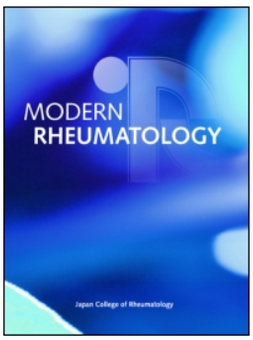

Modern Rheumatology

ISSN: 1439-7595 (Print) 1439-7609 (Online) Journal homepage: http://www.tandfonline.com/loi/imor20

Impact of double positive for anti-centromere and anti-SS-a/Ro antibodies on clinicopathological characteristics of primary Sjögren's syndrome: a retrospective cohort study

Yasunori Suzuki, Hiroshi Fujii, Hideki Nomura, Ichiro Mizushima, Kazunori Yamada, Masakazu Yamagishi & Mitsuhiro Kawano

To cite this article: Yasunori Suzuki, Hiroshi Fujii, Hideki Nomura, Ichiro Mizushima, Kazunori Yamada, Masakazu Yamagishi & Mitsuhiro Kawano (2018): Impact of double positive for anti-centromere and anti-SS-a/Ro antibodies on clinicopathological characteristics of primary Sjögren's syndrome: a retrospective cohort study, Modern Rheumatology, DOI: 10.1080/14397595.2017.1418164

To link to this article: https://doi.org/10.1080/14397595.2017.1418164

Accepted author version posted online: 18 Dec 2017.
Published online: 09 Jan 2018.

MODERN RHEUMATOLOGY, 2017
https://doi.org/10.1080/14397595.2017.1418164

Japan College of Rheumatology
MODERN RHEUMATOLOGY
Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Impact of double positive for anti-centromere and anti-SS-a/Ro antibodies on clinicopathological characteristics of primary Sjögren's syndrome: a retrospective cohort study

Yasunori Suzuki[a], Hiroshi Fujii[a], Hideki Nomura[b], Ichiro Mizushima[a], Kazunori Yamada[a], Masakazu Yamagishi[c] and Mitsuhiro Kawano[a]

[a]Division of Rheumatology, Department of Cardiovascular and Internal Medicine, Kanazawa University Graduate School of Medicine, Kanazawa, Japan; [b]Department of General Medicine, Kanazawa University Hospital, Kanazawa, Japan; [c]Division of Cardiology, Department of Cardiovascular and Internal Medicine, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

ABSTRACT

Objectives: The purpose of our study was to define the clinical characteristics of anti-centromere antibody and anti-SS-A/Ro antibody (ACA/SS-A) double positive Sjögren's syndrome (SS) and to clarify the clinical impact of these antibodies.

Methods: We examined 108 patients (6 males, mean age 57.9 years) with SS who underwent labial salivary gland biopsy. The patients were divided into four groups by ACA and anti-SS-A/Ro antibody positivity. Symptoms, laboratory and pathological data, and scleroderma-related data were compared among the groups.

Results: The cohort consisted of 16 ACA/SS-A double positive, 20 ACA single positive, 67 SS-A single positive, and 5 ACA/SS-A double negative SS. ACA/SS-A double positive SS were significantly older than SS-A single positive SS (mean age 71.1 vs. 53.1 years). They had higher EULAR Sjögren's syndrome disease activity index (ESSDAI) at diagnosis (mean 3.81 vs. 0.50) and higher serum IgG (mean 2009 vs. 1389 mg/dL) than ACA single positive SS. No patients developed skin sclerosis during a mean follow-up period of 45.6 months (range: 1–178).

Conclusion: These results demonstrate that ACA/SS-A double positive SS is distinct from ACA single positive and SSA single positive SS. The combination of ACA and anti-SS-A/Ro antibody in SS should deserve greater attention in clinical practice.

ARTICLE HISTORY
Received 13 November 2017
Accepted 12 December 2017

KEYWORDS
Primary Sjögren's syndrome; anti-centromere antibody; anti-SS-A/Ro antibody; limited scleroderma

Introduction

Sjögren's syndrome (SS) is an autoimmune disease characterized by lymphocyte infiltration in the salivary and lacrimal glands and production of autoantibodies. Anti-SS-A/Ro and anti-SS-B/La antibodies are diagnostic markers that are included in all classification criteria [1–3]. Anti-SS-A/Ro antibody is positive in 3 3–74% of SS, and anti-SS-B/La antibody in 23–52%, meaning on the other hand that some patients with SS are negative for these antibodies [4].

There are some antibodies other than anti-SS-A/Ro and anti-SS-B/La antibody in SS, one of which is anti-centromere antibody (ACA) [5]. Several reports have identified different clinical characteristics in ACA-positive primary SS as compared with ACA-negative SS, making it a distinct subgroup of SS [6–17]. ACA-positive SS has intermediate features between SS and limited scleroderma, with a few such cases evolving to limited scleroderma [10] and has more severe sicca symptoms than ACA-negative SS [16]. Anti-SS-A/Ro antibody positivity in SS is related to hypergammaglobulinemia, rheumatoid factor positivity, and antinuclear antibody positivity, implicating B cell activation [18], although Katano et al. showed that ACA positivity in SS is not related to hypergammaglobulinemia [7]. Thus, clinicopathological features of SS may differ according to the positivity of ACA or anti-SS-A/Ro antibody.

