Concise report

Diagnostic performance and validation of autoantibody testing in myositis by a commercial line blot assay

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Abstract

Objective. Serological testing for myositis-specific or associated autoantibodies [myositis-specific antibody (MSA) and myositis-associated antibody (MAA)] is useful for the diagnosis of idiopathic inflammatory myopathies (IIMs). However, available assays are neither standardized nor validated. The objective is to evaluate the accuracy of a commercial line blot assay for myositis diagnosis.

Methods. IgG antibodies against Jo-1, PL-7, PL-12, PM/Scl, Ku, Mi-2 and Ro52 antigens were detected by a line blot and in-house RNA immunoprecipitation or immunoblot. We tested sera from 208 IIM patients, 50 healthy subjects and 180 control patients (11 non-autoimmune myopathy, 23 muscular dystrophy, 11 UCTD, 68 SLE, 36 SSc, 22 SS and 9 arthropathy).

Results. MSAs or MAAs were detected in 98 (47%) out of the 208 IIM patients by line blot: anti-Jo-1 in 43 (21%), anti-PL-7 or anti-PL-12 in 8 (4%), anti-Mi-2 in 9 (4%), anti-PM/Scl in 9 (4%), anti-Ku in 10 (5%) and anti-Ro52 in 49 (24%). Overall specificity was: 100% for anti-Jo-1, anti-PL-7 or PL-12 and anti-PM/Scl; 96% for anti-Ku; 98% for anti-Mi-2; and 76% for anti-Ro52. In-house testing confirmed line blot results regarding anti-Jo-1, anti-PM/Scl and anti-Ku, while it was more accurate than line blot in detecting anti-Mi-2 (7 vs 4% sensitivity, 100 vs 98% specificity), and anti-aminoacyl-tRNA synthetase (anti-ARS) non-Jo-1 antibodies (11 vs 4% sensitivity, 97 vs 99% specificity).

Conclusions. Line blot could be a suitable serological test in the diagnostic workup for myositis, and it represents a reliable alternative to more time-consuming procedures. Continuous effort is recommended in order to improve its accuracy.

Key words: Autoimmune myositis, Myositis-specific autoantibodies, Serological methods, Diagnostic accuracy, Immunoprecipitation, Immunoblot, Line blot.

Introduction

Over the past 15 years, specific autoantibodies [myositis-specific antibodies (MSAs)] were identified as a phenotypic expression of idiopathic inflammatory myopathies (IIMs) and as specific markers of clinical subsets, disease prognosis and treatment response [1–6]. Main autoantibodies are grouped on the basis of diagnostic specificity: MSAs including anti-a-minoacyl tRNA synthetase (anti-ARS), anti-Mi-2 and anti-signal recognition particle (anti-SRP), and myositis-associated antibodies (MAAs) such as anti-Ro/SSA, anti-U1RNP, anti-PM/Scl and anti-Ku as the prominent ones.

Sero logical testing is analytically complex in myositis more than in other CTDs because major target autoantigens are predominantly localized in the cytoplasm and/or poorly represented in cell extracts. Moreover, current detection methods are not standardized and use widely
variable techniques and antigen preparations [6, 7]. Thus, there is a limitation on the use of these autoantibodies in clinical practice as reliable and validated commercial tests are only available for a few of them, but over the past 10 years, efforts in optimizing serological testing in immune-mediated myositis have been encouraged.

Recently, analytical variability and clinical utility of a commercial line blot assay (Myositis Profile Euroline, Euroimmun, Lübeck, Germany) for the detection of MSA/MAA as a confirmatory test for the diagnosis of autoimmune myositis has been assessed [8], but assay validation against other methods before including it in laboratory diagnostics and collaborative studies has not been performed yet [8, 9]. The aim of this study was to assess the diagnostic accuracy of line blot assay and to validate the test results against those obtained using established autoantibody detection techniques.

Patients and methods

In the present study, a multicentre cohort of patients affected with IIM was enrolled. It encompassed 137 consecutive IIM patients referred to the Division of Rheumatology, University of Padova (Italy), between January 1999 and May 2008, and 71 IIM patients seen at the Rheumatology Unit of Karolinska University Hospital, Stockholm, Sweden, within the above-mentioned period. There were 208 patients affected with definite or probable myositis according to Bohan and Peter criteria [10]: 100 PM, 63 DM, 2 JDM, 27 overlap myositis, 9 cancer-associated myositis (CAM) and 7 anti-synthetase syndrome with subclinical myositis. There were 159 women and 49 men (F : M ratio 3.2 : 1), mean age (S.D.) at disease onset is 48.5 (16.9) years (range 6–84 years), mean age (S.D.) at diagnosis 49.3 (16.7) years and median disease duration from diagnosis to serum sampling is 12 months (range 0–372 months).

The study was approved by the Local Ethics Committees (Comitato Etico per la Sperimentazione dell’Azienda Ospedaliera di Padova; Regionala Ethikprüfungsämter in Stockholm and Internal Reviewing Board of Department of Neurological Sciences and Vision, University of Verona, Italy) and written informed consent was obtained from all patients and controls according to the Declaration of Helsinki.

