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ORIGINAL PAPER

Specific autoantibodies in dermatomyositis: a helpful tool to classify different clinical subsets

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Abstract Autoantibodies are important in the diagnosis of dermatomyositis. They can be divided in two different groups: myositis-associated autoantibodies (MAA) prevailing in overlap syndromes, and myositis-specific autoantibodies (MSA), with diagnostic specificity exceeding 90%. Our purpose was to detect retrospectively the prevalence of the most common MSAs in a group of 19 adult DM patients (13 women, 6 men). A severe DM (SDM), with extensive cutaneous and muscular manifestations, dysphagia, and sometimes pneumopathy, was detected in ten cases. Three patients had a mild DM (MDM), with little muscle and skin impairment, and a short course. Four patients suffered from amyopathic DM (ADM), two from paraneoplastic DM (PDM). Each serum was tested for ANA, ENA, MAAs, MSAs. Myositis-specific autoantibodies were detected in 15 cases. The most frequent was anti-TIF1γ, associated with SDM or PDM in four out of seven cases. Anti-MDA5 antibodies were recorded in a SDM and in a ADM with lung fibrosis. Anti-Mi2 and anti-SRP antibodies were both detected in a MDM and in a SDM, whereas anti-SAE1 in a amyopathic form. Other antibodies (anti-NXP2, -Jo1, -PL7, -PL12, -OJ) were found in single patients with SDM. Our series confirmed that specific autoantibodies could be helpful to classify different clinical subsets, particularly in the case of paraneoplastic forms or association with pneumopathy. Moreover, they can help in predicting the disease evolution and influence therapeutic strategies. A greater number of cases should be useful to highlight the clinical and pathogenic role of these antibodies, and develop a homogeneous protocol for diagnosis and treatment.

Keywords Dermato-polymyositis · Autoimmune myositis · Myositis-associated antibodies · Myositis-specific antibodies · Autoimmunity

Introduction

Dermato-polymyositis are a group of systemic autoimmune connective-tissue diseases characterized by symmetrical proximal muscle weakness and cutaneous signs. From a clinical and histological point of view, the idiopathic inflammatory myositis include eight categories: dermatomyositis (DM) of adults, juvenile DM, amyopathic DM (ADM), cancer-associated DM, polymyositis (PM), overlap myositis, immune-mediated necrotizing myopathy and inclusion body myositis.

The incidence of this group of diseases is approximately 2–15 new cases/year per million of inhabitants, with a predilection for the female sex, with a female/male ratio of about 2.5:1 [11, 41, 46, 49].

Autoantibodies are important for the diagnosis of connective-tissue diseases including DM, since they can be detected in the majority of the patients. To date, it is not clear if they have a pathogenic role or if they are merely an epiphenomenon, and further studies are needed in this regard [19].

G. Merlo and A. Clapasson contributed equally to this manuscript, respectively, for the medical and laboratory section.

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Classically, autoantibodies can be divided into two groups, based on their diagnostic accuracy: the first one is the group of myositis-associated autoantibodies (MAAs), as anti-PM/Scl, -U1, -U2, -U3, -RNP, -Ku, -SSA/Ro and -SSB/La, which are usually found in mixed connective-tissue diseases with myositis as a clinical symptom; the second one is the group of the myositis-specific autoantibodies (MSAs), with diagnostic specificity exceeding 90%. It includes anti-synthetase autoantibodies which are the well known as anti-Jo1 (histidyl-tRNA-synthetase), anti-PL7 (threonyl-tRNA-synthetase), anti-PL12 (alanyl-tRNA-synthetase), anti-EJ (glycyl-tRNA-synthetase), anti-OJ (isoleucyl-tRNA-synthetase), and anti-Mi2 (nucleosome remodeling and histone deacetylase protein complex). Recently other autoantibodies have been described in DM patients, namely anti-TIF1γ (an antigen of 155 kDa, the transcriptional intermediary factor 1γ), anti-NXP2 (a nuclear matrix protein 2), anti-SAE (small ubiquitin-like modifier activating enzyme), anti-MDA5 (melanoma differentiation-associated gene 5) and anti-SRP (signal recognition particle). All these MSAs seem to be important for a precise definition of disease subsets.