ACA-positive SS includes two different groups: ACA and anti SS-A/Ro antibody (ACA/SS-A) double positive SS and ACA-positive and anti-SS-A/Ro antibody-negative SS (ACA single positive SS). Considering the role of SS-A and ACA antibody in SS, these two groups can exhibit different clinical characteristics. However, few data exist regarding the clinical differences between these two groups or on the clinical characteristics of ACA/SS-A double positive or ACA single positive SS. The objective of this study was to evaluate the clinical characteristics of ACA/SS-A double positive SS and to clarify the clinical impact of ACA and anti-SS-A/Ro antibody on SS.

Materials and methods

This study was a retrospective cohort study conducted at a single center in Japan. This study was approved by the

CONTACT Mitsuhiro Kawano sk33166@gmail.com Division of Rheumatology, Department of Internal Medicine, Kanazawa University Graduate School of Medicine, Takara-machi 13-1, Kanazawa, Ishikawa 920-8641, Japan

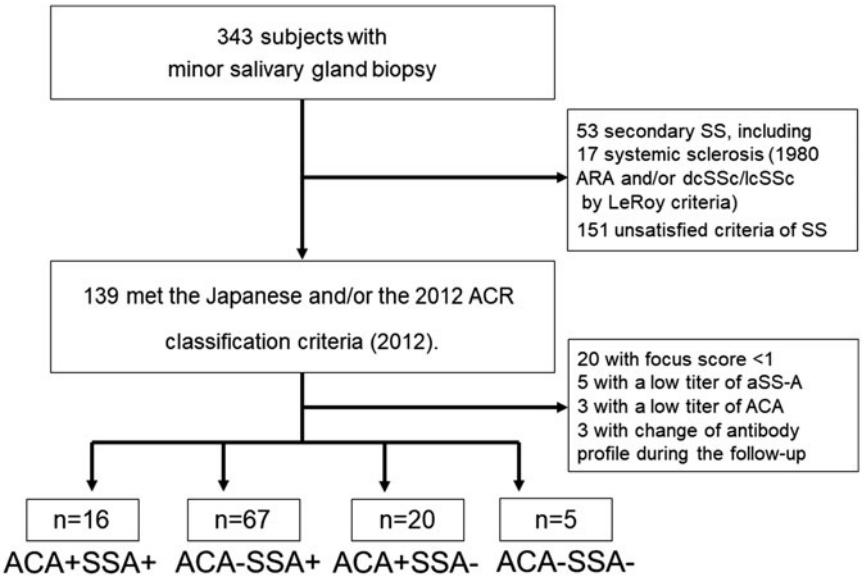

Figure 1. Flowchart of study population. Japanese classification criteria; Japanese Ministry of Health revised criteria [1], ACR classification criteria; the American College of Rheumatology classification criteria [3].

Ethics Committee of Kanazawa University Hospital. Informed consent for use of all data was obtained from each patient, and the research was conducted in compliance with the Declaration of Helsinki.

Patients

We reviewed 343 patients with labial salivary gland biopsy from 1998 to 2015 referring to their medical records. We excluded 151 patients who did not meet the classification criteria of SS, and 53 secondary SS patients with other connective tissue disease, including 17 with systemic sclerosis (Figure 1). One hundred and thirty nine patients met the Japanese Ministry of Health revised criteria [1] and/or American College of Rheumatology criteria [3] with cases with more than 1 focus score selected for this study. All the patients were also evaluated using the 2016 American College of Rheumatology/European League Against Rheumatism classification criteria [19]. Systemic sclerosis was diagnosed according to the 1980 American Rheumatology Association criteria [20] and diffuse or limited scleroderma by LeRoy's criteria [21]. Other connective tissue diseases were diagnosed according to the respective established criteria.

We excluded eight patients who showed low antibody titers (anti-SS-A/Ro antibody: less than 30 EU, anti-SS-B/La antibody: less than 25 EU, and ACA: less than 16 EU), and 3 whose antibody profiles had changed during the follow-up period. Cut-off titer levels for each antibody were defined in the manufacturer's protocol. We designed to exclude severe fibrosis on labial salivary glands suggesting possible overlap with scleroderma [22], but in practice no patients showed this feature. Fibrosis in labial salivary gland tissue was evaluated according to the criteria of Avouac et al. [22].

Clinical and laboratory assessments

Onset of SS was determined by appearance of Raynaud's phenomenon, sicca symptoms, and extraglandular involvement [13], while disease duration was defined as the period from onset to presentation to our department. Follow-up period was defined as the period from first visit to final visit to our hospital until 2015.

