A New Immunodot Assay for Multiplex Detection of Autoantibodies in a Cohort of Italian Patients With Idiopathic Inflammatory Myopathies

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Background: Autoantibody detection has been assessed as tool for the diagnosis and the definition of idiopathic inflammatory myopathies (IIM). The aim of the study was to characterize the autoantibody profiling of a cohort of Italian patients with IIM. **Methods:** Sera of 53 adult patients with definite IIM, according to Bohan–Peter criteria, were tested for anti-nuclear autoantibodies (ANA), using indirect immunofluorescence (IIF) method, and for myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs), using two new commercial immunodot assays. **Results:** MSAs and/or MAAs were detected in 29 of 53 (54.7%) patients with IIM. Twenty-three patients (43.4%) were positive for at least one MSAs: 13 (24.5%) had anti-histidyl-tRNA synthetase autoantibodies (Jo1), 4 (7.5%) had other anti-aminoacyl-tRNA synthetases autoantibodies (anti-ARS), 1 (1.8%) had anti-transcription intermediary factor 1 gamma autoantibodies (anti-TIF1 γ), 2 (3.7%) had

anti-nuclear helicase protein Mi-2 autoantibodies (anti-Mi-2), 4 (7.5%) had anti-small ubiquitin like modifier activating enzyme heterodimer autoantibodies (anti-SAE). Moreover, 17 patients (32%) were positive for at least one MAAs. Coexisting MSAs and MAAs were observed in 9 of 53 (16.9%) patients, anti-Jo1/SS-A autoantibodies in most cases. Overall sensitivity of immunodot assays was 54.7%, the specificity was almost absolute. At cut-off value of 1:160, the sensitivity of ANA-IIF was 52.8%, increasing to 66% if cytoplasmatic fluorescence reaction was reported. Notably, two (5.7%) ANA-IIF negative patients had MSAs, detected only by immunodot assays. **Conclusion:** It was possible to identify MSAs otherwise undetectable because of the use of new assays. Immunodot can reveal MSAs even when IIF results are inconclusive or, in some cases, ANA negative. J. Clin. Lab. Anal. 30:859–866, 2016.

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Key words: idiopathic inflammatory myopathies; myositis-specific autoantibodies; myositis-associated autoantibodies; multiplex assays; indirect immunofluorescence method

INTRODUCTION

Idiopathic inflammatory myopathies (IIM) are a group of rare rheumatic diseases characterized by skeletal muscle inflammation, leading to chronic weakness and disability. According to the current classification criteria, polymyositis (PM), dermatomyositis (DM), and inclusion body myositis (IBM) are the main clinical forms (1–3). Recently, immune-mediated necrotizing myopathy (IMNM)

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TABLE 1. Most Common Myositis-Specific and Myositis-Associated Autoantibodies

Myositis-specific autoantibodies (MSAs)		Myositis-associated autoantibodies (MAAs)
ARS	Non-anti-ARS	
Anti-Jo1	Anti-Mi-2	Anti-SSA
Anti-PL-7	Anti-SAE-1/2	Anti-PM/Scl
Anti-PL-12	Anti-SRP	Anti-Ku
Anti-EJ	Anti-TIF1 γ	Anti-Scl-70
Anti-OJ	Anti-MDA5	Anti-snRNP
Anti-KS	Anti-NXP2	Anti-RNAP III
Anti-HA	Anti-EIF-3	
Anti-ZO	Anti-HMGCR	

MSA, myositis-specific autoantibodies.

Anti-ARS: anti-histidyl-tRNA synthetase (Jo-1), anti-threonyl-tRNA synthetase (PL-7), anti-alanyl-tRNA synthetase (PL-12), anti-glycyl-tRNA synthetase (EJ), anti-isoleucyl-tRNA synthetase (OJ), anti-asparaginyl-tRNA synthetase (KS), anti-tyrosyl-tRNA synthetase (Ha), and anti-phenylalanyl-tRNA synthetase (Zo).

Non-Anti-ARS: Anti-nuclear helicase/ATPase (Mi-2), anti-small ubiquitin-like modifier activating enzyme (SAE), anti-signal recognition particle (SRP), anti-transcriptional intermediary factor 1 gamma (TIF1 γ), anti-melanoma differentiation-associated gene 5 (MDA5), anti-nuclear matrix protein 2 (NXP2), anti-eukaryotic initiation factor 3 complex (EIF-3), and anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR).