We retrospectively detected the prevalence of the most common MSAs in a group of 19 adult patients with DM seen in our section of Dermatology.

Materials and methods

The sera of 19 adult patients diagnosed with DM, seen in our section of Dermatology from May, 2000 to December, 2013 were collected. The mean age at diagnosis was 56.4 years (range from 31 to 79).

The diagnostic criteria, proposed by Bohan and Peter [9, 10] were adopted. They include: (a) pathognomonic skin manifestations (Gottron's sign, Gottron's papules); (b) symmetrical proximal muscle weakness, with or without dysphagia or respiratory muscle involvement; (c) typical muscular and/or skin histopathological alterations; (d) increased serum levels of muscle enzymes (creatine phosphokinase, aldolase, alanine transaminase, lactate dehydrogenase); (e) typical myopathic pattern in electromyography.

We distinguished four clinical subtypes. (1) Severe DM (SDM) included cases of refractory disease and severe manifestations such as marked weakness, myositis, joint involvement and dysphagia. A rapidly progressive interstitial lung involvement or cardiac impairment could also be present [40, 43]. From the dermatological point of view, a great severity of manifestations characterized this form, with extensive erythema, scales, erosion and ulceration, and the possible occurrence of poikiloderma and calcinosis. In addition, Gottron's papules, periungual changes and alopecia could be present [3]. (2) Mild DM was defined by the presence of little muscle and skin manifestations of lesser extent than the first group [3], without other systemic involvement. The course of the disease was usually short and patients had good response to therapy. (3) Amyopathic presentation was characterized by the occurrence of typical skin manifestations, irrespective of their extension, but no evidence of musculoskeletal involvement [13]. (4) Finally, paraneoplastic DM (PDM) refers to the presence of disease associated with a tumor, regardless of the severity of skin and muscle manifestation.

The first serum of each patient, collected at the time of the diagnosis and before any treatment, was studied. The follow-up of all cases was between 2 and 15 years, with an average time of 10 years. During this period it was possible to monitor the clinical course and to evaluate any occurrence of muscular involvement in patients with amyopathic presentation at the first visit, and/or the onset of a neoplasia. Informed consent was obtained from all investigated subjects.

Laboratory evaluations were performed by automated methods. Serum samples were first assayed for antinuclear antibodies (ANA) by indirect immunofluorescence on HEp2 cells (test kits from EUROIMMUN Lübeck, Germany) and after anti extractable nuclear antibodies (ENA: SSB/La, SSA/Ro, RNP, Sm) were detected with standard immunofluorescence and immunoenzymatic methods (ELISA, test kits from Orgentec Diagnostika GmbH, Mainz, Germany).

We considered ANA titer $\geq$1:80 as positive, even if occasionally found also in healthy population, because it could be the predictor of an initial connective disease.

To detect MSAs and MAAs, we used a qualitative in vitro immunoassay in which membrane strips, printed with lines of purified antigens are used as solid phase (Euroimmun AG Lübeck, Germany). In the test, each antigen is coated onto a separate membrane fragment. Since antigen bands are located at defined positions, results can be evaluated visually without the need for additional equipment. We detected anti -Mi2, -Jo1, -PL7, -PL12, -EJ, -OJ, -SRP, -TIF1γ, -MDA5, -NXP2, -SAE1 antibodies. In addition, we detect any presence of particular MAAs, represented by anti-Ku, anti-Ro52 and anti-Pm/Scl-75 and -100 antibodies. Sera from patients were diluted in sample buffer (1:101) and processed according to the manufacturer's protocol. Assays were interpreted with scanning software (EUROLine-Scan, Lübeck, Germany) as negative, borderline, positive or strongly positive.

Results

Clinically, four patients (two men and two women) had an amyopathic presentation, and one of them manifested an additional pulmonary fibrosis. Two patients (both men) had

a paraneoplastic DM (PDM), with the onset of a myeloma and a colon carcinoma. Both of them had severe cutaneous and muscular manifestations. In the remaining 13 patients, a classic DM of adults was appreciated, respectively, with a very severe skin and muscle involvement (SDM) in ten cases (eight women and two men), and with a mild and short course (MDM) in three cases (all women).