Xerophthalmia, xerostomia, presence or absence of Raynaud's phenomenon, the result of Schirmer's test (5 mm or less defined as positive), ocular staining, and Saxon's test (2.0 g or less per 2 min defined as positive) were assessed at the first visit.

Data were classified according to EULAR Sjögren's syndrome disease activity index (ESSDAI) [23] and ESSDAI at diagnosis and the highest ESSDAI during the follow-up was examined. Each item of 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) systemic sclerosis criteria was evaluated in the patients with ACA positivity [24]. Dermatologists determined the presence and severity of any skin sclerosis at diagnosis, and appearance of skin sclerosis during the follow-up was evaluated from the medical records. We also looked for pulmonary hypertension, autoimmune hepatitis, primary biliary cirrhosis, chronic thyroiditis, and malignant lymphoma. Pulmonary hypertension was defined as a mean pulmonary arterial pressure more than 25 mmHg by right heart catheterization or pulmonary artery pressure more than 40 mmHg on Doppler echocardiography. Pathological examinations were necessary to diagnose autoimmune hepatitis and malignant lymphoma, whereas primary biliary cholangitis could be diagnosed on the basis of positive anti-mitochondria antibody and suggestive clinical data. The diagnosis of chronic thyroiditis was based on positive anti-thyroglobulin antibody and/or anti-thyroid peroxidase antibody with hypothyroidism.

Laboratory data and pathological findings of labial salivary gland biopsy were evaluated. Antinuclear antibody was detected by immunofluorescent method and rheumatoid factor by nephelometric assay. We used MESACUP-2test SS-A (MBL, Nagoya, Japan) for anti-SS-A/Ro antibody,

MESACUP-3 test (MBL, Nagoya, Japan) for anti-SS-B/La antibody, and MESACUP-2 test CENP-B (MBL, Nagoya, Japan) for ACA measurement. Standard values were defined by the product manual. All the patients with a low titer of anti-SS-A/Ro antibody were confirmed to be negative by Ouchterlony method (Anti-SSA antibody FR, Fujirebio Inc., Tokyo, Japan). Serum cytokines were measured with FlowCytomixTM multiple analyte detection system (eBioscience, San Diego, CA) according to the manufacturer's protocol. For the analysis of serum cytokines, we selected patients who were not receiving steroids or immunosuppressants because these agents might affect the results.

Statistical analysis

Continuous data were expressed as mean ± SD, Kruskal–Wallis test and Mann–Whitney *U* test were used for the analyses. Categorical data were analyzed by chi-square test and Fisher's exact test. Differences between groups were considered significant at $p < .05$. Unrecorded data or unperformed tests were regarded as missing data and these numbers were clarified in the table. Univariate analysis was performed for three groups other than ACA/SS-A double negative group that included only five patients. The Bonferroni procedure was used as a *post hoc* test. To assess whether autoantibody profiles affect the severity of sicca symptoms, multivariate logistic regression analysis was performed. We used ACA, anti-SS-A/Ro antibody, age at diagnosis, sex, age at onset, and disease duration as predictor variables. In multivariate analysis, all data including the ACA/SSA double negative group were used. Forward stepwise selection was used for selecting variables for a regression model. Statistical analysis was performed with SPSS ver. 21 (IBM Japan, Tokyo, Japan).

Results

Baseline patient profiles

One hundred and eight patients met the inclusion criteria of this study. Mean age of total participants was 57.9 years and 94.4% were female. Thirty-six patients were positive for ACA (33.3%), and 83 positive for anti-SS-A/Ro antibody (76.9%). Sixty-seven patients were SS-A single positive, twenty ACA single positive, sixteen ACA/SS-A double positive, and five ACA/SS-A double negative. Using the 2016 American College of Rheumatology/European League Against Rheumatism classification criteria, 88.9% of the patients in this study were classified as having primary SS.

ACA/SS-a double positive SS vs. SS-a single positive SS

Clinical characteristics of each group were listed in Table 1. ACA/SS-A double positive SS showed older age at diagnosis and at onset than SS-A single positive SS. ACA/SS-A double positive SS also tended to have lower saliva production as assessed by Saxon test and lower tear production assessed by Schirmer's test and higher focus score in the salivary gland biopsy than SS-A single positive SS. Serologically ACA/SS-A double positive SS showed significantly higher antinuclear antibody (ANA) than SS-A single positive SS (Table 1).

With regard to the disease activity of SS, the data were similar in these two groups (Table 2). Typical signs such as lymphadenopathy, salivary gland and lacrimal gland

Table 1. Demographics, clinical, pathological, and laboratory data of the cohort.

