Clin Rheumatol (2017) 36:469–475
DOI 10.1007/s10067-016-3453-0

BRIEF REPORT

Myositis-specific autoantibodies and their association with malignancy in Italian patients with polymyositis and dermatomyositis

Angela Ceribelli[1] · Natasa Isailovic[1] · Maria De Santis[1] · Elena Generali[1] · Micaela Fredi[2] · Ilaria Cavazzana[2] · Franco Franceschini[2] · Luca Cantarini[3] · Minoru Satoh[4] · Carlo Selmi[1,5]

Received: 19 September 2016 / Revised: 5 October 2016 / Accepted: 12 October 2016 / Published online: 20 October 2016

Abstract This study aims to characterize myositis-specific antibodies in a well-defined cohort of patients with idiopathic inflammatory myopathy and to determine their association with cancer. Sera from 40 patients with polymyositis, dermatomyositis, and controls were tested by protein and RNA immunoprecipitation to detect autoantibodies, and immunoprecipitation-Western blot was used for anti-MJ/NXP-2, anti-MDA5, and anti-TIF1γ/α identification. Medical records were re-evaluated with specific focus on cancer. Anti-MJ/NXP-2 and anti-TIF1γ/α were the most common antibodies in dermatomyositis. In six dermatomyositis cases, we found five solid forms of cancer and one Hodgkin's lymphoma in long-term remission. Among patients with cancer-associated dermatomyositis, three were positive for anti-TIF1γ/α, two for anti-Mi-2, and one for anti-MJ/NXP-2. The strongest positivity of anti-TIF1γ was seen in two active forms of cancer, and this antibody was either negative or positive at low titers in the absence of cancer or in the 7-year remission Hodgkin's lymphoma. Four out of twenty (20 %) patients with polymyositis had solid cancer, but no specific association with autoantibodies was identified; further, none of the four cases of antisynthetase syndrome had a history of cancer. No serum myositis-associated autoantibody was observed in control sera, resulting in positive predictive value 75 %, negative predictive value 78.5 %, sensitivity 50 %, specificity 92 %, and area under the ROC curve 0.7083 for the risk of paraneoplastic DM in anti-TIF1γ/α (+) patients. Myositis-specific autoantibodies can be identified thanks to the use of immunoprecipitation, and their association with cancer is particularly clear for anti-TIF1γ/α in dermatomyositis. This association should be evaluated in a prospective study by immunoprecipitation in clinical practice.

Keywords Biomarkers · Cancer · Idiopathic inflammatory myositis · Immunoprecipitation

✉ Carlo Selmi
carlo.selmi@unimi.it

[1] Division of Rheumatology and Clinical Immunology, Humanitas Research Hospital, via A. Manzoni 56, 20089 Rozzano, Milan, Italy

[2] Unit of Rheumatology, Spedali Civili, Brescia, Italy

[3] Research Center of Systemic Autoinflammatory Diseases and Behçet's Disease Clinic, Department of Medical Sciences, Surgery and Neurosciences, University of Siena, Siena, Italy

[4] Department of Clinical Nursing, University of Occupational and Environmental Health, Kitakyushu, Japan

[5] BIOMETRA Department, University of Milan, Milan, Italy

Introduction

Idiopathic inflammatory myopathy (IIM) is characterized by muscle inflammation, skin alterations, and internal organ involvement, resulting in muscle atrophy, skin microangiopathy, and tissue fibrosis [1]. IIMs are divided into several conditions with polymyositis (PM) and dermatomyositis (DM) as the most frequent forms despite being considered rare worldwide [2]. Beyond the clinical and histopathological differences, PM and DM can be further classified into subsets thanks to myositis-specific autoantibodies (MSA) which have diagnostic and prognostic roles [3, 4]. Some MSA have been known for decades, as for the anti-Jo-1 characterizing the antisynthetase syndrome or anti-Mi-2, peculiar for DM [5, 6]. Several MSA have been defined most recently by protein and RNA immunoprecipitation (IP). Paradigmatic MSA include DM-associated anti-MDA5, anti-MJ/NXP-2, and anti-TIF1γ/α which define specific clinical features and predict the

association with cancer [2, 6, 7], sometimes without independent confirmation [8].

