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ORIGINAL ARTICLE

Myositis autoantibody profiles and their clinical associations in Greek patients with inflammatory myopathies

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Abstract

Myositis-specific (MSAs) or-associated autoantibodies (MAAs) have been linked to particular clinical phenotypes of idiopathic inflammatory myopathies (IIM) and appear to aid diagnosis. The objective of this study was to analyze the prevalence of MSAs and MAAs and their possible clinical associations in Greek IIM patients. This study comprised 95 IIM patients classified based on the 2017 EULAR/ACR classification criteria. All patients had MSAs and MAAs measured in their sera by line immunoblot assay. Dermatomyositis was the most prevalent IIM clinical subtype. MSAs were found in 44% of the patients, whereas MAAs in 23%. The most frequently detected MSA was anti-Jo-1 (22%), while the most frequently detected MAA was anti-Ro-52 (30%). The distributions of MSAs/MAAs did not differ between the five IIM subgroups, except for anti-Mi-2 which was only detected in dermatomyositis patients. Patients with at least one MSA and/or MAA positivity showed more frequently IIM characteristic skin rashes, while those presenting solely MAA positivity had more often puffy hands and Raynaud's phenomenon. Anti-Jo1-positive patients presented more frequently lung disease, while anti-Ro52 positivity related to mechanic's hands. Anti-Ro-52 and anti-Jo-1 strongly associated with one another. Prevalence of IIM subtypes and of MSAs/MAAs in our patients is in line with published reports in populations of similar geographic distribution. While MSA and/or MAA positivity did associate with particular clinical manifestations, it did not predict in our cohort specific IIM subgroup as defined by the latest EULAR/ACR classification criteria. Future studies are warranted to conclusively decide if these autoantibodies, measured with a standardized method, should or not be incorporated in every day clinical practice to aid IIM diagnosis.

Keywords Dermatomyositis · Idiopathic inflammatory myopathies · Myositis-associated autoantibodies · Myositis-specific autoantibodies · Polymyositis

Introduction

Idiopathic inflammatory myopathies (IIM) are a group of diseases characterized by muscle inflammation and by a great variety of clinical manifestations from many different organs. IIM were first classified by Bohan and Peter [1, 2] into five distinct clinical groups: polymyositis (PM), dermatomyositis (DM), DM/PM associated with neoplasia, childhood DM/PM associated with vasculitis, and DM/PM associated with collagen-vascular disease [1, 2]. In an effort to include characteristics from muscle biopsy specimens, in 1991, Dalakas [3] formulated IIM diagnostic criteria including specific histopathologic features and incorporated one more clinical entity, the sporadic inclusion body myositis (sIBM), in the IIM groups. A few years later, in the revised criteria [4], the DM spectrum was expanded to include the

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amyopathic dermatomyositis (ADM). Since the description of different clinical subsets and the deeper understanding of IIM pathogenetic aspects, the discovery of autoantibodies that have been linked to specific IIM phenotypes and clinical course, appears to aid the classification, diagnosis, and prognosis of the different IIM groups [5–7]. Autoantibodies found in IIM patients have been classified into myositis-specific autoantibodies (MSAs), which are found exclusively in IIM, and myositis-associated autoantibodies (MAAs), which can be encountered in other connective tissue diseases as well [8]. Nonetheless, to date, studies have not concluded with certainty on the pathogenetic, diagnostic, and prognostic role of all of these autoantibodies, since different patient populations have been evaluated [9, 10] and variable sensitivity and specificity detection methods have been used. This has led to their non-incorporation in the most recent IIM classification criteria [11].

The aim of this study was to detect the prevalence and possible clinical associations of MSAs and MAAs in a cohort of Greek IIM patients who were classified based on the latest IIM criteria [11].

Patients and methods

Study population

Hundred eighteen IIM patients fulfilling the 2017 EULAR/ACR classification criteria [11] were currently followed in four Rheumatology sections (Department of Pathophysiology, First Department of Propaedeutic and Internal Medicine, Medical School, University of Athens; Institute for Autoimmune Systemic and Neurological Diseases, Athens/Greece, and Department of Internal Medicine and Clinical Immunology, Euroclinic Hospital, Athens/Greece). Forty-three had already been tested for MSAs and MAAs (MSAs/MAAs) using a line immunoblot assay during their evaluation process and the remaining 75 patients were asked to participate in the evaluation of autoantibody profile in their sera after receiving informed consent. One patient had passed away within the past 3 months due to pre-existing neoplasia, 22 were either not available or refused to undergo testing, and the remaining 52 were tested for MSAs/MAAs using the immunoblot assay.

The study was approved by the Ethical/Scientific Committee of the Athens Medical, School National and Kapodistrian University (1718024741-19/04/2018).