	ACA + SSA+ $n = 16$	ACA-SSA+ $n = 67$	ACA + SSA− $n = 20$
Age at diagnosis of SS, mean years ± SD	71.1 ± 11.2*	53.1 ± 16.2	61.2 ± 12.3
Female, %	100	92.5	95.0
Disease duration from onset of first symptom, mean years ± SD	9.7 ± 11.8	4.5 ± 7.3	5.8 ± 7.0
Age at onset of SS, mean years ± SD	61.4 ± 15.4*	48.7 ± 15.4	55.0 ± 11.8
Follow up period, mean months ± SD	52.1 ± 52.5	40.4 ± 42.6	40.5 ± 50.2
Subjective xerophthalmia, *n* (%)	9/16 (56.3)	31/67 (46.2)	11/20 (55.5)
Subjective xerostomia, *n* (%)	14/16 (87.5)	51/67 (76.1)	19/20 (95.0)
Chief complaint of sicca symptoms at diagnosis, *n* (%)	11/16 (68.8)	25/67 (37.3)‡	14/20 (70.0)
Schirmer's test (mm), mean ± SD	6.3 ± 3.8 ($n = 12$)	8.7 ± 7.1 ($n = 44$)	5.9 ± 7.2 ($n = 12$)
Positive Schirmer's test, *n* (%)	8/12 (66.7)	23/44 (52.3)	10/12 (81.9)
Positive ocular staining, *n* (%)	9/13 (69.2)	24/35 (68.6)	11/14 (78.6)
Saxon's test (g/2min), mean ± SD	0.45 ± 0.51 ($n = 12$)	1.31 ± 1.41 ($n = 35$)	0.67 ± 0.63 ($n = 14$)
Focus score, mean ± SD	4.29 ± 3.74	2.47 ± 2.27	2.70 ± 1.76
Germinal center-like structure, *n* (%)	4/16 (25.0)	16/67 (23.9)	5/20 (25.0)
aSS-A/Ro(60kDa), *n* (%)	16/16 (100)	67/67 (100)	0/19 (0)
aSS-B/La, *n* (%)	5/14 (35.7)†	22/64 (34.4)‡	0/19 (0)
RF, *n* (%)	6/13 (46.2)	26/57 (45.6)‡	1/16 (6.3)
ANA ≧320, *n* (%)	12/16 (75.0)*	25/66 (37.9)‡	19/20 (95.0)
Both positive RF and ANA≧320, *n* (%)	6/13 (46.2)	17/55 (30.9)	1/16 (6.3)
WBC (per mm^3), mean ± SD	4689 ± 1482	4544 ± 1431‡	5908 ± 1358
Lymphocytes (per mm^3), mean ± SD	1373 ± 448	1489 ± 516‡	1776 ± 549
Serum IgG (mg/dL), mean ± SD	2009 ± 755†	2281 ± 1234‡	1389 ± 282
CH50 (U/mL), mean ± SD	47.5 ± 10.0	47.6 ± 11.1	52.8 ± 9.5
C3 (mg/dL), mean ± SD	102.9 ± 13.8	99.2 ± 16.8	111.3 ± 21.1
C4 (mg/dL), mean ± SD	24.1 ± 8.4	20.4 ± 7.5	24.2 ± 6.3

ACA: anti-centromere antibody; SSA: anti-SSA antibody; SS: Sjögren's syndrome; RF: rheumatoid factor; ANA: anti-nuclear antibody.
*$p < .017$ in ACA + SSA + vs. ACA-SSA+.
†$p < .017$ in ACA + SSA + vs. ACA + SSA−.
‡$p < .017$ in ACA-SSA + vs ACA + SSA− by Mann–Whitney's *U* test or Chi square test.

Table 2. ESSDAI score, organ involvement, treatment of the cohort.

	ACA + SSA+ $n = 16$	ACA-SSA+ $n = 67$	ACA + SSA− $n = 20$
ESSDAI at baseline, mean ± SD	3.81 ± 4.68†	4.69 ± 5.27‡	0.50 ± 0.89
Highest ESSDAI during follow-up, mean ± SD	3.88 ± 4.70†	5.13 ± 5.44‡	0.65 ± 0.99
Any organ involvements, *n* (%)	13/16 (81.3)†	56/67 (83.6)‡	6/20 (30.0)
Increase of ESSDAI score during the follow-up, *n* (%)	1/16 (6.3)	9/67 (13.4)	2/20 (10.0)
Constitutional, *n* (%)	2/16 (12.5)	5/67 (7.5)	0/20 (0)
Lymphadenopathy, *n* (%)	0/16 (0)	4/67 (6.0)	0/20 (0)
Glandular, *n* (%)	0/16 (0)	3/67 (4.5)	0/20 (0)
Articular, *n* (%)	0/16 (0)	12/67 (17.9)	1/20 (5.0)
Cutaneous, *n* (%)	0/16 (0)	3/67 (4.5)	0/20 (0)
Pulmonary, *n* (%)	4/16 (25.0)	3/67 (4.5)	0/20 (0)
Renal, *n* (%)	2/16 (12.5)	3/67 (4.5)	0/20 (0)
Muscular, *n* (%)	0/16 (0)	0/67 (0)	0/20 (0)
Peripheral nervous system, *n* (%)	0/16 (0)	4/67 (6.0)	0/20 (0)
Central nervous system, *n* (%)	0/16 (0)	1/67 (1.5)	0/20 (0)
Hematological, *n* (%)	2/16 (12.5)	20/67 (29.9)	2/20 (10.0)
Biological, *n* (%)	11/16 (68.8)†	50/67 (74.6)‡	5/20 (25.0)
Pulmonary hypertension, *n* (%)	1/16 (6.3)	0/67 (0)	0/20 (0)
Autoimmune hepatitis, *n* (%)	1/16 (6.3)	2/67 (3.0)	0/20 (0)
Primary biliary cholangitis, *n* (%)	3/16 (18.8)	2/67 (3.0)	4/20 (20.0)
Chronic thyroiditis, *n* (%)	2/16 (12.5)	3/67 (4.5)	1/20 (5.0)
Malignant lymphoma, *n* (%)	0/16 (0)	3/67 (4.5)	0/20 (0)
Treatment			
Steroid, *n* (%)	2/16 (12.5)	10/67 (14.9)	0/20 (0)
Saliva stimulant, *n* (%)	4/16 (25.0)	20/67 (29.9)	10/20 (50.0)
Ophthalmic drug, *n* (%)	6/16 (37.5)	18/67 (26.9)	4/20 (20.0)

