Characteristics of idiopathic inflammatory myopathies with novel myositis-specific autoantibodies

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Abstract

Background. In recent years, many novel myositis-specific autoantibodies (MSAs) have been identified. However, their links with the pathogenesis and clinical manifestations of inflammatory myopathies remain uncertain.

Objectives. To characterize the population of adult dermatomyositis (DM) and polymyositis (PM) patients treated at our center for autoimmune diseases using clinical and laboratory measures.

Materials and methods. According to the Bohan and Peter criteria, we retrospectively analyzed patients who fulfilled diagnostic criteria for DM or PM. Myositis-specific autoantibodies and myositis-associated autoantibodies (MAAs) were identified using immunoblot assays.

Results. Fifty-one PM (71% women) and 36 DM (67% women) Caucasian patients with a median age of 58 (range: 21–88) years who met the definite or probable diagnostic criteria for myositis were included in the study. Myositis-specific autoantibodies were identified in 63 (72%) patients, whereas MAAs were observed in 43 (49%) of them. Interstitial lung disease (ILD) was characteristic of PM patients (67%, χ^2 with Yates's correction (χ_c^2) = 13.8078, df = 1, p = 0.0002), being associated with anti-Jo-1 or anti-PL-12 antibodies (fraction comparison test (FCT) 6.4878, p < 0.0001, 6.8354, p = 0.0003, respectively). Interestingly, among patients with anti-MDA5 antibodies (n = 8, 9.2%), all but one had an amyopathic form, with more frequent ILD, skin changes and arthralgias than observed in other patients (FCT 4.7029, p = 0.0228 and p = 7.7986, p = 0.0357, p = 4.7029 and p = 0.0228, respectively). Anti-signal recognition particle (SRP) was strongly associated with the Raynaud's phenomenon (FCT 4.1144, p = 0.0289) and the highest muscle injury markers (Mann–Whitney U test, z = 2.5293, p = 0.0114). Malignancy was recorded in 14 (16%) patients and was equally common in those with PM and DM. The anti-TIF-1γ was the most frequently related to cancer χ^2 = 14.7691, df = 1, p < 0.0001). The anti-Mi-2α, similarly prevalent in DM and PM, was typically accompanied by skin changes (FCT 7.7986, p = 0.0357) but not ILD (FCT 8.7339, p = 0.0026).

Conclusions. Identification of MSAs might help to predict the clinical course of the autoimmune myopathy and malignancy risk. However, these antibodies were absent in about 30% of patients with typical PM or DM manifestations, which encourages further research in this area.

Keywords: myositis-specific antibodies, polymyositis, dermatomyositis, idiopathic inflammatory myopathies

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Background

Dermatomyositis (DM) and polymyositis (PM) belong to the heterogeneous group of rare inflammatory myopathies. The prevalence of these disorders ranges from 5 to 22 per 100,000. Interestingly, in Europe, morbidity significantly increases from the north to the south, likely due to the environmental or genetic factors.[1]

Multiple epidemiological studies have reported an association between inflammatory myopathies and cancer, strongly linked with DM.[2–4] It has been demonstrated that even 1/3 of patients with DM may present malignancy in the 3 years after DM diagnosis. An exact explanation for this relationship remains unknown, although it may be related to altered cellular and humoral immunity.[5,6] Interestingly, the type of associated neoplasm varies between races. In the Asian population, the most frequent is nasopharyngeal and lung cancer, while in Europe and North America, it is ovarian cancer.[7]

Few reports have described the clinical manifestations of inflammatory myopathies over the world. However, the clinical presentation seems to be similar and independent of race. Both diseases are characterized by proximal skeletal muscle weakness and evidence of immune-mediated muscle injury. On the other hand, skin changes are more common in DM and may not be accompanied by laboratory confirmed or clinically diagnosed muscle injury. In turn, interstitial lung disease (ILD), dysphagia and polyarthritis occur with the same frequency in both disorders, together with constitutional symptoms and the Raynaud's phenomenon.[8]

The exact pathogenesis of inflammatory myopathies remains unknown. However, autoantibodies in peripheral blood and T cell muscle infiltrations suggest an autoimmune background with unidentified or heterogenic antigens. It has been postulated that capillary, myofiber and keratinocyte injury in DM might be related to the interferons[9–11] and antigen-antibody complexes.[12,13] On the other hand, common PM findings include the endomysial T cells surrounding and invading myofibers,[14,15] and muscle infiltration of macrophages,[15,16] myeloid dendritic cells[17] and plasma cells.[18]

The PM and DM are diagnosed based on clinical presentations and laboratory findings, including the presence of myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs). Myositis-specific autoantibodies are considered relatively specific for DM/PM, whereas MAAs may also be found in other autoimmune diseases.

