Research paper

Testing for myositis specific autoantibodies: Comparison between line blot and immunoprecipitation assays in 57 myositis sera

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ABSTRACT

Objective: To analyze the performance of a line blot assay for the identification of autoantibodies in sera of patients affected by myositis, compared with immunoprecipitation (IP) as gold standard.
Methods: 66 sera of patients with myositis (23 polymyositis, 8 anti-synthetase syndromes, 29 dermatomyositis and 6 overlap syndromes) were tested by commercial LB (Euroimmun, Lubeck, Germany); 57 sera were analyzed also by IP of K562 cell extract radiolabeled with ^{35}S-methionine. Inter-rater agreement was calculated with Cohen's k coefficient.
Results: Myositis-specific antibodies (MSA) were detected in 36/57 sera (63%) by IP and in 39/66 sera (59%) by LB. The most frequent MSA found by LB were anti-Jo1 and anti-Mi2 found in 15% (10/66) of sera, followed by anti-NXP2 and anti-SRP detected in 106% (7/66) of sera. Anti-TIF1gamma and anti-MDA5 were found in 6 (9%) and 5 sera (7.6%), respectively.
A good agreement between methods was found only for anti-TIF1γ, anti-MDA5 and anti-NXP-2 antibodies, while a moderate agreement was estimated for anti-Mi2 and anti-EJ. By contrast, a high discordance rate for the detection of anti-Jo1 antibodies was evident (k: 0.3).
Multiple positivity for MSA were found in 11/66 (17%) by LB and 0/57 by IP (p: 0001). Comparing the clinical features of these 11 sera, we found total discrepancies between assays in 3 sera (27.3%), a relative discrepancy due to the occurrence of one discordant autoantibody (not confirmed by IP) in 5 cases (45.5%) and a total discrepancy between LB and IP results, but with a relative concordance with clinical features were found in other 3 sera (27.3%). The semiquantitative results do not support the interpretation of the data.
Conclusions: The use of LB assay allowed the detection of new MSA, such as anti-MDA5, anti-MJ and anti-TIF1gamma antibodies, previously not found with routine methods. However, the high prevalence of multiple positivities and the high discordant rate of anti-Jo1 antibodies could create some misinterpretation of the results from the clinical point of view. These data should be confirmed by enlarging the number of myositis cases.

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1. Introduction

Autoantibodies are considered an essential tool for a correct diagnosis of different systemic autoimmune diseases, including idiopathic inflammatory myositides (IIM), in which they are usually divided in myositis-specific (MSA) and myositis associated antibodies (MAA). MAA, namely anti-Ro/SSA, anti-U1RNP, anti-Ku and anti-PM/Scl, are defined as autoantibodies found not only in IIMs but also in other connective tissue diseases, such as Systemic sclerosis (Ssc) or systemic lupus erythematosus (SLE) overlapped with myositis (Targoff, 1992, 2000; Nakashima and Mimori, 2010). On the contrary, MSAs are found almost exclusively in IIMs and allow identifying different subtypes of

spectrum of IIMs with specific clinical features and prognosis (Targoff, 1992, 2000; Love et al., 1991). The clinical associations originally described between different MSAs and IIMs were based on immunoprecipitation (IP) or double immunodiffusion (DID) assays (Mathews and Bernstein, 1983; Nishikai and Reichlin, 1980; Meyer et al., 1987). The detection of protein and/or RNA components of autoantigens by IP, using human cultured cells, allows the screening of most of IIMs, based on their known molecular weight and a unique set of proteins for certain MSA. Most anti-synthetase antibodies (antibodies to aminoacyl tRNA synthetases, ARS), anti-Mi2, anti-SRP and newly identified autoantibodies, such as anti-TIF1γ (transcription intermediary factor-1γ), anti-SAE (small ubiquitin-like molecule activating enzyme), anti-MDA-5 (melanoma differentiation associated gene 5), anti-NXP-2/MJ (nuclear matrix protein 2), are well recognized by IP, though some may require additional tests for confirmation (Satoh et al., 2015). However, IP could

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have some limitations in the interpretations of the precipitation bands: many MSAs immunoprecipitate in the same narrow area of gel electrophoresis (i.e. 100–200 kDa) and some antigens migrate very close in SDS-PAGE (such as MDA5 and NXP2) (Muro et al., 2013).