ESSDAI: EULAR Sjögren's syndrome disease activity index.
*$p < .017$ in ACA + SSA + vs. ACA-SSA+.
†$p < .017$ in ACA + SSA + vs. ACA + SSA−.
‡$p < .017$ in ACA-SSA + vs. ACA + SSA− by Mann–Whitney's *U* test or Chi square test.

Table 3. SSc-related data of the cohort.

	ACA + SSA+ $n = 16$	ACA-SSA+ $n = 67$	ACA + SSA− $n = 20$
Raynaud's phenomenon, *n* (%)	9/16 (56.3)*	8/58 (13.8)	5/20 (25.0)
Puffy fingers, *n* (%)	1/16 (6.3)	ND	3/20 (15.0)
Sclerodactyly, *n* (%)	0/16 (0)	ND	0/20 (0)
Fingertip ulcers, *n* (%)	0/16 (0)	ND	0/20 (0)
Telangiectasia, *n* (%)	3/16 (18.8)	ND	4/20 (20.0)
Abnormal nailfold capillaries, *n* (%)	7/16 (43.8)	ND	6/20 (30.0)
Raynaud's phenomenon and abnormal nailfold capillaries, *n* (%)	2/16 (12.5)	ND	0/20 (0)
Raynaud's phenomenon or abnormal nailfold capillaries, *n* (%)	11/16 (68.8)	ND	8/20 (40.0)
ACR/EULAR SSc criteria score, mean ± SD	6.31 ± 2.21†	ND	4.65 ± 2.18
ACR/EULAR SSc criteria score ≧9, *n* (%)	2/16 (12.5)	ND	1/20 (5.0)

*$p < .017$ in ACA + SSA + vs. ACA-SSA+.
†$p < .05$ in ACA + SSA + vs. ACA + SSA− by Mann–Whitney's *U* test or Chi square test.

swelling, and malignant lymphoma were observed only in SS-A single positive SS (Table 2).

In systemic sclerosis-related data, ACA/SS-A double positive SS showed a higher frequency of Raynaud's phenomenon than SS-A single positive SS (Table 3). Pulmonary involvement was observed more often in ACA/SS-A double positive SS, and was interstitial pneumonia in all cases. One patient with ACA/SS-A double positive SS showed pulmonary hypertension and did not meet 2013 ACR/EULAR systemic sclerosis classification criteria, while none of SS-A single positive SS showed pulmonary hypertension.

ACA/SS-a double positive SS vs ACA single positive SS

Serologically ACA/SS-A double positive SS showed higher serum IgG than ACA single positive SS. Notably, only 6.3% of the patients in ACA single positive group met ACR criteria that required both positive RF and ANA ≧ 1:320 (Table 1).

With regard to the disease activity of SS, ACA/SS-A double positive SS showed higher ESSDAI at diagnosis and maximum ESSDAI score during the follow-up than ACA single positive SS (Table 2). Higher ESSDAI was also noted in SSA single positive SS. These differences were caused especially by biological domain and ACA single positive SS showed no organ involvement requiring steroid or other immunosuppressive drugs.

Although the frequencies of each domain in 2013 ACR/EULAR systemic sclerosis criteria were similar between ACA/SS-A double positive SS and ACA single positive SS, total score was higher in ACA/SS-A double positive SS (Table 3). In 36 ACA positive SS, only three patients (8.3%) met the systemic sclerosis criteria of 2013. No patients newly developed skin sclerosis and/or Raynaud's phenomenon during a maximum of 178 months and a mean 45.6 months follow-up.