Of the 19 tested sera, 18 were positive for ANA, with titles ranging from 1:80 to 1:320, and one serum was negative. We defined ANA titer $\geq$1:80 as positive, even if occasionally found also in healthy population, because it can reveal a predisposition to an autoimmune disease [among the positive ones, 10 had a speckled pattern (title from 1:80 to 1:320), two had both speckled and nucleolar pattern (1:320), two had both speckled and cytoplasmatic pattern (title from 1:160 to 1:320). Furthermore, a patient had a cytoplasmatic and homogeneous pattern (1:320), another one had a speckled and mitochondrial pattern (1:320), a patient presented a speckled pattern with nuclear dots, and finally the last case had nucleolar pattern (1:160)]. Conversely, ENA were detected in three out of 19 sera, directed against SSA/Ro (particularly anti-Ro 52 kDa) in all the cases and against SSB/La in two of these sera. In these patients no sign of overlap syndrome was found. However, a more severe cutaneous and muscular involvement was detected.

Western blot test found MSAs in 15 patients (78.9%). In particular, 12 patients had a reactivity against one antigen, respectively, TIF1γ in five cases, MDA5 in two cases, Mi2 in two cases, and finally NXP2, Jo1, SAE1 in one case each. One of the patients presenting anti TIF1γ, also associated the presence of a MAA, specifically anti-PM/Scl antibody. In two patients two different antibody specificities were detected, specifically anti-TIF1γ and -SRP, anti-TIF1γ and -PL7. Moreover, in one case a triple reactivity against PL12, SRP, OJ was recorded.

None of our patients had anti-EJ or anti-Ku antibodies. Four cases were negative for MSAs.

Comparing the detection of the specific antibodies with the clinical presentation, anti-TIF1γ antibodies were associated with SDM or PDM in four out of seven cases; the remaining cases were two amyopathic and one mild presentation. All paraneoplastic presentations of our series were characterized by severe myositis and extensive skin involvement. Anti-MDA5 antibodies were recorded in a SDM, and in a form of ADM with pulmonary fibrosis. Moreover, anti-Mi2 antibodies were recorded in a MDM and a SDM, whereas anti-SAE1 only in a amyopathic form. Anti-SRP were associated with a MDM and a SDM. Finally, the other detected antibodies (including anti-NXP2, -Jo1, -PL7, -PL12, and -OJ) were found in single cases in patients suffering from SDM.

All these data are summarized in Tables 1 and 2.

Discussion

All our patients were included in the study since they met the clinical, laboratory and imaging criteria proposed by Bohan and Peter [9, 10] for the diagnosis of DM. Because of the higher prevalence of adult patients accessing our Department of Dermatology, we did not record any case of juvenile DM. Moreover, we did not include patients suffering from overlap syndromes, nor from pure PM. We, therefore, decided to distinguish our cases in classic DM, mild or severe, ADM, and cancer-associated DM, paying particular attention to any possible correlation with the detection of specific autoantibodies.

A positive ANA test occurred in the majority of our patients (94.7%), with a prevalence of the speckled pattern. This result is comparable, even superior, to that observed in the literature (80–90%) [33]. Conversely, ENA were positive only in three out of 19 patients, considering that they usually prevail in overlap forms between DM and lupus, as well as systemic sclerosis or Sjögren's syndrome. However, in our series we did not find any case of overlap.

Regarding MSAs, they were found in 15 patients of our study (78.9%). A similar result was detected in literature, where 60–80% of adult patients with DM have at least one identifiable MSA [29, 39].

The MSAs have a diagnostic specificity exceeding 90% and target cytoplasmatic or nuclear ribonucleoproteins involved in key processes of cell biology. They are usually associated with different disease subsets [6, 27, 38].

A comparison between the main clinical association and incidence of the different MSAs in the literature and the results of our series are reported in Table 3.