MAAs: anti-3-ubiquitin ligase (Ro52/SSA), anti-polymyositis-scleroderma antigen (PM/Scl), anti-DNA-dependent protein kinase (Ku), anti-DNA topoisomerase 1 (Scl-70), anti-small nuclear ribonucleoprotein (snRNP), and anti-RNA polymerase III (RNAP III).

has been included as a distinct myositis subset with peculiar histopathological features and specific autoantibodies (4).

The etiopathogenesis of IIM is currently unknown, and both genetic and environmental factors are supposed to contribute to disease susceptibility (5, 6). Muscle biopsy is the gold standard to get the definite diagnosis, although histopathologic findings may frequently be nonspecific or unclear especially in those patients with mild muscle involvement and extra-muscular manifestations. In this context, laboratory investigations may aid to confirm the diagnosis and to predict the prognosis of IIM. Serum autoantibodies are emerging as an important diagnostic tool, especially for contributing to the definition of disease subsets (7).

Myositis correlated autoantibodies are currently subdivided into myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs; Table 1) (8, 9). In Table 2, the autoantigen targets, the biological functions as well as the clinical associations and frequency of the most common MSAs are also summarized (10).

It is however not settled whether MSAs have a pathogenic role or are merely epiphenomena of abnormal immune responses. A possible pathogenic role for anti-Jo1 autoantibodies has recently been suggested, because

these antibodies can form immune complexes that induce type I interferon production by plasmacytoid dendritic cells (11, 12).

Myositis correlated autoantibodies occur in about 60% of patients with IIM, when tested by indirect immunofluorescence (IIF) method on HEp-2 cells, staining frequently a fluoroscopic cytoplasmic pattern (13).

They are highly selective and mutually exclusive autoantibodies, except for sporadic cases of coexistence of two MSAs apparently leading to a more complex and severe disease expression (14, 15).

MSAs are directed against cytoplasmic or nuclear components involved in the regulation of key processes such as protein synthesis, translocation gene transcription, and viral recognition. The three best characterized autoantibodies target mainly the cytoplasmic aminoacyl-tRNA synthetases (anti-ARS) (16), the nuclear helicase protein Mi-2 (17, 18), and cytoplasmic complex signal recognition particle (SRP) (19). Moreover, novel autoantibodies, such as the anti-melanoma differentiation associated gene 5 (anti-MDA5), the anti-transcription intermediary factor 1 gamma (anti-TIF1 γ), the anti-small ubiquitin-like modifier activating enzyme heterodimer (anti-SAE), and the anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR) (20–23), have been recently found as being associated with distinctive myositis subsets, mainly DM subtypes, further potentially expanding the MSAs panel. Epidemiological and clinical studies also suggest that each type of MSAs seems to be related to peculiar clinical features, prognosis, and response to treatment.

The high specificity of traditional MSAs for IIM has been established either toward other connective tissue diseases or other neuromuscular disorders, including muscular dystrophies (24). However, the diagnostic accuracy for IIM of newly conceived MSAs is far from being defined, because these autoantibodies have been only tested a small proportion of patients with restricted ethnogeographic features and they have yet to be extensively studied in nonmyositis populations. The aim of our study was to characterize the autoantibody profile in a cohort of Italian patients with IIM using two new commercially available myositis profiling assays.

PATIENTS AND METHODS

Patients

Sera from 53 IIM adult Caucasian patients have been collected in a single Italian center, according to Bohan and Peter criteria (1, 2). The demographic and clinical characteristics of patients are shown in Table 3.

At the time of sampling, all patients were taking glucocorticoids and immunosuppressive drugs. Only one patient had been in treatment with simvastatin.

TABLE 2. Myositis-Specific Autoantibodies, Autoantigen Target, Biological Function, Clinical Associations, and Frequency

