Anticentromere antibody positive patients with primary Sjögren's syndrome have distinctive clinical and immunological characteristics

Y. Liu[1,2], L. Guo[3], W. Lin[2], X. Ning[2], M. Liu[2], J. Cao[2], Y. Su[2], X. Zheng[2], S. Li[2], F. Li[2], L. Ren[4], G. Song[1,4]

[1]Department of Graduate School, Hebei Medical University, Shijiazhuang; [2]Department of Rheumatology and Immunology, [3]Department of Urology, and [4]Department of Endocrinology, Hebei General Hospital, Shijiazhuang, China.

Abstract

Objective

To investigate the clinical manifestations, immunological characteristics, circulating lymphocyte subsets and risk factors of anticentromere antibody (ACA) positive patients with primary Sjögren's syndrome (pSS).

Methods

Data of 333 patients with newly diagnosed pSS were collected and analysed retrospectively. The demographic features, glandular dysfunction, extraglandular manifestations, laboratory data, peripheral blood lymphocyte profiles and serum cytokines were compared between ACA-positive and ACA-negative pSS patients. Logistic regression analysis was used to evaluate the association between ACA and pSS characteristics.

Results

The prevalence of ACA among pSS patients was 13.5%. ACA-positive pSS patients were older at diagnosis and had longer disease duration. Xerostomia, xerophthalmia, parotid enlargement, Raynaud's phenomenon (RP), lung and digestive system involvement were more common in ACA-positive group, whereas haematological involvement such as leukopenia was more common in the ACA-negative group. Less frequency of rheumatoid factor, hypergammaglobulinaemia, anti-SSA and anti-SSB positivity, as well as higher positivity rate of ANA were observed in ACA-positive pSS patients, who exhibited a lower ESSDAI. In addition, decreased B cells and elevated NK cells were found in ACA-positive patients. Multivariate analysis identified that disease duration longer than 5 years, parotid enlargement, normal immunoglobulin and the absence of anti-SSA antibody were risk factors of ACA-positive pSS.

Conclusion

ACA positive pSS patients have distinctive clinical manifestations and less severe immunological features, present a lower disease activity and lower activation of the humoral immune system. Physicians should pay attention to RP, lung and liver involvement in this subset of pSS.

Key words

primary Sjögren's syndrome, anticentromere antibody, lymphocyte subsets, ESSDAI, B cells

Yixuan Liu, MM
Liuxiong Guo, MM
Wei Lin, MD
Xiaoran Ning, MM
Meilu Liu, MM
Jingjing Cao, MM
Yashuang Su, MM
Xiao Zheng, MM
Shuo Li, MM
Fang Li, MM
Luping Ren, MD
Guangyao Song, MD

Please address correspondence to:
Guangyao Song
Department of Endocrinology,
Hebei General Hospital,
no. 348 Heping West Road,
Shijiazhuang, Hebei 050051, China.
E-mail: sguangyao2@163.com

Received on November 24, 2022; accepted in revised form on March 6, 2023.

Competing interests: none declared.

Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease characterised by lymphocytic infiltration into the exocrine glands such as salivary and lachrymal glands, resulting in sicca symptoms and various extra glandular manifestations (1, 2). As a prototypical systemic autoimmune disease, many evidences suggest B cells participate in the pathogenesis of pSS (3, 4). The involvement of B cells in the pathogenesis of pSS has been suspected as the basis of clinical features that include the presence of serum autoantibodies, hypergammaglobulinaemia, increased level of rheumatoid factor (RF) and increased risk of B cell lymphoma (5, 6). A variety of autoantibodies have been detected in the serum of patients with pSS. Among them, anti-SSA and anti-SSB antibodies are the most common; they are considered as disease markers and included in the classification criteria of pSS (7, 8). However, it is estimated that only 50~70% pSS patients fulfilling the 2016 criteria present anti-SSA positivity (9), implying a considerable number of pSS patients are negative for this traditional antibody. There are other autoantibodies existing in pSS patients, such as anticentromere antibody (ACA), anti-α-fodrin, anti-carbonic anhydrase, anti-cyclic citrullinated peptide antibody and so on (10, 11). ACA is first found in patients with limited cutaneous sclerosis and its prevalence in pSS ranges from 4 to 27% (12, 13). Research showed ACA not only correlated with significant clinical phenotypes in pSS, but also associated with other autoimmune disease such as systemic sclerosis (SSc) (10, 12). It was reported that ACA-positive pSS patients had similar impaired salivary and lacrimal secretion, but different clinical and laboratory features compared to those ACA-negative individuals, such as older age at diagnosis, more common in Raynaud's phenomenon (RP) and liver involvement, lower prevalence of anti-SSA, anti-SSB antibodies and RF, lower frequency of leukopenia and hypergammaglobulinaemia (13-16). These studies suggested that ACA-positive patients were a subset of pSS with specific feature, course and prognosis. But some results related to patients with ACA positive are controversial. Moreover, data on this issue in China is limited.