The sera from 50 age/sex-matched healthy subjects or 180 patients with various diseases, including 11 non-autoimmune myopathy, 23 sporadic or genetic muscular dystrophies, 11 UCMS, 68 SLE, 36 SSC, 22 primary SS (pSS) and 9 arthropathy, were tested as healthy or disease controls, respectively. MSAs and MAAs were detected by a commercially available line immunoassay (Myositis Profile Euroline), and by homemade protein A-assisted immunoprecipitation for RNA and western immunoblot assay [3].

Line immunoassay

Line blot test kit (Myositis Profile Euroline) is a qualitative line immunoassay for the detection of human immunoglobulin G (IgG) autoantibodies to seven myositis antigens in serum or plasma. Test nitrocellulose strips are coated with parallel lines of affinity-purified native mammalian Jo-1 and recombinant full-length antigens (Mi-2, Ku, PL-7, PL-12 and Ro52) derived from the corresponding human cDNAs expressed in baculovirus-infected insect cells.

Protein A-assisted immunoprecipitation

Serum MSAs including anti-ARS antibodies (anti-Jo-1 and others) and anti-SRP together with anti-RoRNP and anti-UsnRNP were searched by protein A-assisted immunoprecipitation for cognate RNAs as previously described [3].

Immunoblot analysis for anti-Mi-2 antibody determination

IgG antibodies to Mi-2 240 kDa immunodominant protein were detected by immunoblot on nuclear extract from Raji cells (a human B lymphoid cell line) [11]. The nuclear protein-enriched extract was electrophoresed on 7% SDS–PAGE and electrotransferred to nitrocellulose membranes. Antibody binding was visualized by high-sensitivity chemiluminescence.

Immunoblot analysis for MAA determination

Briefly, nuclear or cytoplasmic protein-enriched lysates from Raji cells were electrophoresed on 12.5 and 15% SDS–PAGE gels, respectively, transferred to nitrocellulose membranes, and probed with diluted patient and reference sera, as previously reported [12, 13]. Details of extensive methods on autoantibody testing are reported as supplementary data (available at Rheumatology Online).

Statistical analysis

Data were statistically analysed using SPSS 15.0I statistical package (Windows version). Differences in frequencies of dichotomatous variables were analysed by chi-squared test or Fisher’s exact test, when appropriate; P-values <0.05 were considered to be statistically significant. Concordance between methods was evaluated by contingency tables.

Diagnostic accuracy for IIM was measured by sensitivity, specificity, positive- and negative-predictive values for any antibody specificity investigated. Sensitivity (%) for IIM = (number of MSA/MAA-positive patients with IIM/total patients with IIM) × 100. Specificity for IIM (with respect to healthy subjects) = (number of MSA/MAA-negative healthy subjects/total healthy subjects) × 100. Specificity for IIM (with respect to disease controls) = (number of MSA/MAA-negative disease controls/total disease controls) × 100.

Results

The prevalence of prominent clinical and serological features of the 208 IIM patients included in the study is reported in supplementary table 1 (available as supplementary data at Rheumatology Online). No differences in clinical parameters or serological findings have been observed between Italian and Swedish IIM patients,
Myositis serological profile in a large cohort of IIM patients was investigated in parallel by a commercial line blot assay and in-house testing, and the results obtained with both the methods were compared. Our study, for the first time, clinically validates line blot assay against other methods as a confirmatory test for autoimmune myositis. In addition, we tested the myositis serological profile in other CTDs and non-inflammatory myopathies.

Autoantibodies in myositis are very different in antigen specificity and characteristics; thus, different in-house laboratory methods for their detection have been used to date. Recently, a single multi-analytic line blot assay has been developed representing a promising methodological approach for MSA and MAA testing [9]. Differently from in-house techniques that employ extracted native antigens as substrates, in line blot as well as in other commercial blot assays, highly purified recombinant or synthetic antigens are immobilized on nitrocellulose [9].

Line blot has recently been reported as a valid screening test for autoantibodies in IIM [8, 9, 14], but the authors recommended its validation against other methods in order to comparatively quantify its accuracy. As displayed in Table 1, line blot positive test for anti-Jo-1, PL-7, PL-12 or PM/Scl is highly specific for autoimmune myositis. Conversely, anti-Ku and especially anti-Ro52 antibodies detected were frequently detected in IIM by line blot, overall accounting for up to 30% positivity, but not specific for them. Anti-Ro52 was more prevalent in other CTDs, including pSS (81%), SLE (43%) and SSc (18%) confirming previous studies [15, 16]. Anti-Ku antibodies were also found in some SLE patients (~10%) and it is known that anti-Ku antibodies could be present in primary myositis as well as in a wide variety of other CTDs [17], often associated with signs of myositis [7], and our data.