To validate the proposed clinical associations, we utilized IP for MSA in a well-characterized cohort of patients with PM and DM from two clinical centers, with particular focus on the specificities identified in recent years and their association with cancer.

Materials and methods

Patients

The study included IIMs patients followed at the outpatient clinic at Humanitas Research Hospital (Rozzano, Milan, Italy) and Spedali Civili (Brescia, Italy) in the period 2013–2016. We included sera from 20 patients with PM, 2 with antisynthetase syndrome, 18 with DM, and controls represented by healthy subjects (NHS; $n = 12$) and patients with systemic sclerosis (SSc; $n = 79$), Behçet's disease (BD; $n = 45$), and psoriatic arthritis (PsA; $n = 145$). We used established criteria for the diagnosis of PM/DM, SSc, BD, and PsA and collected clinical and laboratory data at enrollment.

The study was approved by the Institutional Review Board of the hospitals and informed consent was obtained from all subjects.

Methods for autoantibody analysis

Patients' sera were isolated from whole blood through centrifugation at 2000*g* for 15 min, and then stored in −20 °C freezer until use. MSA were first screened by protein-IP using ^{35}S-methionine-labeled K562 cell extract followed by SDS-PAGE and autoradiography, and by RNA-IP using unlabeled K562 cell extract followed by urea-PAGE and silver staining [9, 10]. MSA were determined using reference sera obtained from the Autoantibody Standardization Committee (www.autoab.org) and from internal controls.

Candidates for anti-MJ/NXP-2 and anti-MDA5 were tested by IP-Western Blot (IP-WB) based on IP of a 140-kD protein, while candidates for anti-TIF1γ/α were selected based on bands at 155–140 kD by protein-IP. In detail, 8 μl of candidate sera were cross-linked with protein-A Sepharose beads and then immunoprecipitated with cell extract from 10^7 K562 cells. Proteins were then fractionated by 8 % SDS-PAGE and transferred to a nitrocellulose filter, probed with 1 μg/ml of anti-MORC3 mouse polyclonal antibody (Abnova, Taipey City, Taiwan) for MJ/NXP-2, followed by horseradish peroxidase (HRP) goat anti-mouse IgG (1:5000 dilution) (ThermoFisher, Waltham, MA, USA) and developed using Immobilon Western Chemiluminescent HRP substrate (Millipore, Darmstadt, Germany). The same procedure was used for anti-MDA5 antibodies using 1:1000 rabbit anti-MDA5 antibody (Millipore, Darmstadt, Germany) followed by 1:5000 HRP-conjugated goat anti-rabbit Ig light chain antibody (Jackson Immunoresearch, West Grove, PA, USA), and developed using Supersignal West Femto (ThermoFisher, Waltham, MA, USA). For TIF1γ IP-WB, we used 1:1000 mouse monoclonal anti-TIF1γ antibody (Abcam, Cambridge, UK), followed by 1:10.000 HRP goat anti-mouse IgG (ThermoFisher, Waltham, MA, USA), and developed using Immobilon Western Chemiluminescent HRP substrate (Millipore, Darmstadt, Germany).

Statistical analysis

All comparisons were performed by Mann-Whitney test and Pearson Chi square test using Stata 13.1 for Macintosh (StataCorp, 2013, CollegeStation, Texas, USA) and Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was accepted as $p < 0.05$.

