Diagnostic associations in a large and consecutively identified population positive for anti-SSA and/or anti-SSB: the range of associated diseases differs according to the detailed serotype

I Peene, L Meheus, E M Veys, F De Keyser

Objective: To determine the diagnostic distribution in a consecutive anti-SSA and/or anti-SSB positive population.

Methods: A total of 15,937 serum samples from 10,550 consecutive patients were analysed for antinuclear antibodies (ANA) on HEp-2 cells. Serum samples positive for ANAs were analysed by immunodiffusion and line immunoblot assay with recombinant SSA-Ro52, natural SSA-Ro60, and recombinant SSB.

Results: Among ANA positive patients in whom clinical information was available, 181 consecutive patients with anti-SSA and/or anti-SSB antibodies were identified. Disease associations were systemic lupus erythematosus (SLE) (45.3%), primary Sjögren’s syndrome (pSS) (14.4%), scleroderma (8.8%), RA (7.7%), cutaneous lupus (7.7%), and dermatomyositis (2.2%). The ratio of diagnoses differed according to the anti-SSA/anti-SSB serotype. Scleroderma and dermatomyositis were enriched among mono-Ro52 reactive serum samples (54.2% and 10.5% respectively). Single reactivity towards Ro60 or anti-Ro60 with anti-Ro52 predisposed for SLE (80.0% and 52.2% respectively). Tricple reactivity towards Ro52, Ro60, and SSB was primarily linked with SLE (55.8%) followed by pSS (20.9%). Anti-SSA on immunodiffusion increased the chance for SLE (62.8%), whereas isolated anti-SSB reactivity on immunodiffusion was less indicative for SLE (14.3%) and predisposed more for cutaneous lupus (23.8%) and pSS (33.3%).

Conclusion: The diagnostic range associated with anti-SSA or anti-SSB reactivity differs significantly according to the detailed serotype defined by line immunoblot assay and immunodiffusion.

Patients
A total of 15,937 serum samples from 10,550 consecutive patients were referred to the rheumatology laboratory (Ghent University Hospital) over a three year period (1996–9) for ANA detection and identification. These samples were referred by in house rheumatologists (25% of the samples), internal medicine specialists (15%), gastroenterologists (7%), dermatologists (5%), neurologists (3%), nephrologists (3%), and external hospitals or laboratories (23%).

Serum samples positive for ANA were further analysed in parallel by double immunodiffusion with thymus/spleen nuclear extract (mammalian extracted nuclear antigen, Immunoconcepts, Sacramento, CA, USA) and by line immunoblot assay coated with nuclear antigens, including recombinant Ro52 and SSB, and natural Ro60 (INNO LIA ANA K1090, Innogenetics, Gent, Belgium). For each patient showing anti-SSA (Ro52 and/or Ro60) and/or anti-SSB reactivity, clinical information was asked from the doctor who had ordered the test. Thus, diagnostic information could be obtained in 181 patients. Patients who were classified as having SLE, rheumatoid arthritis (RA), scleroderma (Scl), primary SS (pSS), or dermatomyositis (DM) met the classification criteria for the respective diseases.

Abbreviations: ANA, antinuclear antibodies; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; CLE, cutaneous lupus erythematosus; DM, dermatomyositis; ELSIA, enzyme linked immunosorbent assay; pSS, primary Sjögren’s syndrome; RA, rheumatoid arthritis; Ro52, 52 kD protein; Ro60, 60 kD protein; Scl, scleroderma; SLE, systemic lupus erythematosus; SS, Sjögren’s syndrome
respective diseases. Patients classified with cutaneous lupus erythematosus (CLE) had CLE established by biopsy but did not meet the criteria for SLE.

**Indirect immunofluorescence on HEp-2 cells**

Serum diluted 1:40 in phosphate buffered saline (PBS) was overlaid onto fixed HEp-2 cells (Medica inc, Carlsbad, CA, USA) for 30 minutes at room temperature. Slides were washed twice for five minutes each with PBS, overlaid with fluoresceinated total immunoglobulin, and incubated for an additional 30 minutes. After washing twice, a coverslip was placed over the slide, and the slides were read using a fluorescence microscope at 40× power.

**Double immunodiffusion**

Precipitating antibodies against extractable nuclear antigens were detected by double immunodiffusion on Ouchterlony plates with thymus/spleen nuclear extract (mammalian extracted nuclear antigen, Immunoconcepts, Sacramento, CA, USA). Antibody specificity was determined by comparison with a reference serum.

**Line immunoassay**

A line immunoassay coated with nuclear antigens, including full size *Escherichia coli* derived recombinant Ro52, recombinant SSB, and natural Ro60 (INNO-LIA ANA K1090), was used. The test was performed according to the manufacturer's instructions. Briefly, the nylon strips were incubated with serum at a 1:200 dilution. A goat antihuman IgG labelled with alkaline phosphatase was allowed to bind to the antigen-antibody complex. The enzyme substrate and chromogen 5-bromo-4-chloro-3-indolyl phosphatase (BCIP) produces a dark brown colour in proportion to the amount of specific autoantibody in the test sample. Sulphuric acid stops the colour development (fig 1).

**Statistics**

Percentages and their corresponding 95% confidence intervals (95% CIs) (one binomial) and Fisher's exact test were performed by StatXact.

