Anticentromere Antibodies Identify Patients with Sjögren’s Syndrome and Autoimmune Overlap Syndrome

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ABSTRACT. Objective. To assess the prevalence and clinical and immunological significance of anticentromere antibodies (ACA) in patients with primary Sjögren’s syndrome (pSS).

Methods. We retrospectively investigated the prevalence of ACA in patients with SS. We compared ACA-positive SS patients with ACA-negative pSS patients.

Results. The prevalence of ACA among patients with pSS was 4.7% (10/212). Among the patients with SS and an associated autoimmune disease, 10 patients had ACA and limited cutaneous sclerosis (SSc). Clinical and immunological patterns did not differ between the 10 pSS patients with ACA alone and the 10 SS patients with ACA and SSc, except for presence of limited cutaneous SSc (lcSSc). Moreover, all ACA-positive sera recognized centromere protein-B on ELISA, regardless of the presence of SSc. The entire SS-ACA group (n = 20) showed greater frequency of Raynaud’s phenomenon, objective xerophthalmia, peripheral neuropathy, and additional autoimmune disorders, especially primary biliary cirrhosis, compared to pSS patients without ACA (p = 0.005, p = 0.04, p = 0.001, p = 0.05, p < 0.0001, respectively). SS patients with ACA less frequently showed anti-SSA or anti-SSB antibodies than those without ACA (p = 0.0002, p = 0.01, respectively) but greater prevalence of autoantibodies other than anti-SSA/SSB or ACA (p = 0.001).

Conclusion. Clinical and immunological features of SS were largely similar among SS patients with ACA with and without SSc. However, the presence of ACA among patients with SS allows identification of a subset of patients with “SS overlap syndrome,” who show a wide diversity of autoimmunity, encompassing but not limited to SSc. (First Release Oct 15 2007; J Rheumatol 2007;34:2253–8)

Key Indexing Terms: Sjögren’s SYNDROME ANTICENTROMERE ANTIBODIES AUTOIMMUNE OVERLAP SYNDROME

Sjögren’s syndrome (SS) is an “autoimmune epithelitis” disease characterized by sicca syndrome but also extraglandular manifestations. SS can occur alone as primary SS (pSS) or accompany other autoimmune disorders as secondary SS (sSS)1-4.

We previously demonstrated that patients with SS associated with systemic sclerosis (SSc) more frequently show anticentromere antibodies (ACA) and a spreading of autoimmunity, with additional autoantibodies and autoimmune diseases5. However, ACA detected by immunofluorescence in Hep-2 cells are not specific to SSc alone and might be detected in pSS without evidence of SSc; the presence of ACA is observed in 5% of other autoimmune disorders. According to published series, such antibodies were found in nearly 30% of patients with Raynaud’s disease with primary biliary cirrhosis (PBC), 4% of systemic lupus erythematosus (SLE), 8% of mixed connective tissue disease, and 1% to 7% of rheumatoid arthritis6-12. In pSS the prevalence of ACA is variable from one study to the other.

In SSc, ACA recognize 3 centromeric proteins (CENP) identified as autoantigens localized at the kinetochore plates: CENP-A, CENP-B, and CENP-C. In pSS, the specific target of ACA is unknown.

We aimed to assess the prevalence of ACA and their centromeric targets in a cohort of patients with pSS and to determine the clinical and immunological significance of their presence.

MATERIALS AND METHODS

This was a retrospective, comparative study in a single tertiary-referral clinic. Selection of patients. The prevalence of ACA in pSS was evaluated among the patients with SS from the cohort followed in the Department of
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Rheumatology of Bicêtre Hospital between 2000 and 2006; 480 patients were successively referred to the Rheumatology Department for dry symptoms. A primary diagnosis of SS, SS associated with another autoimmune disease, or sicca syndrome polyalgia syndrome (SAPS) was performed by 2 clinician experts (XM and FD). Two hundred thirty-nine patients had SAPS, 212 had pSS, including 10 with ACA without scleroderma, and 29 patients had SS associated with another autoimmune disease, including 10 ACA-positive patients with SS and limited scleroderma.

