as the first-line therapy because these drugs exhibit corticosteroid-sparing effects.[25,26] Immunosuppressive agents should be considered in PM/DM patients, particularly when ILD severely or progressively develops, to reduce the side effects of corticosteroids and improve the response to treatment. Combination therapy with corticosteroids and immunosuppressive agents is commonly used, although there have been no large randomized clinical

trials (RCTs) in ILD with PM/DM patients.[27,28] However, it is difficult to conduct RCTs because ILD with PM/DM is a relatively rare disease and is occasionally progressive and fatal. Herein, we review each agent that was previously reported as effective for the treatment of ILD with PM/DM. Table 2 summarizes the characteristics of each agent. We initially describe conventional agents for the treatment of ILD with PM/DM. Subsequently, we review the efficacy of novel agents from recent studies that provided evidence for the treatment of ILD with PM/DM, especially tacrolimus (TAC) and rituximab (RTX).

Conventional Agents

Corticosteroids. Frazier and Miller first reported the use of oral corticosteroids in ILD with PM/DM in 1974.[29] Oral high-dose corticosteroids (>1 mg/kg/day of prednisone) or pulse therapy of methylprednisolone (1000 mg intravenously for 3 days) are still used as a first-line therapy for ILD with PM/DM. Approximately half of patients respond well to initial corticosteroid therapy.[2,30–33] However, there is a difference in the response to corticosteroid monotherapy between ILD with PM patients and ILD with DM patients. Fujisawa et al investigated the differential responses of 28 ILD with PM/DM patients (16 PM and 12 DM) to the therapy.[2] Corticosteroid monotherapy achieved favorable responses in six (37.5%) ILD with PM patients but only one (8.3%) ILD with DM patient. The overall 2.5-year survival rate of ILD with DM patients was 58%, and the 5-year survival of ILD with PM patients was 81%. RP-ILD is generally not responsive to corticosteroid monotherapy.[8,9,14,34–36] RP-ILD is frequently complicated with CADM. Nawata et al reported that ILD with PM/DM patients with normal creatine kinase (CK) exhibited significantly more resistance to corticosteroid therapy and poorer prognosis than patients with high CK (1-year survival, 31% vs. 89%).[32] Patients with normal CK levels may present with the clinical manifestation of CADM. The efficacy of corticosteroids alone as an initial therapy for ILD is limited. Clinicians should also consider the corticosteroid-sparing effects of the immunosuppressive agents described below, although corticosteroids remain the standard therapy for ILD with PM/DM.

Table 2. Characteristics of each drug therapy for ILDs with PM/DM.

DRUG THERAPY	CHARACTERISTICS
Corticosteroids	First-line therapy for ILD with PM/DM.
	Corticosteroids monotherapy is generally not effective for RP-ILD with PM/DM.
MTX	Inhibitor of folic acid metabolism.
	Useful for corticosteroid-sparing agents.
AZA	A prodrug of 6-mercaptopurine, inhibition of purine synthesis.
	Useful for corticosteroid-sparing agents.
CY	Alkylating agent, nitrogen mustard derivative. IVCY is administered in RP-ILD or refractory ILD.
MMF	Anti-metabolite that blocks de novo purine synthesis and the production of B and T cells.
	Efficacy for corticosteroid-resistant ILD with PM/DM has been shown.
IVIG	The mechanism of drug action is variable.
	Efficacy for refractory myositis has been demonstrated.
	Efficacy was also found in several PM/DM cases with ILD.
CSA	CNI, one of the T-cell-targeting therapies.
	Cornerstone for the PM/DM-ILD treatment.
	Usually administered in antisynthetase syndrome or RP-ILD.
TAC	CNI. TAC has a 100-fold greater potency than CSA in inhibiting T-cell activation.
	Efficacy for CSA-refractory ILD in PM/DM has been demonstrated.
RTX	Chimeric monoclonal anti-CD20 antibody, B-cell targeting agent.
	Efficacy for refractory ILD or antisynthetase syndrome has been shown.

Abbreviation: CD, cluster of differentiation.

Methotrexate/azathioprine. Methotrexate (MTX) is an inhibitor of folic acid metabolism and suppresses T-cell activation and adhesion molecule expression.[37] MTX is widely used in the treatment of arthritis and myositis as an adjunctive agent after corticosteroid failure[38,39] or as a corticosteroid-sparing agent in PM/DM patients. There is no evidence of pulmonary-specific efficacy, although MTX is accepted in the treatment of ILD with PM/DM.[40,41] Azathioprine (AZA) is a prodrug of 6-mercaptopurine that inhibits purine synthesis. AZA prevents lymphocyte proliferation, which suppresses the production of antibodies and cytokines. AZA has been widely used in ILD with PM/DM patients as a corticosteroid-sparing agent in maintenance therapy after IVCY.[23,40,42,43]

Cyclophosphamide. Cyclophosphamide (CY) is used in RP or refractory ILD. CY is generally administered orally or intravenously and is commonly used in combination with corticosteroids. IVCY is a recent standard treatment for lupus and PM/DM because there are fewer adverse effects associated with monthly IVCY than the daily oral administration of CY. The efficacy of CY for the treatment of ILD with PM/DM was demonstrated in several case studies.[43–47] Yamasaki et al demonstrated the efficacy of IVCY in refractory ILD with PM/DM in a small open-label trial.[46] IVCY (300–800 mg/m^2, at least six times every 4 weeks) was administered in combination with corticosteroids (0.5–1 mg/kg/day) in 17 cases of refractory ILD with PM/DM. Eight of the 17 patients exhibited improvement in vital capacity, and 9 of 17 patients showed improved findings on HRCT. Mok et al reported improvement when CY was administered orally followed by AZA as a maintenance therapy in RP-ILD with DM patients.[48] CY has also

been used with other immunosuppressive agents in refractory ILD.[45,49] Kameda et al demonstrated the efficacy of combination therapy that included prednisolone (PSL), IVCY (10–30 mg/kg, every 3–4 weeks), and cyclosporin A (CSA, 2–4 mg/kg/day).[49] In this study, 10 DM patients with acute/subacute ILD were initially given combination therapy with IVCY, PSL, and CSA, and this group exhibited significantly lower mortality (50% vs. 75%) over 3 months compared to treatment with corticosteroids alone. However, five patients died of respiratory failure within 3 months. Biweekly IVCY administration with corticosteroids and calcineurin inhibitors (CNIs) was recently recommended in Japanese patients with RP-ILD who harbor hyperferritinemia and/or anti-MDA5.[50] Nakashima et al demonstrated that the 6-month survival rate was increased to 75% in patients with an early intensive combination therapy with corticosteroids, IVCY, and CNI than patients treated with conventional therapy. Therefore, early intensive combination therapy may improve prognosis in RP-ILD with PM/DM patients.