Table 1: Frequencies of MSA and MAA detected by line blot and in-house assays in patients with IIM or healthy/disease controls

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>Total controls</th>
<th>Healthy subjects</th>
<th>Non-immune myopathy</th>
<th>CTDs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total patients</td>
<td>Healthy/disease controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>DM</td>
<td>Overlap</td>
<td>CAM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Jo-1</td>
<td>208</td>
<td>100</td>
<td>65</td>
<td>34</td>
</tr>
<tr>
<td>Anti-ARS non Jo-1</td>
<td>11</td>
<td>4</td>
<td>13</td>
<td>0.3</td>
</tr>
<tr>
<td>Anti-Mi-2</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anti-SRP</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Ro52</td>
<td>24</td>
<td>21</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Anti-Ku</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Anti-PM/Scl</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Findings are reported as n (%) of positivity; LB: Line blot assays; H: in-house assays; Overlap: overlap myositis.
The sensitivity of anti-Mi-2 in myositis was lower when the antibody was detected by line blot than by in-house testing. Unexpectedly, by using line blot, anti-Mi-2 was also found in a proportion of SLE patients (8%), thus reducing its specificity for myositis. Indeed, native or recombinant origin of Mi-2 antigen as substrate could influence the method’s diagnostic performance [18]. Notably, anti-Mi-2-positive SLE patients had active disease with cutaneous involvement at the time of serum sampling, but no overt muscle involvement.

Also the sensitivity of anti-ARS non-Jo-1 in myositis was lower when the antibodies were detected by line blot than by in-house testing, but this result is attributable to the limited number of anti-ARS specificities detectable by line blot at present (i.e. PL-7 and PL-12). Conversely, immunoprecipitation can potentially detect any anti-ARS antibody specificity.

Furthermore, it is noteworthy that both line blot and in-house testing resulted negative in patients with non-immune myopathy or dystrophy as well as in healthy subjects, apart from sporadic anti-PL-7 or anti-PL-12 borderline positivity in blood donors. The assessed specificity of line blot testing against muscular dystrophy has never been described and is of relevance since the differential diagnosis of PM with sporadic muscular dystrophy could be difficult in some instances even in the presence of characteristic histopathological findings [19].

As reported in Table 2, both assays demonstrated an overall sensitivity between 38 and 51%, which is in agreement with that reported in previous IIM cohort studies. This sensitivity is not low since many myositis sera do not contain known autoantibodies. In addition, the clinical accuracy of both the testings is dependent on the inclusion of anti-Ro52 antibody. In fact, by considering anti-Ro52 positivity, overall specificity is unacceptable due to the low antibody specificity for myositis [9]. Conversely, by excluding anti-Ro52 detection, both the methods performed better, achieving a specificity higher than 90%, but without losing sensitivity (40%). Even overall concordance rates improve by excluding anti-Ro52 antibody detection.

Furthermore, our results demonstrated that anti-SRP antibody, which is not yet available on the line blot assay, improves the potential of laboratory diagnostics in myositis. Indeed, the high specificity of anti-SRP antibody for necrotizing immune-mediated myopathy has been assessed [20], even though only in case reports or limited patient cohorts. In this regard, the manufacturer has recently begun the commercialization of a new line blot assay, implemented according to present suggestions and included SRP in the platform together with other additional myositis antigens (EJ, OJ, U1RNP, PM/Scl75).

Our study confirms that MSAs more than MAAs, as represented here by anti-Ro52 and anti-Ku, are useful tools for the diagnosis and classification of IIM, and a simple/rapid test as a confirmatory serological test in the suspected autoimmune myositis is proposed. The current testing approach based on immunoblot and the gold standard immunoprecipitation assay is analytically powerful, but conversely technically complex and time consuming, and it cannot be applied on a large scale in the routine diagnostic setting.

In our study, the line blot test is an accurate serological test and positive MSAs included in the test support the diagnosis of myositis and with a high likelihood rule out other myopathies or systemic rheumatic diseases, thus representing a reliable alternative to more complex procedures. On the basis of our findings, we can suggest an algorithm to be included in the clinical setting of suspected immune-mediated myopathies: line blot can be used as an initial screen, and if patients are seronegative by line blot testing, then proceed to in-house testing.

**TABLE 2** Overall comparative evaluation of line blot and in-house testing performance by including anti-Ro52 antibody detection or by excluding anti-Ro52 antibody detection from the analysis

<table>
<thead>
<tr>
<th>Anti-Ro52 antibody included</th>
<th>Anti-Ro52 antibody excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line blot testing</td>
<td>In-house testing</td>
</tr>
<tr>
<td>Overall sensitivity, %</td>
<td>47</td>
</tr>
<tr>
<td>Overall specificity, %</td>
<td>69</td>
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</tbody>
</table>

Anti-SRP antibody was not considered in the analysis because it was not included in line blot testing panel.

**Rheumatology key messages**

- A commercial line blot is validated for the serologic-al confirmation of autoimmune myositis.
- Line blot represents a reliable alternative to more time-consuming procedures.
- Anti-Ro52 antibody has a poor diagnostic value in immune-mediated myositis.

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References