Primary SS was defined according to the American-European Consensus group criteria14 and SSc according to the American Rheumatism Association (ARA) and LeRoy, et al criteria1,4,15. Limited cutaneous SSc was defined by skin thickening in areas solely distal to the elbows and knees, with or without facial involvement; diffuse SSc was defined by the presence of skin thickening proximal and distal to the elbows and knees, with or without facial or trunk involvement15.

Data collection. Demographic features were collected for all patients of each group.

Patterns of SS. We collected data on subjective dry eyes and mouth, keratoconjunctivitis sicca and objective xerostomia, purpura, and parotid gland enlargement. Objective xerostomia corresponded to an unstimulated salivary flow < 0.1 ml/min or abnormal results on parotid sialography or scintigraphy of salivary glands with 99mTc. Keratoconjunctivitis sicca was defined by an abnormal Schirmer’s test result (≤ 5 mm in 5 min) or Van Bijsterveld score (≥ 4 after Lissamine green coloration). Previous or present extraglandular complications of SS were defined as arthritis with objective synovitis, purpura, renal involvement (glomerulonephritis, interstitial nephritis, renal insufficiency), pulmonary involvement (bronchiectasis, interstitial pneumonitis) as assessed by chest radiography, nephropathy based on the clinical and electrophysiologic presence of sensitive or motor involvement, or lymphoma documented by biopsy. Biopsy results of minor salivary glands were classified according to Chisholm and Mason16.

Immunological data of SS in medical files included antinuclear antibodies (detected by indirect immunofluorescence), anti-Ro/SSA, La/SSB antibodies (by ELISA), and rheumatoid factor (RF; by nephelometry). The ACA were detected by indirect immunofluorescence.

Patterns of SSc. For all patients with ACA, we collected data on the presence of sclerodactyly, Raynaud’s phenomenon, calcinosis, telangiectasia, esophageal involvement, lung interstitial syndrome, digital ulcerations or pitting scars, and vascular abnormalities seen on nailfold capillaroscopy. Moreover, antibodies to CENP-B were measured by ELISA (Varelisa™ Ana profile, Phadia, Uppsala, Sweden) in sera from ACA-positive patients.

Other autoimmune disorders and antibodies. For all groups, we investigated the presence of additional autoimmune disorders such as SLE, PBC, or autoimmune thyroiditis (AIT) and presence of other antibodies such as antimitochondrial, antihyroyglobulin, antithyroid peroxidase, and IgG antibodies to double-stranded DNA by ELISA17.

Statistical analysis. Chi-square testing, with Yates’s correction when appropriate, was used to compare the difference in prevalence for qualitative variables. Analysis of variance or Mann-Whitney tests, as appropriate, were used to assess quantitative variables. A p < 0.05 was considered significant.

RESULTS

Patient characteristics. The demographic characteristics of the 3 patient groups (pSS-ACA, SS-ACA with SSc, and pSS without ACA) are summarized in Tables 1 and 2.

Prevalence of ACA in patients with pSS. Among the 212 patients with pSS, 10 (4.7%) had ACA detected by indirect immunofluorescence. These 10 patients did not have symptoms of SSc as described in Table 1. However, 4 of them had Raynaud’s phenomenon without specific abnormality on the nailfold capillaroscopy. Patients with SS and lcSSc (according to the ARA and LeRoy criteria) were recruited among patients with SS associated with another autoimmune disease.

Comparison of SS-ACA patients with and without SSc (Table 1). We compared the 10 pSS-ACA patients with the 10 SS-ACA patients with lcSSc. Patients with ACA and without scleroderma did not present clinical characteristics of scleroderma (sclerodactyly, calcinosis, esophageal dysmotility), whereas they often expressed Raynaud’s phenomenon and other clinical and immunologic features that are often observed in patients with SSc. A nailfold capillaroscopy was performed in the 4 pSS-ACA patients with Raynaud’s phenomenon: 2 were normal and the 2 others showed a nonspecific microangiopathy. Followup data could be obtained for 8 of the 10 ACA-positive patients with pSS. After a median followup of 3.5 years (range 1 to 7 yrs), none of them developed any clinical feature of scleroderma.