Mycophenolate mofetil. Mycophenolate mofetil (MMF) is an antimetabolite that blocks de novo purine synthesis and targets the production of activated B and T lymphocytes and fibroblasts. Several case series revealed the potential efficacy of MMF in the stabilization of progressive ILD and the reduction of corticosteroid dose in ILD with connective tissue disease (CTD) patients, including PM/DM.[42,51–58] An open trial of MMF was conducted in 28 patients with ILD in CTD, including five patients with PM/DM. These patients received MMF (30 mg/kg/day), although pulmonary function tests (FVC and diffusion lung capacity for carbon monoxide [DLco]) showed no significant improvement at 18-month follow-up.[52] In another case series, three patients with ILD in PM/DM who received corticosteroids and MMF showed improvement in ILD.[53]

A retrospective study was conducted to identify differences in the efficacy of AZA, CY, and MMF for corticosteroid-resistant ILD with PM/DM.[42] Thirteen, 24, and 9 patients were treated with AZA, CY, and MMF, respectively. There were no differences in baseline pulmonary function in each subset. Pulmonary function improved and the severity of dyspnea decreased in each subset at the 6-month assessment. The corticosteroid dose was also reduced. This result suggests that the addition of immunosuppressive agents is useful for refractory cases and corticosteroid-sparing effects in ILD with PM/DM patients.

Intravenous immunoglobulin. Intravenous immunoglobulin (IVIG) is widely used for the treatment of numerous autoimmune diseases. IVIG is variously involved in the suppression or neutralization of autoantibodies and cytokines and complements the blockade of several cell surface molecules and specific immune cell surface receptors.[59] The efficacy of IVIG in the treatment of refractory myositis has been demonstrated,[60,61] although its efficacy for ILD with PM/DM is not certain. One case series treated five patients with severe and refractory ILD in PM/DM with IVIG as a salvage therapy in combination with conventional treatments, including corticosteroids, IVCY, and CNIs.[62] Another case report demonstrated improvements in pulmonary function tests and chest CTs following the use of IVIG alone without other immunosuppressive therapies, including corticosteroids, in a patient with ILD in PM/DM.[63]

Plasmapheresis/hemoperfusion. Plasma exchange is used to remove circulating autoantibodies, cytokines, and immune complexes. There have been two case reports of the efficacy of plasmapheresis in patients with antisynthetase syndrome who were refractory to corticosteroids and other immunosuppressive therapies.[64,65] Lee et al reported a case series of DAD associated with PM/DM[66] in which plasmapheresis was performed, although all three patients died within 5 months after the development of ILD.

Recent reports suggest that hemoperfusion is promising in certain cases with ILD associated with PM/DM.[67–70] Direct hemoperfusion using a polymyxin B–immobilized fiber column (PMX-DHP) exhibits efficacy for Gram-negative–induced sepsis. PMX-DHP reduces endotoxin levels and inflammatory chemical mediators, such as cytokines.[71] Ichiyasu et al reported three cases of RP-ILD with CADM, in which the PaO_2/FiO_2 ratio and CT findings improved, and all patients survived after treatment with PMX-DHP.[67] The mechanism of the efficacy of PMX-DHP in RP-ILD is not sufficiently known, although PMX-DHP likely inhibits monocyte activation.[72]

Novel Agents

TAC and RTX have been the focus of novel treatments of ILD with PM/DM in the last several years. TAC is a CNI, and RTX is a biologic agent. The efficacies of other CNIs and biologic agents have been reported in ILD with PM/DM patients. These CNIs and biologic agents are described below.

Calcineurin inhibitors. CNIs are T-cell–targeting agents that may become the cornerstone for the treatment of ILD with PM/DM based on clinical findings. CNIs inhibit interleukin (IL)-2 production and T-cell proliferation. CNIs were recently indicated as an appropriate choice for the early phase of ILD with PM/DM. Zou et al. reported that CADM-acute ILD patients treated with CNIs exhibited a significantly better outcome than patients who received treatment without CNIs.[73] CNIs may improve the outcome of CADM patients with acute ILD. CSA and TAC are described below.

Cyclosporin A. Several retrospective and open-label studies analyzed the efficacy of CSA in ILD with PM/DM patients.[2,31,32,74,75] Takada et al reported a retrospective multicenter study of 38 cases with acute ILD with PM/DM. Patients who received a combination therapy of corticosteroids and CSA as a first-line therapy had a better survival rate than patients who received corticosteroids alone. Nine cases with ILD in PM and five cases with chronic ILD in DM exhibited good CSA efficacy and a good prognosis, whereas 17 cases with RP-ILD in DM showed poor prognosis, and 7 of these 17 patients died.[75] A further analysis of 32 cases with RP-ILD in DM demonstrated that 9 of 13 (69%) patients who

started CSA within 2 weeks of initial corticosteroid treatment survived, whereas all 17 cases who received only corticosteroids for more than 2 weeks as the initial therapy died within 9 months from the initiation of therapy. Another study revealed that the early use of cyclosporine was beneficial for survival outcome in DM-associated ILD. The mortality rate was significantly lower in the initial treatment group compared to the delayed-treatment group (0.02 person-years vs. 0.18 person-years; $P = 0.0092$, log-rank test).[76] These results suggest that combination therapy with CSA and corticosteroids during the early phase of ILD is superior to corticosteroid monotherapy in the treatment of ILD with PM/DM.