Autoantibodies	Autoantigen target	Biological function	Clinical associations	Frequencies (%)
Anti-ARS	Aminoacyl-tRNA synthetases	Esterification of the specific aa to its cognate tRNA	Anti-synthetase syndrome	Overall: 30–40
Jo1	Histidyl		Myositis	15–20
PL7	Threonyl		ILD	<5
PL12	Alanyl		Raynaud phenomenon	<5
OJ	Isoleucyl		Arthritis	<5
EJ	Glycyl		Mechanic's hands	<5
KS	Asparagynyl		Fever	<5
Ha	Tyrosyl			<1
Zo	Phenylalanyl			<1
Anti-Mi-2	Nucleosome remodeling deacetylase complex (NuRD)	Remodeling of nucleosomes	DM	<10
Anti-p155/140	Transcriptional intermediary factor 1 gamma (TIF1 γ)	Nuclear transcription + cellular differentiation unknown	JDM: DM/ulceration Adults: DM/malignancy	13–21
Anti-p140	Nuclear matrix protein 2 (NXP2)	Nuclear transcription + RNA metabolism unknown	JDM: DM and calcinosis Adults: DM and ILD	<5
Anti-SAE	Small ubiquitin-like modifier activating enzyme (SAE)	SAE1, SAE2: involved in posttranslational modification	DM	<5
Anti-CADM-140	Melanoma differentiation-associated gene 5 (MDA5)	Innate immune response against viral infections unknown	CADM and ILD	50–73 CADM (not in Caucasian)

ILD, interstitial lung disease; CADM, clinically amyopathic dermatomyositis; JMD, juvenile dermatomyositis. Modified from (10).

TABLE 3. Demographic and Clinical Characteristics of Patients

Sex	
No. of female (%)	45 (84.9)
No. of male (%)	8 (15.1)
Female to male ratio	5.6:1
Age, mean range (years)	59.7, 32–85
Disease duration (median years)	5.7
At the onset of disease; no. of patients (%)	12 (22.6)
Disease classification (no. %)	
Polymyositis	33 (62.2)
Dermatomyositis	20 (37.7)

As control population sera, 49 patients with other autoimmune systemic diseases were selected, 19 with rheumatoid arthritis (RA), 3 with systemic sclerosis (SSc), 3 with undifferentiated connective tissue disease, 22 with systemic lupus erythematosus (SLE), 1 with Sjogren syndrome, and 1 with mixed connective disease (mean age, 51 years; range, 19–78 years; 40 female and 9 male). Twenty healthy subjects, 12 female and 8 male (mean 49 years, range 33–53) were also included.

Methods

A total of 22 autoantibodies were screened using two new kits provided from Alphadia s.a./n.v. Diagnostic Products, Wavre, Belgium.

With “Sclero-Poly-Synthetase Profile 12 DOT” it was possible to detect anti-Jo1, anti-PL-7, anti-PL-12, anti-EJ, anti-SRP-54, anti-Mi-2, anti-MDA5, anti-TIF1 γ , anti-Ku, anti-PM-Scl 100, anti-Scl-70, and anti-SSA/Ro52 autoantibodies (Fig. 1a).

With “BlueDot Polymyositis IgG” it was possible to detect anti-OJ, anti-KS, anti-HA, anti-ZO, anti-NXP2, anti-SAE-1 and SAE-2, anti-EIF-3, anti-HMGCR, and anti-RNAP-III autoantibodies (Fig. 1b).

Briefly, the strips were first incubated with diluted patients' sera. Human autoantibodies, if present, bound the corresponding specific antigen(s) on the membrane. Unbound or excess autoantibodies were removed by washing. Upon further incubation into AP-conjugated goat autoantibodies against human IgG, the enzyme conjugate bound the antigen–antibody complexes. After a second washing step in order to remove excess conjugate, substrate solution was added. Enzyme activity, if present, led to the development of purple dots on the membrane pads. The intensity of the coloration was directly proportional to the amount of autoantibody present in the sample.

Each sample strip contained a cut-off and a positive control. The BlueDiver Instrument (Alifax Spa, Padova, Italy) was used to perform the experiment in total automation. Then, the strips were digitalized using a camera and the dots' intensity was determined by a computer program (Dr. Dot software, Alifax Spa).

Fig. 1. (a) Patient's sera showed specific autoantibodies' reactivity using the "Sclero-Poly-Synthetase Profile 12 DOT assay." (b) Two patient's sera showed a specific reactivity against SAE-1 and SAE-2 antigens using the "BlueDot Polymyositis IgG assay."

Anti-nuclear autoantibodies (ANA) were assayed using IIF methods on HEP-2 substrate cells (Euroimmun, Lübeck, Germany) at a starting serum dilution of 1:80, following the manufacturers' instructions. The slides were visually read by two independent expert operators (T.M. and F.A.), who worked independently. Samples were scored positive if a fluorescence reaction was observed

at 1:80 sample dilution. Cytoplasmatic fluorescence reaction was also detected.