The MSA group consists of antibodies directed against aminoacyl-transfer RNA synthetases, such as anti-Jo-1, anti-PL-7, anti-PL-12, anti-OJ, and anti-EJ. The other identified antigens for MSAs include a signal recognition particle (anti-SRP antibody), nuclear helicase Mi-2 (anti-Mi-2 antibody), 155-kD nuclear protein transcriptional intermediary factor 1 gamma (anti-TIF-1γ antibody), RNA helicase encoded by the melanoma differentiation-associated gene 5 (anti-MDA5 antibody), nuclear matrix protein 2 (anti-NXP-2 antibody), and small ubiquitin-like modifier activating enzyme (anti-SAE1 antibody). To date, a few publications have suggested that the type of detected MSA may indicate PM and DM specificity, and thus may help to predict clinical prognosis, including cancer risk.[19–21] However, there is a deficiency of large-scale studies characterizing patients with inflammatory myopathies in the Caucasian population.

Objectives

This study aimed to analyze the population of adult DM and PM patients with particular MSAs and MAAs treated at our large center for autoimmune diseases in southern Poland to determine whether the presence of specific antibodies is associated with certain clinical and laboratory features.

Materials and methods

Study design

A retrospective analysis of clinical and laboratory data was carried out.

Setting

This study was conducted in the Department of Allergy and Clinical Immunology, University Hospital, Kraków, Poland, from October 1, 2014 to September 30, 2019.

Participants

All included patients fulfilled the diagnostic criteria for DM or PM. The diagnosis of myositis was established according to the Bohan and Peter criteria, which include: 1) symmetrical, progressing muscle weakness of the limb-girdle muscles; 2) muscle biopsy evidence of myositis; 3) increased serum levels of muscle-associated enzymes (creatine kinase (CK), aldolase, lactate dehydrogenase (LDH), transaminases); 4) electromyographic features of primary muscle damage; and 5) skin rashes, typical for DM. If the patient fulfilled the first 4 criteria, "definite PM" was diagnosed. If they met 3 of the first 4 criteria then "probable PM" was diagnosed, while in the case of 2, "possible PM" was established. On the other hand, "definite DM" was diagnosed if the patient had a rash and 3 of the elective criteria listed above, "probable DM" was diagnosed in those with a rash and 2 elective criteria, and "possible DM" was established when rash and any myositis criterion were observed. Only those who met "definite" or "probable" myositis criteria were included in the study.

Variables

The complete medical history of patients, results of laboratory tests, spirometry and echocardiographic investigations, and high resolution computed tomography (HRCT) of the chest scans were recorded. Potential confounders such as obesity, smoking, hypertension, hypercholesterolemia, diabetes mellitus, heart failure, coronary artery disease, kidney disease, liver failure and other autoimmune diseases were taken into account in the statistical analysis.

Data sources/measurement

Antinuclear antibodies (ANAs) were detected with an indirect immunofluorescence assay using Hep-2 cell lines. The MSAs and MAAs were identified with immunoblotting (Euroline, Lübeck, Germany). The MSAs analyzed included anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, anti-SRP, anti-Mi-2α, anti-Mi-2β, anti-TIF-1γ, anti-MDA5, anti-NXP-2, and anti-SAE1. The MAAs were also identified, including anti-PM-Scl 75, anti-PM-Scl 100, anti-Ku, anti-SSB/La, anti-SSA/Ro, and anti-Ro-52 kDa.

Interstitial lung disease was diagnosed by a radiologist based on interstitial lung infiltration (ground glass and reticular opacities, or honeycombing) demonstrated on HRCT scans. Constitutional symptoms included fever, weight loss and fatigue. The high probability of pulmonary hypertension (PH) was assessed based on echocardiography when pulmonary artery systolic pressure was above 45 mm Hg. This approach has a 95% specificity compared to right heart catheterization, which is considered the gold standard for PH diagnosis.

Quantitative variables

Laboratory features of muscle injury were defined as creatine kinase, myoglobin high-sensitive (hs) I troponin levels above the upper limit of the normal range (>180 U/L, ≥110 μg/L and ≥47.3 μg/L, respectively). An ANA titer higher than 1:160 was considered positive.

Bias

Two researchers checked the accuracy and relevance of the database.

Study size

In order to obtain the appropriate number of patients with rare diseases such as DM and PM treated at 1 center, the enrollment of patients into the study lasted for 5 years.

