Clinical and Laboratory Features of Anticentromere Antibody Positive Primary Sjögren’s Syndrome

KENICHI KATANO, MITSUHIRO KAWANO, ICHIRO KONI, SUSUMU SUGAI, and YOSHINAO MURO

ABSTRACT. Objective. To determine whether the clinical and laboratory characteristics of anticentromere antibody (ACA) positive, anti-SSA/Ro antibody (SSA) negative primary Sjögren’s syndrome (SS) differ from SSA positive, ACA negative primary SS.

Methods. Twelve patients with ACA positive primary SS (ACA SS) and 19 patients with SSA positive primary SS (SSA SS) were examined. We compared the age, laboratory data, proportion with Raynaud’s phenomenon (RP), activity of natural killer cells (NK), titer of antibodies against Epstein-Barr virus, and histological findings of minor labial salivary glands. The presence of anti-chromo antibodies (AChA) was evaluated by immunoblotting of patients’ sera.

Results. The mean age of the ACA SS group was higher than that of the SSA SS group (p < 0.05). Serum IgG level was lower in ACA SS than in SSA SS (p < 0.0001). Serum IgG level of the ACA SS group with one exception was close to the normal range. Leukopenia was less frequently observed in ACA SS than in SSA SS (p < 0.05). RP was seen more frequently in the ACA SS group than the SSA SS group (p < 0.05). NK activity of the ACA SS group was higher than that of the SSA SS group (p < 0.05). Most of the ACA SS patients’ NK activity was normal, in contrast to the tendency for NK activity in SS to be low. Virus capsid antigen IgA titer of the ACA SS group was lower than that of the SSA SS group (p < 0.05). Histological findings of minor labial salivary glands of both groups showed a similar severity of lymphocytic infiltrates, destruction of normal structures, and pattern of infiltrating lymphocyte subsets. AChA was positive in 11 of the 12 sera of ACA SS patients.

Conclusion. The results confirm that ACA positive primary SS differs from SSA positive classic SS in several significant respects. (J Rheumatol 2001;28:2238–44)

Key Indexing Terms: Sjögren’s Syndrome Antichromo Antibodies

Anticentromere Antibodies Epstein-Barr Virus

Certain types of autoantibodies are specific for the diagnosis of autoimmune diseases and are clinically used as diagnostic markers. In Sjögren’s syndrome (SS), anti-SSA/Ro antibodies are detected in the vast majority of cases, often accompanied by anti-SSB/La antibodies. It is thought that about 71% of primary SS cases are SSA/Ro positive and about 67% are SSB/La positive. Now anti-SSA antibodies and anti-SSB antibodies are recognized as useful diagnostic markers of SS.

In SS patients without SSA and SSB, a small group of anticentromere antibody (ACA) positive patients have been reported. However, whether ACA positive and SSA negative SS has clinically distinct characteristics from SSA positive ACA negative SS is not clear. In addition, analysis of ACA by ELISA using recombinant 80 kDa centromere antigen of human chromosomes (CENP-B) in pSS has rarely been reported.

To clarify these issues, we analyzed the clinical and laboratory characteristics of ACA positive primary SS compared to SSA positive SS.

MATERIALS AND METHODS

Patients. From 1994 to 2001, 69 patients were diagnosed as having primary SS in the Second Department of Internal Medicine, Kanazawa University Hospital and affiliated hospitals. The percentage of patients with SSA positive primary SS in this group was 65.2% including 2 ACA positive patients. The percentage of ACA positive primary SS was 24.6%. Among these cases, 12 patients with ACA positive SSA negative primary SS (all female, age range 52 to 84 yrs, mean age 68.4 ± 7.9) and 19 patients with SSA positive ACA negative SS (all female, age range 23 to 77 yrs, mean age 54.6 ± 16.2) were studied. Minor salivary gland biopsy was performed in all SSA positive patients and 10 of the ACA positive patients. The remaining 2 ACA positive patients refused the biopsy test, and we employed scintigraphy and rose bengal testing to establish their diagnosis. All patients fulfilled preliminary criteria for primary SS. To differentiate the coexistence of CREST...
syndrome, esophageal function was examined by barium study and/or endoscopies. Esophageal manometry was not employed because all patients had no gastroesophageal complaints and did not agree to undergo this test. Abnormality of lower gastrointestinal motility was also ruled out by the absence of clinical symptoms and distinctive radiographic findings. Skin thickness was assessed by experienced physicians and the absence of sclerodactyly was confirmed. Existence of calcinosis was evaluated with hand and chest radiographs, and was confirmed absent in all the ACA positive patients. The mean followup duration of these 12 ACA positive patients was 59.8 months (range 6 to 185 mo), and we could find no CREST related clinical symptoms or signs except for Raynaud’s phenomenon (RP) in these patients during the followup periods.