Results

Through protein- and RNA-IP, we identified serum autoantibodies in IIMs as illustrated in Fig. 1a. None of our control sera were positive for MSA. Before using IP and IP-WB, only 6/18 (33 %) DM and 4/20 (20 %) PM cases had autoantibodies detected by routine autoimmunity tests, particularly anti-Ro/SSA, La/SSB, Mi-2, and -Jo-1. Thanks to IP analysis, we confirmed this positivity and identified additional MSA in patients with positive antinuclear antibodies (ANA) by indirect immunofluorescence but negative autoantibody for extractable nuclear antigens (ENA). We did not observe double autoantibody positivity in our cohort, except for the association of Ro/SSA, La/SSB, and Jo-1 as reported [11]. Protein- and RNA-IP confirmed two less common antisynthetase antibodies as anti-EJ (anti-glycyl tRNA synthetase) and anti-PL-12 (anti-alanyl tRNA synthetase) in one PM and one antisynthetase syndrome case, respectively (Fig. 1a). Using RNA-IP, we identified the 7SL RNA band characteristic of anti-SRP antibodies, in association with anti-Ro/SSA in one patient with PM (Fig. 1b), and in both cases necrotizing myositis was seen at muscle biopsy. Eight cases (1 DM and 7 PM) remain seronegative by IP, while in six DM cases, we identified bands at different molecular weight by protein-IP but their antigenic significance is still unknown (data not shown). Nine samples (5 IIMs and 4 SSc) had one band detectable around 140 kD by protein IP, and were tested by IP-WB for anti-MJ/NXP-2 and -MDA5 antibodies to identify the specificity corresponding to this band. In three DM cases, we