Statistical methods

Statistical analyses were performed using STATISTICA Tibco v. 13.3 software (StatSoft, Tulsa, USA). The Shapiro–Wilk test was used to evaluate the data distribution. According to the data distribution, continuous variables are shown as the mean and standard deviation (SD) or median and interquartile range (IQR), as appropriate. Categorical variables are given as numbers and percentages. In comparison to other MSA types, patient subsets were compared using the χ^2 test with the Yates's correction. The Mann–Whitney U test and the fraction comparison test (FCT; i.e., standard z-test comparing proportions or its equivalent) were also applied depending on the number of elements. The considered proportions were obtained from conditional probability distributions. A value of $p < 0.05$ was considered statistically significant.

Results

A total of 87 patients were identified, with 51 (58.6%) meeting the PM criteria and 36 (41.4%) meeting the DM criteria. All patients were of the white Caucasian race. The demographic, clinical and laboratory characteristics of these patients are provided in Table 1. Women (n = 60) constituted a group more than twice as large as men (n = 27). The median age of the analyzed individuals was 58 (range: 21–88) years, while the median disease duration since the onset was 3.5 (range: 0.7–8) years. Statistical analysis did not confirm the influence of potential confounders such as obesity, smoking, hypertension, hypercholesterolemia, diabetes, heart failure, coronary artery disease, kidney disease, liver failure and other autoimmune diseases on the observed relationships.

As expected, ILD was significantly more common in patients with PM (n = 34, 66.7% of PM patients) than DM (n = 9, 25% of DM patients, $\chi_c^2 = 13.8078$, df = 1, p = 0.0002). Cancer was diagnosed in 14 (16%) subjects with 7 cases in each subgroup. The majority of patients were treated with corticosteroids (n = 83, 95.4%). Thirty (34.4%) individuals also received methotrexate, 29 (33.3%) azathioprine, while mycophenolate mofetil was used by 13 (14.9%) subjects. Patients with a more severe disease received cyclophosphamide and/or rituximab (n = 32 (36.8%) and n = 8 (9.2%) for PM and DM, respectively).

Immunological characteristics of PM and DM patients

Detectable ANAs were reported in 73 (83.9%) patients, MSAs in 63 (72.4%) and MAAs in 43 (49.4%) of them. The most frequent was anti-Ro-52 (n = 32, 36.8%) followed by anti-Jo-1 (n = 17, 19.5%), and both of these antibodies often coexisted ($\chi_c^2 = 5.0633$, df = 1, p = 0.0244). Figure 1 depicts the MSA frequency in the cohort.

Associations of MSAs with clinical and laboratory presentations

Table 2 presents the leading associations between MSAs and clinical symptoms, as well as imaging and laboratory investigations. These associations are also briefly described in the following paragraphs.

Table 1. Clinical characteristics of subjects studied

Variable	Polymyositis (PM) n = 51	Dermatomyositis (DM) n = 36	PM compared to DM p-value
Age, mean (range) [years]	59 (30–88)	59 (21–79)	Mann–Whitney U test, z = 0.4047 p = 0.9624
Females, n (%)	36 (71)	24 (67)	χ_c^2 = 0.0238, df = 1 p = 0.8775
Constitutional symptoms, n (%)	20 (39)	12 (33)	χ_c^2 = 0.0565, df = 1 p = 0.8122
Elevated muscle injury markers, n (%)	36 (71)	24 (67)	χ_c^2 = 0.0328, df = 1 p = 0.8564
Shoulder/pelvic girdle weakness, n (%)	36 (71)	30 (83)	χ_c^2 = 1.2407, df = 1 p = 0.2653
Interstitial lung disease, n (%)	34 (67)	9 (25)	χ_c^2 = 13.8078, df = 1 p = 0.0002
Pulmonary artery systolic pressure >45 mm Hg, n (%)	6 (12)	2 (6)	χ_c^2 = 0.0620, df = 1 p = 0.8033
Pulmonary artery systolic pressure 31–45 mm Hg, n (%)	22 (43)	8 (22)	χ_c^2 = 0.5313, df = 1 p = 0.4661
Cutaneous involvement, n (%)	14 (27)	34 (94)	χ_c^2 = 38.0997, df = 1 p = 0.0000
Mechanic's hands, n (%)	10 (20)	5 (14)	χ_c^2 = 0.1223, df = 1 p = 0.7265
Gottron's sign, n (%)	1 (2)	9 (25)	χ_c^2 = 9.2023, df = 1 p = 0.0024
Heliotrope rash, n (%)	4 (8)	19 (53)	χ_c^2 = 20.5417, df = 1 p < 0.0001
Shawl sign, n (%)	1 (2)	11 (31)	χ_c^2 = 12.6573, df = 1 p = 0.0004
Thigh rash, n (%)	0 (0)	4 (11)	χ_c^2 = 3.8075, df = 1 p = 0.0510
Raynaud's phenomenon, n (%)	14 (27)	4 (11)	χ_c^2 = 2.3243, df = 1 p = 0.1274
Heart involvement, n (%)	2 (4)	2 (6)	χ_c^2 = 0.0244, df = 1 p = 0.8758
Dysphagia, n (%)	3 (6)	8 (22)	χ_c^2 = 3.8043, df = 1 p = 0.0511
Malignancy, n (%)	7 (14)	7 (19)	χ_c^2 = 1.2350, df = 1 p = 0.539
Treatment used			
Glucocorticoids, n (%)	47 (92)	36 (100)	χ_c^2 = 1.4416, df = 1 p = 0.2299
Cyclophosphamide, n (%)	22 (43)	10 (28)	χ_c^2 = 1.5315, df = 1 p = 0.2159
Azathioprine, n (%)	18 (35)	11 (31)	χ_c^2 = 0.0533, df = 1 p = 0.8174
Methotrexate, n (%)	14 (27)	16 (44)	χ_c^2 = 1.9977, df = 1 p = 0.1575
Mycophenolate mofetil, n (%)	8 (16)	5 (14)	χ_c^2 = 0.0054, df = 1 p = 0.941
Rituximab, n (%)	7 (14)	1 (3)	χ_c^2 = 1.8599, df = 1 p = 0.1726