Multivariate analysis

Multivariate logistic regression analysis showed disease duration and age at onset to be associated with lower salivary

Table 4. Multivariate logistic regression analysis for factors related to salivary flow rate and lacrimal secretion.

Predictor valuables	OR	95%CI	*p*
Predictor valuables for more than 1.0 g per 2 min for Saxon's test			
Age at onset	0.954	0.913–0.996	.034
Disease duration	0.836	0.745–0.939	.002
Predictor valuables for more than 5 mm per 5 min on Schirmer's test			
Anti-SS-A/Ro antibody	3.981	1.003–15.806	.050
Age at diagnosis	0.966	0.935–0.998	.040

Table 5. Serum cytokine data of the cohort.

	ACA + SSA+ $n = 5$	ACA-SSA+ $n = 15$	ACA + SSA− $n = 9$
IFN-γ (<1.6 pg/ml)	51.4 ± 111.3	67.4 ± 93.2	6.3 ± 10.9
IL-1β (<4.2 pg/ml)	39.4 ± 78.6	43.5 ± 46.6	5.0 ± 2.3
IL-2 (<16.4 pg/ml)	88.1 ± 105.3	71.0 ± 60.2	69.9 ± 72.0
IL-4 (<20.8 pg/ml)	84.6 ± 52.3	86.0 ± 78.7	40.6 ± 24.7
IL-5 (<1.6 pg/ml)	57.4 ± 90.0	31.3 ± 90.4	44.0 ± 64.5
IL-6 (<1.2 pg/ml)	2.1 ± 2.1	3.4 ± 5.0	29.6 ± 85.2
IL-10 (<1.9 pg/ml)	24.0 ± 31.3	16.5 ± 26.3	14.1 ± 28.5
IL-12 p70 (<1.5 pg/ml)	1.5 ± 12.4	1.5 ± 3.2	1.5 ± 0
IL-13 (<4.5 pg/ml)	45.7 ± 71.3	44.4 ± 46.9	55.0 ± 62.5
IL-17A (<2.5 pg/ml)	82.8 ± 110.6	28.4 ± 48.2	103.4 ± 122.0
TNF-α (<3.2 pg/ml)	22.2 ± 42.5	46.6 ± 62.0	33.2 ± 48.2

flow rate, defined as 1.0 g or less per 2 min for Saxon's test. Also, age at diagnosis and absence of anti-SS-A/Ro antibody were associated with lower lacrimal secretion, defined as 5 mm or less per 5 min on Schirmer's test (Table 4). ACA was not associated with either parameter.

Serum cytokines

To explore factors underlying the differences noted in clinical characteristics, we examined representative Th1, Th2, and Th17 serum cytokines of 5 ACA/SS-A double positive SS, 15 SS-A single positive SS, and 9 ACA single positive SS. Although there was no significant difference, serum levels of IFN-γ, IL-1β, and IL-4 were high in SS-A positive SS. IL-6 was high in SS-A negative SS and IL-17A was high in the ACA positive group (Table 5). In the comparison of ACA positive SS and ACA negative SS, ACA positive SS showed significantly lower levels of IL-1 β (<4.2 pg/ml) [17.2 ± 46.9 vs. 43.5 ± 46.6 ($p = .035$)].

Discussion

This is the first study to focus on the clinical features of ACA/SS-A double positive SS. Although it was conducted in a single center, 16 ACA/SS-A double positive SS patients and 20 ACA single positive SS patients were available for analysis, representing a relatively large number in comparison with previous analyses of ACA positive SS. The results showed that ACA/SS-A double positive SS was characterized by high age at diagnosis and onset, and higher ESSDAI than that of ACA single positive SS.

Higher ESSDAI was observed not only in ACA/SS-A double-positive SS but also in SS-A single-positive SS. These results were attributed to increased organ involvement and higher scores in the biological domain of the ESSDAI than those of ACA single positive SS. The presence of anti-SS-A/Ro antibody in SS is related to activation of humoral immunity [18], which might explain higher ESSDAI in ACA/SS-A double-positive SS and SS-A single-positive SS [25].

ACA has been reported as a sicca-associated antibody, and in support of this contention Baer et al reported that ACA-positive SS showed more severe salivary and lacrimal gland dysfunction than ACA-negative SS [16]. ACA-positive SS in this study also showed a tendency to lower Saxon's test and lower Schirmer's test scores, while more patients with ACA complained of sicca symptoms than those without ACA at presentation, although these differences were not statistically significant (Table 1). In addition, there were no differences in subjective dry eye or dry mouth between ACA/SSA double positive SS and ACA single positive SS. Therefore, severe sicca symptoms may be characteristic of ACA-positive SS, with further investigation needed to confirm this point.