In our experience, anti-TIF1γ was the most frequently expressed antibody, being positive in seven patients (36.84%), respectively, in five cases alone, in one case associated with the presence of anti-SRP antibodies, and in another patient associated with anti-PL7 antibodies. Furthermore, in a woman suffering from SDM, the presence of anti-TIF1γ antibodies was accompanied by the presence of a MAA, anti-Pm/SCL, which usually prevails in overlap syndromes. Our case had no signs of overlap with other autoimmune diseases. However, she presented an extensive skin involvement, a severe muscle weakness with myositis and joint involvement, and a disease refractory to therapy. In literature anti-TIF1γ antibodies have been found in variable percentages from 9 to 41% according to different studies and reviews [6, 8, 23, 24, 27, 50, 52]. They target the nuclear transcription factor TIF1γ, involved in the TGFβ signaling pathway, and are often associated with malignancy, mainly in adult male patients. In particular, their findings have a positive predictive value of 42% for the association with cancer, whereas its negativity has a

Table 1 Clinical and antibody characteristics of 19 patients with DM

Patient	Sex	Clinical form (DM)	ANA (IgG)	ENA (U/ml)	MSAs and MAAs anti
1	F	ADM	Speckled (1:160)	Negative	TIF1γ
2	F	ADM	Speckled + mitochondrial (1:320)	Negative	TIF1γ
3	M	ADM	Speckled + cytoplasmatic (1:320)	Negative	SAE1
4	M	ADM + fibrosis	Speckled (1:160)	Negative	MDA5
5	F	MDM	Speckled + nucleolar (1:320)	Negative	TIF1γ, SRP
6	F	MDM	Speckled (1:80)	Negative	–
7	F	MDM	Speckled (1:80)	Negative	Mi2
8	M	PDM	Negative	Negative	–
9	M	PDM	Speckled (1:160)	Negative	TIF1γ
10	F	SDM	Speckled + nucleolar (1:320)	Positive: SSA/Ro 98, SSB/La 107	NXP2
11	F	SDM	Nucleolar (1:160)	Positive: SSA/Ro 65	TIF1γ, PM/Scl
12	F	SDM	Speckled (1:320)	Negative	Jo1
13	F	SDM	Speckled (1:160)	Negative	–
14	F	SDM	Homogeneous + cytoplasmatic (>1:320)	Negative	TIF1γ
15	F	SDM	Speckled (1:80)	Negative	TIF1γ, PL7
16	F	SDM	Speckled (1:320)	Negative	Mi2
17	M	SDM	Speckled + cytoplasmatic (1:160)	Negative	SRP, PL12, OJ
18	M	SDM	Speckled (>1:320)	Positive: SSA/Ro 131, SSB/La 135	MDA5
19	F	SDM	Speckled + nucleardots	Negative	–

ADM amyopathic dermatomyositis, *ANA* antinuclear antibodies, *DM* dermatomyositis, *ENA* anti extractable nuclear antibodies, *F* female, *M* male, *Jo1* histidyl-tRNA-synthetase, *MDA5* melanoma differentiation-associated gene 5, *Mi2* nucleosome remodeling and histone deacetylase protein complex, *MDM* mild dermatomyositis, *MSA* myositis-specific autoantibodies, *NXP2* nuclear matrix protein 2, *OJ* isoleucyl-tRNA-synthetase, *PL7* threonyl-tRNA-synthetase, *PL12* alanyl-tRNA-synthetase, *PDM* paraneoplastic dermatomyositis, *SAE1* small ubiquitin-like modifier activating enzyme subunit 1, *SDM* severe dermatomyositis, *SRP* signal recognition particle, *TIF1γ* transcriptional intermediary factor 1γ

negative predictive value of 97% [1, 17]. Recently, some authors observed that age is associated with an increased risk for malignancy in these patients, since the older patients have a higher probability of developing cancer [23, 24]. From the dermatological point of view, these antibodies are usually detected in severe cutaneous forms, both in adult and young patients. Fiorentino et al. described several significant findings in anti-TIF1γ-positive patients, including hyperkeratotic Gottron's papules, psoriasiform lesions, hypopigmented and telangiectatic patches and diffuse photoerythema. These antibodies also showed to be significantly associated with lower prevalence of arthritis/arthralgia and of interstitial pulmonary disease [23].