Clinical and laboratory assessments. The presence or absence of anti-SSA/Ro antibodies was evaluated by ELISA using the Mesacup SSA/Ro test kit (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan), which can detect both anti-60-kDa and anti-52-kDa SSA/Ro antibodies using purified SSA/Ro antigens and recombinant 60 kDa SSA/Ro proteins. After screening for anticentromere antibody determined by immunofluorescence methods using HEp-2 cells, the titer of ACA in all sera of the ACA positive group and 17 sera of the SSA positive group was measured by ELISA using the Mesacup CENP-B test kit (Medical & Biological Laboratories). The presence of anti-chromo antibodies (AChA), which are thought to exist exclusively in ACA positive sera, was evaluated by immunoblotting with HeLa nuclear extract and a recombinant chromo p25 antigen in all sera of ACA positive patients and 17 sera of the SSA positive patients. The recombinant protein was expressed as a fusion protein of the 106 N-terminal amino acids of p25, which included the major autoepitope “chromo domain” of p25, with glutathione S-transferase. We compared serum levels of IgG, IgM, IgA, counts of white blood cells, lymphocytes, activity of natural killer (NK) cells, and the proportion with RP, between the ACA positive and SSA positive groups. Leukocytopenia was regarded as counts under 4000/mm³. Lymphocytopenia was regarded as counts below 1500/mm³. NK activity was determined using 51Cr labeled immunoblotting with HeLa nuclear extract and a recombinant chromo p25 “chromo domain” of p25, with glutathione S-transferase. We compared serum levels of IgG, IgM, IgA, counts of white blood cells, lymphocytes, activity of natural killer (NK) cells, and the proportion with RP, between the ACA positive and SSA positive groups. Leukocytopenia was regarded as counts under 4000/mm³. Lymphocytopenia was regarded as counts below 1500/mm³. NK activity was determined using 51Cr labeled K562 cells as the target cells. To assess the relation between Epstein-Barr virus (EBV) and SSA positive SS or ACA positive SS, we measured the titer of antibodies against EBV in each group by immunofluorescence study. Measured antibodies were antiviral capsid antigen (VCA) IgG (VCA-IgG), anti-VCA-IgA, anti-VCA-IgM, anti-early antigen (EA) IgG (EA-IgG), anti-EA-IgA, and anti-EV nuclear antigen (EBNA). Histological analysis of salivary glands. Minor salivary glands obtained from each patient were stained with hematoxylin-eosin, immunostaining methods, ELISA against 80 kDa recombinant CENP-B protein, and Western blotting. All patients were positive for anti-CENP-B antigen and Western blotting with HeLa nuclear extract, and the mean ELISA titer was 238 ELISA units. In contrast, we confirmed the absence of ACA in the 17 SSA positive patients using these 3 methods. The absence of ACA was evaluated in the remaining 2 sera using only indirect immunofluorescence. Anti-chromo antibody (AChA) was positive in 11 of the 12 sera of ACA positive patients (Figure 1). In contrast, 17 sera of SSA positive patients did not have AChA. These results were confirmed by the analysis with the recombinant p25 protein (data not shown). Table 3 shows a comparison between the ACA positive group and the SSA positive group. The age of the ACA positive group was higher than the SSA positive group (68.4 ± 7.9 vs 54.6 ± 16.2 yrs; p < 0.05). Serum IgG level of the