Figure 2a, b, c, and d shows different staining patterns in patients with IIM. All patients' and controls sera were also performed with the routine available method (Chemiluminescence ImmunoAssay, CLIA) for detection of the autoantibodies' specificity, anti-Jo1, and anti-SS-A. The

Fig. 2. Immunofluorescent staining on HEp-2 cells. (a) Serum from a patient with anti-Jo1 autoantibodies. The serum showed low fluorescence reactivity judged as negative finding. (b) Serum from a patient with anti-TIF1 γ autoantibodies. The serum showed fine granular cytoplasmic staining without perinuclear reinforcement. (c) Serum from a patient with anti-Mi-2 autoantibodies. The serum showed homogeneous nuclear staining with nucleolar sparing. (d) Serum from a patient with anti-PL-12 autoantibodies. The serum showed fine cytoplasm condensed around the nucleus, which diminished toward the periphery.

study was conducted according to the ethical standards as it was formulated in the Helsinki Declaration.

Statistical Analysis

Diagnostic sensitivity was calculated in 53 patients with IIM, while the specificity was calculated in 49 controls for each method. The sensitivity is defined as the proportion of true positives among patients that are correctly identified by the test, while the specificity corresponds to the proportion of true negatives among controls that are correctly identified by the test (25). Cohen's kappa with

95% confidence interval (95% CI) was used to evaluate the analytical agreement between the CLIA method and the immunodot assay for the search of anti-Jo1 autoantibodies. Differences in dichotomic variables were analyzed by chi-squared or Fisher's test. *P* values, <0.05 , were considered statistically significant. Statistical analyses were done using the SPSS (version 20) statistical software.

RESULTS

Autoantibody profiles of IIM patients are shown in Table 4. Twenty-eight of 53 (52.8%) IIM patients were

TABLE 4. Frequency of ANA-IFI, MSAs and MAAs in Patients With Polymyositis and Dermatomyositis

	Polymyositis	Dermatomyositis
Number (%)	33 (62.2)	20 (37.7)
ANA (HEp-2-IIF)		
Positive title $\geq 1/160$	14 (42.4)	14 (70)
ANA negative cytoplasmatic	6 (31.5)	1 (16.6)
Homogeneous	3 (21.4)	-
Speckled	5 (35.7)	12 (85.7)
Nucleolar	3 (21.4)	1 (7.1)
Diffuse grainy	2 (14.2)	-
Dense fine speckled	1 (7.1)	1 (7.1)
Immunodot method		
Anti-Jo1	9 (27.2)	4 (25)
Anti-EJ	1 (3)	-
Anti-PL-12	2 (6)	-
Anti-KS	1 (3)	-
Anti-TIF $_1$ γ	-	1 (5)
Anti-SAE 1 e 2	-	4 (20)
Anti-Mi-2	-	2 (10)
Total MSA	13 (39.4)	12 (60)
Anti-Ro52	8 (24.2)	5 (25)
Anti-PM/Scl 100	5 (15)	-

positive for ANA-IIF. ANA were detected in 14 of 33 (42.4%) PM patients and in 14 of 20 (70%) DM patients ($P = 0.09$). The most common pattern of fluorescence was speckled and reported in 61% of cases, mainly of patients with DM (12/20; $P = 0.002$). Seven ANA-IIF negative patients had positive cytoplasmatic fluorescence reaction, six patients with PM and one with DM ($P = 0.85$). If cytoplasmatic fluorescence reaction is reported, all of the IIM patients result positive using IIF method (Table 4).

In total, 73.5% (36/49) of disease controls were positive to ANA-IIF. Indeed at a cut-off value of 1:160, the sensitivity and the specificity of ANA-IIF method were 66% and 34.5%, respectively.

The use of immunodot assays showed that 29 of 53 (54.7%) patients with IIM were positive for one or more MSAs or MAAs. The autoantibodies' positivity is shown in Table 4.

Twenty-three (43.4%) patients were positive for at least one MSAs: anti-Jo1 in 13 (24.5%), other anti-ARS in 4 (7.5%), anti-TIF $_1$ γ in 1 (1.8%), anti-Mi-2 in 2 (3.7%), and anti-SAE in 4 (7.5%).