Among our patients, the detection of these antibodies was associated with severe cutaneous manifestations, characterized by necrotic, ulcerative and edematous lesions, in three cases, and with a paraneoplastic form in one case. Eventually, two patients had ADM and the last had a mild cutaneous involvement. Overall, the association in approximately 60% of our patients with severe DM lines up with the literature data [24].

Anti-synthetase autoantibodies are directed against specific enzymes which catalyze the ATP-dependent binding of each amino acid to its tRNA in the protein synthesis. They include different subtypes, among which anti-Jo1 is by far the most common, being found in 10–40% of patients with PM/DM [6, 12, 36, 54]. In literature, the detection of anti-synthetase antibodies is frequently associated with the presence of a characteristic clinical phenotype, also known as "anti-synthetase syndrome", characterized by interstitial lung disease, myositis, non-erosive polyarthritis and/or polyarthralgias, Raynaud's phenomenon, heliotrope rash, Gottron's papules and hyperkeratosis on the knuckles and the back of the hands (the so-called "mechanic hands"). The presence of anti SSA/Ro antibodies (especially anti Ro 52 kDa) often accompanies anti-Jo-1 in these patients, and is associated with severe lung involvement [47, 55]. In our survey, this specific syndrome has not been reported; however, in all patients these antibodies were associated with a SDM, with important skin manifestations. Anyways, the detection of anti-synthetase autoantibodies requires a periodic muscular and even more respiratory surveillance, as they would seem to be predictive of a possible pulmonary interstitial idiopathic disease, which may progress to fibrosis and death.

Antibodies against Mi-2 recognize the nuclear protein complex NURD (nucleosome remodeling histone deacetylase) involved in transcription of DNA. They have a

Table 2 Correlation between antibody positivity and clinical presentation in 19 patients with DM

Antibodies anti	Clinical form			
	ADM	MDM	SDM	PDM
TIF1γ	2	1	3	1
Mi2		1	1	
SRP		1	1	
MDA5	1		1	
SAE1	1			
NXP2			1	
Jo1			1	
PL7			1	
PL12			1	
OJ			1	
PM/Scl			1	
MSA neg.		1	2	1

ADM amyopathic dermatomyositis, *Jo1* histidyl-tRNA-synthetase, *MDA5* melanoma differentiation-associated gene 5, *MDM* mild dermatomyositis, *Mi2* nucleosome remodeling and histone deacetylase protein complex, *MSA* myositis-specific autoantibodies, *NXP2* nuclear matrix protein 2, *OJ* isoleucyl-tRNA-synthetase, *PDM* paraneoplastic dermatomyositis, *PL7* threonyl-tRNA-synthetase, *PL12* alanyl-tRNA-synthetase, *SAE1* small ubiquitin-like modifier activating enzyme subunit 1, *SDM* severe dermatomyositis, *SRP* signal recognition particle, *TIF1γ* transcriptional intermediary factor 1γ

high sensitivity and characterize classical forms of DM with typical cutaneous manifestation, both in adults and in children [53], usually benign course and a good response to steroid therapy. Furthermore, these MSAs seem to be associated with a lower risk of paraneoplastic form, thus being a good prognostic factor. Moreover, there would be no correlation with amyopathic form [34, 37, 53].

Anti-Mi2 antibodies can be found in 10–30% of DM patients [6, 26, 28, 32] and in 8–12% of idiopathic myositis cases [34, 53]. In our small series, the detection of anti-Mi 2 correlated, in only one case out of two, to a DM with a benign course, such as that described in the literature. Certainly, an extension in the number of the patients is recommended to expand our data.