Therefore, we aimed to clarify the clinical and immunological characteristics of pSS patients presenting with ACA positivity. For this purpose, the demographic features, clinical manifestations, laboratory findings were compared the between ACA-positive and ACA-negative group. We tried to seek evidence of immune disturbance, so the lymphocyte subsets and serum cytokines in the peripheral blood were performed.

Materials and methods

Patients

A retrospective analysis was performed on 333 newly diagnosed patients with pSS that were hospitalised in Hebei General Hospital from September 2016 to March 2019. All candidates were fulfilling the classification criteria of 2002 American-European Consensus Group (AECG) (7). Patients combined with other systemic autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), SSc and so on were excluded from inclusion of the cohort. The additional exclusion criteria were chronic hepatitis C, human immunodeficiency virus infections, previous lymphoproliferative disease, sarcoidosis, graft-versus-host disease, amyloidosis and immunoglobulin G (IgG) 4-related disease. pSS patients were classified into two groups according to the presence or absence of ACA, named as ACA-positive and ACA-negative group respectively. There were 45 subjects were positive for ACA, while the remaining 288 were not. Informed consent was obtained from all participants before enrolment. This study was approved by the Ethics Board of Hebei General Hospital (approval ID: no. 2016070).

Clinical and laboratory assessments

We obtained the clinical and laboratory variables at the time of cohort enrolment in this study. Demographic data such as gender, age at diagnosis, age at onset of pSS and disease duration were collected. Clinical features were rigor-

ously assessed by experienced rheumatologist, including sicca symptoms, rampant caries, salivary gland enlargement, RP and extra glandular manifestations of pSS. The medications used for patients were also recorded.

The definition of each type of system involvement was based on 2010 EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) (17): 1) Articular involvement was defined as arthralgias accompanied by morning stiffness over 30 minutes or synovitis in joints; 2) Mucocutaneous involvement was indicated as cutaneous vasculitis including urticarial vasculitis, diffuse purpura, ulcers related to vasculitis and so on; 3) Haematological involvement was defined as the appearance of neutropenia, lymphopenia, anaemia and/or thrombocytopenia; 4) Lung involvement included the presence of interstitial lung disease (ILD) shown by high-resolution computed tomography (HRCT) with shortness of breath or abnormal lung function tests; 5) Renal involvement was indicated by the presence of renal tubular acidosis with or without renal failure, glomerular involvement combined with proteinuria over 0.5 g/day; 6) Digestive involvement was including the appearance of symptoms and signs associated with impaired digestive function, autoimmune hepatitis (AIH), autoimmune cholangitis or primary biliary cholangitis (PBC); 7) Nervous system involvement included peripheral nervous system (PNS) involvement and central nervous system (CNS) involvement. The former was including pure sensory axonal polyneuropathy shown by nerve conduction studies (NCS) or trigeminal (V) neuralgia and so on. The latter was including cerebral vasculitis with cerebrovascular accident or transient ischaemic attack, seizures, transverse myelitis, lymphocytic meningitis and so on; 8) Lymphatic system involvement was defined as abnormal enlargement of the lymph nodes and/or splenomegaly, with the exclusion of infection. Disease activity was evaluated by the ESSDAI (18). Lymphoma was confirmed histologically by a lymph node biopsy.

The laboratory parameters were recorded by inquiring the medical records, including routine blood test, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), antinuclear antibody (ANA), anti-SSA antibody, anti-SSB antibody, anti-Ro-52, ACA, anti-mitochondrial antibody type 2 (AMA-M2), anti-RNP antibody, immunoglobulins (IgG, IgM, IgA), complement (C3 and C4) and rheumatoid factor (RF). The results of Schirmer's test (less than 5 mm/5 min defined as positive), ocular staining, and tear break-up time (BUT, less than 10s defined as positive) were obtained at first visit. The pathological grade of labial salivary gland biopsy was evaluated. The focus score ≥1 evaluated by histopathologist was defined as positivity for minor salivary gland biopsy.