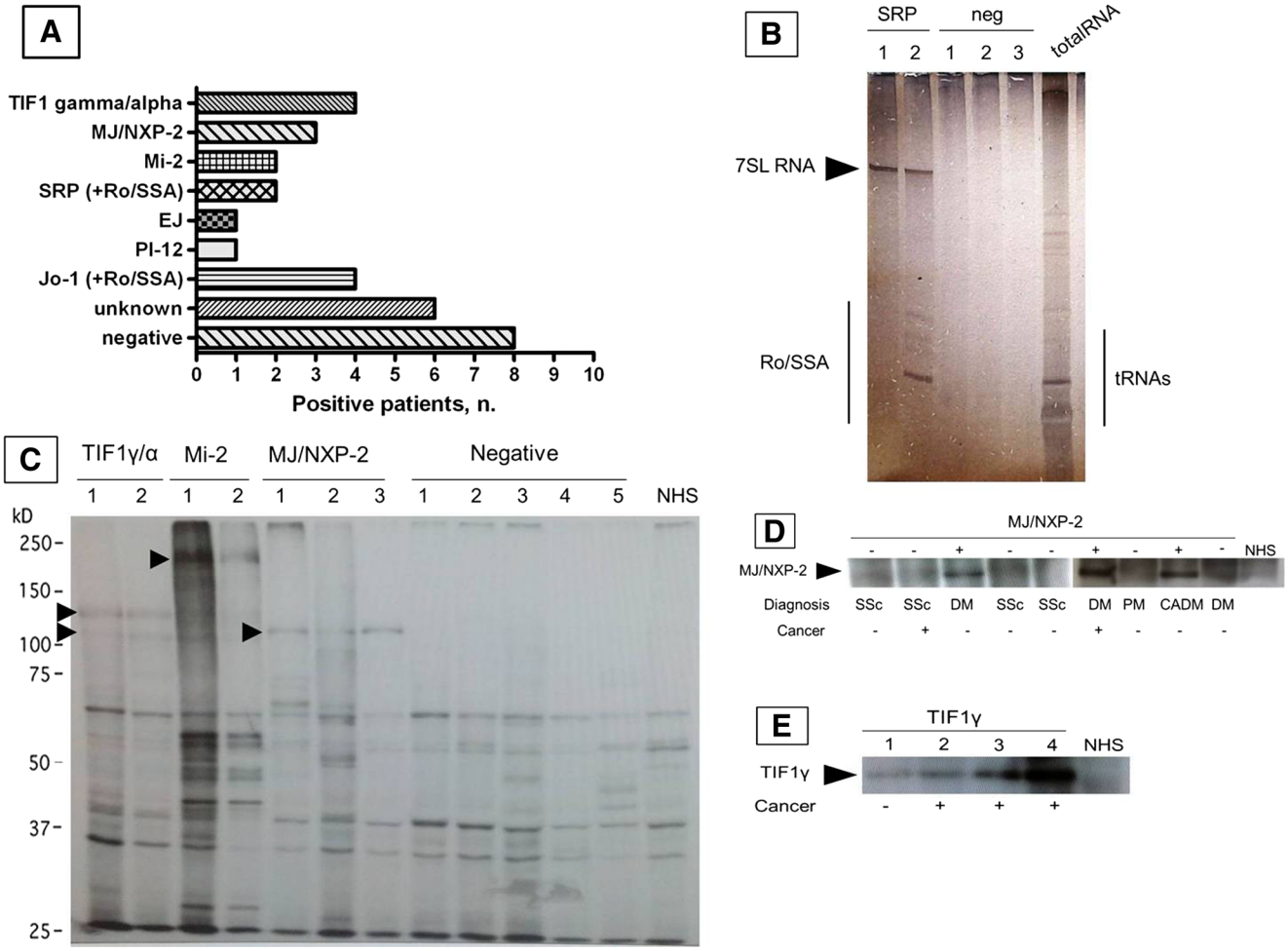

Fig. 1 MSA identified in our cohort of Italian PM/DM through the use of protein-IP, RNA-IP, and IP-WB. **a** Bar graph showing the specific autoantibodies and the number of corresponding cases identified in our cohort of PM/DM patients. **b** RNA-IP of two positive SRP samples, recognized by the band corresponding to 7SL RNA (*black arrow*). In one case, association with anti-Ro/SSA antibodies was identified (*black vertical line*) and supported by protein-IP (data not shown). The three negative RNA-IP samples shown are the anti-MJ/NXP-2 (+) patients reported in **c** and **d**, that typically do not show reactivity by RNA-IP. Total RNA was used as positive control. **c** Protein-IP (8 % SDS-PAGE gel) of representative PM/DM patients and corresponding autoantibodies: two anti-TIF1γ/α (+) cases shown by the bands at 155/140 kD (*black arrows*; two additional cases not shown in this protein-IP gel were identified based on the mobility of the same bands), two anti-Mi-2 (+) cases shown by the 240, 150, 72, 65, 63, 50, and 34 kD bands (*black arrow* for the 240 kD band), and the three anti-MJ/NXP-2 (+) cases identified by the 140 kD band (*black arrow*). Five DM cases negative for MSA are also shown, and one NHS (normal human serum) is present in the last lane. **d** IP-WB for anti-MJ/NXP-2 positive cases. The three anti-MJ/NXP-2 (+) cases shown in the protein-IP gel in **c** were tested together with other samples (myositis and SSc) that had 140 kD band at protein-IP; no SSc sample had positivity for MJ/NXP-2. This panel also represents in which cases an association with cancer was present, as described in Table 2. **e** IP-WB for anti-TIF1γ positive cases. The four anti-TIF1γ (+) cases identified by protein-IP through the detection of the 155/140 kD bands were positive by IP-WB as shown in this panel. The weakest sample (#1) was the only one not associated with cancer until the moment of evaluation of the patient, sample #2 had a diagnosis of Hodgkin's Lymphoma 7 years before DM onset and it is now considered in remission, sample#3 has active lung cancer, and sample#4 has advanced ovary cancer. One normal human serum (*NHS*) is represented in the last lane

confirmed the anti-MJ/NXP-2 positivity (Fig. 1c, d), while no MSA was detected in our control population.