Among other MSAs, anti-SRPs are rarer but just more important than the previous. They are directed against a cytoplasmic protein involved in the protein translocation across the rough endoplasmic reticulum [56]. These antibodies are present in 4–8% of patients affected by PM/DM and usually characterize mild forms, both from a clinical and a prognostic point of view [4, 6, 26, 29]. However, they can sometimes represent the serological markers of a specific syndrome named "anti-SRP antibodies syndrome"; this is a rare form of immune-mediated necrotizing myositis, clinically characterized by a sudden motor deficit, a progressive muscle weakness, sometimes a cardiac involvement, and an increase of serum muscle enzymes. The response to immunosuppressive therapy is usually poor and incomplete, leading to an unfavorable prognosis, since the 5-year survival is about 25% [18, 31, 51, 57]. The histology reveals a massive necrosis of muscular fibers, with degeneration and repair processes, and usually with poor inflammation. The differential diagnosis should consider other neuromuscular diseases, such as muscular dystrophy [4, 26].

In our study, no case of necrotizing myopathy was described. Instead, in a patient the detection of these antibodies was associated with a mild form, as suggested in literature [31, 51], and in another patient with a more aggressive DM.

Anti-MDA5 antibodies target a protein of 140 kDa, encoded by the MDA5 gene, which seems to be involved in the innate antiviral immune response. These antibodies are considered a specific marker of ADM and they have been found in about 20% of DM patients, particularly in Asian patients [6, 14, 21, 26, 42]. In these patients some typical cutaneous manifestations often occur, including ulcerations, palmar pustules, and even panniculitis, which usually lack in the classical form. Furthermore, anti-MDA5 antibodies are specifically associated with an interstitial lung involvement, characterized by a rapidly progressive fibrosis, which can dramatically complicate the prognosis of the patient [14, 42, 45]. In fact, the mortality rate of these patients can reach nearly 50%, despite aggressive immunosuppressive therapy and even lung transplantation. Sato et al. found that the antibody titer correlates to disease activity and predicts the outcome, so it can be used to monitor the disease [48].

Furthermore, some authors suggested an association between these autoantibodies and a severe skin vasculopathy in adult DM patients, characterized by vascular fibrin deposition and variable perivascular inflammation [16, 21, 30].

In our experience, only two patients had anti-MDA5 antibodies, respectively, a patient suffering from SDM with severe cutaneous and muscular involvement, and the other with ADM associated with pulmonary fibrosis, reflecting the data collected from the literature [14, 42]. The detection of these antibodies should, therefore, be accompanied by a periodic clinical monitoring, as to make possible the early detection of any respiratory symptoms.

Only in one of our patients we found anti SAE1 antibodies. In literature they are described in approximately 5–8% of DM patients [6, 7, 25, 26, 44] and seem to be related to a severe cutaneous involvement, with mild muscular manifestations [5, 50]. Some patients complain of dysphagia, but the prognosis is usually favorable. As

Table 3 Comparison between our series and the data reported in literature

Antibodies anti	In our series		In literature	
	Incidence	Clinical form	Incidence	Clinical form
TIF1γ	7/19 (36.8%)	2 ADM 1 MDM 3 SDM 1 PDM	13–31% [6] 9% [8] 41% [23] 16.8% [24] 20-30% [27] 13-21% [50] 21% [52]	PDM SDM
Anti-synthetase autoantibodies	3/19 (15.8%): 1 Jo1 1 PL7 1 PL12 and OJ	3 SDM, but no anti-synthetase syndrome	Jo1 9-24% [6] Other <5% [6] Jo1 18% [12] Other 3% [12] Jo1 11% [36] Other 10% [36] Jo1 18–20% [54] Other <5% [54]	Anti-synthetase syndrome
Mi2	2/19 (10.5%)	1 MDM 1 SDM	9–24% [6] 10–15% [26] Up to 30% [28] 20–30% [32]	MDM
SRP	2/19 (10.5%)	1 MDM 1 SDM	4–6% [4] 5% (Caucasian patients) [6] 8–13% (Asian/African patients) [6] 4–6% [26] 5% [29]	MDM Anti-SRP antibodies syndrome
MDA5	2/19 (10.5%)	1 ADM + lung fibrosis 1 SDM	0–13% (Caucasian patients) [6] 10–48% (Asian patients) [6] 9.4% [14] 13% [21] 20% (Asian patients) [26] 11% (Asian patients) [42]	ADM SDM Interstitial lung involvement
SAE1	1/19 (5.3%)	1 ADM with severe skin lesions	6–8% (Caucasian patients) [6] 2% (Asian patients) [6] 8% [7] 8.4% [25] 8% [26] 1.8% [44]	Severe cutaneous involvement, with mild muscular manifestations
NXP2	1/19 (5.3%)	1 SDM	1–17% [6] <5% [8] 17% [22] 20% [26] 25% [27]	SDM PDM