Table I. Demographic characteristics and clinical manifestations of ACA-positive and ACA-negative pSS patients.

	ACA-positive	ACA-negative	*p*-value
Demographic characteristics			
Female (%)	95.56	92.71	0.701
Age at diagnosis (years)	58.8 ± 12.1	53.2 ± 13.5	0.009
Age at onset of pSS (years)	49.9 ± 13.1	47.2 ± 13.3	0.201
Disease duration (months)	120 [42-120]	36 [12-120]	<0.001
Clinical manifestations (n, %)			
Dry mouth	45/45, (100%)	245/288, (85.07%)	0.005
Dry eye	42/45, (93.33%)	204/288, (70.83%)	0.001
Rampant caries	15/45, (33.33%)	109/288, (37.85%)	0.56
Salivary gland enlargement	10/45, (22.22%)	28/288, (9.72%)	0.014
Raynaud's phenomenon	10/45, (22.22%)	28/288, (9.72%)	0.014
Systemic involvements (n, %)			
Articular involvement	18/45, (40%)	128/288, (44.44%)	0.576
Mucocutaneous involvement	15/45, (33.33%)	70/288, (24.31%)	0.196
Haematological involvement	14/45, (31.11%)	171/288, (59.38%)	<0.001
Leukopenia	3/45, (6.67%)	61/288, (21.18%)	0.022
Lymphopenia	11/45, (24.44%)	77/288, (26.74%)	0.746
Thrombocytopenia	1/45, (2.22%)	19/288, (7.06%)	0.417
Lung involvement	15/45, (33.33%)	56/288, (19.44%)	0.034
Renal involvement	2/45, (4.44%)	20/288, (6.94%)	0.76
Digestive involvement	6/45, (13.33%)	11/288, (3.82%)	0.02
Nervous system involvement	7/45, (15.56%)	34/288, (11.81%)	0.476
Lymphatic system involvement	3/45, (6.67%)	30/288, (10.42%)	0.607
Lymphoma (n, %)	0/45, (0%)	1/288, (0.35%)	1.000
ESSDAI	5 [1-11]	8 [4-13]	0.006

Values are presented as n, number (%), mean (S.D.), or median (interquartile range).
ACA: anticentromere antibody; pSS: primary Sjögren's syndrome.

Immunophenotyping of lymphocyte subsets

Flow cytometric immunophenotyping was performed to determine the percentage and absolute number of the mature human lymphocyte subsets in peripheral whole blood including T lymphocytes ($CD3^+$), helper/inducer T lymphocytes ($CD3^+CD4^+$), suppressor/cytotoxic T lymphocytes ($CD3^+CD8^+$), B lymphocytes ($CD19^+$) and natural killer (NK) lymphocytes ($CD16^+CD56^+$). Cells were incubated with fluorochrome-labelled antibodies against leucocyte surface antigens. All the antibody conjugates were procured from Becton Dickinson. Flow-cytometric analysis was measured by FACS Canto flow cytometer (Becton Dickinson, San Jose, CA, USA) using FlowJo Software (Treestar, Ashland, OR, USA).

According to the results of measurement, absolute numbers of lymphocyte subsets were divided into below normal range, within normal range and above normal range; defined as low, normal and high respectively.

Serum cytokines

Sera derived from patients were collected and examined at once. Serum cytokines including interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 10 (IL-10), tumour

necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin 17A (IL-17A) were detected by flow cytometry (Becton Dickinson FACS Canto, San Jose, CA, USA) according to the manufacturer's instructions. Cytokine detection reagents were provided by Jiangxi Cellgene Biotechnology Limited Company.