The main clinical and laboratory features of patients are described in Table 1. The diagnosis of myositis was confirmed by muscle biopsy and/or electromyography only in 13/18 (72 %) of DM patients, coined clinically amyopathic DM. No significant difference was detected for organ involvement, laboratory tests abnormalities, and ongoing therapies in DM and PM patients, while the expression of anti-TIF1γ/α antibodies was significantly associated to DM patients (p = 0.04) as shown in Table 1. The ANA pattern reported by routine autoimmunity tests was very variable for titer and pattern, and in some cases also defined as "negative" (Tables 1 and 2), thus it was necessary to proceed with further testing by IP for the identification of MSA. Two anti-MJ/NXP-2 necessary to proceed with further testing by IP for the identification of MSA. Two anti-MJ/NXP-2 (+) DM patients had severe diffuse calcinosis that required surgical

Table 1 Main demographic and clinical features of our cohort of DM and PM patients, for which we performed serum IP analysis. Two anti-synthetase cases are not included

	DM (n = 18)	PM (n = 20)	p
Demographic features			
Female:Male	13:5	14:6	–
Mean age at enrollment, years (range)	49 (21–75)	59 (29–83)	0.05
Mean age at myositis onset, years (range)	42.5 (15–71)	53.5 (24–78)	ns
Clinical features			
Myositis (%)*	13 (72)	20 (100)	0.01
Raynaud's phenomenon (%)	7 (39)	4 (20)	ns
Arthritis (%)	5 (28)	5 (25)	ns
Interstitial lung disease (%)	2 (11)	5 (25)	ns
Dysphagia (%)	2 (11)	7 (35)	ns
Cancer (%)	6 (33)	4 (20)	ns
Use of steroid therapy (%)	17 (94)	19 (95)	ns
Use of immunosuppressants (%)	16 (89)	16 (80)	ns
Laboratory features			
Median CK at myositis onset, U/l (25th–75th percentile)	1605 (92–5160)	1400 (842–3295)	ns
ANA positive titer ≥1:320 (%)	9 (50)	11 (55)	ns
ENA identified by IP			
Anti-TIF1γ/α	4 (22)	0	0.04
Anti-MJ/NXP-2	3 (17)	0	ns
Anti-Mi-2	2 (11)	0	ns
Anti-SRP	1+Ro/SSA(5)	1 (5)	ns
Anti-Jo-1	0	4+Ro/SSA (20)	ns
Anti-EJ	0	1 (5)	ns
Anti-PL-12	0	1 (5)	ns
Anti-HMGCR	0	0	–
Anti-MDA5	0	0	–

ANA anti-nuclear antibodies, *CK* creatine kinase, *ENA* extractable nuclear antigen, *GI* gastro-intestinal, *IP* immunoprecipitation, *ns* not significant

*Confirmed by electromyography and/or muscle biopsy

removal in one case, and ongoing therapy with pamidronate infusions in one case of clinically amyopathic DM [12]. The association with cancer was present only in one DM case positive for this autoantibody (Fig. 1d). No serum was positive for anti-MDA5 antibodies, and in fact, no patient in our PM/DM cohort had symptoms such as rapidly progressive interstitial lung disease that are usually associated with this autoantibody [6]. The four anti-TIF1γ (+) sera have history of cancer in the three strongest positive cases (Fig. 1e), while the weakest positive case is the only one without cancer history until the moment of our clinical evaluation.

Cumulatively, we calculated the positive predictive value (75 %), negative predictive value (78.5 %), sensitivity (50 %), specificity (91.6 %), and area under the ROC curve (0.7083) for the risk of paraneoplastic DM in anti-TIF1γ/α (+) patients and these were compared to previous reports.

Discussion

The routine use of protein- and RNA-IP may increase the detection rate of rare autoantibodies in clinical practice, particularly in rare conditions such as PM and DM, thus maximizing the diagnostic and prognostic power of these biomarkers. In fact and despite the low incidence and prevalence worldwide, IIMs are characterized by wide clinical phenotype variability, mirrored by a significant number of MSA. We thus utilized the sensitive and specific IP to identify autoantibody prevalence and clinical significance in a well-defined cohort of Italian patients affected by PM/DM, with particular focus on cancer associations.