Autoantibody detection

Myositis autoantibody profile in all patients included in the study was assessed by line immunoblot assay (EUROLINE: Autoimmune Inflammatory Myopathies 16 Ag, EUROIMMUN, Lübeck, Germany) according to the manufacturer's protocol. This assay detects the following MSAs: anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, anti-Mi-2 alpha, anti-Mi-2 beta, anti-TIF1γ, anti-MDA5, anti-NXP2, anti-SAE1, and anti-SRP as well as the MAAs: anti-PM-Scl100, anti-PM-Scl75, anti-Ku, and anti-Ro-52. The results were defined for each autoantibody as negative, weakly, moderately, or strongly positive by two independent researchers (FNS and CPM) who were blind to the patients' clinical data. Only the moderate or strong reactivity results were taken into account.

Patients' characteristics

Demographic, clinical, laboratory/serological, and treatment-related data were obtained from the patients' medical records. More specifically, clinical symptoms at disease onset were grouped based on organ involvement. For skin involvement, presence of heliotrope rash, Gottron's papules or sign, puffy hands, V-sign rash, mechanic's hands, facial erythema, Shawl sign, calcinosis, or Raynaud's phenomenon was recorded. For articular involvement, the presence of arthralgia or arthritis was noted. Lung involvement in the form of interstitial lung disease (ILD) was considered present when characteristic lung parenchymal findings, such as linear opacities, ground-glass opacities, reticulation, patchy areas of consolidation and peribronchovascular thickening, or their combination, were documented on chest high-resolution computed tomography. Serum levels of creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) higher than the respective upper normal limit on at least two separate occasions were considered as elevated, provided that there was no other obvious cause for their increase. Treatment-related data were recorded and presented as therapeutic modalities ever received from the time of diagnosis until the time of the study.

Statistical analysis

Statistical analysis was performed using SPSS 24 software. Qualitative data are presented as count (percentage) and quantitative data as the mean ± SD. Qualitative variables regarding the prevalence and clinical associations of autoantibodies in different clinical subsets of IIM were compared using Chi square and Fisher's exact tests and quantitative variables were compared using Student's t test. P value ≤ 0.05 was considered statistically significant.

Results

IIM classification, patient characteristics, and clinical manifestations

The study cohort included 95 patients, women in their majority (76%), with a mean age of 58.3 ± 14.3 years and mean disease duration 6.8 ± 5.3 years. Based on the 2017 EULAR/ACR criteria web calculator [11], 60% of the patients were classified as definite IIM (mean probability 97.6 ± 6.1%, minimum score without muscle biopsy 8.8/with muscle biopsy 9.3), 37% as probable IIM (mean probability 71.7 ± 14.4%, minimum score without muscle biopsy 6.5/with muscle biopsy 7.3), and 3% as possible IIM (mean probability 52.3 ± 1.5%, minimum score without muscle biopsy 5.4). As to the subgroups of IIM, 46% classified as DM, 31% as PM, 18% as ADM, 4% as juvenile DM (JDM), and 1% as IBM. Regarding the different IIM clinical manifestations in these five subgroups, symmetrical proximal muscle weakness of upper and/or lower limbs was equally frequent in DM, PM, JDM, and IBM patients, while, as expected, was absent in ADM patients ($P < 0.001$). On the other hand, the classical cutaneous manifestations of DM (heliotrope rash, Gottron's papules and Gottron sign) were significantly more prevalent in DM, ADM, and JDM, while they were not seen in PM and IBM patients ($P < 0.001$, $P = 0.0029$, $P = 0.003$ respectively). V-sign rash and facial erythema were documented in similar frequency in DM and ADM patients, less frequently in PM patients, and was absent in JDM and IBM patients ($P = 0.048$, $P = 0.020$ respectively). Muscle enzymes, at disease diagnosis, were significantly less elevated in ADM patients compared to other IIM subgroups ($P < 0.001$) (Table 1). Neoplasia preceding or following IIM diagnosis was equally frequently attested in DM and PM patients, while none of the ADM, JDM, or IBM patients had such history before or during the years of follow-up (Table 1). Therapeutic modalities ever used to treat the patients in the different IIM subgroups did not differ (Table 1).

Myositis autoantibodies and IIM subgroups

MSA/MAAs were positive in 77% patients. Of those, 44% had only MSA positivity, 23% had only MAA positivity, 33% had MSA and MAA positivity, while 23% were MSA and MAA negative. Among MSAs, the anti-synthetase antibodies (ASAs) (anti-Jo1, anti-PL-12, anti-PL-7 anti-EJ, and anti-OJ) were the most prevalent autoantibodies (27%). Among the ASAs, anti-Jo1 was most frequently detected (80%), while it was the second most frequent autoantibody in the entire cohort (22%). The MAA anti-Ro52 was the most frequently present (31%) autoantibody in the entire cohort. In the different IIM subgroups, prevalence of MSAs and/or MAAs did not differ significantly. Interestingly, the appearance of anti-Jo1 antibody was uniformly distributed among DM, PM, ADM, and JDM, while the rest of the ASAs were only detected in the DM subgroup; anti-Mi2 was detected only in the DM subgroup, while anti-SAE and anti-Ku were more prevalent in ADM patients. JDM patients were positive only for anti-Jo1, anti-Ro52, and anti-PMScl75, while the single IBM patient included in the study had no autoantibody positivity (Table 2).

