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 (ANAs) 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 (34.2% and 10.5% respectively). Single reactivity towards Ro60 or anti-Ro60 with anti-Ro52 predisposed for SLE (80.0% and 52.2% respectively). Triple 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 AND METHODS

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 (5%), 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 disease.

Abbreviations: ANA, antinuclear antibodies; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; CLE, cutaneous lupus erythematosus; DM, dermatomyositis; ELISA, 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 nucleic 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.

<table>
<thead>
<tr>
<th>CLE</th>
<th>% (95% CI)</th>
<th>SLE</th>
<th>% (95% CI)</th>
<th>Other</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA+ and/or SSB+</td>
<td>181</td>
<td>45.3 (47.1 to 62.1)</td>
<td>34.2 (19.6 to 51.4)</td>
<td>17.4 (9.4 to 31.8)</td>
<td>13.8 (9.1 to 19.7)</td>
</tr>
<tr>
<td>Line immunoassay result: Ro52+</td>
<td>38</td>
<td>15.8 (6.0 to 31.3)</td>
<td>10.5 (4.9 to 20.3)</td>
<td>10.5 (4.9 to 20.3)</td>
<td>10.5 (4.9 to 20.3)</td>
</tr>
<tr>
<td>Ro52− Ro60+ SSB−</td>
<td>10</td>
<td>80.0 (44.4 to 97.5)</td>
<td>66.7 (9.4 to 99.2)</td>
<td>66.7 (9.4 to 99.2)</td>
<td>66.7 (9.4 to 99.2)</td>
</tr>
<tr>
<td>Ro52− Ro60− SSB+</td>
<td>10</td>
<td>40.0 (12.2 to 73.8)</td>
<td>20.0 (2.5 to 55.6)</td>
<td>20.0 (2.5 to 55.6)</td>
<td>20.0 (2.5 to 55.6)</td>
</tr>
<tr>
<td>Ro52+ Ro60+ SSB−</td>
<td>23</td>
<td>52.2 (30.6 to 73.2)</td>
<td>52.2 (30.6 to 73.2)</td>
<td>52.2 (30.6 to 73.2)</td>
<td>52.2 (30.6 to 73.2)</td>
</tr>
<tr>
<td>Ro52+ Ro60− SSB+</td>
<td>86</td>
<td>55.8 (44.7 to 66.5)</td>
<td>55.8 (44.7 to 66.5)</td>
<td>55.8 (44.7 to 66.5)</td>
<td>55.8 (44.7 to 66.5)</td>
</tr>
<tr>
<td>Ro52− Ro60− SSB−</td>
<td>2</td>
<td>50.0 (1.3 to 98.7)</td>
<td>50.0 (1.3 to 98.7)</td>
<td>50.0 (1.3 to 98.7)</td>
<td>50.0 (1.3 to 98.7)</td>
</tr>
</tbody>
</table>

ID+, cutaneous lupus without systemic involvement; ID, immunodiffusion; PM/DM, polymyositis/dermatomyositis; pSS, primary Sjögren’s syndrome; RA, rheumatoid arthritis; SCI, systemic sclerosis; SLE, systemic lupus erythematosus.

Figure 1 Different combinations of reactivities towards Ro52, Ro60, and SSB defined by line immunoassay. Lane a: anti-Ro52 antibodies; lane b: anti-Ro60 antibodies; lane c: anti-SSB antibodies; lane d: anti-Ro52 and anti-Ro60 antibodies; lane e: anti-Ro60 and anti-SSB antibodies; lane f: anti-Ro52 and anti-SSB antibodies; lane g: anti-Ro52 and anti-Ro60 and 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 2

<table>
<thead>
<tr>
<th>n</th>
<th>SLE % (95% CI)</th>
<th>CLE % (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>7.7</td>
<td>8.8</td>
<td>7.7</td>
<td>2.2</td>
<td>14.4</td>
</tr>
<tr>
<td>ID SSA+ and ID SSB+</td>
<td>27</td>
<td>66.7 (46.0 to 83.5)</td>
<td>3.7 (0.1 to 19.0)</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>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>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>ID SSB+ and ID SSB−</td>
<td>21</td>
<td>14.3 (3.0 to 36.3)</td>
<td>23.8 (8.2 to 47.2)</td>
<td>0</td>
<td>14.3 (3.0 to 36.3)</td>
<td>0</td>
<td>33.3 (14.6 to 57.0)</td>
</tr>
<tr>
<td>ID SSB−</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>
<td>4.8 (1.0 to 13.3)</td>
</tr>
</tbody>
</table>

CLE, cutaneous lupus without systemic involvement; ID, immunodiffusion; PM/DM, polymyositis/dermatomyositis; pSS, primary Sjögren’s syndrome; RA, rheumatoid arthritis; SCl, scleroderma; 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 3