Table 1. Clinical and laboratory features of patients with ACA positive primary Sjögren’s syndrome.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, Sex</th>
<th>WBC, per mm³</th>
<th>Lymphocytes, per mm³</th>
<th>IgG, mg/dl</th>
<th>NK Activity</th>
<th>Raynaud’s Phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66 F</td>
<td>6400</td>
<td>2000</td>
<td>1945</td>
<td>23.8</td>
<td>34.0</td>
</tr>
<tr>
<td>2</td>
<td>84 F</td>
<td>7700</td>
<td>2400</td>
<td>1706</td>
<td>19.9</td>
<td>28.7</td>
</tr>
<tr>
<td>3</td>
<td>67 F</td>
<td>3700</td>
<td>700</td>
<td>1260</td>
<td>7.1</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>76 F</td>
<td>4200</td>
<td>2200</td>
<td>1962</td>
<td>13.5</td>
<td>23.8</td>
</tr>
<tr>
<td>5</td>
<td>62 F</td>
<td>4700</td>
<td>2200</td>
<td>2929</td>
<td>16.0</td>
<td>31.6</td>
</tr>
<tr>
<td>6</td>
<td>71 F</td>
<td>5800</td>
<td>2100</td>
<td>1780</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>7</td>
<td>64 F</td>
<td>6500</td>
<td>1400</td>
<td>1770</td>
<td>18.2</td>
<td>30.1</td>
</tr>
<tr>
<td>8</td>
<td>67 F</td>
<td>5200</td>
<td>2200</td>
<td>1579</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>9</td>
<td>67 F</td>
<td>6000</td>
<td>2300</td>
<td>1545</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td>67 F</td>
<td>9700</td>
<td>2000</td>
<td>1751</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>11</td>
<td>52 F</td>
<td>5700</td>
<td>1300</td>
<td>1496</td>
<td>17.1</td>
<td>27.5</td>
</tr>
<tr>
<td>12</td>
<td>74 F</td>
<td>10400</td>
<td>1600</td>
<td>1741</td>
<td>8.4</td>
<td>12.7</td>
</tr>
</tbody>
</table>

WBC: white blood cells; NK: natural killer.
The ACA positive group was significantly lower than the SSA positive group (1789 ± 408 vs 2790 ± 672 mg/dl; p < 0.0001). Serum IgG level did not correlate with age (data not shown). Leukocytopenia was less frequently observed in the ACA positive group than in the SSA positive group (8.3 vs 42.1%; p < 0.05). NK activity of the ACA positive group was significantly greater than the SSA positive group (NK activity 10:1, 15.5 ± 5.6% vs 8.6 ± 2.3%, p < 0.05; NK activity 20:1, 25.2 ± 8.1% vs 15.2 ± 4.5%, p < 0.05). Most of the ACA positive patients' NK activity was normal, in contrast to the tendency for NK activity in primary SS to be low. RP was seen more frequently in the ACA positive group than the SSA positive group (50.0% vs 5.3%; p < 0.05). Figure 2 shows the titers of VCA-IgA, VCA-IgG, and EA-IgG of each group. The titer of VCA-IgA of the ACA positive group was lower than the SSA positive group (p < 0.05). The titer of the VCA-IgG and EA-IgG of the ACA positive group tended to be lower than that of the SSA positive group, but this difference was not statistically signifi-
cant. We also measured the titers of VCA-IgM, EA-IgA, and EBNA in each group, but no differences were observed (data not shown).

**Histological analysis and immunostaining of minor salivary glands.** Figure 3 shows the histological findings of an ACA positive patient’s minor salivary glands. Lymphocytic infiltrates and destruction of normal structures were seen. In immunostaining, OPD4+ cells were more numerous than CD8+ cells, and almost all infiltrating T cells were HLA-DR positive. CD20+ cells were also observed. Eight of 12 ACA positive patients showed histological findings of similar severity to those illustrated in Figure 3. These findings were the same as those of the SSA positive patients.

**DISCUSSION**

ACA was first detected in sera from patients with scleroderma in 1980, and has since been widely accepted as a diagnostic marker of the CREST variant of scleroderma. However, ACA positivity is not always associated with sclerodactyly in autoimmune patients. Raynaud’s phenomenon alone is another well known condition in ACA positive patients, while primary Sjögren’s syndrome is also accepted as a representative variant of ACA positive autoimmune diseases. Vlachoyiannopoulos, et al analyzed 41 autoimmune patients with ACA and found that 7 (17%) were diagnosed with primary SS, with a low incidence of parotid gland enlargement and anti-SSB antibodies.