df – degrees of freedom; χ_c^2 – χ^2 with Yates's correction.

Anti-Jo-1 antibody

Anti-Jo-1 antibodies were detected in 17 (19.5%) patients, 76.5% of whom were female. As expected, these antibodies were detected more frequently in PM than in DM cases (15 compared to 2 cases, χ_c^2 = 6.8971, df = 1, p = 0.0086). All but 1 had radiological signs of ILD (FCT 6.4878, p < 0.0001). These patients were also characterized

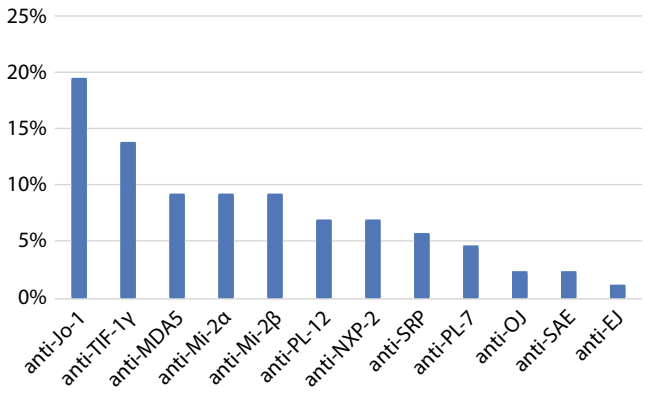

Fig. 1. Prevalence of specific autoantibodies in idiopathic inflammatory myopathies

by severe constitutional symptoms, including fever and weight loss reported in 9 (53%) anti-Jo-1-positive patients, and arthralgia or arthritis documented in 12 (71%) of them. Six (35%) individuals in this group had mechanic's hands as a unique skin manifestation of the disease. Laboratory features of muscle injury were recorded in 11 (65%) of anti-Jo-1-positive patients, while proximal muscle weakness was observed in 15 (88%) of them.

Anti-PL-12 antibody

Six patients (6.9% of all individuals) had circulating anti-PL-12 antibodies. Five of these patients were diagnosed with PM and 1 with DM. All had ILD (FCT 6.8354, p = 0.0003) and 5 (83% of anti-PL-12-positive patients) had severe constitutional symptoms, including recurrent fever (FCT 7.8243, p = 0.0105). Three (50%) patients in this group complained of arthralgia and arthritis, and the same number had laboratory features of muscle injury.

Anti-PL-7 antibody

Anti-PL-7 antibodies were detected in 4 cases (4.6% of all patients), of which 50% were female. Three of these patients were diagnosed with PM, and 1 had systemic lupus erythematosus (SLE)/myositis overlap syndrome. All but 1 had ILD and 1 had pericarditis. Only 1 patient presented laboratory features of muscle injury.

Anti-OJ and anti-EJ antibodies

Anti-OJ antibodies were reported in 2 female patients (2.3% of all patients). In 1 subject, these antibodies co-existed with anti-Jo-1 and were associated with a very severe PM manifestation. The 2nd anti-OJ patient had an amyopathic form of PM. Both patients had ILD and a heliotrope rash. An amyopathic form of PM with ILD and arthritis also characterized the only individual with anti-EJ antibodies.

