Autoantibody testing in patients with myositis: clinical accuracy of a multiparametric line immunoassay

Sirs,

Increasing evidence assesses the value of serum autoantibody testing in the diagnostic workup of idiopathic inflammatory myopathies (IIM) (1-3). Myositis autoantibodies are categorized into two groups: myositis-specific antibodies (MSA) and myositis-associated antibodies (MAA). Besides established MSA, i.e. anti-aminocytu tRNA synthetases, anti-SRP and anti-Mi-2 antibodies, novel autoantibodies have been identified, which target constitutive proteins involved in gene transcription, post-translational modifications, or innate immunity, such as transcription intermediary factor 1-gamma (TIF1-γ), nuclear matrix protein 2 (NXP2), melanoma differentiation associated protein 5 (MDA5), and SUMO activating enzymes (SAE) (4-6). The MAA, including anti-PM/Scl and anti-Ku, mostly occur in myositis-overlap syndromes (3,7).

Since our paper in 2010 (8), which assessed the diagnostic accuracy of a line immunoassay (LIA) for the detection of 7 MSA/MAA targets, i.e. Jo-1, PL-7, PL-12, PM/Scl, Ku, Mi-2 and Ro52, commercial LIA has been improved by virtue of human recombinant autoantigens’ disposal. Nowadays, a wide spectrum of myositis-related autoantibodies can be determined by last generation LIA, which needs validation before inclusion in clinical setting.

We assessed the clinical accuracy of a multiparametric LIA for the determination of 15 myositis autoantibodies in patient’s serum. We tested sera from 267 IIM patients, 55 healthy subjects, and 203 disease controls (11 non-autoimmune myopathies, 27 muscular dystrophy, 9 undifferentiated connective tissue disease, 89 systemic lupus, 35 systemic sclerosis, 22 Sjögren’s syndrome and 10 arthropathy). Patients with IIM were classified according to Bohan and Peter criteria (9): 115 polymyositis, 88 dermatomyositis (DM), 52 overlap myositis, 12 cancer-associated myositis. The study was approved by the Local Ethics Committees according to the Helsinki Declaration, and written informed consent was obtained from each patient. IgG autoantibodies towards myositis antigens were detected by LIA (Euroimmun, Lübeck, Germany). Test nitrocellulose strips are pre-coated with parallel lines of affinity-purified mammalian Jo-1 or recombinant full-length SRP, PL-7, PL-12, EJ, OJ, Mi-2, Mi-2β, TIF1-γ, MDA5, NXP2, SAE1, Ku, PM-Scl100 and PM-Scl100 proteins, derived from respective human cDNAs. Statistical analysis was performed using Fisher’s exact test.

Overall, MSA and/or MAA positivity by LIA was found in 166/267 (62%) IIM patients and in 59/258 (23%) controls (p<0.0001, Odds ratio 5.5, 95% Confidence Interval 3.7-8.1, +Likelihood ratio 2.7). MSA positivity was found in 48/258 (19%) controls. Forty-five (27%) myositis sera had more than one autoantibody: a MSA/MAA association was observed in 80% of cases. Novel autoantibodies overall accounted for a 24% increment in sensitivity compared with the assay confined to 7 analytes. The test’s specificity was: 100% for anti-Jo-1, anti-OJ and anti-SAE1; 99% for anti-PL-7 and anti-PM-Scl100; 98% for anti-EJ, anti-TIF1-γ and anti-MDA5; 96.5% for anti-PL-12 and anti-Mi-2β; 95% for anti-SRP, anti-Mi-2α and anti-NXP2; 94% for anti-PM-Scl75; 92% for anti-Ku. Anti-Mi-2β and anti-PM-Scl100 antibodies were more specific than the antibodies against Mi-2α or PM-Scl75 subunits. The ability of each antibody marker to distinguish myositis from controls is presented in Table I. Noteworthy, the clinical accuracy of each antibody can vary widely, from very good for anti-Jo-1, anti-Mi-2αβ, anti-TIF1-γ, anti-MDA5, anti-SAE1, and anti-PM-Scl100, to less good for anti-SRP, anti-NXP2, anti-PM/Scl75 and anti-Ku.