Statistical analysis

SPSS 26.0 (IBM, Armonk, NY, USA) was used for statistical analysis. The normally distributed continuous data were presented as means ($_{S.D.}$) and analysed by Student's *t*-test. Whereas the non-normally distributed measurement data were presented as medians and interquartile ranges (IQR), Mann-Whitney U was used for comparisons. Categorical data were summarised as frequencies and percentages, analysed by chi-squared test or Fisher's exact test as appropriate. Multivariate logistic regression analysis was performed to calculate OR value and 95% confidence interval (95% CI) to identify risk factors associated with ACA in pSS patients. The correlations between variables were evaluated with Spearman rank correlation coefficient, with an "r" representing linear correlation. $p<0.05$ was considered statistically significant.

Results

Demographic characteristics

As described in the methods, 45 of the 333 patients in our study were ACA-positive, with a positivity rate of 13.5%. The ACA-positive pSS patients were older at diagnosis (58.8±12.1 years vs. 53.2±13.5 years, $p<0.05$) and had apparently longer disease duration (median, 120 months (IQR, 42-120) *vs*. median, 36 months (IQR, 12-120), $p<0.001$) compared with those in ACA-negative group. The predominance of female was observed in both groups (95.56% and 92.71%, respectively). However, there were no differences in age at onset and female percentage between the two groups (Table I).

Clinical manifestations

Compared with ACA-negative group, the ACA-positive group was more common in dry mouth (100% *vs*. 85.07%, $p<0.01$), dry eye (93.33% *vs*. 70.83%, $p=0.001$), parotid enlargement (22.22% *vs*. 9.72%, $p<0.05$) and RP (22.22% *vs*. 9.72%, $p<0.05$). While the rampant caries did not differ between the ACA-positive and ACA-negative patients (Table I). Although the subjective sicca symptoms were more frequent in ACA-positive patients, the objective tests related to ocular dryness such as Schirmer's test, tear BUT, ocular staining showed no differences between the two groups (Table II).

Systemic involvements of each group were listed in Table I. Patients in ACA-positive group presented less frequency of haematological involvement (31.11% *vs*. 59.38%, $p<0.001$), especially a lower prevalence of leucopoenia (6.67% *vs*. 21.18%, $p<0.05$). The frequent of lung (33.33% *vs*. 19.44%, $p<0.05$) and digestive system involvement (13.33% *vs*. 3.82%, $p<0.05$) were significantly higher in ACA-positive patients than the ACA-negative ones. Among the six patients with liver involvement in the ACA-positive group, four had PBC, two had AIH. No significant differences were observed in arthritis, mucocutaneous, renal, nervous involvement, lymphatic system involvement and lymphoma between the groups (Table I).

Table II. Laboratory data of ACA-positive and ACA-negative pSS patients.

	ACA-positive	ACA-negative	*p*-value
White blood cell ($\times10^9$/L)	5.02 [4.21-6.46]	4.91 [3.85-6.24]	0.44
Neutrophil ($\times10^9$/L)	3.08 [2.16-4.48]	2.93 [2.18-4.24]	0.681
Lymphocyte ($\times10^9$/L)	1.59 [1.15-1.89]	1.49 [1.09-1.86]	0.618
Haemoglobin (g/L)	127.00 [115.50-139.50]	120.50 [108.25-131.00]	0.016
Platelet ($\times10^9$/L)	219.00 [176.00-249.50]	224.50 [178.00-274.75]	0.378
ESR (mm/h)	12.50 [7.00-25.25]	21.00 [10.00-36.50]	0.003
CRP (mg/L)	2.41 [0.96-3.3]	3.30 [1.13-4.97]	0.089
RF (IU/L)	10.60 [10.60-13.0]	25.65 [10.60-86.63]	<0.001
RF (+) (n, %)[a]	5/43, 11.63%	145/268, 54.10%	<0.001
IgG (g/L)	13.34 [10.90-15.05]	16.00 [12.80-20.73]	<0.001
Hyper-IgG (n, %)	5/45, 11.11%	112/282, 39.72%	<0.001
IgA (g/L)	2.31 [1.82-3.01]	2.86 [1.97-3.82]	0.012
IgM (g/L)	1.16 [0.79-1.39]	1.14 [0.81-1.61]	0.662
C3 (g/L)	1.07 [0.92-1.22]	1.05 [0.91-1.20]	0.529
C4 (g/L)	0.20 [0.14-0.25]	0.19 [0.16-0.24]	0.892
ANA (+) (n, %) [b]	44/45, 97.78%	219/288, 76.04%	0.001
Anti-RNP (+) (n, %)	4/45, 8.89%	35/288, 12.15%	0.527
Anti-Ro52 (+) (n, %)	22/45, 48.89%	178/288, 61.81%	0.100
Anti-SSA (+) (n, %)	9/45, 20%	178/288, 61.81%	<0.001
Anti-SSB(+) (n, %)	3/45, 6.67%	76/288, 26.39%	0.004
AMA-M2 (n, %)	4/45, 8.89%	22/288, 7.64%	1.000
Labial gland biopsy (+), (n, %)	43/44, 97.73%	266/279, 95.34%	0.746
Positive ocular staining (n, %)	20/37, 54.05%	157/253, 62.06%	0.351
Positive Schirmer's test (n, %)	31/37, 83.78%	209/253, 82.61%	0.86
Positive BUT test (n, %)	36/37, 97.30%	239/253, 94.47%	0.742