Anti-TIF-1γ antibody

Twelve patients (13.8%, 9 DM, 2 PM and 1 SLE/myositis overlap syndrome) were positive for anti-TIF-1γ antibodies. The majority of these patients reported proximal muscle weakness and all but 2 had elevated markers of muscle injury. In this subgroup, skin changes were also common, predominantly shawl sign and/or a heliotrope rash (n = 9, 75%, FCT 6.1568, p = 0.007). On the other hand, ILD and joint involvement were rare (i.e., in 1 (8%) and 4 (33%) of anti-TIF-1γ-positive patients, respectively). Interestingly, more than half of these subjects were diagnosed with malignancy (χ_c^2 = 19.3782, df = 1, p < 0.0001).

Anti-MDA5 antibody

Five DM and 3 PM patients (9.2%) had anti-MDA5 antibodies. Interestingly, all of them complained of severe proximal muscle weakness, although all but 1 had an amyopathic form of the disease (χ_c^2 = 10.7787, df = 1, p = 0.001). The only individual who exhibited laboratory features of muscle injury was characterized by a coexistence of anti-TIF-1γ and various MAAs, such as anti-Ku and anti-PM-Scl 100. Interstitial lung disease was detected in 3/4 of these patients, similar to joint involvement (FCT 6.5504, p = 0.0228). Half of these patients complained of severe constitutional symptoms, whereas skin involvement, such as Gottron's and shawl signs, and heliotrope rash was demonstrated in 5 of anti-MDA5-positive patients (62.5%, FCT 7.798, p = 0.0357). Three (37.5%) patients in this group died during the follow-up due to severe ILD, lymphoma and cancer.

Anti-Mi-2α and anti-Mi-2β antibodies

Eight patients (18.4% of all individuals) had anti-Mi-2α and anti-Mi-2β antibodies each. These patients were alike in PM and DM. All of them reported proximal muscle weakness and all but 2 (88%) had laboratory signs of muscle injury. Interstitial lung disease was reported only in 1 (12.5%) of anti-Mi-2α and in 2 (25%) of anti-Mi-2β-positive patients (FCT 6.1456, p = 0.0026, 3.3763, p = 0.0392, respectively; Table 2). Skin lesions occurred in 5 (62.5%) anti-Mi-2α and 4 (50%) of anti-Mi-2β-positive subjects. One woman with anti-Mi-2α antibodies accompanied by Ro-52 had heart involvement in the form of heart failure with preserved ejection fraction.

Anti-NXP-2 antibody

Anti-NXP-2 antibodies were observed in 6 patients (6.9%; 4 DM and 2 PM). All complained of proximal muscle weakness and half of them had general muscle weakness. In 1 case, heart involvement was recorded, in 2 dysphagia (33%) and 3 (50%) of anti-NXP-2-positive subjects had typical DM skin changes.