Myositis autoantibodies and demographic/clinical/laboratory associations

Stratifying IIM patients based on whether they tested positive for any MSAs/MAAs, the IIM patients in the autoantibody-positive group were significantly more women (81%) compared to the autoantibody-negative group (59%, $P = 0.049$). From all clinical manifestations studied, the MSA-/MMA-positive patients did only differ in the prevalence of IIM characteristic skin rashes (heliotrope rash, Gottron's papules or sign, puffy hands, V-sign rash, mechanic's hands, facial erythema, Shawl sign) compared to the MSA-/MMA-negative patients (79% versus 59% respectively, $P = 0.020$).

When considering only MSA positivity, the MSA-negative IIM patients demonstrated more frequently elevated serum muscle enzymes at presentation, compared to the MSA-positive IIM patients (85% versus 62%, $P = 0.011$). From the MSAs, when evaluating the ASAs, ASAs-positive patients presented more frequently with Gottron's papules (18% versus 8%, $P = 0.038$), mechanic's hands (8% versus 4%, $P < 0.001$), facial erythema (43% versus 12%, $P = 0.014$), and ILD (44% versus 19%, $P = 0.015$) whereas tended to have more dysphagia/esophageal dysmotility ($P = 0.058$) and arthralgias ($P = 0.067$) compared to the ASAs-negative patients. IIM patients who presented solely MAA positivity tended to have more often puffy hands (23% versus 8%, $P = 0.054$) and Raynaud's phenomenon (35% versus 15%, $P = 0.058$) compared to the MAA negative. When associating the more prevalent MSAs/MAAs in our cohort to all clinical parameters studied, anti-Ro52-positive patients tended to present more frequently with mechanic's hands (Fig. 1). Anti-Jo1-positive patients presented more frequently arthralgias and ILD and less frequently facial erythema. Anti-SRP and anti-Ku positivity did not contribute significantly to any particular clinical characteristic. Anti-PMScl75-positive patients had more frequently puffy hands and Raynaud's phenomenon. Anti-TIF1γ positivity tended to associate with more frequent Gottron's papules, myalgias/muscle tenderness, and muscle weakness of proximal upper extremities. Anti-Mi2α positivity is associated with more frequent presentation of Gottron sign and puffy hands. Anti-SAE1 reactivity is associated with the presence of Gottron sign and dysphagia/esophageal dysmotility (Fig. 1).

Table 1 Demographic, clinical, and laboratory characteristics as well as treatment modalities ever used for the entire cohort of the IIM patients and for the five IIM subgroups separately. All parameters are expressed as frequencies and percentages for the categorical variables or as means ± standard deviations (SD) for the continuous variables