Except for signs of scleroderma, SS-ACA patients with or without SSc did not significantly differ in clinical and immunological measures (Table 2). In these patients with ACA, an echocardiography was performed in 6 patients. No pulmonary hypertension was detected in 4 of them (2 patients with limited scleroderma and 2 patients with pSS-ACA). But for 2 patients with limited scleroderma, a moderate pulmonary hypertension was detected.

Primary SS-ACA patients without SSc showed additional autoimmune disorders (4 PBC and 2 AIT). In addition to showing lcSSc, 2 patients with SS also had PBC fulfilling the diagnosis of Raynaud’s syndrome18. No significant difference was observed between the 2 groups regarding the presence of autoantibodies other than ACA (Table 1). We also investigated the antigen targets of ACA in 18 ACA-positive patients. All patients (with and without SSc) had antibodies against CENP-B.

Given the clinical and immunological similarity of the 20 ACA-positive patients, regardless of presence of SSc, we compared the whole group of patients with SS and ACA with the 202 pSS patients without ACA to investigate whether the presence of ACA could allow identification of a specific subset of patients with SS.

Comparison of patients with SS and without ACA, regardless of SSc (Table 2). SS patients with and without ACA regardless of SSc did not differ in subjective evidence of sicca syndrome, objective evidence of xerostomia and histologic patterns of minor salivary gland biopsies, except in mean age at diagnosis of pSS, which was higher in the group with ACA. Among patients with ACA, 17 biopsy results were reassessed for the presence of fibrosis: 2/8 biopsies for the group with cutaneous limited SSc showed fibrosis, as did 5/9 for the group without SSc. Patients with ACA had significantly more frequently objective evidence of xerophthalmia and Raynaud’s phenomenon (p = 0.04 and p = 0.005, respectively) than patients without ACA.

Overall, patients with ACA did not show more complications of SS than patients without ACA (p = 0.95). Nevertheless, peripheral neuropathic features were more com-
mon in patients with ACA than in those without ACA (25% vs 2.4%; p = 0.001). Among the patients with neuropathy, only one had a type II mixed cryoglobulinemia (IgM kappa).

Patients with ACA had an additional autoimmune disorder (other than lcSSc) more frequently than patients without ACA (40% vs 18.8%, respectively; p = 0.05), especially PBC for 6 patients (p < 0.0001). The diagnosis of PBC was retained for these patients on the basis of the presence of cholestasis, antimitochondrial antibodies (for 5 of them), and typical histological lesions on liver biopsy for 2 patients. The other autoimmune disease was AIT in 2 patients (including that without any antimitochondrial antibodies).

Regarding immunological features, the prevalence of anti-SSA and anti-SSB antibodies differed significantly between the 2 groups, with a significantly higher frequency of these autoantibodies in the pSS group without ACA (p = 0.0002 and p = 0.01, respectively). RF was also more common in pSS patients without ACA (Table 2).

Interestingly, the prevalence of autoantibodies other than anti-SSA, anti-SSB, ACA, and RF was significantly higher in the patients with ACA than in those without ACA (50% vs 16.8%; p = 0.001). These were antimitochondrial (n = 5 with confirmed PBC), IgG to double-stranded DNA (n = 4), antifilaggrin (n = 2), antithyroglobulin, antineutrophil cytoplasmic, anti-Jo1, and antiphospholipid (1 patient each) antibodies. No patient with IgG to double-stranded DNA had clinical features suggestive of SLE.

DISCUSSION

In our study, we found a prevalence of 4.7% (10/212) of ACA (identified by indirect immunofluorescence) among patients with pSS. Except for presence of lcSSc, clinical and immunological features did not differ between 10 pSS patients with ACA and 10 other SS patients with ACA and lcSSc. Moreover, all ACA-positive sera from SS-ACA patients with and without SSc recognized the same target, CENP-B.

The clinical and immunological similarity of ACA-positive patients, regardless of the presence of associated SSc, allowed us to compare the whole group of ACA-positive patients with ACA-negative patients. Sjögren’s syndrome with ACA differed from pSS without ACA in different clinical and immunological features. First, diagnosis was made later; Raynaud’s phenomenon, objective xerophthalmia, and peripheral neuropathy were more frequent in the ACA group than the

### Table 1. Comparison of clinical and biological features of patients with primary SS and anticentromere antibodies (pSS-ACA), with and without systemic sclerosis (SSc).