Many commercial kits including enzyme-linked immunosorbent assay (ELISA), line blot (LB) or dot blot assays using recombinant antigens are available on the market. Some studies showed a good performance and good concordance of the data obtained by these assays and by original IP, when single autoantibody reactivity was considered. The widespread use of these assays has allowed non-research laboratories to detect the majority of the MSAs. However, the clinical associations between different subtypes of IIMs and MSA, detected by commercial assays, are not completely investigated, so far.

The aim of the present study is to analyze the performance of a commercial LB assay on sera of patients affected by well-known IIMs, previously characterized by protein and RNA IP, in order to evaluate the correlations between clinical features and MSA and concordance or discrepancy between the two methods.

2. Material and methods

2.1. Patients

Sixty-six adult European Caucasian patients with myositis followed-up in the Rheumatology Unit in Brescia (Spedali Civili, Brescia, Italy) between 2010 and 2012 were enrolled in this retrospective study. Polymyositis (PM) and dermatomyositis (DM) were diagnosed according to Bohan and Peter's criteria (Bohan and Peter, 1975); the anti-synthetase syndrome was defined as the triad of arthritis, myositis and interstitial lung disease associated with ARS (Imbert-Maseau et al., 2003); overlap syndromes included patients affected by PM and Sjogren's syndrome (SS) (Vitali et al., 2002), systemic sclerosis (SSc) (Masi et al., 1980; LeRoy et al., 1988), systemic lupus erythematosus (SLE) (Tan et al., 1982), or rheumatoid arthritis (RA) (Arnett et al., 1988). Patients' clinical data were independently re-viewed by two authors (M.F. and F.F.), in order to confirm or change the diagnoses previously made. Serum samples, taken from each patient during 2012, were tested by IP and LB.

The study was approved by the Institutional Review Board of the Hospital. This study meets, and is in compliance with, all ethical standards of medicine, and informed consent was obtained from all patients in accordance with the Helsinki Declaration of 1975/83/2013.

2.2. Methods

Antinuclear antibodies (ANA) were detected by indirect immunofluorescence (IIF) using HEp-2 cells (BioRad, Hercules, CA, USA) with starting dilution of 1:160. Anti-ENA antibodies were tested by counterimmunoelectrophoresis (CIE) using calf thymus and bovine spleen extracts as antigen sources as described (Bernstein et al., 1982; Clark et al., 1969; Venables et al., 1983). Fifty-seven sera were analyzed by protein-IP of K562 cell extract radiolabeled with ^{35}S-methionine, as previously described, and autoantibodies were determined using reference sera (Yamasaki et al., 2006). Sixty-six sera were tested also by commercial line blot assay (Euroline Autoimmune Inflammatory Myopathies 15 Ag (IgG) Euroimmun, Lubeck, Germany). The results were arbitrarily defined as negative (0), borderline ((+)), positive (+ or ++), and strong positive (+++) as indicated by the manufacturer. Every strip included recombinant human proteins for Mi-2 alpha, TIF1γ, MDA5, NXP-2, SAE, Ku, PM-Scl75/100, SRP, PL-7, PL-12, EJ, OJ and native purified antigen for Jo-1.

2.3. Statistical analysis

Statistical analysis for categorical variables was performed with Chi-square or Fisher's exact tests. Inter-rater agreement was calculated with Cohen's kappa coefficient: values of 0–0.2 are considered as slight agreement, 0.21–0.4 as fair, 0.41–0.6 as moderate, 0.61–0.8 as substantial, 0.81–1 as almost perfect agreement.