Our most relevant findings include that anti-MJ/NXP-2 and -TIF1γ/α antibodies are the two most frequent MSA in DM cases previously anti-ENA negative at routine tests.

Table 2 Main characteristics of the anti-MJ/NXP-2 (+) and TIF1γ/α (+) cases identified in our cohort of PM/DM patients. The cases described in this table are shown in Fig. 1d, e

	Anti-MJ/NXP-2 case 1	Anti-MJ/NXP-2 case 2	Anti-MJ/NXP-2 case 3	Anti-TIF1γ/α case 1	Anti-TIF1γ/α case 2	Anti-TIF1γ/α case 3	Anti-TIF1γ/α case 4
Demographic data							
Sex	Female	Female	Male	Male	Female	Female	Female
Age (years)	33	21	21	72	40	59	54
Diagnosis	DM	CADM	DM	DM	DM	CADM	DM
Age at onset (years)	19	15	18	67	22	58	52
Clinical data							
Skin lesions	+ (Gottron's papules, erythematosus rash)	+ (Gottron's papules, erythematosus rash)	+ (V-neck erythema)	+ (Gottron's papules, erythematosus rash)	+ (Gottron's papules, heliotrope rash)	+ (Gottron's papules, erythematosus rash)	+ (Gottron's papules, erythematous rash)
Calcinosis	+++	+++	−	−	−	−	−
Myositis	+	−	+	+	+	−	+
Arthritis	−	−	−	−	−	−	−
Raynaud's phenomenon	−	−	−	−	−	−	+
Interstitial lung disease	−	−	−	−	−	−	−
Cancer	+	−	−	−	+	+	+
Cancer location	Thyroid	−	−	−	Hodgkin's lymphoma	Lung adenocarcinoma	Ovary
Age at cancer onset (years)	31	−	−	−	15	56	52
Immunosuppressive therapy*	+ (PDN, HCQ, MTX, CsA, IV Ig, AZA)	+ (PLQ)	+ (PDN, PLQ, MTX, IV Ig, AZA, CsA)	+ (PDN, MMF)	+ (PDN, HCQ, CTX, AZA)	−	+ (PDN, MTX, IV Ig)
Laboratory data							
Increased CK at myositis onset	+	−	+	+	+	−	+
CK at last visit	+	Normal	Normal	Normal	Normal	Normal	+
ANA	1:640 nuclear dots	1:160 speckled	1:80 speckled	Negative	1:160 speckled	Negative	>1:640 speckled

ANA anti-nuclear antibodies, *AZA* azathioprine, *CADM* clinically amyopathic DM, *CK* creatine kinase, *CsA* cyclosporine, *CTX* cyclophosphamide, *DM* dermatomyositis, *HCQ* hydroxychloroquine, *IV Ig* intravenous immunoglobulins, *MMF* mycophenolate mofetil, *MTX* methotrexate, *PDN* prednisone

*The order of these therapies corresponds to the chronological order they were used by the patients

Accordingly, to what reported in the literature, our three anti-MJ/NXP-2 (+) DM cases have juvenile onset DM with typical skin DM features, no internal organ involvement, and the worst clinical manifestation is severe calcinosis [13]. All these cases required immunosuppressive therapy beyond steroids to control muscle inflammation, but in one case, DM was not completely controlled and this unresponsive patient had a diagnosis of papillary thyroid cancer 12 years after the onset of DM. In fact, cancer has been reported in adult anti-MJ/NXP-2(+) DM patients despite not being confirmed in our previous publication on a different Italian cohort [10, 14]. The identification of anti-MJ/NXP-2 antibodies was based on the first observation of a common band of 140 kD molecular weight by protein-IP, but it was then necessary to perform IP-WB to have a positive result for MJ/NXP-2. Anti-MDA5 antibodies also migrate in the same molecular weight range, but no sample tested positive by IP-WB and it was concordant with the clinical observation that these samples did not show the suggestive clinical features (i.e., rapidly progressive interstitial lung disease) that are commonly referred to anti-MDA5 positivity.