Values are presented as n, number (%), or median (interquartile range).
ACA: anticentromere antibody; pSS: primary Sjögren's syndrome; RF: reumatoid factor; ANA: antinuclear antibody.
[a] positive RF >20 IU/ml; [b] positive for ANA titres ≥1:320.

Immunological features

There were no significant differences in absolute white blood cell count as well as the subsets between pSS patients with and without ACA. The median haemoglobin concentration of the ACA-positive group was higher than ACA-negative group. While the ESR ($p<0.001$) and IgA ($p<0.05$) level were lower in the ACA-positive group. In addition, lower serum RF ($p<0.001$) and IgG level ($p<0.001$), less frequency of RF positivity (11.63% *vs*. 54.10%, $p<0.01$) and hyperimmunoglobulinaemia (11.11% *vs*. 39.72%, $p<0.001$) were also observed in ACA-positive pSS patients. As to the autoantibodies that evaluated in our study, the presence of ANA (97.78% *vs*. 76.04%, $p=0.001$) was more frequent, whereas the anti-SSA (20% *vs*. 61.81%, $p<0.001$) anti-SSB antibody (6.67% *vs*. 26.39%, $p<0.01$) were less frequent in ACA positive pSS patients. Moreo-

ver, no statistical difference was found in the positivity of labial gland biopsy between the two groups (Table II).

Disease activity and treatment

ACA-positive pSS patients showed a lower ESSDAI score (5(1-11) *vs*. 8(4-13), $p<0.01$) compared with ACA-negative individuals, indicating a lower disease activity in those patients (Table I). Therefore, we speculated the treatment regard to the ACA-positive patients should be more mildly. Then the current medications used for pSS patients were compared between the two groups. As shown in Table III, results indicated although the glucocorticoids and immunosuppressants were less prescribed in patients with ACA, the differences were not statistically significant. In 45 ACA-positivity pSS patients, only 11 individuals received immunosuppressive therapy, 9 were treated with leflunomide, 1 with iguratimod and 1 with cyclophosphamide (Table III). No patient developed skin sclerosis and/or SSc during a mean follow-up of 38.55±12.61 months (range 26–49 months).

Table III. Treatment of ACA-positive and ACA-negative pSS patients.

Treatment (n, %)	ACA-positive	ACA-negative	*p*-value
Prednisone/methylprednisolone	8/45, 17.78%	69/288, 23.96%	0.36
Immunosuppressants	11/45, 24.44%	103/288, 35.76%	0.137
Methotrexate	0/45, 0%	2/288, 0.69%	1
Leflunomide	9/45, 20.00%	73/288, 25.35%	0.439
Iguratimod	1/45, 2.22%	13/288, 4.51%	0.754
Mycophenolate mofetil	0/45, 0%	6/288, 2.08%	1
Cyclosporin A	0/45, 0%	8/288, 2.78%	0.543
Cyclophosphamide	1/45, 2.22%	3/288, 1.04%	0.442
Tacrolimus	0/45, 0%	1/288, 0.35%	1
Hydroxychloroquine	34/45, 75.56%	222/288, 77.08%	0.821

ACA: anticentromere antibody; pSS: primary Sjögren's syndrome.