Caramaschi, et al indicated that a high frequency of RP and low frequency of leukocytopenia, polyclonal hypergammaglobulinemia, rheumatoid factor, and anti-SSA antibodies were prominent features of ACA positive primary SS in comparison with SS with typical serologic features.

We analyzed the laboratory features of 12 patients with ACA positive primary SS to confirm the results of the previous studies and identify additional characteristics indicating that ACA positive SS is an independent clinical entity. The frequency of RP was significantly higher in ACA positive SS than in ACA negative SS. Moreover, in accord with a previous study, leukocytopenia and hypergammaglobulinemia were found less frequently in ACA positive SS than in ACA negative SS. In addition, we found that ACA positive patients tend to be older and to have normal NK activity. Several studies have revealed that decreased NK activity is a common feature of primary SS and may be related to the immunological abnormalities characteristic of SS. That NK activity is normal in ACA positive SS suggests that the etiology of this condition differs from that of SSA positive SS.

EBV is thought to be related to SS. EBV encoded early antigen was noted in salivary gland biopsies from 57% of SS patients, and EBV DNA was detected in parotid saliva samples from 40% of SS patients. In addition, B lymphocytes derived from SS patients’ peripheral blood often generate many copies of EBV without stimulation. In our

---

*Figure 2. Comparison of antibody titers against EBV between ACA positive (ACA (+) SSA (–)) and ACA negative and anti-SSA positive SS (SSA (+) ACA (–)). The titer of VCA-IgA of the ACA (+) SSA (–) group was lower than that of the SSA (+) ACA (–) group (p < 0.05). VCA: viral capsid antigen, EA: early antigen.*
analysis, expression of EBER in labial minor salivary gland lesions could not be detected using the *in situ* hybridization method in either ACA positive or ACA negative patients (data not shown). However, we found that the frequency of anti-VCA-IgA antibody, usually detected in chronic active EBV infections, was significantly higher in SS with typical serologic features than ACA positive SS. This finding argues against a role for EBV as the pathogenic agent in ACA positive SS, in contrast to SSA positive SS.

AChA are autoantibodies against 23 kDa and 25 kDa nuclear proteins (p23 and p25, respectively) and have been found in 36% of sera containing ACA. In these autoantigens, Saunders, *et al* have shown that the p25 antigen is a human homolog of Drosophila melanogaster heterochromatin protein 1 (HP1) by DNA sequence analysis. Although the clinical significance of AChA positive autoimmune status is still unclear, we analyzed the features of AChA positive patients with various autoimmune diseases,
and found that the frequency of AChA was higher in patients with nonsystemic sclerosis than in those with systemic sclerosis\(^4\). Moreover, AChA were present in 62.5% of sera from patients with primary SS with ACA\(^4\). In the present study, AChA were detected in 11 of the 12 sera from primary SS patients with ACA, suggesting that primary SS represents an AChA positive subgroup of ACA positive autoimmune disease different from the well known ACA positive CREST variant scleroderma.

In histological analysis of minor salivary glands from SS patients, CD4\(^+\) T cells are usually predominant and comprise 60–70% of the infiltrating mononuclear cells\(^8\). In our analysis, the predominance of CD4\(^+\) T lymphocytes was a common feature of ACA positive and ACA negative SS. Similarly, the severity of the destruction of normal salivary gland structures was not different between these groups. Thus, ordinary immunohistochemical analysis could not discriminate ACA positive SS from SSA positive SS.

Our study identified additional laboratory findings that discriminate ACA positive primary Sjögren’s syndrome from classic SS. Further studies are needed to confirm whether ACA positive SS is a clinically independent disease entity and to determine whether subclinical SS is present in unclassified ACA positive autoimmune diseases.
ACKNOWLEDGMENT
We are indebted to Prof. H. Mabuchi and J. S. Gelblum for their critical reading of this manuscript and to Y. Kubo for her secretarial assistance.

REFERENCES