ADM amyopathic dermatomyositis, *Jo1* histidyl-tRNA-synthetase, *MDA5* melanoma differentiation-associated gene 5, *MDM* mild dermatomyositis, *Mi2* nucleosome remodeling and histone deacetylase protein complex, *MSA* myositis-specific autoantibodies, *NXP2* nuclear matrix protein 2, *OJ* isoleucyl-tRNA-synthetase, *PDM* paraneoplastic dermatomyositis, *PL7* threonyl-tRNA-synthetase, *PL12* alanyl-tRNA-synthetase, *SAE1* small ubiquitin-like modifier activating enzyme subunit 1, *SDM* severe dermatomyositis, *SRP* signal recognition particle, *TIF1γ* transcriptional intermediary factor 1γ

described in the literature, also a patient of ours was suffering from ADM with severe skin involvement [5, 50].

Finally, anti-NXP2 antibodies are rare in adults DM patients, being reported in variable percentages from 5 to about 20%. [6, 8, 22, 26, 27] of the cases. In these patients, they are frequently associated with severe forms, with possible paraneoplastic evolution, particularly in males. In this regard, a recent study conducted by Fiorentino et al. provided the evidence that anti-NXP2 and -TIF1γ antibodies identify overall, the vast majority of the patients with cancer-associated DM [22]. Instead, these antibodies are more frequent in juvenile forms (23–25%), typically with specific cutaneous manifestations, including calcinosis, an early onset and an aggressive course [10, 15, 20, 50]. In our series, the positivity of these antibodies was associated, in the only reported case, to a SDM with an aggressive skin involvement, but without neoplastic evolution.

Overall, the results we observed are largely similar to those of the literature, although our number of patients is small. Anti-TIF1γ antibodies proved to be involved in more than half of the cases to severe manifestations or association with cancer. Anti-synthetase autoantibodies were found in forms with extensive and severe general involvement, though we did not observe any cases of "anti synthetase syndrome". Moreover, anti-MDA5 antibodies showed a match with the literature in one out of two cases, since we found them in an amyopathic presentation with a severe lung fibrosis. The anti-SAE1, usually observed in the literature in extensive cutaneous forms with mild muscle involvement, were reported in a patient with ADM and severe skin lesions. Furthermore, anti-NXP2 antibodies were detected in a patient with an aggressive form as suggested by the literature, whereas anti-Mi2 in half of the cases showed similarity with the literature, appearing in a patient with mild DM. Finally, anti-SRP antibodies, commonly associated with mild manifestations or with immune-mediated necrotizing myositis, in our series were found both in a severe and in a mild presentation, but no necrotizing myositis was detected.

Our series confirmed that DM patients usually have a specific antibody profile, and revealed that SDM are the clinical forms with a higher reactivity to MSAs, whereas ADM, MDM, and PDM have lower reactivity. These antibodies showed a very high sensitivity (approximately 80% in our series) and can be used to classify different clinical subsets. Furthermore, they can help in predicting the evolution of the disease and influence therapeutic strategies. The limitation of our study is represented by the small number of patients, which does not allow to provide significant results and to draw definitive conclusions on what we observed. However, it may be a good starting point to expand the information and knowledge we have. Dermato-polymyositis are in fact rare diseases, therefore the study of a larger number of patient sera by different specialized centers could help to confirm the association of MSAs with distinct clinical subsets, to highlight their clinical and pathogenic role, and to develop a homogeneous screening, diagnosis and treatment.

Compliance with ethical standards

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