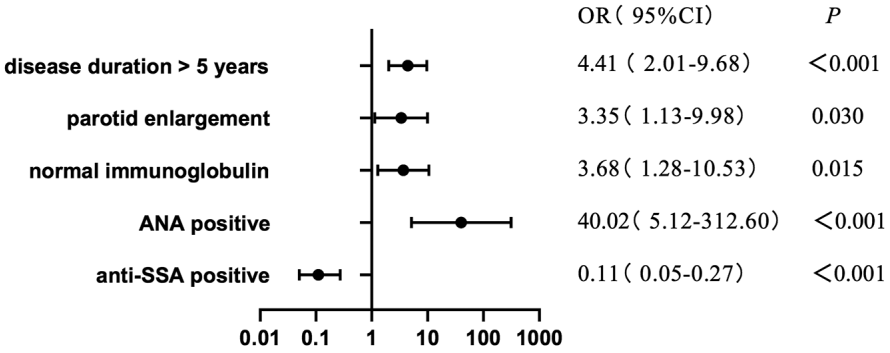

Fig. 1. Risk factors related to ACA in pSS patients.
CI: confidence interval; OR: odds ratio; ANA: antinuclear antibody; ACA: anticentromere antibody; pSS: primary Sjögren's syndrome.

Potential risk factors

Multivariate logistic regression analysis was performed to identify risk factors associated with ACA in pSS patients. Age, disease duration, parotid enlargement, RP, lung and digestive system involvement, ANA, anti-SSA, anti-SSB and variables examined by monofactor analysis found to be significantly different were included in the assessment. Logistic regression analysis illustrated disease duration longer than 5 years, parotid enlargement, normal immunoglobulin, absent of anti-SSA to be positively associated with ACA in pSS patients (Fig.1 and Supplementary Table S1).

Lymphocyte subsets in peripheral blood

There was no statistical difference in circulating lymphocyte between ACA positive and negative group. We further detected the lymphocyte subsets in peripheral blood by flow cytometry to investigate their immunological status. The distributions of T cells, B cells and NK cells in the two groups were listed in Figure 2 and Supplementary Tables S2, S3, S4. We found the absolute number and prevalence of $CD19^+$ B cells were significantly decreased ($p<0.05$), while the absolute number of $CD16^+$ $CD56^+$ NK cells were significantly increased ($p<0.05$) in ACA-positive group. Whereas $CD3^+$ T cells, $CD4^+$ T cells and $CD8^+$ T cells were comparable between the two groups ($p>0.05$). We also found most pSS patients in both groups had normal values in $CD19^+$ B cells (90.91% and 90.14%, respectively). The absolute number of $CD16^+$ $CD56^+$ NK cells were within the normal range in all of the ACA-positive patients. Besides, the ESSDAI inversely correlated with the absolute number of $CD3^+$ T cells, $CD4^+$ T cells, $CD16^+$ $CD56^+$ NK cells and $CD19^+$ B cells (Fig. 2).

Serum cytokines

To explore factors underlying the differences noted in clinical characteristics, we examined serum cytokines in ACA-positive and ACA-negative pSS patients. Results showed IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17A did not differ between the two groups. (Table IV).

Discussion

ACA has been regarded as the specific autoantibody of the CREST variant of scleroderma since first detected (19). However, it also has been found in patients with SSc, Sjögren's syndrome (SS), PBC, SLE and RA. At recent decade, ACA has been suggested as a marker related to pSS due to its consistent detection in the sera of pSS patients. The present study was the first to evaluate the differences between ACA positive and negative patients with pSS in China. Our study uncovered that patient with ACA-positive had distinctive demographic characteristics and circulating lymphocyte profiles; they presented with less severe immunological features and lower disease activity than those with ACA-negative.

In our study, 13.5% of the pSS patients were ACA-positive. The positivity rate of ACA was within the range of literature reports, similar with two recent