Differences between ACA-positive SS and scleroderma should be discussed carefully. We defined scleroderma by 1980 ARA criteria [19] whereas LeRoy relied on limited scleroderma criteria [20] and excluded skin sclerosis extending proximal to the metacarpophalangeal joints. Only 3 (8.3%) of 36 ACA-positive SS cases in this study satisfied ACR/EULAR systemic sclerosis criteria and no case had sclerodactyly. These results suggest that ACA-positive SS shows mild symptoms of limited scleroderma consistent with the findings of Baldini et al. [13]. There have been numerous reports on sicca symptoms in limited scleroderma accompanied with labial salivary gland fibrosis [21,26]. Meanwhile, lymphocyte infiltration in labial glands is a characteristic finding of primary SS [21,26]. We excluded patients with a focus score of 1 or less and severe fibrosis on lip biopsy. In this regard, we recruited 'pathologically diagnosed' SS. Reliance on these inclusion criteria may lead to a result that no ACA-positive patient developed skin sclerosis during the long term follow-up differing from previously reported ones in which 15 (16.7%) of a total 90 ACA-positive SS patients developed limited scleroderma [5,6,9,10,15,27]. In this study, only 2 patients (5.6%) had both Raynaud's phenomenon and capillary abnormalities. Furthermore, 22 patients (61.1%) did not have Raynaud's phenomenon in ACA-positive SS. Raynaud's phenomenon and capillary abnormalities are reported to be predictive of the development of systemic sclerosis [28], which implies that few of the ACA positive SS cases in our study will develop limited scleroderma. Collectively, ACA-positive SS appears to be a different entity from limited scleroderma.

The frequency of ACA-positive SS in our study was higher than that noted in previous studies (33.3% vs. 3.7 to 24.6%) [5–16,29]. Although the adoption of different inclusion criteria precludes simple comparisons of the results of previous studies with ours regarding the frequency of ACA-positive SS, ACA-positive SS may likely have been underestimated in the past studies.

In this study, we measured serum cytokines in each group. No significant difference was noted between any of the three groups probably due to the small number of patients. However, when divided into two groups,

ACA-positive SS and ACA-negative SS, the former had lower serum IL-1β, which is reported to be related to the fatigue of SS [30]. In this regard, IL-1β may be implicated in the clinicopathological differences between ACA positive and negative patients.

The design of this study, namely, a retrospective observational study conducted in a single center, is a major limitation of this study. In addition, some selection biases were present because cases were selected from the labial salivary gland biopsy cohort in our institute. Nevertheless, this is the first study to describe ACA/SS-A double positive SS including ESSDAI, although further prospective study is necessary to confirm the present results and clinical implications.

Conclusion

In conclusion, we identified several distinct clinical characteristics of ACA/SS-A double positive SS. Affected patients were older than those with SS-A single positive SS, and had higher disease activity of SS according to ESSDAI and higher serum IgG than those with ACA single positive SS. Anti SS-A antibody positivity was related to the features of B cell activation such as elevated serum IgG, and ACA positivity with features of scleroderma such as Raynaud's phenomenon. Nevertheless, a low frequency of capillary abnormalities and absence of sclerodactyly in ACA positive SS were salient characteristics which clearly distinguish the group from scleroderma. The combination of SS-A and ACA define the clinical phenotype of SS, and so the combination of these antibodies should attract attention in clinical practice.

Acknowledgements

We thank John Gelblum for his critical reading of the manuscript.

Conflict of interest

None.