The monitoring of serum CSA concentrations is important for achieving maximum efficacy and reducing toxicity. There is marked interpatient variability in CSA absorption. Nagai et al. suggested that preprandial once-daily administration of CSA is beneficial, rather than twice daily, because C0 was significantly lower and adverse effects may be reduced using a once-daily administration of CSA.[77] The 2-hour postdose level (C2) was correlated with the therapeutic effect.[77,78] Recent studies indicated that the C2 level should reach 1000 ng/mL to achieve a maximal immunosuppressive effect.[79]

Tacrolimus. TAC has a 100-fold greater potency than CSA for the inhibition of T-cell activation. The medication concentration in blood is also more stable, and dose adjustments of medication are easier in TAC than CSA. Therefore, TAC is more often used than CSA in recent treatments of CTD, including ILD with PM/DM, especially in Japan. TAC was previously used in refractory ILD with PM/DM as an alternative to CSA. Several case series and retrospective studies demonstrated the efficacy and tolerability of TAC in ILD in PM/DM patients, including patients who were refractory to CSA.[75,80–85] Kurita et al reported the efficacy of TAC for the treatment of ILD with PM/DM. Forty-nine patients were treated with the addition of TAC to conventional therapy (25 cases) or conventional therapy alone (24 cases, PSL, IVCY, and/or CSA). The group treated with TAC exhibited significantly longer survival than the other group, although the concomitant use of IVCY was more frequent in the group treated with TAC than the other group. This study encourages the use of TAC in progressive or refractory ILD in which conventional treatments, such as corticosteroids and other immunosuppressive agents, have no efficacy.

TAC also appears more effective in ILD with anti-ARS patients.[81–83,86] Wilkes et al retrospectively assessed TAC efficacy in 13 patients with ILD harboring anti-ARS.[82] The authors suggested that TAC is a well-tolerated and effective therapy for the management of ILD with anti-ARS. Labirua-Iturburu et al demonstrated the efficacy of CNIs (TAC or CSA) for ILD management in 15 patients with anti-ARS.[86] A greater than 10% increase in FVC was observed in 13 patients treated with CNIs. Taken together, these reports demonstrate that CNIs are effective in refractory cases and as a first-line therapy in ILD with PM/DM patients.

Biologic agents. Biologic agents, such as anti-tumor necrosis factor (anti-TNF), anti–IL-6 receptor, and anti-CD20, have exhibited sufficient efficacies in improvements of disease status in rheumatoid arthritis. These agents were also used in PM/DM patients. The anti-CD20 antagonist RTX improved clinical outcome in PM/DM patients. Herein, we review recent studies of the efficacy of RTX or other biologics in PM/DM patients.

Rituximab. RTX is a biologic agent consisting of a chimeric monoclonal anti-CD20 antibody. This molecule targets B cells and results in B-cell depletion.[87] Several case reports and case series reported RTX efficacy in patients with refractory myositis or ILD in PM/DM.[88–94] Sem et al demonstrated the short-term efficacy of RTX in 11 patients with antisynthetase syndrome, including severe and progressive ILD, in a retrospective case series.[88] RTX stabilized or improved the disease activity of ILD in 7 of 11 patients during the first 6 months. Krystufková et al demonstrated that serum levels of B-cell–activating factor (BAFF) were significantly higher in patients with PM/DM, especially those patients with anti–Jo-1, DM, or ILD.[95] BAFF is necessary for B-cell maturation and function. These findings indicate that BAFF may also be a potential therapeutic target in patients with ILD in PM/DM. Aggarwal et al investigated predictors of clinical improvement in PM/DM patients treated with RTX.[96] Patients with anti–Mi-2 or anti-ARS exhibited greater improvement than patients with other MSAs, such as anti-signal recognition particle (anti-SRP), anti–TIF-1γ, and anti-MJ. Andersson et al reported the efficacy of ILD with anti-ARS in 24 ILD patients with anti-ARS.[97] Sixteen patients were treated with glucocorticoid steroids and/or immunosuppressive agents prior to RTX administration. Acute onset/exacerbation of ILD was revealed in 50% of these 24 patients. Pulmonary function, the extent of ILD, and myositis improved after RTX treatment in most patients with anti-ARS. These findings indicate that RTX was more effective in patients with antisynthetase syndrome with acute-onset ILD. RTX administration should be considered in patients with anti-ARS.

Anti-TNF agents. RCTs of anti-TNF agents were conducted to confirm improvements in myositis,[98] although the efficacy of anti-TNF agents is controversial. There have been no large RCTs of anti-TNF agents to investigate the efficacy of these agents in ILD with PM/DM. Therefore, only small case series are described below.

Infliximab (IFX) is a chimeric monoclonal antibody against soluble and membrane-bound TNF-α with a murine Fv region. There is only one relevant case report and a literature review that described a retrospective study of 14 patients with DM and acute ILD (10 CADM, 4 DM). These 14 patients received conventional immunosuppressive therapies and IFX (5 mg/kg).[99] IFX was administered at 5 mg/kg intravenously once weekly at weeks 0, 2, and 6, and every 8 weeks thereafter in available patients. Ten cases (71.4%) exhibited favorable responses in chest CT findings. These 10 patients were treated with IFX at an early stage of the disease. The other four

patients were treated with IFX after respiratory failure was progressive. These four patients died of respiratory failure.

Adalimumab is a fully human monoclonal antibody that successfully improved ILD with DM in a case report and exhibited marked improvements in DLco and radiographic findings.[100]

PM/DM may be induced or exacerbated in chronic inflammatory diseases, such as RA, during anti-TNF therapy.[101] One literature review revealed that 20 cases, including 17 RA patients, developed new PM/DM during anti-TNF therapies,[98] and myositis and ILD with anti-ARS was complicated in 6 cases. Thus, physicians should carefully consider the use of anti-TNF in PM/DM.