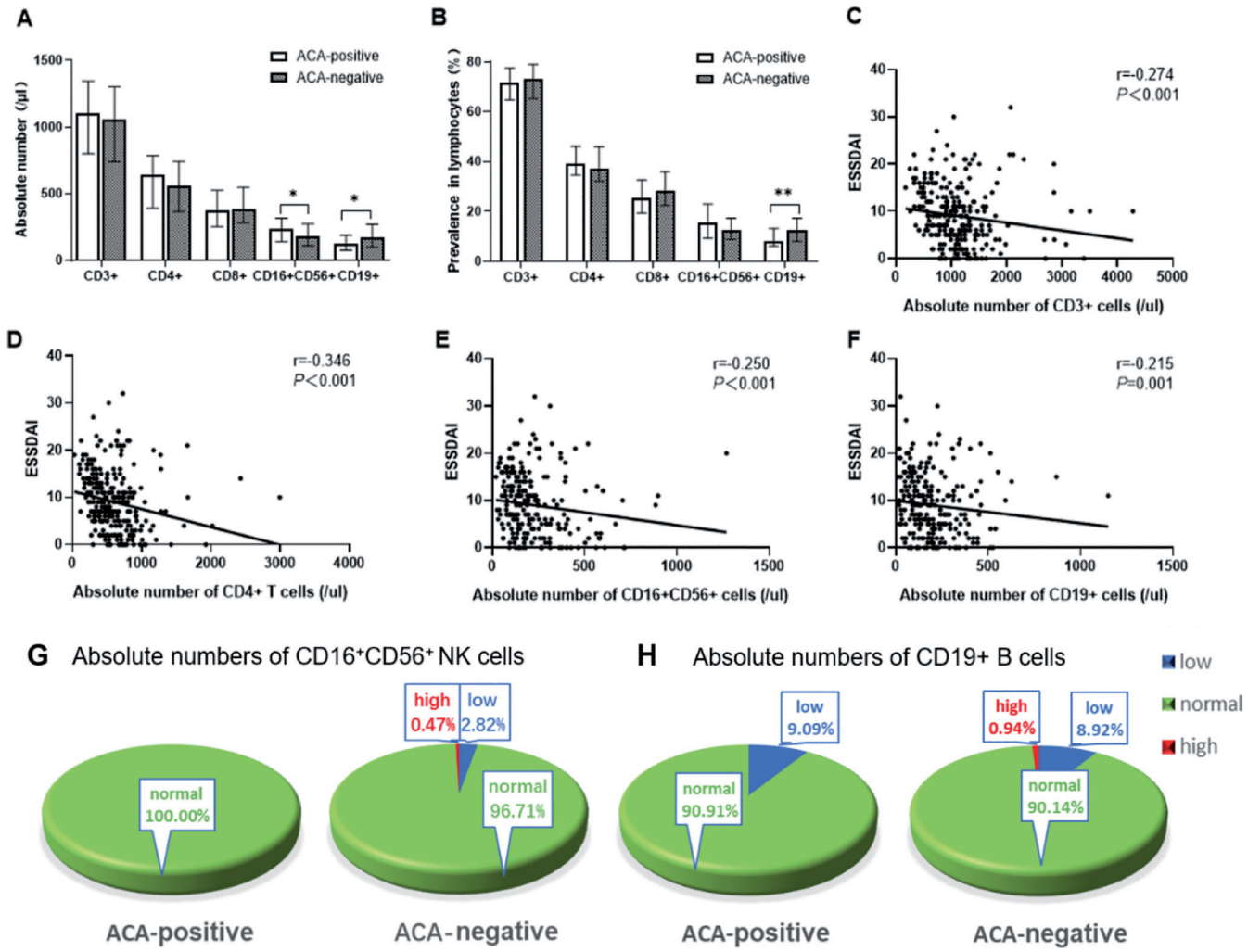

Fig. 2. Immunological status of ACA-positive and ACA-negative pSS patients.
A: Absolute numbers of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD16^+CD56^+$ NK cells and $CD19^+$ B cells in the ACA-positive and ACA-negative pSS patients.
B: Prevalence of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD16^+CD56^+$ NK cells and $CD19^+$ B cells in the lymphocytes of ACA-positive and ACA-negative pSS patients.
C-F: Correlation of the ESSDAI with the absolute numbers of $CD3^+$ T cells, $CD4^+$ T cells, $CD16^+CD56^+$ NK cells and $CD19^+$ B cells in pSS patients.
G-H: The proportions of below, within and above normal range of $CD16^+CD56^+$ NK cells and $CD19^+$ B cells in pSS patients with and without ACA.
*p<0.05, **p<0.01.

Table IV. Serum cytokine of ACA-positive and ACA-negative pSS patients.