	All patients ($n = 95$)	DM ($n = 44$)	PM ($n = 29$)	ADM ($n = 17$)	JDM (n = 4)	IBM (n = 1)	P
Female gender	72 (76)	33 (75)	21 (72)	15 (88)	3 (75)	0 (0)	ns
Age of disease onset, years (mean ± SD)	48.2 ± 16	49.5 ± 15.3	52.3 ± 13.8	43.8 ± 13.8	16.2 ± 0.9	71	< 0.001
Current age, years (mean ± SD)	58.3 ± 14.3	60.0 ± 14.7	61.0 ± 4.9	53.5 ± 13.0	35.2 ± 5.1	77	0.002
Disease duration, years (mean ± SD)	6.8 ± 5.3	7.8 ± 6.9	6.10 ± 4.9	5.1 ± 8.1	9.5 ± 4.4	1	ns
Clinical manifestations at disease onset							
Weight loss	7 (7)	4 (9)	3 (10)	0 (0)	0 (0)	0 (0)	ns
Fever	15 (16)	8 (18)	4 (14)	0 (0)	3 (75)	0 (0)	0.007
Myalgias/muscle tenderness	55 (58)	31 (70)	15 (52)	5 (29)	3 (75)	1 (100)	0.038
Neoplasia preceding/following IIM diagnosis	12 (13)	7 (16)	5 (17)	0 (0)	0 (0)	0 (0)	ns
Heliotrope rash	35 (37)	24 (54)	0 (0)	9 (53)	2 (50)	0 (0)	< 0.001
Gottron's papules	16 (17)	9 (20)	0 (0)	6 (35)	1 (25)	0 (0)	0.029
Gottron sign	25 (26)	17 (39)	0 (0)	6 (35)	2 (50)	0 (0)	0.003
V-sign rash	29 (30)	17 (39)	4 (14)	8 (47)	0 (0)	0 (0)	0.048
Facial erythema	33 (35)	18 (41)	6 (17)	10 (59)	0 (0)	0 (0)	0.020
Shawl sign	6 (6)	4 (9)	0 (0)	2 (12)	0 (0)	0 (0)	ns
Mechanic's hands	6 (6)	1 (2)	2 (7)	3 (18)	0 (0)	0 (0)	ns
Calcinosis	5 (5)	4 (9)	1 (3)	0 (0)	0 (0)	0 (0)	ns
Raynaud	118 (19)	8 (18)	5 (17)	3 (17)	2 (50)	0 (0)	ns
Puffy hands	10 (10)	6 (14)	2 (7)	2 (12)	0 (0)	0 (0)	ns
Oral mucosa ulcers	6 (7)	5 (11)	0 (0)	1 (6)	0 (0)	0 (0)	ns
Musculoskeletal manifestations							
Arthralgias	35 (37)	15 (34)	12 (41)	5 (29)	3 (75)	0 (0)	ns
Non-erosive arthritis	7 (7)	2 (4)	4 (14)	1 (6)	0 (0)	0 (0)	ns
Symmetrical muscle weakness of proximal upper extremities	59 (62)	33 (75)	21 (72)	0 (0)	4 (100)	1 (100)	< 0.001
Symmetrical muscle weakness of proximal lower extremities	71 (75)	40 (91)	26 (90)	0 (0)	4 (100)	1 (100)	< 0.001
Proximal leg muscles weaker that distal	59 (62)	31 (70)	24 (83)	0 (0)	4 (100)	0 (0)	< 0.001
Dysphagia or esophageal dysmotility	18 (19)	9 (20)	7 (24)	1 (6)	1 (25)	0 (0)	ns
Lung involvement-interstitial lung disease	25 (27)	13 (29)	7 (24)	5 (33)	0 (0)	0 (0)	ns
Elevated serum levels CK or LDH or SGOT or SGPT	74 (78)	36 (82)	27 (93)	6 (35)	4 (100)	1 (100)	< 0.001
Thyroid dysfunction at the time of diagnosis	6 (6)	1 (2)	3 (11)	2 (12)	0 (0)	0 (0)	ns
Statin use at the time of diagnosis	7 (7)	1 (2)	4 (14)	2 (12)	0 (0)	0 (0)	ns
Treatment with GC	85 (89)	33 (59)	26 (90)	14 (87)	4 (100)	1 (100)	ns
Treatment with MTX	75 (79)	38 (86)	22 (76)	11 (6)	4 (100)	0	ns
Treatment with CyS-A	15 (16)	10 (23)	2 (7)	1 (6)	2 (50)	0	ns
Treatment with AZA	27 (29)	17 (39)	7 (24)	3 (19)	0	0	ns
Treatment with RTX	21 (23)	13 (30)	4 (14)	4 (25)	0	0	ns
Treatment with IVIg	42 (45)	23 (53)	12 (41)	3 (19)	3 (75)	1 (100)	ns
Treatment with CYC	16 (17)	10 (23)	4 (14)	2 (16)	0	0	ns
Treatment with MMF	9 (10)	4 (9)	4 (14)	1 (6)	0	0	ns

IIM idiopathic inflammatory myopathies, *DM* dermatomyositis, *PM* polymyositis, *ADM* amyopathic dermatomyositis, *JDM* juvenile dermatomyositis, *IBM* inclusion body myositis, *CK* creatine kinase, *LDH* lactate dehydrogenase, *SGOT* aspartate aminotransferase, *SGPT* alanine aminotransferase, *GC* glucocorticoids, *MTX* methotrexate, *Cys-A* cyclosporine-A, *AZA* azathioprine, *RTX* rituximab, *IVIg* intravenous immunoglobulins, *CYC* cyclophosphamide, *MMF* mycophenolate mofetil

Table 2 Prevalence of the myositis (specific and associated) autoantibodies in the entire cohort and in the five IIM subgroups. Results are expressed as frequencies and percentages

Myositis autoantibodies	All patients ($n = 95$)	DM ($n = 44$)	PM ($n = 29$)	ADM ($n = 17$)	JDM ($n = 4$)	IBM ($n = 1$)	*P*
Positive myositis autoantibodies	73 (77)	37 (84)	20 (69)	14 (82)	2 (50)	0 (0)	ns
Anti-Ro-52	29 (30)	14 (32)	9 (31)	4 (23)	2 (50)	0 (0)	ns
Anti-Jo-1	21 (22)	8 (18)	8 (28)	4 (23)	1 (25)	0 (0)	ns
Anti-SRP	12 (13)	7 (16)	3 (10)	2 (12)	0 (0)	0 (0)	ns
Anti-PM-Scl75	9 (9)	4 (9)	3 (10)	1 (6)	1 (25)	0 (0)	ns
Anti-TIF1γ	7 (7)	1 (14)	3 (10)	3 (10)	0 (0)	0 (0)	ns
Anti-Mi-2α	6 (6)	5 (11)	0 (0)	1 (6)	0 (0)	0 (0)	ns
Anti-Mi-2β	6 (6)	6 (14)	0 (0)	0 (0)	0 (0)	0 (0)	ns
Anti-SAE1	6 (6)	3 (7)	1 (3)	2 (12)	0 (0)	0 (0)	ns
Anti-Ku	6 (6)	3 (7)	1 (3)	2 (12)	0 (0)	0 (0)	ns
Anti-NXP2	4 (4)	3 (7)	1 (3)	0 (0)	0 (0)	0 (0)	ns
Anti-PM-Scl100	4 (4)	2 (4)	1 (3)	1 (6)	0 (0)	0 (0)	ns
Anti-PL-12	3 (3)	3 (7)	0 (0)	0 (0)	0 (0)	0 (0)	ns
Anti-PL-7	1 (1)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	ns
Anti-MDA5	1 (1)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	ns
Anti-EJ	1 (1)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	ns
Anti-OJ	1 (1)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)	ns
Anti-aminoacyl-tRNA synthetases	2 (2)	1 (2)	1 (3)	0 (0)	0 (0)	0 (0)	ns