Symptomatic treatment. Symptomatic treatment should be considered in patients with irreversible lesions in the lung, such as severe pulmonary fibrosis, and patients with poor response to immunosuppressive therapy. Home oxygen therapy (HOT) is a valuable option for patients suffering from hypoxia associated with ILD, and HOT can improve functional performance in daily life. Pulmonary rehabilitation may also improve respiratory muscle strength and functional daily performance levels in patients with ILD.[102]

Therapeutic Strategy for ILD with PM/DM

Clinicians should determine when and how patients with ILD should be treated in PM/DM. However, there are no large controlled trials to confirm the efficacy of treatments in ILD with PM/DM patients. Figure 1 provides a flowchart that illustrates the process for determining the optimal therapeutic strategy based on our experiences and the findings described above. The clinical course of ILD shows a less rapid progression in Caucasians than in East Asians. For example, ILD with anti-MDA5 more frequently shows rapid progression in Asian patients than in Westerners. Clinical manifestations and treatment responses for each MSA may depend on race, although MSAs are useful predictors of clinical manifestations and prognoses. Thus, therapeutic strategies should be considered individually in each race.

Corticosteroids and immunosuppressive agents should be co-administered as soon as possible in RP-ILD with PM/DM patients. However, other causes of pneumonia or pneumonitis, such as infections and drug use, should be excluded before treatment initiation. The development of RP-ILD should be considered particularly in Asian patients with anti-MDA5 and/or hyperferritinemia, which is defined as serum ferritin levels greater than 500 ng/mL. Moreover, RP-ILD patients with hyperferritinemia and/or anti-MDA5 have a poorer prognosis than other patients with PM/DM in Japan. Therefore, a combination therapy of corticosteroids, IVCY, and CNIs, such as CSA and TAC, should be immediately administered in RP-ILD patients with hyperferritinemia and/or anti-MDA5. However, ILD is not generally RP in Caucasian patients with anti-MDA5.

CNIs or IVCY should be added to corticosteroids in patients with chronic progressive ILD if pulmonary function is gradually deteriorating and/or the lesions of ILD are spreading on CT findings. CNIs should be administered with PSL in ILD with anti-ARS patients to prevent the progression and recurrence of ILD. The administration of steroid-sparing agents (eg, MTX, AZA, CNIs, and MMF) should

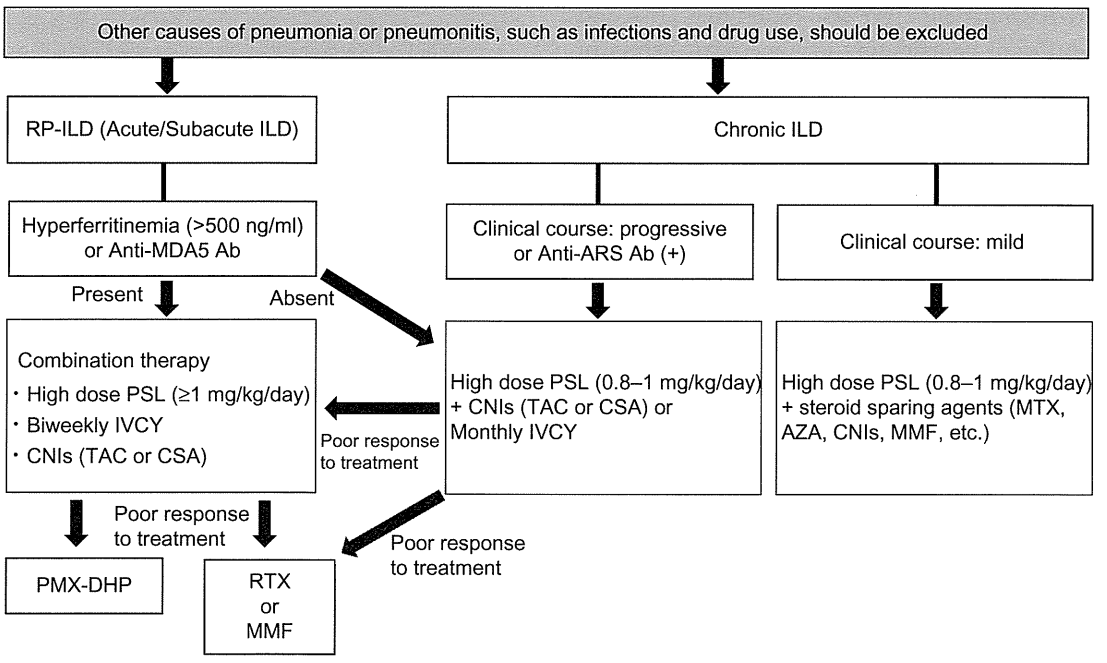

Figure 1. Therapeutic strategy for ILDs associated with PM/DM.
Abbreviation: Ab, antibodies.

be also considered in patients with chronic mild ILD. RTX or MMF administration may be considered when RP-ILD or chronic ILD with anti-ARS is refractory to the therapies described above.

Perspectives for Future Therapies for ILD with PM/DM

The agents described above are not sufficient to improve the clinical outcome of ILD in PM/DM. For example, approximately 20%–40% of RP-ILD with anti-MDA5 patients die within 6 months after diagnosis, even if a combination therapy of corticosteroids, IVCY, and CNIs is immediately administered.[14]

Therefore, there is a need to elucidate the pathophysiology of ILD with PM/DM in greater detail to improve the outcome of ILD. Recent studies demonstrated novel findings in the pathophysiology of PM/DM. Th17, $CD28^{null}$ T cells, BAFF, IL-1, IL-6, type 1 interferon, high-mobility group box 1, and leukotriene B4 are potential molecular targets for the treatment of patients with PM/DM.[103] Anti-BAFF, belimumab, and anti–IL-6 receptor tocilizumab are commonly administered in lupus and RA patients, respectively. These agents may be relatively easy to use as a novel therapy in PM/DM in the future.

Recent differences in cytokine profiles were demonstrated between RP-ILD (ILD with anti-MDA5) and the chronic form ILD (ILD with anti-ARS) in PM/DM.[104] Serum IL-8 levels were significantly higher in ILD with anti-MDA5 patients than ILD with anti-ARS patients, although IL-6, TNF-α, and IP-10 levels were high in both subsets. Therefore, IL-6–targeting therapy, such as tocilizumab, may be efficacious in ILD with anti-MDA5 and ILD with anti-ARS patients. We also found that IL-6 and IL-8 were significant contributors to hyperferritinemia in PM/DM-ILD.[105] Inflammatory alveolar macrophages synthesize ferritin and become activated in lung, liver, spleen, and bone marrow in RP-ILD with anti-MDA5 patients.[106] These findings suggest that activated alveolar macrophages and inflammatory cytokines are more selectively and strongly regulated to improve clinical outcome in RP-ILD with PM/DM.