Our data support the clinical utility of implemented LIA as a confirmatory test for the diagnosis of myositis. It can represent a powerful alternative to throughput yet complex gold standard immunoprecipitation. Novel putative DM-specific antibodies were almost restricted to DM. The association of anti-TIF1-γ with CAM was confirmed (10), being observed in 4 out of 7 patients with cancer-associated DM, thus providing a tool for the diagnosis of prognostically unfavourable DM. Crucial for the testing accuracy is the quality and epitope integrity/availability of blot-immunobilised antigens. Improvement of the test’s accuracy, especially pertaining to anti-SRP and anti-NXP2 antibodies, is recommended by the manufacturer.

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Table I. Clinical accuracy of each MSA or MAA for IIM versus controls.

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>+LR</th>
<th>PPV %</th>
<th>NPV %</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Jo-1</td>
<td>19</td>
<td>100</td>
<td>nd</td>
<td>100</td>
<td>54</td>
<td>&lt;0.0001</td>
<td>123 (7.5-2006)</td>
</tr>
<tr>
<td>Anti-SRP</td>
<td>8</td>
<td>95</td>
<td>1.6</td>
<td>60</td>
<td>50</td>
<td>n.s.</td>
<td>1.7 (0.8-3.4)</td>
</tr>
<tr>
<td>Anti-ARS non Jo-1</td>
<td>7</td>
<td>95</td>
<td>1.5</td>
<td>60</td>
<td>50</td>
<td>n.s.</td>
<td>1.5 (0.7-3.1)</td>
</tr>
<tr>
<td>Anti-PL-7</td>
<td>18</td>
<td>96.5</td>
<td>1.3</td>
<td>60</td>
<td>50</td>
<td>&lt;0.0001</td>
<td>10.6 (4.5-24.8)</td>
</tr>
<tr>
<td>Anti-Mi-2αβ(DM)*</td>
<td>11</td>
<td>98</td>
<td>1.3</td>
<td>60</td>
<td>50</td>
<td>&lt;0.0001</td>
<td>10.0 (2.8-35.3)</td>
</tr>
<tr>
<td>Anti-TIF1-α(DM)*</td>
<td>6</td>
<td>100</td>
<td>1.3</td>
<td>58</td>
<td>50</td>
<td>0.0052</td>
<td>6.3 (1.7-22.5)</td>
</tr>
<tr>
<td>Anti-PL-12</td>
<td>11</td>
<td>98</td>
<td>1.3</td>
<td>60</td>
<td>50</td>
<td>0.0037</td>
<td>20.7 (6.1-67.2)</td>
</tr>
<tr>
<td>Anti-NXP2</td>
<td>18</td>
<td>96.5</td>
<td>1.3</td>
<td>60</td>
<td>50</td>
<td>&lt;0.0001</td>
<td>0.55 (0.2-1.5)</td>
</tr>
<tr>
<td>Anti-Ku</td>
<td>9</td>
<td>95</td>
<td>1.3</td>
<td>58</td>
<td>50</td>
<td>0.001</td>
<td>6.1 (1.8-21.1)</td>
</tr>
<tr>
<td>Anti-PM-Scl100</td>
<td>12</td>
<td>100</td>
<td>1.3</td>
<td>58</td>
<td>50</td>
<td>n.s.</td>
<td>1.8 (0.5-5.4)</td>
</tr>
</tbody>
</table>

*Findings are referred to the target form of IIM, DM in these cases.

MSA: myositis-specific antibody; MSA: myositis-associated antibody; IIM: idiopathic inflammatory myopathy; ARS: aminocytu tRNA synthetase; DM: dermatomyositis; +LR: positive likelihood ratio; PPV: positive predictive value; NPV: negative predictive value; p: statistical significance; OR: odds ratio; CI: confidence interval; nd: not determined; n.s.: not significant.
References