Co-existence of MSAs and MAAs

Among the MSA-/MAA-positive patients, 20% had dual autoantibody positivity, 11% had triple autoreactivity, while only 5% had more than three positive autoantibodies. Few MSAs were detected in combination with MAAs. ASAs and anti-Ku autoantibodies were found in four patients ($P = 0.042$), while ASAs and anti-PMScl75 in three patients ($P = 0.034$). Anti-

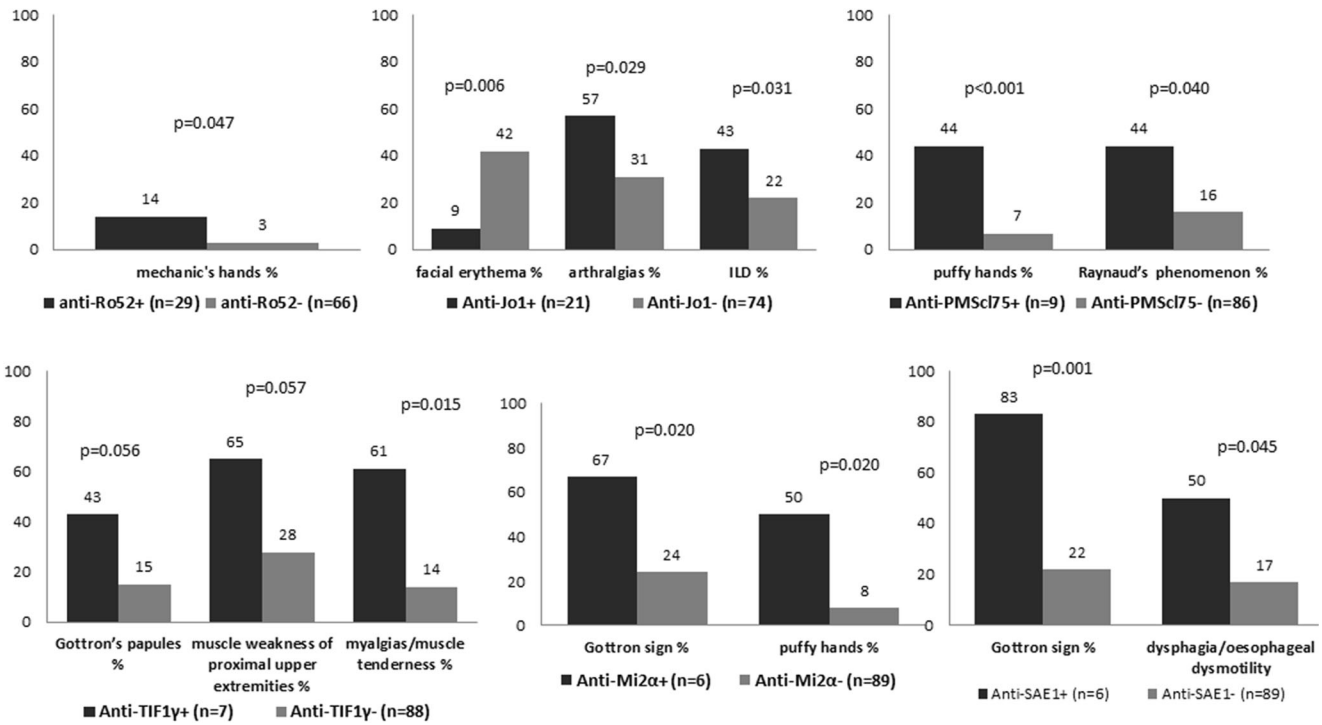

Fig. 1 Association of myositis autoanibodies with particular clinical manifestations

Ku is associated with anti-PL12 ($P < 0.001$) and anti-Mi2β in two patients ($P = 0.005$) and with anti-SRP in three patients ($P = 0.004$). The most apparent association was found between anti-Ro52 and anti-Jo-1 ($P = 0.05$) as well as between anti-Ro-52 and ASAs ($P = 0.025$).