Conclusion

ILD is associated with poor prognosis in PM/DM. The appropriate management of ILD is very important for improving the prognosis of patients with PM/DM. ILD activity and severity depend on the disease subtype. Therefore, clinicians should determine therapeutic strategies according to the subtype in each patient. Anti-MDA5 antibody and hyperferritinemia predict the development and severity of RP-ILD, particularly in East Asians. Combination therapy with corticosteroids, IVCY, and CNIs should be started immediately in RP-ILD patients. In contrast, ILD patients with anti-ARS exhibit a better response to corticosteroid monotherapy in the short term, although these patients often experience a recurrence and progression of ILD. Therefore, CNIs or RTX may be added to corticosteroids if pulmonary function gradually deteriorates.

Large-scale, multicenter RCTs should be conducted to confirm that the agents described above exhibit efficacy for ILD with PM/DM. We should also elucidate the pathophysiology of ILD with PM/DM in greater detail. Investigations of the pathophysiology of these conditions may lead to the development of novel agents to improve the prognosis in patients with ILD with PM/DM.

Author Contributions

Conceived and designed the experiments: HK, TG, YK, HY. Analyzed the data: HK. Wrote the first draft of the manuscript: HK. Contributed to the writing of the manuscript: TG. Agree with manuscript results and conclusions: HK, TG, YK, HY. Jointly developed the structure and arguments for the paper: HK, TG. Made critical revisions and approved final version: HK, TG, YK, HY. All authors reviewed and approved of the final manuscript.

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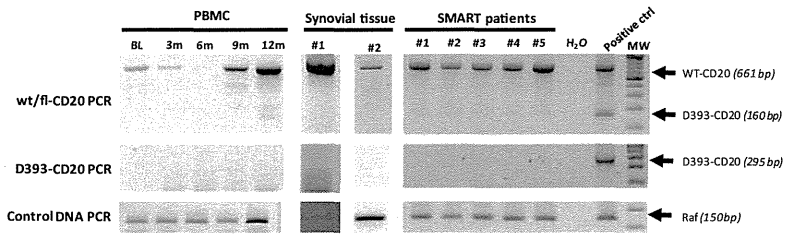
Fig. 1 Alternative CD20 transcript variant expression in PBMCs and synovial tissue from patients with RA



Representative qualitative RT-PCR analysis of *wt/fl cd20* and *d393-cd20* transcripts performed on cDNA from PBMCs of a RA patient, sampled at baseline (BL) and 3, 6, 9 and 12 months after RTX treatment, from two synovial tissues sampled during arthroplasty and from five PBMC samples representative of the SMART cohort (non-responder RTX-treated patients). *fl/wt-cd20* PCR allowed amplification of both *fl/wt-cd20* and *d393-cd20* transcripts, whereas *d393-cd20* PCR amplified specifically the *d393-cd20* transcripts, using a primer spanning the splicing junction. H_2O was used as negative control and cDNA from a B-cell line (positive ctrl) was used as positive control. MW: 100 bp molecular marker. PBMC: peripheral blood mononuclear cells.

Rheumatology key message

- The alternative CD20 transcript is not a marker for resistance to rituximab in RA.

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High incidence of cancer in anti-small ubiquitin-like modifier activating enzyme antibody-positive dermatomyositis

Sir, The idiopathic inflammatory myopathies (IIMs) are a group of systemic autoimmune diseases that include PM and DM [1]. Several myositis-specific autoantibodies, which have been regarded as mutually exclusive, are associated with certain clinical forms of IIM.

Since autoantibodies to small ubiquitin-like modifier activating enzyme (SAE) in patients with DM were described [2, 3], a few studies on anti-SAE antibodies in DM have been published from Italy [4], Japan [5] and Hungary [6]. We analysed serum samples from 110 DM patients and 2 were found to be anti-SAE positive [7]. The frequency of anti-SAE antibodies in DM overall was 1.5–5.7%. Nearly all patients with anti-SAE antibodies had skin and muscle symptoms, and most of them had skin disease before the muscle disease; however, the clinical features of the patients with anti-SAE antibodies are

not conclusive. We aimed to establish a quantitative assay for measuring anti-SAE antibodies and to clarify the clinical features of DM patients with these antibodies.

We screened 134 consecutive Japanese patients with DM (12 children, 122 adults) followed at Nagoya University Hospital, Nagoya, Japan. The serum samples were from 85 patients with DM and the remaining 49 samples were from patients with clinically amyopathic DM (CADM). An additional 16 adult patients with DM, including 11 with CADM, were also screened because their doctors introduced them for investigation of DM-marker autoantibodies. Of these 150 patients (male:female ratio 41:109), 67 patients were complicated with interstitial lung disease as diagnosed by chest radiograph or chest CT scan and 22 patients were diagnosed with cancer-associated DM. The definitions of DM, CADM and cancer-associated DM are as defined in our previous study [7]. This study was approved by the ethics committee of Nagoya University. All the patients and healthy individuals provided written informed consent according to the Declaration of Helsinki.

The full-length cDNA clones of SAE1 and SAE2 were purchased from Thermo Scientific Open Biosystems (Waltham, MA, USA). Biotinylated recombinant proteins were produced from the cDNA, using the SP6 Quick Coupled Transcription/Translation System (Promega, Madison, WI, USA). Antibodies against SAE1 and SAE2 were tested by antigen-capture ELISA according to our published protocols [8]. Cut-off values were determined as the mean (+ 5 S.D.) of the units obtained from 36 control serum samples from healthy individuals. Anti-MDA5, anti-Mi-2, anti-NXP-2 and anti-TIF1γ antibodies were also measured.