We detected serum anti-TIF1γ/α antibodies in four DM cases, only in one case there was no history of cancer despite extensive screening exams and it was the weakest positive case. All the other three cases have cancer history. In one case, this autoantibody was present in a DM patient with Hodgkin's

lymphoma diagnosed and treated 7 years prior to the onset of DM features, and considered in remission at the time of the blood draw. This is in contrast with previous reports of anti-TIF1γ/α antibodies not found in juvenile DM cases associated with cancer and of the highest associated risk of malignancy during the year prior to and the year after IIMs diagnosis [15]. The two strongest samples positive for anti-TIF1γ/α antibodies have active cancer unresponsive to treatment at the time of blood drawn. It is also important to highlight the fact that both our two anti-Mi-2(+) DM cases had a history of breast cancer prior to DM onset but no higher risk of cancer has been reported in association with this autoantibody [4]. Our estimate of the positive and negative predictive values, sensitivity, specificity, and area under the ROC curve for the risk of paraneoplastic DM in anti-TIF1γ/α (+) patients were concordant to previous reports [16–21].

In our PM cohort, four cases of cancer were reported, in three being diagnosed several years prior to and in one concomitant to the onset of PM; in all cases, solid forms affecting the thyroid, colon, breast, skin, and only two of them had a known autoantibody signature at routine tests represented by anti-Ro/SSA antibodies. No tumor was reported in the four cases affected by antisynthetase syndrome, and no MSA was identified in SSc, BD, PsA cases with or without a history of cancer. No PM case showed positivity for anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibodies despite the onset of necrotizing myopathy after the use of statin [3]. Other autoantibodies that were reported by routine autoimmunity laboratories through techniques such as immunoblotting were not confirmed by IP, as for anti-PM/Scl (PM100) and PL-7.

We collected data relative to indirect immunofluorescence ANA patterns reported by routine laboratory tests, and we observed that the most frequent ANA pattern reported in our anti-MJ/NXP-2(+) and anti-TIF1γ/α(+) cases is speckled, and in one anti-MJ/NXP-2(+) case, the presence of nuclear dots suggestive for promyelocytic leukemia nuclear bodies was reported, thus needing further evaluation [10]. Despite the use of the most sensitive techniques, eight patients with IIMs remained negative for both ANA and ENA, while in six additional cases, we could identify bands by protein-IP, but no clear specificity [22]. These gaps underline the existing limitations in the identification of autoantibodies in rheumatic diseases such as PM/DM which are mainly due to lack of standardization for ANA and ENA, low number of positive cases studied for autoantibodies clinical association, identification of rare and new autoantibodies through time and labor-consuming techniques such as IP, and the lack of commercially available techniques that may help in the identification of rare autoantibodies in a clinical setting [23] and shed light on PM/DM pathogenesis [2]. We acknowledge that the efforts of international registries such as Euromyositis or the Autoantibody Standardization Committee are expected to minimize the frequency of seronegative cases and to provide a clear estimate of the prevalence of rare autoantibodies [24].

Acknowledgments This work was supported by the JSPS KAKENHI (Grants-in-Aid for Scientific Research) Grant Number 15K08790 for MS.

Compliance with ethical standards

Disclosures None.