**RESULTS**

Testing for ANA consecutively performed on 15 937 serum samples from 10 550 patients referred to our laboratory over a three year period, was positive in 4691 samples from 2669 patients. Anti-SSA and/or anti-SSB reactivity was found in 11.8% of ANA positive serum samples. We identified 181 consecutive patients with anti-SSA and/or anti-SSB antibodies.
Distribution of diagnoses in the anti-SSA and/or anti-SSB positive population according to the fine reactivity defined by immunodiffusion

<table>
<thead>
<tr>
<th>n</th>
<th>SLE % (95% CI)</th>
<th>Scl % (95% CI)</th>
<th>RA % (95% CI)</th>
<th>PM/DM % (95% CI)</th>
<th>pSS % (95% CI)</th>
<th>Other % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA+ and/or SSB+</td>
<td>181</td>
<td>45.3</td>
<td>8.8</td>
<td>7.7</td>
<td>2.2</td>
<td>14.4</td>
</tr>
<tr>
<td>ID SSA+ and IDSSB+</td>
<td>27</td>
<td>66.7 (46.0 to 83.5)</td>
<td>3.7 (0.1 to 19.0)</td>
<td>7.4 (0.9 to 24.3)</td>
<td>0</td>
<td>18.5 (6.3 to 38.1)</td>
</tr>
<tr>
<td>SSA− ID SSA and ID SSB+</td>
<td>70</td>
<td>61.4 (49.0 to 72.8)</td>
<td>5.7 (1.6 to 14.0)</td>
<td>4.3 (0.9 to 12.0)</td>
<td>0</td>
<td>15.7 (8.1 to 26.4)</td>
</tr>
<tr>
<td>SSA− and ID SS−B</td>
<td>63</td>
<td>28.6 (17.9 to 41.3)</td>
<td>6.3 (1.8 to 15.5)</td>
<td>23.8 (14.0 to 36.2)</td>
<td>9.5 (3.6 to 19.6)</td>
<td>6.3 (1.8 to 15.5)</td>
</tr>
<tr>
<td>Other diagnoses</td>
<td>26</td>
<td>51.9</td>
<td>42.2 (18.5 to 78.8)</td>
<td>51.1 (16.7 to 73.2)</td>
<td>0</td>
<td>18.5 (6.3 to 41.1)</td>
</tr>
</tbody>
</table>

CLE, cutaneous lupus without systemic involvement; ID, immunodiffusion; LIA, line immunoassay; PM/DM, polymyositis/dermatomyositis; pSS, primary Sjögren’s syndrome; RA, rheumatoid arthritis; Scl, systemic sclerosis; SLE, systemic lupus erythematosus.

Distribution of diagnoses in the population positive for anti-Ro52, anti-Ro60, and anti-SSB as defined by line immunoassay in relation to the immunodiffusion result

<table>
<thead>
<tr>
<th>n</th>
<th>Ro52+</th>
<th>Ro60+</th>
<th>SSB+</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA+ and/or Ro52+</td>
<td>86</td>
<td>55.8</td>
<td>5.8</td>
</tr>
<tr>
<td>ID SSA+ and ID SS−B</td>
<td>26</td>
<td>69.2 (48.2 to 85.7)</td>
<td>0</td>
</tr>
<tr>
<td>SSA− Ro52− and ID SS−B</td>
<td>39</td>
<td>39.4 (27.2 to 52.8)</td>
<td>51.1 (16.7 to 73.2)</td>
</tr>
<tr>
<td>SSA− and ID SS−B</td>
<td>15</td>
<td>33.3 (14.3 to 60.3)</td>
<td>33.3 (14.3 to 60.3)</td>
</tr>
</tbody>
</table>

Combining immunodiffusion and line immunoassay had only additional diagnostic value in the case of combined reactivity towards Ro52, Ro60, and SSB (table 3). A positive test result for anti-SSA on immunodiffusion in this group gave a probability for SLE of 69.2% (95% CI 48.2 to 85.7) and 64.1% (95% CI 47.2 to 78.8) and a significantly lower chance for pSS (19.2%, 95% CI 6.6 to 39.4 and 15.4%, 95% CI 5.9 to 30.5). On the contrary, the finding of a precipitin line on immunodiffusion for SSB but not for SSA in this profile decreased the probability for SLE (13.3%, 95% CI 1.7 to 40.5) whereas in that case there was a tendency towards an increased chance for pSS (46.7%, 95% CI 21.3 to 73.4; table 3).

DISCUSSION

The description of a large, consecutively identified cohort of anti-SSA and/or anti-SSB positive serum samples in the routine setting of a rheumatology laboratory offers the opportunity to look at a realistic representation of the diagnostic range associated with this type of autoreactivity. Most of the work on the value of autoantibodies has been carried out by...
testing samples from selected patients with well defined clinical disease.\textsuperscript{15,17} By contrast, by looking at sensitivity and specificity of autoantibody markers such as anti-SSA and anti-SSB, the current study rather provides clues for estimating the probability for a certain diagnosis given the anti-SSA/anti-SSB status, taking into account that the a priori probabilities can differ according to the type of clinical practice and the specialty of the doctor ordering the test. Serum samples in our laboratory had a mixed origin, with about one third of the ANA positive samples coming from the rheumatology department. A positive ANA result itself has only weak diagnostic value than line immunodiffusion. The phenomenon of anti-Ro52 antibodies in the 26 patients. Previous evidence has been presented that to Ro52, Ro60, and anti-SSB on line immunoassay, we found that additionally performed immunodiffusion discriminates between SLE and pSS. An additional advantage of the line immunoassay technique in clinical practice is that with one test result information can be obtained on the range of simultaneous occurrence of autoantibodies in connective tissue disease. Besides anti-Ro52 and anti-Ro60, this assay also detects antitriplex reactivity towards the different antigenic determinants of the RNP-antigen (RNP-A, C, and 70) and the Sm antigen (SmB and SmD).\textsuperscript{18,19} A major challenge for autoantibodies in general and for anti-SSA/anti-SSB in particular is now to find out whether reactivities to subtypes of antigens orientate towards a specific diagnosis or a specific feature common to different clinical entities, as well as to understand which mechanisms induce these different reaction patterns in autoimmune patients.

**References**


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