References

1. Fujibayashi T, Sugai S, Miyasaka N, Hayashi Y, Tsubota K. Revised Japanese criteria for Sjögren's syndrome (1999): availability and validity. Mod Rheumatol. 2004;14:425–34.
2. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61:554–8.
3. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's international collaborative clinical alliance cohort. Arthritis Care Res. 2012;64:475–87.
4. Bournia VK, Vlachoyiannopoulos PG. Subgroups of Sjogren syndrome patients according to serological profiles. J Autoimmun. 2012;39:15–26.
5. Ramos-Casals M, Nardi N, Brito-Zeron P, Aguilo S, Gil V, Delgado G, et al. Atypical autoantibodies in patients with primary Sjögren syndrome: clinical characteristics and follow-up of 82 cases . Semin Arthritis Rheum. 2006;35:312–21.
6. Caramaschi P, Biasi D, Carletto A, Manzo T, Randon M, Zeminian S, et al. Sjögren's syndrome with anticentromere antibodies. Rev Rhum Engl Ed. 1997;64:785–8.
7. Katano K, Kawano M, Koni I, Sugai S, Muro Y. Clinical and laboratory features of anticentromere antibody positive primary Sjögren's syndrome. J Rheumatol. 2001;28:2238–44.
8. Hsu TC, Chang CH, Lin MC, Liu ST, Yen TJ, Tsay GJ. Anti-CENP-H antibodies in patients with Sjogren's syndrome. Rheumatol Int. 2006;26:298–303.
9. Salliot C, Gottenberg JE, Bengoufa D, Desmoulins F, Miceli-Richard C, Mariette X. Anticentromere antibodies identify patients with Sjogren's syndrome and autoimmune overlap syndrome. J Rheumatol. 2007;34:2253–8.
10. Bournia VK, Diamanti KD, Vlachoyiannopoulos PG, Moutsopoulos HM. Anticentromere antibody positive Sjogren's Syndrome: a retrospective descriptive analysis. Arthritis Res Ther. 2010;12:R47.
11. Nakamura H, Kawakami A, Hayashi T, Iwamoto N, Okada A, Tamai M, et al. Anti-centromere antibody-seropositive Sjogren's syndrome differs from conventional subgroup in clinical and pathological study. BMC Musculoskelet Disord. 2010;11:140.
12. Kitagawa T, Shibasaki K, Toya S. Clinical significance and diagnostic usefulness of anti-centromere antibody in Sjogren's syndrome. Clin Rheumatol. 2012;31:105–12.
13. Baldini C, Mosca M, Della Rossa A, Pepe P, Notarstefano C, Ferro F, et al. Overlap of ACA-positive systemic sclerosis and Sjogren's syndrome: a distinct clinical entity with mild organ involvement but at high risk of lymphoma. Clin Exp Rheumatol. 2013;31:272–80.
14. Fujimura T, Fujimoto T, Hara R, Shimmyo N, Kobata Y, Kido A, et al. Subclinical articular involvement in primary Sjogren's syndrome assessed by ultrasonography and its negative association with anti-centromere antibody. Mod Rheumatol. 2015;25:871–5.
15. Lee KE, Kang JH, Lee JW, Wen L, Park DJ, Kim TJ, et al. Anti-centromere antibody-positive Sjogren's syndrome: A distinct clinical subgroup?. Int J Rheum Dis. 2015;18:776–82.
16. Baer AN, Medrano L, McAdams-DeMarco M, Gniadek TJ. Anti-centromere antibodies are associated with more severe exocrine glandular dysfunction in Sjogren's syndrome: analysis of the Sjogren's international collaborative clinical alliance cohort. Arthritis Care Res (Hoboken). 2016;68:1554–9.
17. Tanaka N, Muro Y, Suzuki Y, Nishiyama S, Takada K, Sekiguchi M, et al. Anticentromere antibody-positive primary Sjogren's syndrome: epitope analysis of a subset of anticentromere antibody-positive patients. Mod Rheumatol. 2017;27:115–21.
18. Quartuccio L, Baldini C, Bartoloni E, Priori R, Carubbi F, Corazza L, et al. Anti-SSA/SSB-negative Sjogren's syndrome shows a lower prevalence of lymphoproliferative manifestations, and a lower risk of lymphoma evolution. Autoimmun Rev. 2015;14:1019–22.
19. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European league against rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. Ann Rheum Dis. 2017;76:9–16.
20. Subcommittee for scleroderma criteria of the American rheumatism association diagnostic and therapeutic criteria committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum. 1980;23:581–90.
21. LeRoy EC, Medsger TA. Jr., Criteria for the classification of early systemic sclerosis. J Rheumatol. 2001;28:1573–6.
22. Avouac J, Sordet C, Depinay C, Ardizonne M, Vacher-Lavenu MC, Sibilia J, et al. Systemic sclerosis-associated Sjögren's syndrome and relationship to the limited cutaneous subtype: results of a prospective study of sicca syndrome in 133 consecutive patients. Arthritis Rheum. 2006;54:2243–9.

23. Seror R, Bowman SJ, Brito-Zeron P, Theander E, Bootsma H, Tzioufas A, et al. EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. RMD Open 2015;1:e000022.
24. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum. 2013;65: 2737–47.
25. Takada K, Suzuki K, Okada M, Nakashima M, Ohsuzu F. Salivary production rates fall with age in subjects having anti-centromere, anti-Ro, and/or anti-La antibodies. Scand J Rheumatol. 2006;35:23–8.
26. Osial TA, Jr., Whiteside TL, Buckingham RB, Singh G, Barnes EL, Pierce JM, et al. Clinical and serologic study of Sjogren's syndrome in patients with progressive systemic sclerosis. Arthritis Rheum. 1983;26:500–8.
27. Miyawaki S, Asanuma H, Nishiyama S, Yoshinaga Y. Clinical and serological heterogeneity in patients with anticentromere antibodies. J Rheumatol. 2005;32:1488–94.
28. Koenig M, Joyal F, Fritzler MJ, Roussin A, Abrahamowicz M, Boire G, et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. Arthritis Rheum. 2008;58:3902–12.
29. Collins K, Mitchell S, Griffiths B, Bowman SJ, Ng WF. United Kingdom Primary Sjogren's Syndrome Registry. Potential diagnostic utility of anti-centromere antibody in primary Sjogren's syndrome in the UK. Clin Rheumatol. 2012;31:1147–8.
30. Norheim KB, Harboe E, Gøransson LG, Omdal R. Interleukin-1 inhibition and fatigue in primary Sjögren's syndrome–a double blind, randomised clinical trial. PLoS One. 2012;7:e30123.