Among the anti-ARS antibodies, anti-Jo1 were found in 9 of 33 (27.2%) PM patients and in 4 of 20 (20%) DM patients ($P = 0.53$). Anti-EJ, anti-PL-12, and anti-KS were exclusively observed in patients affected by PM (12.1%), while autoantibodies against Mi-2, TIF $_1$ γ , SAE-1 and SAE-2 were exclusively found in patients affected by DM (35%).

Moreover, 17 patients (32%) were positive for at least one MAAs: anti-SSA/Ro52 was found in eight patients with PM (24.2%) and in five patients with DM (25%).

TABLE 5. Frequency of Autoantibody Positivity in Function of the Number of Associations and Type of Autoantibody Found Detected for Each Single Case Studied

Polymyositis patients	
Number of positive cases (%)	18/33 (54.5)
Anti-Jo1 + Ro52	5 (27.7)
Anti-PL-12 + Ro52	1 (5.5)
Anti-Jo1	3 (16.6)
Anti-PL-12	1 (5.5)
Anti-EJ	1 (5.5)
Anti-KS	1 (5.5)
Anti-PM/Scl 100	2 (11.1)
Anti-Ro52	1 (5.5)
Anti-PM/Scl 100 + Scl-70	2 (11.1)
Anti-PM/Scl 100 + Jo1 + Ro52	1 (5.5)
Dermatomyositis patients	
Number of positive cases (%)	11/20 (55)
Anti-Mi-2	2 (18.2)
Anti-SAE	2 (18.2)
Anti-TIF $_1$ γ	1 (9)
Anti-Jo1 + Ro52	2 (18.2)
Anti-Jo1	1 (9)
Anti-Ro52	1 (9)
Anti-SAE + Ro52	1 (9)
Anti-SAE + Ro52 + Jo1	1 (9)

Five patients were positive for anti-Pm/Scl and were all affected by PM (9.4%).

Sixteen of 53 (30.1%) IIM patients had a single autoantibody's positivity. The simultaneous presence of MSAs and MAAs was observed in 9 of 53 (16.9%) patients, showing anti-Jo1/SS-A autoantibodies in most cases. Two of 53 (3.7%) had a triple combination of autoantibodies. Six patients (11.3%) only had MAAs. None of IIM patients had autoantibodies against OJ, HA, NXP2, EIF-3, HMGCR, RNAP-III, PL-7, and SRP-54 e Ku.

Table 5 shows the frequency of each autoantibody found either alone or in associations with others, else comparing PM to DM subsets. Among control patients with RA, one had anti-SRP autoantibodies and one anti-MDA5 autoantibodies. Among control patients with SSc, one had anti-TIF $_1$ γ and another one anti-EJ autoantibodies. Among control patients with SLE, one had anti-Mi-2, one had anti-PL-12, and one had anti-NXP2 autoantibodies.

Overall sensitivity of immunodot assays was 54.7%; the specificity for each autoantibody was included in a range between 98% and 100%. The overall concordance between the results obtained with CLIA method and immunodot assay for anti-Jo1 autoantibodies detection was 94.3%, with a positive agreement of 92.3% and a negative agreement of 97.4%. The Cohen's kappa (0.85) demonstrated a very good agreement.

We also verified if clinical features such as myositis, interstitial lung disease (ILD), Raynaud's phenomenon, arthritis, heliotrope rash, Gottron's papules, V sign,

mechanic's hands, dysphagia, dysphonia, heart involvement, and malignancy occurred more frequently in presence of MSA or MAA. No statistically significant correlation has been noticed. In total, 92.9% and 84.7% of patients affected by ILD had anti-Jo1 and anti-Ro52 autoantibodies, respectively, rising to 100% in case of simultaneous presence of both autoantibodies. Anti-Mi-2 and SAE positive patients exhibited heliotrope rash and Gottron's papules. The patient with anti-TIF1 γ autoantibodies did not have current neoplasia.

DISCUSSION AND CONCLUSIONS

Autoantibody detection is a very important tool for IIM diagnosis and the definition of autoantibody profiles enables the distinction between PM and DM. Most relevant autoantibodies are currently detected by the IIF method, however recognition and interpretation of patterns can reveal themselves to be challenging and errors are not infrequent due to the lack of test standardization and to the operator-dependency of the technique. The American College of Rheumatology (ACR) drew up a consensus statement containing guidelines for appropriate and effective autoantibody testing and notably, the roles of IIF as a reference method and the importance of defining nuclear and cytoplasmic pattern of staining were emphasized (26). Concerning the diagnosis of IIM, IIF recognition and interpretation of patterns can be challenging because of the variability of the patient's serological samples. Furthermore, cytoplasmic structures often show low reactivity fluoroscopy which is not always judged as positive finding. This reaction makes the assignment of the pattern and the titer quite difficult, considering that both of them are useful criteria for the search of autoantibodies' specificity.