Discussion

In the present study, we re-classified consecutive Greek IIM patients based on the latest IIM classification criteria [11] and we evaluated the prevalence and possible clinical and laboratory/serological associations of MSAs and MAAs detected by a commercially available line immunoblot assay. In the last few years, measurement of MSAs/MAAs has been increasingly introduced in the clinical practice. For the past decades, immunoprecipitation (IP) has been considered a reliable assay for the detection of the majority of MSAs; nevertheless, it has been performed only at a limited number of research laboratories and never became a routine assay in clinical practice. With the growing need to introduce detection of MSAs/MAAs in the classification criteria [11, 12] as well as in the clinical routine, the use of line immunoblot assays as an alternative to IP has been tested. Although results are limited and at times still controversial, there is some evidence that line immunoblot assays could represent a reliable alternative [13–15]. Nonetheless, more studies from larger registries are needed to harmonize these assays and improve their accuracy.

In our cohort, DM was the most prevalent IIM subgroup, which is in accordance with the results from the large EuroMyostis registry [16] and with previous studies reporting an increased prevalence of DM in southern Europe [10]. MSAs were positive in approximately half of the patients and MAAs in one third.

These numbers are somewhat higher than those reported in studies of patients from similar geographic origin [9, 17]. This could be attributed to different assays used to detect myositis autoantibodies [18] as well as to different IIM subgroups included in these studies. More precisely, in European patients with DM, PM, and IBM [9], MSAs/MAAs were detected by immunoblot, enzyme-linked immunosorbent assay (ELISA), and/or IP. In this study, MSAs were detected in approximately one third of patients, yet not all autoantibodies currently available were measured at that time. In a series of 88 Mediterranean patients, 30% were positive for at least one MSA and 43% for at least one MAA, measured by ELISA and IP [17]. In a recent study [19] of Indian patients with DM, PM, JDM, and connective tissue disease-associated myositis, the overall prevalence of MSAs/MAAs detected by line immunoblot, as we have used, was similar to the prevalence of our cohort. Although the use of different autoantibody detection methods in various studies, including ours, creates a limitation to proper MSA/MAA prevalence comparison and clinical association in patients with IIM, the more frequently detected MSAs and MAAs in our cohort (anti-Jo-1, anti-Mi2, anti-SRP, anti-TIF1-γ, anti-Ro-52, anti-PMScl75, anti-Ku, anti-SAE1) are not different and in general have similar prevalence to those found in other cohorts, irrespectively of the antibody detection method used [9, 17, 19, 20]. The only MSA that was more prevalent in our cohort compared to the already published series was anti-SRP [9, 17, 19, 20]. Anti-SRP autoantibodies are known to be associated with immune-mediated necrotizing myositis (IMNM) and are more frequently found in Asian origin than in European patients. Of note, in our cohort, the 12 patients that showed anti-SRP reactivity did not have IMNM. Nevertheless, the prevalence of anti-SRP varies highly among studies (0–54%) [7] and there is a strong heterogeneity of anti-SRP positivity in IMNM and non-IMNM subgroups [21]. Whether these differences are due to genetic or environmental factors, or different immunoassays used is still questionable [7, 18], therefore, it has been suggested that both indirect immunofluorescence and dot immunoassay are necessary to confirm the diagnosis of anti-SRP-associated myositis [21].

When considering association of autoantibody positivity with particular clinical manifestations, there are several classical MSAs (anti-Jo-1, PL-7, PL-12, EJ, OJ, Mi-2, and SRP) known for years to be specific for PM/DM among other systemic autoimmune rheumatic diseases [7]. In our cohort, in line with the literature [8, 9, 17, 20], from the classical MSAs, ASA positivity related to a collection of clinical manifestations depicting the anti-synthetase syndrome (ILD, arthritis, Raynaud's phenomenon, mechanic's hands) and anti-Jo1 positivity associated independently with ILD. Moreover, anti-Mi2, in our cohort, was solely detected in DM patients, in agreement with already published reports [7, 18].

Some newer antibodies such as anti-TIF1-γ and anti-MDA5 have the strongest evidence to also be considered as MSAs [22, 23] and are both associated with distinct clinical subsets of DM; anti-TIF1-γ with cancer-associated DM and anti-MDA5 with ADM often complicated by rapidly progressive ILD [22, 23]. Malignancy in our cohort ever occurred in 13% of the total patient population, a prevalence identical to other large IIM cohorts [16]. Neoplasia can develop before, concurrently, or subsequently to the onset of IIM in a period of time as long as 10 years, but is usually recognized within (before or after) 3 years of IIM diagnosis [24]. Although it has been shown that the prevalence of neoplasia in anti-TIF1-γ-positive adult DM patients is high in comparison to all other MSAs [8, 24, 25], in our cohort, anti-TIF1-γ positivity did not associate to cancer, probably either because the mean follow-up time after IIM diagnosis in our cohort is less than 10 years or because the mean age of our cohort is less than 60 years of age and it has been

postulated that in such IIM patients, the risk of cancer is somewhat lower [8, 25].