References

1. Lundberg IE et al (2016) Diagnosis and classification of idiopathic inflammatory myopathies. J Intern Med 280(1):39–51
2. Ceribelli A. et al. (2016) *The Immune Response and the Pathogenesis of Idiopathic Inflammatory Myositis: a Critical Review.* Clin Rev Allergy Immunol
3. Gunawardena H. (2015) *The Clinical Features of Myositis-Associated Autoantibodies: a Review.* Clin Rev Allergy Immunol
4. Satoh M. et al. (2015) *A Comprehensive Overview on Myositis-Specific Antibodies: New and Old Biomarkers in Idiopathic Inflammatory Myopathy.* Clin Rev Allergy Immunol
5. Targoff IN (1992) Autoantibodies in polymyositis. Rheum Dis Clin N Am 18(2):455–482
6. Ceribelli A et al (2014) Prevalence and clinical significance of anti-MDA5 antibodies in European patients with polymyositis/dermatomyositis. Clin Exp Rheumatol 32(6):891–897
7. Satoh M et al (2012) Autoantibodies to transcription intermediary factor TIF1beta associated with dermatomyositis. Arthritis Res Ther 14(2):R79
8. Fiorentino DF et al (2016) PUF60: a prominent new target of the autoimmune response in dermatomyositis and Sjogren's syndrome. Ann Rheum Dis 75(6):1145–1151
9. Ceribelli A et al (2010) Anti-Th/To are common antinucleolar autoantibodies in Italian patients with scleroderma. J Rheumatol 37(10):2071–2075
10. Ceribelli A et al (2012) Anti-MJ/NXP-2 autoantibody specificity in a cohort of adult Italian patients with polymyositis/dermatomyositis. Arthritis Res Ther 14(2):R97
11. Yamasaki Y et al (2016) Clinical subsets associated with different anti-aminoacyl transfer RNA synthetase antibodies and their association with coexisting anti-Ro52. Mod Rheumatol 26(3):403–409
12. Palaniappan P, Lionel AP, Kumar S (2014) Successful treatment of calcinosis cutis in juvenile dermatomyositis with pamidronate. J Clin Rheumatol 20(8):454–455
13. Rider LG et al (2013) The myositis autoantibody phenotypes of the juvenile idiopathic inflammatory myopathies. Medicine (Baltimore) 92(4):223–243
14. Ichimura Y et al (2012) Anti-NXP2 autoantibodies in adult patients with idiopathic inflammatory myopathies: possible association with malignancy. Ann Rheum Dis 71(5):710–713
15. Tiniakou, E. and A.L. Mammen, *Idiopathic Inflammatory Myopathies and Malignancy: a Comprehensive Review.* Clin Rev Allergy Immunol, 2015.
16. Selva-O'Callaghan A et al (2010) Malignancy and myositis: novel autoantibodies and new insights. Curr Opin Rheumatol 22(6):627–632
17. Targoff IN et al (2006) A novel autoantibody to a 155-kd protein is associated with dermatomyositis. Arthritis Rheum 54(11):3682–3689

18. Gunawardena H et al (2008) Clinical associations of autoantibodies to a p155/140 kDa doublet protein in juvenile dermatomyositis. Rheumatology (Oxford) 47(3):324–328
19. Kaji K et al (2007) Identification of a novel autoantibody reactive with 155 and 140 kDa nuclear proteins in patients with dermatomyositis: an association with malignancy. Rheumatology (Oxford) 46(1):25–28
20. Chinoy H et al (2007) The diagnostic utility of myositis autoantibody testing for predicting the risk of cancer-associated myositis. Ann Rheum Dis 66(10):1345–1349
21. Fujikawa K et al (2009) Association of distinct clinical subsets with myositis-specific autoantibodies towards anti-155/140-kDa polypeptides, anti-140-kDa polypeptides, and anti-aminoacyl tRNA synthetases in Japanese patients with dermatomyositis: a single-centre, cross-sectional study. Scand J Rheumatol 38(4):263–267
22. Selmi C et al (2016) Serum antinuclear and extractable nuclear antigen antibody prevalence and associated morbidity and mortality in the general population over 15 years. Autoimmun Rev 15(2): 162–166
23. Cavazzana I et al (2016) Testing for myositis specific autoantibodies: comparison between line blot and immunoprecipitation assays in 57 myositis sera. J Immunol Methods 433:1–5
24. Chan EK et al (2016) Report on the second International Consensus on ANA Pattern (ICAP) workshop in Dresden 2015. Lupus 25(8): 797–804