Recently, different immunoblot assays have been introduced as confirmatory serological tests for the diagnosis of IIM. They may be a very promising tool and have been clinically validated (27, 28). The immunoblot assays were comparable in their handling, analytical parameters and clinical performance and correlated with IIF method (28, 29). They are continually implemented in order to replace conventional techniques reducing the time of execution and increasing the number of potentially detectable autoantibodies' reactivity.

In this study, we evaluated the autoantibody profiles in a cohort of IIM patients using both the established IIF method and two multiplex immunodot assays. Our data showed that myositis correlated autoantibodies occur in 66% of patients with IIM, when tested by IIF method on HEp-2 cells, reporting also fluoroscopic cytoplasmic pattern. On the other hand, in two (5.7%) ANA-IIF negative patients, autoantibodies anti-Jo1 were detected only by immunodot assay. Thus, our results confirm the usefulness of an association of the additive analytical method

to IIF in order to identify anti-Jo1 autoantibodies when IIF results are inconclusive.

The autoantibody anti-Jo1 is common in IIM patients and the results showed a good agreement between CLIA method and immunodot assay, but if it is true that the autoantibody anti-Jo1 is the most frequent (15–20%) in patients with PM (16), this autoantibody is just one of eight autoantibodies aminoacyl tRNA synthetase described (16). Although the others are present with a frequency much lower (3–5%), they are equally important from the clinical point of view for their high specificity, all being markers of the “anti-synthetase” (30). In our study the immunodot assay enabled the identification of two patients positive for autoantibody anti-PL-12, one for anti-EJ and one for anti-KS autoantibodies otherwise undetectable. The ANA-IIF test in these cases was found to be positive at low titer or with cytoplasmic reaction. All these patients had ILD.

Autoantibodies anti-aminoacyl-tRNA synthetase (ARS) were exclusively found in PM patients (39%), while autoantibodies against Mi-2, TIF1 γ , SAE-1, and 2 were exclusively found in patients affected by DM (35%). The anti-Mi-2 autoantibody is a specific marker of DM and 95% of anti-Mi-2 positive patients show characteristic skin features of DM (17, 18). The frequency of anti-Mi-2 in our cohort of patients was similar to that reported in literature; all the anti-Mi-2 positive patients had a DM with no signs of ILD, confirming the high specificity of the antibody.

Furthermore, anti-SAE patients are also known to present with cutaneous manifestations and develop systemic features, including dysphagia (31, 32). We found four anti-SAE patients with exclusive cutaneous manifestations.

Recently, the anti-TIF1 γ antibodies have emerged as strongly associated to malignancy as well as to severe DM in adult patients, having a prognostic role especially in older people. In our cohort, only one patient tested positive for anti-TIF1 γ , but the diagnostic investigations for neoplasia were negative and the patient was referred to the clinical follow-up.

In our cohort, MAAs were often observed in combination with at least one MSAs. Since the sensitivity and specificity are low, the clinical significance of MAAs is yet to be defined. Notably, anti-SSA/Ro52 was the only autoantibody found in almost 25% (13) of IIM patients, both alone (2/13) and in combination with anti-Jo1 (7/13), anti-PL-12 (1/13), anti-SAE (1/13), anti-SAE + anti-Jo1 (1/13), and anti-Jo1 + anti-Pm/Scl (1/13). Eleven of 13 (84%) anti-SSA/Ro52 positive patients had active myositis and ILD. In agreement with literature, we observed that MSAs and MAAs are associated to specific clinical features, although the linear regression analysis did not support a statistically significant correlation

probably because of the low number of patients for each subgroup.

In conclusion, the multiplex immunodot assay showed a higher sensitivity than IIF method for autoantibodies anti-Jo1 and higher specificity for detection of other MSAs. Our study suggests this could meet the increasing demand for cost-efficient detection and profiling of myositis autoantibodies and particularly it candidate as alternative screening method in patients with IIM.

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