<table>
<thead>
<tr>
<th>Feature</th>
<th>pSS-ACA without SSc, n = 10</th>
<th>SS-ACA with SSc, n = 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis of pSS, mean yrs ± SD (range)</td>
<td>54.9 ± 8.1 (44–71)</td>
<td>63.3 ± 12.8 (42–77)</td>
<td>0.09</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>1.00</td>
</tr>
<tr>
<td>Clinical patterns of pSS, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjective xerostomia and dry eyes</td>
<td>9 (90)</td>
<td>9 (90)</td>
<td>1.00</td>
</tr>
<tr>
<td>Objective xerostomia*</td>
<td>6/9 (66.6)</td>
<td>3/4 (75)</td>
<td></td>
</tr>
<tr>
<td>Keratoconjunctivitis sicca**</td>
<td>8/9 (88.8)</td>
<td>7/7 (100)</td>
<td></td>
</tr>
<tr>
<td>Histologic patterns of minor salivary gland biopsy: % with grade 3 or 4</td>
<td>9 (90)</td>
<td>7/9 (77.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>Clinical patterns of SSc, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>4 (40)</td>
<td>10 (100)</td>
<td></td>
</tr>
<tr>
<td>Sclerodactyly</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Calcinosis</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Esophageal involvement</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Lung interstitial syndrome</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Digital ulcerations or pitting scars</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Vascular abnormalities seen on nailfold capillaroscopy</td>
<td>0</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>Serological patterns, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Ro/SSA antibodies</td>
<td>2 (20)</td>
<td>2 (20)</td>
<td>1.00</td>
</tr>
<tr>
<td>Anti-La/SSB antibodies</td>
<td>1 (10)</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Centromere protein B antibody</td>
<td>10/10 (100)</td>
<td>8/8 (100)</td>
<td>1.00</td>
</tr>
<tr>
<td>At least one other antibody††</td>
<td>7 (70)</td>
<td>3 (30)</td>
<td>0.18</td>
</tr>
<tr>
<td>Additional autoimmune disorder***, n (%)</td>
<td>6 (60)</td>
<td>2 (20)</td>
<td>0.18</td>
</tr>
<tr>
<td>Primary biliary cirrhosis, n (%)</td>
<td>4 (40)</td>
<td>2 (20)</td>
<td>0.62</td>
</tr>
<tr>
<td>Sensory neuropathy, n (%)</td>
<td>3 (30)</td>
<td>2 (20)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Defined as unstimulated salivary flow < 0.1 ml/min or an abnormal test result on parotid sialography or scintigraphy of salivary glands with99-m Tc. ** Defined as an abnormal Schirmer’s test result (≤ 5 mm in 5 min) or Van Bijsterveld score ≥ 4 after Lissamine green coloration. *** Other than SSc and SS. † Chisholm and Mason classification16. †† Other than ACA, RF, or anti-SSA and anti-SSB antibodies.
The presence of anti-SSA and anti-SSB antibodies was significantly lower in the ACA group (20% and 5%, respectively) than in the non-ACA group. Second, in the ACA group, autoantibodies other than ACA, anti-SSA and anti-SSB, or RF were detected in 50% of patients; this proportion was significantly higher than in the non-ACA group (p = 0.001). This diversification of autoimmunity accompanied an “SS overlap syndrome” (i.e., a syndrome other than SS + SSc) in 40% of cases, with a frequent association between SS and PBC.

Concerning the prevalence of ACA in patients with SS, results in the literature are conflicting. When patients with ACA were investigated for SS, the prevalence of SS was 15% to 37%9,11,19,20, whereas that of ACA, as detected by indirect immunofluorescence, among patients with pSS was 2% to 24.6%.11,21-24. This discrepancy could be explained by the recent change in diagnostic criteria of SS13. Two recent publications found a prevalence of 2% and 7% of pSS according to the American-European Consensus Group criteria, which is in accord with our results23,24.

Despite the small size of each group (SS-ACA with and without SSc) and because this comparison did not show any significant difference concerning SS features, we decided to pool these groups and to compare them with patients without ACA. This shows that ACA-positive patients represent a homogeneous group. Nevertheless, some of the less powerful associations could be the result of over-testing without correction.