2.4. Results

Among 66 enrolled IIMs patients, 23 were affected by PM (34.8%), 8 had anti-synthetase syndrome (12.1%), 29 DM (44%, including 1 amyopathic DM and 1 cancer-related DM), and 6 overlap syndromes: 2 PM-SS, 2 PM-SSc; 1 PM-SLE; 1 PM/RA. The female to male ratio was 3.7:1; the mean age at onset was 45.5 years (SD: 17.3 years), median 46 years (range 6–73 years). Patients were followed-up for a mean of 9 years (SD: 5.9). 3.1 Autoantibodies' analysis IP was performed in 57 sera: ANA were positive in 74% (37/50) cases, with a prevalent speckled pattern detected in 28/37 (75.7%). CIE detected at least one positivity in 36.8% of sera (21/57), represented by anti-Jo1 in 10 cases, anti-Ro in 5 anti-Ro + La in 3, anti-Ku in 2, anti-PM/Scl and anti-Ki in one serum each MSA/MAA positivity was found in 48 cases (84.2%) and MSA in 36 sera (63.1%). The most frequent autoantibody specificity detected by IP was anti-Jo1 (14%) and anti-NXP2 (14%). Autoantibodies' positivity by LB was detected in 44/66 sera (66.7%) and MSA in 39 cases (59%): anti-Jo1 and anti-Mi2 were found in 15% (10/66) of sera, followed by anti-NXP2 and anti-SRP detected in 10.6% (7/66) of sera. Anti-TIF1gamma were found in 6/66 (9%), and anti-MDA5 in 5/66 (7.6%). On these 66 sera ANA were positive in 71.2% (42/59) cases, with a prevalent speckled pattern detected in 36/42 (85.7%). CIE detected at least one positivity in 34.8% of sera (23/66), represented by anti-Jo1 in 11 cases, anti-Ro in 7, anti-Ro + La in 2, anti-Ku in 3, anti-PM/Scl and anti-Ki in one serum each.

2.5. Comparison between LB and IP

Comparing the results of 57 sera analyzed by the two methods, we found an overall concordance rate of 77% with a fair agreement (Cohen's k: 0.30). When we analyzed the concordance rate for the single specificity, a good agreement was found only for anti-TIF1 γ (k: 0.78), anti-MDA5 (k: 0.63) and anti-NXP-2 antibodies (k: 0.61) as shown in Table 1. The two assays showed a moderate agreement for anti-Mi2 (k: 0.5) and anti-EJ (k:0.48) and a fair agreement for anti-Jo1 (k: 0.3).

2.5.1. Anti-Jo-analysis

LB and IP showed a high discordance rate for the detection of anti-Jo1 antibodies (80%: 12 discordant on 15 positive sera), as shown in Table 2. Among 11 LB anti-Jo1 positive sera, 3 sera were IP +, 1 was not examined by IP (excluded from Table 2), 7 resulted discordant for IP (5 positive and 2 negative for other antibodies): 4 discordant sera showed multiple specificities by LB, 3 sera showed a single anti-Jo1 reactivity by LB.

Table 1
Comparison between IP (immunoprecipitation) and line blot (LB) assays in 57 sera of patients affected by IIMs (idiopathic inflammatory myopathies).

MSA	IP	LB	Cohen's k
	n. 57 (%)	n. 57 (%)	
Anti-Jo-1	8 (14)	10 (17.5)	0.3
Anti-PL-7	0	2 (3.5)	0.00
Anti-PL-12	0	3 (5.2)	0.00
Anti-EJ	3 (5.2)	1 (1.7)	0.48
Anti-OJ	2 (3.5)	0	0.00
Anti-SRP	3 (5.2)	4 (7%)	0.00
Anti-Mi-2α/β	3 (5.2)	8 (15.7)	0.50
Anti-NXP-2	8 (14)	7 (12.3)	0.61
Anti-TIF1gamma	4 (7)	6 (10.5)	0.7
Anti-MDA5	4 (7)	5 (8.7)	0.63
Anti-SAE	2 (3.5)	1 (1.7)	0.00
Anti-SMN	1 (1.8)	NA	NA