<table>
<thead>
<tr>
<th>n</th>
<th>Ro52+</th>
<th>Ro60+</th>
<th>SSB+</th>
<th>Ro52+ and/or Ro60+</th>
<th>SSA+ and/or SSB+</th>
<th>ID SSA+ and ID SSB+</th>
<th>ID SSA+ and ID SSB−</th>
<th>ID SSB+ and ID SSB−</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>86</td>
<td>26</td>
<td>39</td>
<td>55.8</td>
<td>55.8</td>
<td>69.2 (48.2 to 85.7)</td>
<td>0 (0.1 to 19.0)</td>
<td>5.8 (0.1 to 19.0)</td>
</tr>
<tr>
<td>SSA+ and/or SSB+</td>
<td>101</td>
<td>4.3</td>
<td>3.7</td>
<td>58.5</td>
<td>58.5</td>
<td>96.0 (44.0 to 92.8)</td>
<td>5.7 (1.6 to 14.0)</td>
<td>5.7 (1.6 to 14.0)</td>
</tr>
<tr>
<td>ID SSA+ and ID SSB+</td>
<td>27</td>
<td>6.7</td>
<td>3.7</td>
<td>73.7</td>
<td>73.7</td>
<td>0 (0.1 to 19.0)</td>
<td>7.4 (1.6 to 14.0)</td>
<td>7.4 (1.6 to 14.0)</td>
</tr>
<tr>
<td>ID SSA+ and ID SSB−</td>
<td>70</td>
<td>62.9</td>
<td>5.7</td>
<td>3.7</td>
<td>3.7</td>
<td>14.3 (8.2 to 24.3)</td>
<td>0 (0.1 to 19.0)</td>
<td>0 (0.1 to 19.0)</td>
</tr>
<tr>
<td>ID SSB+ and ID SSB−</td>
<td>21</td>
<td>14.3</td>
<td>23.8</td>
<td>0 (0.1 to 19.0)</td>
<td>0 (0.1 to 19.0)</td>
<td>10.0 (4.3 to 19.8)</td>
<td>0 (0.1 to 19.0)</td>
<td>0 (0.1 to 19.0)</td>
</tr>
<tr>
<td>ID SSB−</td>
<td>63</td>
<td>28.6</td>
<td>6.3</td>
<td>23.8</td>
<td>23.8</td>
<td>10.0 (4.3 to 19.8)</td>
<td>0 (0.1 to 19.0)</td>
<td>0 (0.1 to 19.0)</td>
</tr>
</tbody>
</table>

CLE, cutaneous lupus without systemic involvement; ID, immunodiffusion; PM/DM, polymyositis/dermatomyositis; pSS, primary Sjögren’s syndrome; RA, rheumatoid arthritis; SCl, scleroderma; SLE, systemic lupus erythematosus.

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. 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 predictive value for diagnosing SLE or other connective tissue diseases, even in a group whose serum samples are specifically referred for ANA testing. Identification of more specific antinuclear reactivities significantly increases the predictive diagnostic value up to a level that is of real diagnostic value in specialist practice.

Anti-SSA and/or anti-SSB reactivity were identified in 11.8% of the ANA positive patients. The most prevalent disease associated with anti-SSA/SSB autoantibody was SLE. Especially, the combined triple reactivity (anti-Ro52, anti-Ro60, and anti-SSB) and anti-Ro60 with or without anti-Ro52 reactivity makes this diagnosis highly probable. Our data confirm that anti-Ro60 reactivity without anti-Ro52 and anti-Ro52 reactivity is very indicative for SLE. By contrast, none of the 26 patients with pSS had only antibodies to Ro60, whereas anti-Ro52 reactivity was present in 25 of the 26 patients. Previous evidence has been presented that the major anti-SSA response consists of anti-Ro52 antibodies in pSS and anti-Ro60 antibodies in SLE. Patients with Scl or DM rarely present with combined anti-Ro52, anti-Ro60, or anti-SSB antibodies. Our present study, representing a consecutive series of samples, suggests that the finding of an isolated response to Ro52 predisposes most for systemic sclerosis and almost equally for SLE and DM. All patients diagnosed with DM were also encountered in this serotype group. The phenomenon of anti-Ro52 antibodies in DM/Pm and Scl, without concomitant anti-Ro60 and anti-SSB antibodies, has been described previously. Two of our patients with DM had anti-Jo1 reactivity. None of the patients with DM and only one patient with scleroderma were identified by immunodiffusion.

To our knowledge, no other studies examined the diagnostic range associated with the detailed anti-SSA/anti-SSB serology in a large consecutive ANA-positive cohort. This analysis underscores the interest in identifying the detailed reactivity of anti-SSA/SSB autoantibodies, as this alters the ratios of associated diagnoses, and thus the diagnostic probabilities. Evidence has been provided that patients with undifferentiated connective tissue disease and antibodies to SSA can progress in a relatively short period to well defined connective tissue diseases. The possibility exists that some of our patients classified as “other” will evolve to defined connective tissue diseases over time.

It seemed that immunodiffusion had somewhat higher diagnostic value than line immunoblotting (table 2). However, 63 serum samples positive on line immunoblotting were not identified by immunodiffusion versus two serum samples that were solely retrieved by immunodiffusion. Most of these 63 patients had a defined connective tissue disease. The higher sensitivity of the line immunoblotting could mainly be attributed to the earlier described better performance of this assay in detecting anti-Ro52 and anti-SSB antibodies. Based on this higher detection level and on the fact that the diagnostic range associated with anti-SSA/anti-SSB reactivity differs significantly according to the fine serotype, we suggest screening for anti-SSA/anti-SSB reactivity by line immunoblot. When confronted with triple reactivity to Ro52, Ro60, and SSB on line immunoblot, we found that additionally performed immunodiffusion discriminates between SLE and pSS. An additional advantage of the line immunoblot 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 autoantibody towards the different antigen determinants of the RNP-antigen (RNP-A, C, and 70) and the Sm antigen (SmB and SmD). 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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