Serum samples that were positive for anti-SAE by ELISA were analysed with IIF using the Fluoro HEPANA Test (MBL, Nagoya, Japan). The samples were also screened by ELISA kits for antibodies against SS-A/Ro60, SS-B, U1-RNP, Sm, CENP-B, ds-DNA and aminoacyl tRNA synthetases consisting of a mixture of EJ, Jo-1, KS, PL-7 and PL-12 (MBL). Anti-SS-A/Ro52 antibodies were measured by using the ELISA kit of Orgentec (Mainz, Germany). Fisher's exact probability tests were used for comparison of frequencies. Correlations between two parameters were analysed by Spearman's correlation coefficients.

In the first cohort, consisting of 134 serum samples from consecutive patients, 4 (3.0%) patients were positive for both anti-SAE1 and anti-SAE2 antibodies (supplementary Fig. S1A, available at *Rheumatology* Online). Serum samples from two patients had been shown to be positive for anti-SAE antibodies by immunoprecipitation and western blotting [7]. In an additional cohort of 16 patients, 3 had anti-SAE1 antibodies, and 2 of these also had anti-SAE2 antibodies. Anti-SAE1 and anti-SAE2 titres had mutually significant positive correlations ($R = 0.807$, $P < 0.0284$). After the initial screening by ELISA, we investigated anti-SAE antibodies in the serum of five new anti-SAE-positive candidate patients for their ability to immunoprecipitate biotinylated recombinant SAE1 and SAE2. All of the candidates immunoprecipitated recombinant SAE1 and SAE2 (supplementary Fig. S1B, available at *Rheumatology* Online). According to these results, we concluded that we found five new serum samples that were positive for anti-SAE antibodies. All seven of the anti-SAE antibody-positive serum samples exhibited nuclear speckled patterns by IIF analysis (Table 1). Surprisingly, ELISA and immunoprecipitation using

TABLE 1 Clinical characteristics of DM patients with anti-small ubiquitin-like modifier activating enzyme antibody

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Total ($n = 7$), %
Age at onset, years	57	70	65	55	65	77	66	Mean, 65
Sex	Female	Male	Female	Male	Male	Male	Female	4 male:3 female
Heliotrope	+	+	–	+	–	–	–	43
Gottron's sign	+	+	+	+	+	+	+	100
Periungual lesions	+	–	+	+	+	+	NA	83
Mechanic's hands	–	–	–	+	+	+	–	43
V-neck sign	+	–	+	+	–	+	+	71
Shawl sign	+	–	+	+	–	–	+	43
Dysphagia	–	–	–	–	+	+	+	43
Muscle weakness	+	+	+	+	+	–	+	86
Creatine kinase, IU/l	542	429	662	6133	311	5187	1084	Elevated, 100
Interstitial lung disease	+	–	–	+	+	+	–	57
Arthritis	–	–	–	–	NA	–	–	0
Malignancy[a]	–	Rectum	Uterus	–	Oesophagus	Colon	–	57
RP	–	–	–	–	NA	–	–	0
Calcinosis	–	–	–	–	NA	NA	–	0
Other features	PH	–	–	Dysphonia	–	–	–	
Presentation (months)	S (2)	S/M	S (2)	S/M	S (7)	S/M	S (2)	S (mean, 1.9)
Other autoantibodies	Ro52	–	NXP-2, Ro60	Ro60	–	–	–	Ro60 (2), Ro52 (1), NXP-2 (1)

[a]Malignancy associated with DM was defined as that occurring within 3 years of the DM diagnosis. NA: not available; PH: pulmonary hypertension; S: skin disease presented first; S/M: skin and muscle disease presented together.

recombinant NXP-2 protein clarified that one patient also had anti-NXP-2 antibody (data not shown).

Seven anti-SAE-positive patients were diagnosed with adult DM, and all had internal involvement, such as interstitial lung disease, cancer and/or dysphagia, except for patient 7. The frequency of cancer in the anti-SAE-positive patients was significantly higher than in the anti-SAE-negative patients (4/7 *vs* 18/143, $P < 0.0093$). Since anti-NXP-2 antibodies in adult patients with DM are associated with cancer [1], we recalculated the association between anti-SAE antibodies and cancer when the patient with both anti-SAE and anti-NXP-2 antibodies was excluded. The significant association was still confirmed (3/6 *vs* 18/143, $P < 0.0369$).

Previous studies reported the frequency of cancer in anti-SAE-positive patients as 14–25% [3–6]. Interestingly, the cumulative results including our data showed that there were significantly more male patients in the anti-SAE-positive adult cancer-associated myositis group than in the myositis group without cancer (supplementary Table S1, available at *Rheumatology* Online). Multivariate analysis using a large cohort will be needed to clarify whether anti-SAE antibodies independently contribute to the specific clinical characteristics.

Rheumatology key message

- Risk of malignancy should be considered in anti-small ubiquitin-like modifier activating enzyme antibody-positive adult DM patients.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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Tocilizumab in the treatment of a polyostotic variant of fibrous dysplasia of bone

Sir, Fibrous dysplasia of bone (FDB) is a benign disease leading to the slow replacement of normal bone by fibrous tissue, without osteoblastic rimming [1]. Three-quarters of FDB cases are monostotic and occur mainly in craniofacial bones, ribs, femurs and tibias. Polyostotic forms involve, in decreasing order of frequency, femurs, tibias, skull and facial bones, humerus and cervical spine. When associated with café-au-lait macules and hyperfunctioning endocrinopathies, the disease is identified as McCune–Albright syndrome [2]. Bone homeostasis is regulated by the balance between osteoblasts, which build up bone, and osteoclasts, which degrade bone. Pathophysiology of FDB is secondary to an activating mutation in the gene *GNAS* that leads to undifferentiated bone marrow stromal cell

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RESEARCH ARTICLE Open Access

Anti-PM/Scl antibodies are found in Japanese patients with various systemic autoimmune conditions besides myositis and scleroderma

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Abstract

Introduction: Anti-PM/Scl antibodies are associated with polymyositis (PM)/systemic scleroderma (SSc) overlap syndromes and are also found in other systemic autoimmune diseases. Although anti-PM/Scl reactivity is found in 3-11% of PM or SSc patients and in approximately 25% of PM/SSc overlap patients, previous large studies of Japanese patients with scleroderma reported that anti-PM/Scl are not found in Japanese patients at all. The PM/Scl autoantigen complex comprises 11–16 different polypeptides; ELISA with PM1-α peptide, which is a major epitope of the PM/Scl complex, has frequently been used for the detection of these antibodies in recent studies. However, no ELISA kit is commercially available in Japan.