Definition of MAAs is more vague than of MSAs, as the former are considered autoantibodies that can be found in IIM but are not specific for this diagnosis and may also be found in other systemic autoimmune rheumatic diseases [7]. MAAs (anti-PM-Scl, anti-Ku, anti-U1 ribonucleoprotein (RNP), and U1/U2RNP) are known to be associated with PM/DM-overlap syndromes. They can also be found in cases of muscular involvement in systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) even if they are not considered PM/DM-overlap syndromes. In our cohort, anti-PMScl75-positive patients showed more frequently puffy hands and Raynaud's phenomenon, common clinical manifestations of overlap syndromes [8]. Anti-Ro52 antibody can be found in IIMs and is classified as a MAA; however, the significance of anti-Ro52 appears to be different from others in this category [26]. Anti-Ro52 specificity can be found in unselected populations or healthy individuals relatively frequently (~ 0.5–1%) in contrast to rare occurrence (< 0.1%) of MSAs and other MAAs that are associated with overlap syndromes [27]. Anti-Ro52 is frequently associated with other MSAs, in particular ASAs (anti-Jo-1, PL-7, PL-12) [28], but is also found frequently in patients with SLE, SSc, Sjögren's syndrome, and other autoimmune diseases. It has been shown that anti-Ro52 is often present in patients with ILD, its presence could probably precede development of autoimmune disease and therefore separate and repeated detection of anti-Ro52 antibodies might be useful in anti-synthetase syndrome-related diagnosis [26]. In our cohort, anti-Ro52-positive patients presented more frequently mechanic's hands, an isolated clinical manifestation of the anti-synthetase syndrome, and as already shown in other series [9, 20, 28], anti-Ro52 positivity was strongly associated with anti-Jo-1 reactivity.

In conclusion, in this cohort of Greek consecutive IIM patients classified with the latest myositis criteria [11], although MSA and/or MAA positivity did not predict a specific IIM subgroup, their different subtypes and prevalence as well as their clinical associations are in line with previous reports of cohorts of similar geographic distribution. It is apparent that an international effort should be undertaken in order to conclusively decide whether these autoantibodies, measured with a standardized method, should be included in every day clinical practice to aid diagnosis and prognosis of IIM.

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Compliance with ethical standards

The study was approved by the Ethical/Scientific Committee of the Athens Medical, School National and Kapodistrian University (1718024741-19/04/2018).

Disclosures None.

References

1. Bohan A, Perter J, Peter JB (1975) Polymiositis and dermatomyositis (first of two parts). N Engl J Med 292:344–347. https://doi.org/10.1016/B978-012595961-2/50037-8
2. Bohan APJ (1975) Polymyositis and dermatomyositis (second of two parts). N Engl J Med 292:403–407. https://doi.org/10.1056/NEJM198109243051303
3. Dalakas MC (1991) Polymyositis, dermatomyositis and inclusion-body myositis. N Engl J Med 325:1487–1498
4. Dalakas MC, Hohlfeld R (2003) Polymyositis and dermatomyositis. Lancet 362:971–982. https://doi.org/10.1016/S0140-6736(03)14368-1
5. Troyanov Y, Targoff IN, Tremblay JL, Goulet JR, Raymond Y, Senécal JL (2005) Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. Medicine (Baltimore) 84:231–249. https://doi.org/10.1097/01.md.0000173991.74008.b0
6. Betteridge Z, McHugh N (2016) Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 280:8–23. https://doi.org/10.1111/joim.12451
7. Satoh M, Tanaka S, Ceribelli A, Calise SJ, Chan EKL (2017) A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. Clin Rev Allergy Immunol 52:1–19. https://doi.org/10.1007/s12016-015-8510-y
8. Gunawardena H (2017) The clinical features of myositis-associated autoantibodies: a review. Clin Rev Allergy Immunol 52:45–57. https://doi.org/10.1007/s12016-015-8513-8
9. Brouwer R, Hengstman GJD, Vree Egberts W et al (2001) Autoantibody profiles in the sera of European patients with myositis. Ann Rheum Dis 60:116–123. https://doi.org/10.1136/ard.60.2.116
10. Hengstman GJD, Van Venrooij WJ, Vencovsky J et al (2000) The relative prevalence of dermatomyositis and polymyositis in Europe exhibits a latitudinal gradient. Ann Rheum Dis 59:141–142. https://doi.org/10.1136/ard.59.2.141
11. Lundberg IE, Tjärnlund A, Bottai M, Werth VP, Pilkington C, Visser M, Alfredsson L, Amato AA, Barohn RJ, Liang MH, Singh JA, Aggarwal R, Arnardottir S, Chinoy H, Cooper RG, Dankó K, Dimachkie MM, Feldman BM, Torre IGDL, Gordon P, Hayashi T, Katz JD, Kohsaka H, Lachenbruch PA, Lang BA, Li Y, Oddis CV, Olesinska M, Reed AM, Rutkowska-Sak L, Sanner H, Selva-O'Callaghan A, Song YW, Vencovsky J, Ytterberg SR, Miller FW, Rider LG (2017) 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis 76:1955–1964. https://doi.org/10.1136/annrheumdis-2017-211468
12. Malaviya AN, Delhi N, Kunj V, et al (2017) 2017 EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: little emphasis on autoantibodies, why? 2017 EULAR/ACR classification criteria for adult and juvenile idiopathic inflammator. 2017–2018. https://doi.org/10.1136/annrheumdis-2017-212701
13. Ghirardello A, Rampudda M, Ekholm L, Bassi N, Tarricone E, Zampieri S, Zen M, Vattemi GA, Lundberg IE, Doria A (2010) Diagnostic performance and validation of autoantibody testing in myositis by a commercial line blot assay. Rheumatology 49:2370–2374. https://doi.org/10.1093/rheumatology/keq281
14. Rönnelid J, Barbasso S, Storfors H et al (2009) Use of a commercial line blot assay as a screening test for autoantibodies in inflammatory myopathies. Autoimmun Rev 9:58–61. https://doi.org/10.1016/j.autrev.2009.03.005