Table 2. Clinical features of patients with myositis-specific antibodies

Number of patients (n)	Anti-Jo-1 n = 17 n (%)	Anti-PL-12 n = 6 n (%)	Anti-PL-7 n = 4 n (%)	Anti-OJ n = 2 n (%)	Anti-SRP n = 5 n (%)	Anti-Mi-2α n = 8 n (%)	Anti-Mi-2β n = 8 n (%)	Anti-TIF-1γ n = 12 n (%)	Anti-MDA5 n = 8 n (%)	Anti-NXP-2 n = 6 n (%)	Anti-SAE n = 2 n (%)
Interstitial lung disease	16 (94)	6 (100)	3 (75)	2 (100)	3 (60)	1 (13)	2 (25)	1 (8)	6 (75)	1 (17)	0 (0)
Pulmonary artery systolic pressure >45 mm Hg	2 (12)	0 (0)	1 (25)	0 (0)	1 (20)	1 (13)	0 (0)	1 (8)	1 (13)	0 (0)	0 (0)
Pulmonary artery systolic pressure 31–45 mm Hg	5 (29)	2 (33)	2 (50)	1 (50)	2 (40)	0 (0)	5 (63)	3 (25)	4 (50)	3 (50)	0 (0)
Arthritis/arthralgia	12 (71)	3 (50)	2 (50)	0 (0)	1 (20)	3 (38)	3 (38)	4 (33)	6 (75)	1 (17)	2 (100)
Elevated muscle injury markers	11 (65)	2 (33)	1 (25)	1 (50)	4 (80)	7 (88)	7 (88)	10 (83)	1 (13)	5 (83)	2 (100)
Shoulder/pelvic girdle	15 (88)	4 (67)	3 (75)	1 (50)	4 (80)	8 (100)	8 (100)	11 (92)	8 (100)	6 (100)	2 (100)
Dysphagia	2 (12)	0 (0)	0 (0)	0 (0)	1 (20)	1 (13)	1 (13)	1 (8)	2 (25)	2 (33)	0 (0)
Heart involvement	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)
Skin lesions	7 (41)	1 (17)	1 (25)	2 (100)	2 (40)	5 (63)	4 (50)	9 (75)	5 (63)	3 (50)	0 (0)
Gottron's sign	1 (6)	0 (0)	0 (0)	1 (50)	1 (20)	1 (13)	1 (13)	2 (17)	2 (25)	1 (17)	0 (0)
Heliotrope rash	1 (6)	1 (17)	0 (0)	2 (100)	0 (0)	4 (50)	2 (25)	4 (33)	5 (63)	1 (17)	0 (0)
Shawl sign	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	1 (13)	0 (0)	5 (42)	2 (25)	1 (17)	0 (0)
Thigh rash	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	1 (13)	0 (0)	3 (25)	0 (0)	0 (0)	0 (0)
Mechanic's hands	6 (35)	1 (17)	1 (25)	1 (50)	1 (20)	2 (25)	1 (13)	1 (8)	0 (0)	0 (0)	0 (0)
Raynaud's phenomenon	2 (12)	3 (50)	0 (0)	0 (0)	4 (80)	2 (25)	2 (25)	1 (8)	1 (13)	1 (17)	0 (0)
Constitutional symptoms	9 (53)	5 (83)	0 (0)	1 (50)	0 (0)	1 (13)	4 (50)	3 (25)	4 (50)	1 (17)	0 (0)
Malignancy	2 (12)	1 (17)	1 (25)	0 (0)	1 (20)	0 (0)	1 (13)	7 (58)	2 (25)	1 (17)	0 (0)

Anti-SRP antibody

Five females (5.7% of all patients) were anti-SRP-positive. Four of these patients were diagnosed with PM and 1 with DM. One patient was characterized by the coexistence of other antibodies and had clinical signs of systemic sclerosis, rheumatoid arthritis, antiphospholipid syndrome, and PM.

Interstitial lung disease was present in 3 (60%) of anti-SRP-positive patients and Raynaud's phenomenon in 4 of them (80%, FCT 4.1144, p = 0.0289). Furthermore, all but 1 complained of severe proximal muscle weakness and had the highest muscle injury markers among all PM patients (Mann–Whitney U test, z = 2.5293, p = 0.0114). Interestingly, in our data, the anti-SRP antibody was also recorded in non-myositis patients, such as in muscular dystrophy, undifferentiated connective tissue disease and cold agglutinin disease.

Anti-SAE antibody

Anti-SAE was reported in only 2 (2.3%) subjects (both PM) who were characterized by arthritis/arthralgia and proximal muscle weakness with elevated creatine kinase levels. No ILD or skin lesions were observed.

Coexistence of MSAs and MAAs

Myositis-specific autoantibodies coexisted with MAAs in 35.6% of cases. The most common coexisting antibody was anti-Ro-52, which was detected, for example, in 11 patients with anti-Jo-1 antibodies (64.7% of all anti-Jo-1-positive patients, $\chi_c^2 = 5.0633$, df = 1, p = 0.0244) and in 3 patients with anti-PL-7 antibodies. Anti-Ro-52 also accompanied anti-SRP in 3 PM patients who, interestingly, all had ILD. A 55-year-old woman had anti-Jo-1, anti-PL-7, anti-MDA5, anti-NXP-2, and anti-TIF-1γ antibodies. In this patient, we also identified anti-dsDNA, anti-SSA, anti-SSB, anti-centromere B, anti-nucleosomes, anti-histones, and anti-Ro52 antibodies, with a very high ANA titer (higher than 1:20,480). This patient was diagnosed with SLE/myositis overlap syndrome with no laboratory signs of muscle injury but proximal muscle weakness, ILD and arthritis. Furthermore, 1 interesting case was observed with coexisting anti-Jo-1 and anti-OJ related to severe PM with ILD, rhabdomyolysis, mechanic's hands, Gottron's sign, and heliotrope rash. To our knowledge, this case with the coexistence of those antibodies is only the 2nd one described in the literature. In both cases, ILD was the prominent manifestation.[22]

In 1 typical DM patient, anti-SRP coexisted with anti-Mi-2α antibodies, whereas in 1 PM case, anti-SRP was accompanied by anti-NXP-2. The latter is the 1st such case described in the literature with severe muscle injury and

dysphagia. Interestingly, this female patient was also diagnosed with papillary thyroid cancer and endometrial adenocarcinoma 13 and 5 years before autoimmune disease onset, respectively.