Methods: In this study, we developed an immunoassay for measuring antibodies against recombinant PM/Scl-100 and PM/Scl-75 polypeptides, which are the two major targets of the complex, and we investigated their presence in 600 Japanese patients with various systemic autoimmune conditions. Immunoprecipitation analysis using the recombinants in addition to traditional radiolabeled cell extracts were also applied to ELISA-positive sera.

Results: In ELISA, 11 patients were positive for anti-PM/Scl-100 antibodies and 7 of these 11 patients were also positive for anti-PM/Scl-75 antibodies. Immunoprecipitation analysis using the recombinants in addition to traditional radiolabeled cell extracts confirmed that 9 out of these 11 patients immunoprecipitated the typical sets of PM/Scl proteins. In total, 4/16 (25%) undifferentiated connective tissue disease (UCTD) patients, 3/126 (2.4%) dermatomyositis patients, 1/223 (0.4%) SSc patients, 1/88 (1.1%) Sjögren's syndrome patients, 0/123 patients with systemic lupus erythematosus, 0/17 patients with overlap syndrome and 0/7 patients with PM were judged to be positive for anti-PM/Scl antibodies.

Conclusions: This is the first report of Japanese autoimmune patients with anti-PM/Scl antibodies. In Japanese patients, anti-PM/Scl antibodies are only very rarely found, and they are not always specific for dermatomyositis (DM) or SSc; they are also present in various autoimmune conditions with the highest prevalence being in UCTD. All anti-PM/Scl-positive DM cases are complicated with interstitial lung disease and/or cancer, while no life-threatening involvement was found in other anti-PM/Scl-positive cases. Further studies on larger cohorts are necessary to define the clinical significance of anti-PM/Scl antibodies in autoimmune diseases.

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Introduction

A characteristic feature of patients with systemic autoimmune diseases is the presence of autoantibodies in their sera that target intracellular components [1]. Some of these autoantibodies are useful diagnostic markers for various systemic autoimmune diseases [1-3]. Some autoantibodies have great diversity in their prevalence among different races and countries [4-6].

Anti-PM/Scl antibodies, first described as 'anti-PM-1' in 1977, were found in patients with overlap syndrome of polymyositis (PM) and scleroderma (Scl) [7]. Anti-PM/Scl antibodies produce a homogenous nucleolar pattern in indirect immunofluorescence (IIF) staining and recognize the PM/Scl complex, which is the human counterpart of the yeast exosome and consists of 11 to 16 polypeptides [8]. Most anti-PM/Scl antibodies recognize two components, PM/Scl-100 and PM/Scl-75 [9-11], and are found mostly in patients with overlap syndrome (OL) of PM and systemic scleroderma (SSc) (approximately 25%) [12], as well as in PM or SSc patients (3% to 13%) [13]; however, they are rarely found in other diseases, such as Sjögren's syndrome (SS) [14]. For the detection of anti-PM/Scl antibodies, several techniques have been utilized: double immunodiffusion, immunoprecipitation (IPP), enzyme-linked immunosorbent assay (ELISA) and line immunoassay (LIA) [15]. ELISA using the PM-1α synthetic peptide, a major epitope of PM/Scl-100 composed of an alpha helical structure located at amino acid 231 to 245 of PM/Scl-100 [16], was used in a recent multicenter study that elucidated the diagnostic and prognostic relevance of anti-PM/Scl antibodies in SSc clinics [17]. Unfortunately, this ELISA kit is not available in Japan.

The frequencies of some autoantibodies vary by ethnicity. For example, in a U.S. SSc cohort, in African-American patients, anti-U3-RNP (fibrillarin) antibodies were found in 30% of patients; meanwhile anti-Th/To antibodies were found in only 4% [4]. In white patients, however, anti-Th/To antibodies were found in 9%, whereas anti-U3-RNP antibodies were found in only 3% [4]. Another example is that anti-RNA polymerase III antibodies were less prevalent in French patients than in U.S. patients [7]. Although anti-PM/Scl antibodies are found in certain populations of patients in Western countries, as stated above, clinical studies on Japanese autoimmune patients to detect these antibodies have not been reported. Surprisingly, in two large SSc cohorts from two Japanese centers, no anti-PM/Scl-positive patients were found among 272 and 316 patients, respectively [18].

We recently developed a method that allows for the rapid conversion of cDNAs to a chemiluminescent ELISA to detect autoantibodies in human sera [19]. In this study, we constructed an ELISA for measuring anti-PM/Scl-100 and also anti-PM/Scl-75 antibodies, in order to screen these antibodies in 600 patients with various autoimmune conditions from a single center in Japan, and we investigated their clinical significance in Japanese patients.

Methods

Serum samples

Serum samples were collected from 600 Japanese patients, consisting of 223 with SSc, 126 with dermatomyositis (DM), 123 with systemic lupus erythematosus (SLE), 88 with SS, 17 with OL, 7 with PM and 16 with undifferentiated connective tissue disease (UCTD), between 1994 and 2014 at Nagoya University Hospital. SSc was diagnosed according to the classification of the American College of Rheumatology (ACR) [20] or the ACR/European League Against Rheumatism (EULAR) 2013 classification criteria [21]. Of the SSc patients, 185 were classified as diffuse cutaneous and 85 as limited cutaneous, according to the criteria of LeRoy and colleagues [22]. The DM patients (76 with adult DM, 12 with juvenile DM (JDM) and 38 with clinically amyopathic DM (CADM)) and PM patients fulfilled Bohan and Peter's criteria [23], except for CADM, which was defined by Sontheimer's criteria [24]. SLE was diagnosed by the ACR criteria for SLE [25]. SS was diagnosed based on Japanese diagnostic criteria [26]. OL, including 11 patients with PM + SSc, was diagnosed as cases that fulfilled the criteria for two systemic autoimmune diseases. UCTD was diagnosed according to the preliminary classification criteria proposed by Mosaca and colleagues [27]. Interstitial lung disease (ILD) was diagnosed by chest radiograph or chest computed tomography (CT) scan. Clinical information was collected retrospectively by reviewing their medical charts. Our cohort consisted of newly diagnosed incipient patients, except for a few patients with juvenile DM. As for patients with UCTD, serum samples were collected at the first visit. These patients were confirmed, by follow-up with doctors, as not fulfilling the criteria for defined CTD for at least three years from the beginning of symptoms according to the criteria of UCTD [27]. As control samples, serum samples from 72 healthy volunteers were also used. This study was conducted with the approval of the ethics committees of the Nagoya University Graduate School of Medicine and the Kyoto University Graduate School of Medical Science. All patients gave written consent to participate in the study.