Regarding the immunological pattern of ACA-positive patients, the antigenic target of ACA was restricted to CENP-B, with a lower prevalence of anti-SSA/SSB antibodies. The exclusive detection of anti-CENP antibodies is in agreement with the results of Miyawaki, et al11 and Katano, et al21. In Miyawaki’s study, among 40 patients with SS and ACA as detected by indirect immunofluorescence, all sera recognized CENP-B on ELISA and 75% of patients showed an “SS overlap syndrome.” However, for Gelber, et al24, among 10 patients with pSS and antibodies to centromere proteins, sera testing revealed CENP-B in only 3 patients with ACA. For the 7 others, sera testing revealed CENP-C alone but without ACA detected by indirect immunofluorescence. Both CENP-B and CENP-C were more frequent in SS patients with lcSSc with ACA (83%)22,24. The differences from Gelber’s results are explained by the different definition of ACA in the 2 studies. We defined ACA by positive immunofluorescence results, and with this same definition, Gelber, et al found only 3 patients with ACA, whose sera all recognized CENP-B. Thus, the pattern of recognition of ACA in these studies was the same as in our study, regardless of associated SSc.

This common immunological pattern of ACA is a supplementary argument to consider together all patients with SS and ACA as a particular subset of patients with SS and over-
lap syndrome. Concerning the low prevalence of anti-SSA and anti-SSB antibodies in these patients, the results are conflicting: Katano, et al did not find these antibodies in 12 patients23, whereas Vlachoyiannopoulos, et al showed a relatively close prevalence (57% of anti-SSA and 14% of anti-SSB) to that described with pSS25.

Six previous studies evaluated the clinical patterns of patients with pSS and ACA11,21,24-27, for a total of 67 cases, including our cases. No patients had SSc symptoms, but the prevalence of Raynaud’s phenomenon was high (50% to 85%)21,24,25, which led one group to recommend routine testing for ACA in pSS patients with Raynaud’s phenomenon28. Autoantibodies might be present many years before the clinical onset of autoimmune diseases, such as SLE29 and rheumatoid arthritis30. Interestingly, among the 8 well-described SSc-ACA patients Ramos-Casals, et al followed over 6 years, on average, development of limited scleroderma was observed in 726, and among the 10 patients described by Caramaschi, et al, 4 developed a lcSSc during followup27. Thus, the possibility that SSc might develop in our ACA-positive patients cannot be ruled out. However, in all our patients for whom followup data could be obtained, no clinical feature of scleroderma appeared. Lastly, we observed that peripheral neuropathy, an uncommon manifestation of SSc, was significantly higher in patients with ACA than in those without ACA, which has never been described.

The most interesting finding of our study is the wide diversity of autoimmunity in patients with ACA, regardless of the presence of SSc, in comparison with patients without ACA. Indeed, 50% of patients had at least one other antibody (other than ACA, RF, and anti-SSA and anti-SSB antibodies) and 40% had an additional autoimmune disease (other than SSc), particularly PBC. Genetic factors often contribute to the spreading of the autoimmune response rather than to the autoimmune disease itself, as demonstrated for HLA31,32, or cytokine polymorphisms in pSS33. The demonstration that the same antigenic target (CENP-B) was recognized by all tested ACA-positive patients strongly suggests an association with genetic polymorphisms. Interestingly, genetic polymorphisms predisposing to presence of ACA in SSc are quite different from those predisposing to presence of anti-SSA/SSB antibodies. Indeed, ACA are associated with HLA-DR4, DR8, DQB1, and TNF-863A34-36 in SSc, anti-SSA antibodies alone with HLA-DR15 (previously called DR2), and anti-SSA plus anti-SSB antibodies with the HLA-A1B8DR3DQ2 haplotype and TNF-308A31,33 in pSS. The HLA genotype of ACA-positive patients with SS now deserves to be investigated.

Clinical and immunological features of SS were similar between patients with ACA with and without SSc, except for presence of lcSSc. Interestingly, the presence of ACA allows identification among SS patients of a subset with “SS overlap syndrome,” who show a wide diversity of autoimmunity, encompassing but not limited to SSc.

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