Among the 12 anti-TIF-1γ-positive patients, 3 also had anti-MDA5 antibodies. One of these patients fulfilled the criteria for DM and the other one for PM. Interestingly, both had a neoplastic disease in anamnesis. The 3rd patient had SLE/myositis overlap syndrome without malignancy.

Myositis-specific autoantibodies and myositis-associated autoantibodies-negative patients

Nine patients (5 DM and 4 PM, 10.3% of all patients) had no detectable MSAs or MAAs. Neoplasm and ILD were found in 1 (11%) of these patients, whereas skin changes and arthralgia were observed in 7 (77.8%) of these cases (FCT 5.4177, p = 0.0091, both).

Myositis-specific autoantibodies and their associations with malignancy

As expected, neoplastic diseases were frequent in this cohort. Fourteen (16% of all patients) cancer or lymphoma cases were documented, equally common in DM and PM. In half of the cases, the neoplasm was diagnosed before myositis. Malignancy was strongly associated with anti-TIF-1γ antibodies (χ_c^2 = 19.3782, df = 1, p < 0.0001). Neoplasms occurred in 7 (58.3%) of anti-TIF-1γ-positive patients, 6 (85.7%) of them were diagnosed with DM and 1 with PM.

Two of these patients had lung cancer and the others presented with urethral, endometrial and renal cell carcinoma, diffuse large B-cell lymphoma, or carcinoma of unknown primary. Two of the anti-TIF-1γ-positive malignancy patients had coexisting anti-MDA5 antibodies. Bladder cancer and cervical cancer in patients with anti-Jo-1 antibodies were also observed, and ovarian cancer and prostate cancer in those with anti-PL-7 and anti-PL-12 coexisting with anti-Mi-2β antibodies were documented. No tumors were recorded in anti-Mi-2α-positive patients.

Discussion

In this study, we characterized 87 adult Caucasian patients with autoimmune inflammatory myopathies using clinical and laboratory measures. The majority of these patients presented with MSAs or MAAs, indicating that both of these antibody groups are valuable biomarkers for determining disease course and malignancy risk. However, the presence or absence of these antibodies cannot be used to differentiate DM from PM. Furthermore, a specific MSA may accompany other MSAs or MAAs and be associated with different clinical presentations, such as those seen in SLE or systemic scleroderma in the current data. Some of these antibodies may also be seen in other immune or nonimmune disorders. For example, an anti-SRP antibody has been documented in association with muscular dystrophy or cold agglutinin disease at our center.

The role of MSAs in the pathogenesis of inflammatory myopathies is not entirely understood. These antibodies likely sustain inflammatory processes, although their pathological links with muscle injury remain unknown.[23] The anti-Jo-1 antibody is the most common MSA, as confirmed by the current data.[8] However, this antibody was detected in only 19.5% of patients, thus indicating the immunologic heterogeneity of autoimmune myopathies. Typically, this type of antibody is linked with the presence of ILD, the Raynaud's phenomenon, arthritis, and mechanic's hands,[24,25] findings confirmed by the present study. Furthermore, similar to our data, anti-Jo-1 often accompanies anti-Ro-52 antibodies. According to the literature, such a coincidence may be a risk factor for a more severe disease course and cancer.[26,27] Interestingly, 1 of our 2 anti-Jo-1-positive cancer patients had coexisting anti-Ro-52, and in both cases anti-PM-Scl-75 was also detected.

Anti-Jo-1, anti-PL-12, anti-PL-7, anti-EJ, and anti-OJ antibodies are associated with the common anti-synthetase syndrome. However, several recent studies have suggested a clinical heterogeneity, particularly regarding ILD or muscle injury signs.[28] Some authors documented that antibodies other than anti-Jo-1 may be related to a poorer clinical prognosis and more aggressive ILD.[29,30] On the other hand, Hamaguchi et al. reported that muscle injury was closely associated with anti-Jo-1, anti-EJ and anti-PL-7 antibodies.[31] At the same time, ILD might have been observed in all of these patients, while skin changes, such as heliotrope rash and Gottron's sign, were less common but also frequent.[31] The anti-OJ has also been shown to be associated with ILD, often being the sole manifestation of idiopathic inflammatory myopathy. However, if recorded, myositis seems to be more severe than with other anti-synthetase antibodies.[25,32] The anti-PL-12 antibodies were also documented in linkage with ILD and, to a lesser extent, with muscle injury and arthritis.[28,33–35] In turn, pericardial effusion may be a characteristic of anti-PL-7-positive patients.[25] Our data are in line with the presented reports, indicating that all anti-synthetase antibodies have a strong relationship with ILD and PM, but not with the typical DM skin changes. Also, 1 patient with heart involvement in the current cohort had an anti-PL-7 antibody.