Recombinant antigens for ELISA and immunoprecipitation

The full-length cDNA clones of PM/Scl-100 (product No. FXC03779) and PM/Scl-75 (product No. FXC22044) were purchased from Flexi® ORF Clone (Promega, Madison, WI, USA). Biotinylated recombinant proteins were produced from the cDNA, using the T7 Quick Coupled Transcription/Translation System (Promega) according to our published protocol [28]. In short, 800 μl transcription

and translation (TnT) Quick Master Mix, 20 μl 1 mM methionine, 30 μl transcend biotin-lysyl-tRNA, 120 μl water and 30 μl DNA (1 μg/μl) were mixed and then incubated at 30°C for 60 minutes.

ELISA

Antibodies against PM/Scl-100 and PM/Scl-75 were tested by antigen-capture ELISA according to our published protocols [19]. Briefly, a 96-well Nunc™ Immobilizer™ Streptavidin Plate (Thermo Scientific Nunc, Roskilde, Denmark) was incubated with 1 μl/well of *in vitro* TnT reaction mixture including biotinylated recombinant protein. Wells were then incubated with 1:1000 diluted sera and probed with anti-human immunoglobulin G (IgG) antibody conjugated with horseradish peroxidase (HRP) (Dako, Glostrup, Denmark) (1:30,000 dilution). After incubation with SuperSignal® ELISA Femto Maximum Sensitivity Substrate (Thermo Scientific Pierce, Rockford, IL, USA), the relative luminescence unit (RLU) was determined using the GloMax®-Multi Detection System (Promega). Each serum sample was tested in duplicate, and the mean RLU with the background subtracted was used for data analysis. The RLU of the samples was converted into units using a standard curve created by a prototype positive serum. As a standard, the high-titer anti-PM/Scl-100 (patient A in Figure 1) or anti-PM/Scl-75 (patient E in Figure 1) antibody-positive sera diluted 1:5 serially, starting from 1:500, was run. Units correlated with the titers of antibodies: 1:500 dilution, 625 units; 1:2,500, 125 units; 1:12,500, 25 units; 1:62,500, 5 units; 1:312,500, 1 unit; 1:1,562,500, 0.2 units. The cutoff values (4.4 units for anti-PM/Scl-100 antibody and 2.1 units for anti-PM/Scl-75 antibody) were determined as the mean of the units obtained from 36 control sera from healthy volunteers + 5 standard deviations (SD).

Immunoprecipitation

IPP was performed using TnT products as previously described [28] and using radiolabeled extracts of HeLa cells [29]. Prototype sera containing anti-PM/Scl, anti-MDA5, anti-TIF1γ or anti-Mi-2 antibodies from TM's laboratory were also used.

Laboratory tests and serological assay

Sera that were positive for anti-PM/Scl by ELISA were analyzed with an IIF laboratory kit using HEp-2 cells (Fluoro HEPANA Test; MBL, Nagoya, Japan) [30]. The samples were also screened by ELISA for antibodies against CCP, SS-A, SS-B, U1-RNP, Sm, CENP-B, ribosomal P, aminoacyl tRNA synthetase (ARS) and ds-DNA with commercial kits (MBL, Nagoya, Japan). This anti-SS-A kit detects only anti-SS-A/Ro60 and not anti-SS-A/Ro52/TRIM21.

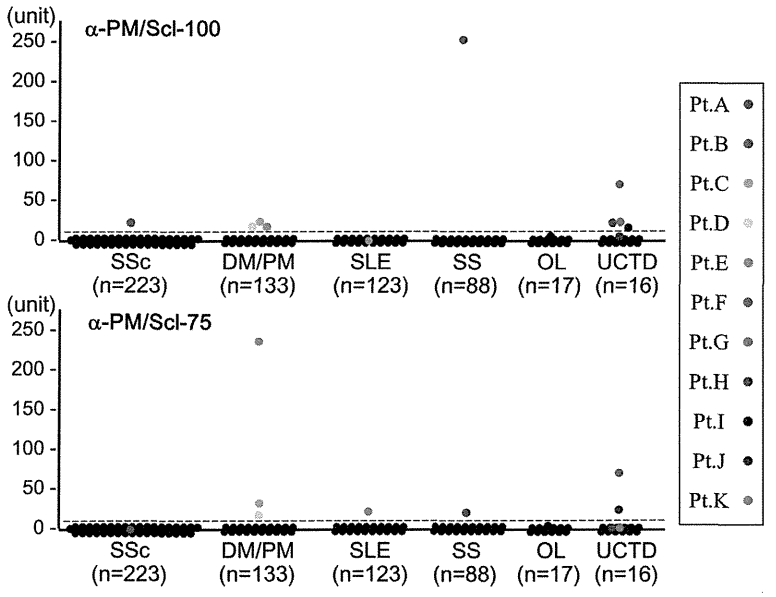

Figure 1 Qualitative measurement of anti-PM/Scl antibodies in ELISA. ELISA units of anti-PM/Scl-100 and anti-PM/Scl-75 antibodies are shown for a total of 600 serum samples from patients with various diseases. The antibody units are calculated from relative luminescence units using a standard curve obtained from serial concentrations of serum samples: patient A's serum for anti-PM/Scl-100 ELISA and patient E's serum for anti-PM/Scl-75 ELISA. The broken line indicates the cutoff value, which is the mean value of 36 healthy controls + 5 standard deviations. DM, dermatomyositis; OL, overlap syndrome; PM, polymyositis; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSc, systemic scleroderma; UCTD, undifferntiated connective tissue disease.