15. Vulsteke J-B, De Langhe E, Claeys KG, Dillaerts D, Poesen K, Lenaerts J, Westhovens R, Van Damme P, Blockmans D, De Haes P, Xavier B (2018) Detection of myositis-specific antibodies. 1–4. https://doi.org/10.1136/annrheumdis-2017-212915
16. Lilleker JB, Vencovsky J, Wang G, et al (2018) The EuroMyositis registry: an international collaborative tool to facilitate myositis research. 30–39. https://doi.org/10.1136/annrheumdis-2017-211868
17. Selva-O'Callaghan A, Labrador-Horrillo M, Solans-Laque R et al (2006) Myositis-specific and myositis-associated antibodies in a series of eighty-eight mediterranean patients with idiopathic inflammatory myopathy. Arthritis Care Res 55:791–798. https://doi.org/10.1002/art.22237
18. Palterer B, Vitiello G, Carraresi A, Giudizi MG, Cammelli D, Parronchi P (2018) Bench to bedside review of myositis autoantibodies. Clin Mol Allergy 16:1–17. https://doi.org/10.1186/s12948-018-0084-9
19. Srivastava P, Dwivedi S, Misra R (2016) Myositis-specific and myositis-associated autoantibodies in Indian patients with inflammatory myositis. Rheumatol Int 36:935–943. https://doi.org/10.1007/s00296-016-3494-3
20. Cruellas M, Viana V, Levy-Neto M, Souza FH, Shinjo SK (2013) Myositis-specific and myositis-associated autoantibody profiles and their clinical associations in a large series of patients with polymyositis and dermatomyositis. Clinics 68:909–914. https://doi.org/10.6061/clinics/2013(07)04
21. Picard C, Vincent T, Lega JC, Hue S, Fortenfant F, Lakomy D, Humbel RL, Goetz J, Molinari N, Bardin N, Bertin D, Johanet C, Chretien P, Dubucquoi S, Streichenberger N, Desplat-Jégo S, Bossuyt X, Sibilia J, Abreu I, Chevailler AFN (2016) Heterogeneous clinical spectrum of anti-SRP myositis and importance of the methods of detection of anti-SRP autoantibodies: a multicentric study. Immunol Res 64:677–686. https://doi.org/10.1007/s12026-015-8774-6
22. Fujimoto M, Hamaguchi Y, Kaji K et al (2012) Myositis-specific anti-155/140 autoantibodies target transcription intermediary factor 1 family proteins. 64:513–522. https://doi.org/10.1002/art.33403
23. Sato S, Hoshino K, Satoh T et al (2009) RNA helicase encoded by melanoma differentiation – associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis association with rapidly progressive interstitial lung disease. 60:2193–2200. https://doi.org/10.1002/art.24621
24. Dankó K, Ponyi A, Molnar AP, András C, Constantin T (2009) Paraneoplastic myopathy. Curr Opin Rheumatol 21:594–598. https://doi.org/10.1097/BOR.0b013e3283317fa5
25. Fiorentino DF, Chung LS, Christopher-Stine L, Zaba L, Li S, Mammen AL, Rosen A, Casciola-Rosen L (2013) Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1γ. Arthritis Rheum 65:2954–2962. https://doi.org/10.1002/art.38093
26. Ghillani P, André C, Toly C, Rouquette AM, Bengoufa D, Nicaise P, Goulvestre C, Gleizes A, Dragon-Durey MA, Alyanakian MA, Chretien P, Chollet-Martin S, Musset L, Weill B, Johanet C (2011) Clinical significance of anti-Ro52 (TRIM21) antibodies non-associated with anti-SSA 60 kDa antibodies: results of a multicentric study. Autoimmun Rev 10:509–513. https://doi.org/10.1016/j.autrev.2011.03.004
27. Satoh M, Chan EKL, Ho LA et al (2012) Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. 64:2319–2327. https://doi.org/10.1002/art.34380
28. Yamasaki Y, Satoh M, Mizushima M et al (2015) Clinical subsets associated with different anti- aminoacyl transfer RNA synthetase antibodies and their association with coexisting anti-Ro52. 7595. https://doi.org/10.3109/14397595.2015.1091155