The another interesting antibody that relates to PM and DM is anti-SRP. Targoff et al. reported a classical PM manifestation with a low prevalence of ILD, arthritis and Raynaud's phenomenon in these subjects.[36] However, some of these cases were severe and/or rapid in onset.[28,36] The current results do not entirely confirm these findings. Although most of anti-SRP-positive patients in this study complained of proximal muscle weakness and had

very high creatine kinase, more than half of them had ILD or Raynaud's phenomenon. Recent studies have reported a histologically unique necrotizing myopathy, which might explain the particularly high levels of muscle injury biomarkers in anti-SRP-positive patients.[37–39]

In contrast to anti-SRP antibodies, anti-MDA5 is likely related to the amyopathic PM/DM form but with rapidly progressive ILD, acute respiratory failure and poor clinical prognosis.[40,41] Indeed, most of our anti-MDA5 patients had aggressive ILD leading to rapid and severe lung fibrosis, and even to a related death in 1 case.

In the literature, anti-Mi-2 and anti-NXP-2 antibodies have mainly been associated with DM.[42,43] Anti-Mi-2 was shown to be associated with Gottron's sign or papules, and heliotrope rash, lung-sparing and an excellent clinical response to corticosteroids.[43] Surprisingly, in the current study, the PM and DM distribution among anti-Mi-2-positive patients was almost equal. However, ILD was rare.

Anti-NXP-2 autoantibodies are associated with a young-onset DM with subcutaneous edema, skin calcinosis and severe muscle involvement with dysphagia.[42,44,45] Moreover, these antibodies may be linked with malignancy.[46,47] Our data partially mirror these reports, including the typical skin changes reported in half of the current patients.

One of the rarest antibody types in the current cohort was anti-SAE1, identified in only 2 individuals. Previous reports demonstrated that these patients may have severe skin changes, mild muscle involvement and ILD.[48,49] Surprisingly, our anti-SAE-1 subjects had no skin lesions or ILD, but proximal muscle weakness, laboratory signs of muscle injury and arthritis. This observation points to the heterogeneity of autoimmune myopathies and the need for further research on this subject.

The last issue that merits comment is the relation of PM, DM, and MSAs to malignancy. Surprisingly, in the current data, a strong association was demonstrated only with anti-TIF-1γ, which is in line with the data in the literature.[28,50,51] In this subgroup of patients, 58% had documented malignancy. Moreover, patients with anti-TIF-1γ antibodies had a lower prevalence of fever, Raynaud's phenomenon, arthritis, ILD, and mechanic's hands. At the same time, these patients have more frequent DM-typical skin changes, particularly shawl sign rash.[28,52] The current study confirms these observations. The presence of anti-TIF-1γ antibodies was highly associated with malignancy, while typical skin rash was reported in 3/4 of these patients. Only 1 patient had ILD, while joint involvement was demonstrated in 1/3 of them.

Among patients with no detectable MSAs or MAAs, ILD was rarely observed, in contrast to skin changes and arthralgia. One patient in this group was diagnosed with cancer. There are limited data on the clinical course of these patients in the available literature.

Limitations

The current study has several limitations. First, it was retrospective in nature. In addition, the number of subjects studied was relatively small, especially with regard to some MSA types, such as anti-OJ, anti-EJ and anti-SAE antibodies. Therefore, future multicenter studies characterizing patients with DM and PM are needed.

Conclusions

The MSA type cannot differentiate DM from PM, although some antibodies may be more prevalent in PM (e.g., anti-Jo1) and others in DM (e.g., anti-TIF-1γ). Interstitial lung disease was common in patients with anti-synthetase antibodies, particularly anti-Jo-1 and anti-PL-12, but also in those with anti-MDA5 antibodies. The majority of ILD patients fulfilled PM criteria. Patients with anti-SRP were characterized by the presence of the Raynaud's phenomenon and the highest serum concentration of muscle injury markers. In turn, an amyopathic form characterized anti-MDA5-positive individuals. Malignancy was highly associated with anti-TIF-1γ antibodies, but also with anti-Jo-1, anti-PL-7, anti-PL-12, anti-SRP, anti-MDA-5, anti-NXP-2, and anti-Mi-2β antibodies.

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