	ACA-positive n=12	ACA-negative n=65	p-value
IL-2	0.46 [0.29-0.65]	0.36 [0.33-0.69]	0.608
IL-4	0.60 [0.34-0.96]	0.62 [0.38-0.88]	0.637
IL-6	1.70 [0.99-6.62]	1.93 [0.89-4.76]	0.861
IL-10	0.87 [0.63-1.50]	0.78 [0.58-1.49]	0.694
TNF-α	0.68 [0.47-0.89]	0.78 [0.49-0.99]	0.710
INF-γ	0.62 [0.39-0.73]	0.67 [0.34-0.93]	0.684
IL-17A	1.05 [0.52-3.89]	0.99 [0.72-2.50]	0.844

ACA: anticentromere antibody; pSS: primary Sjögren's syndrome.

researches in South Korea (13.4% and 16.7% respectively) (15, 20). Our study found that patients with ACA had distinctive demographic features; they had significant longer disease duration and older diagnosis age than those without ACA, which were agreed with the previous reports (21, 22). Moreover, a strikingly lower ESSDAI and ESR in ACA-positive pSS patients suggesting a less severe disease activity in these patients. The possible explanation was the relatively mild clinical phenotype of ACA-positive patients may lead to the delay of visiting doctors, so the disease duration of this subset was longer.

However, in terms of symptoms of pSS, especially the secretory dysfunction of the exocrine glands, the results of previous studies were controversial. In a cross-sectional analysis of 1361 pSS patients from the Sjögren's International Collaborative Clinical Alliance (SICCA), a more severe exocrine glandular dysfunction that demonstrated by Schirmer's test and unstimulated whole saliva was observed in ACA-positive patients (23). Park *et al*. inves-

tigated 318 patients with pSS recruited from the Korean Initiative of primary Sjögren's Syndrome (KISS), including 53 patients with ACA positive. These patients presented higher xerostomia inventory scores. But there were no statistically differences in subjective sicca symptoms between groups (15). Li *et al*. was agreed with the present study, showed the symptoms of dry mouth and dry eye were more frequent in ACA-positive patients (24). Therefore, severe sicca symptom may be the characteristic of ACA-positive pSS, but further investigation was needed to confirm this point.

In addition, the literature consistently reported RP was more common in ACA-positive patients (13-15, 20-22), our research confirmed this as well. Lin *et al*. indicated a higher positivity of ACA was observed in pSS patients with RP (25). All these evidences demonstrated the close relationship between ACA and RP in pSS patients.

Our results were consistent with previous researches, suggested that pSS patients with ACA positive tend to have higher frequency of digestive system involvement, especially liver involvement, such as PBC, AIH and so on (15, 16, 21). The lower prevalence of blood system involvement, especially leukopenia, was also observed in other cohorts (15, 22). However, the results of other system involvements in patients with ACA were different in previous reports. Our study found the incidence of lung involvement in ACA-positive group was higher, which was consistent with the findings of Notarstefano *et al*. (16). Although ACA-positive pSS patients shared some similar features, cohorts from different ethnic did not present the same manifestations, suggesting that genetic, environmental and socioeconomic variations were involved in the biological and immunological responses.

An important finding in our study comes from the lower disease activity in ACA-positive pSS patients. The result was attributed to the decreased organ involvement and lower scores in the biological domain of ESSDAI than those of ACA-negative patients. In line with previous published reports (10, 12), we found ACA-positive pSS patients had less frequent of anti-SSA and anti-SSB antibody. Additionally, it has been demonstrated that the presence of anti-SSA antibody in pSS is related to activation of humoral immunity, result in longer disease duration, more frequency of extra glandular manifestations and higher intensity of the lymphocytic infiltrates invading the minor salivary glands (10, 26, 27). Recently a study from Japan demonstrated that ACA/SSA double positive SS had higher ESSDAI at diagnosis than ACA single positive SS (28). Therefore, the lower ESSDAI in ACA-positive patients may be related to the lower positivity of anti-SSA antibody in this subgroup.

The serum IgG and RF levels were also lower in the ACA-positive patients in our study, implicating a lower degree of chronic B-cell activation. Therefore, for the first time, we compared the distribution of circulating lymphocyte subsets to illustrate the immune status. As expected, we found ACA-positive patients had a significantly reduced B lymphocyte count and percentage compared with ACA-negative ones. This may partly shed light on the reason behind the marked disturbances in the serological aspects of the pSS patients with ACA, as reduced B cells could result to lower frequency of hypergammaglobulinaemia, decreased positivity of RF, anti-SSA and anti-SSB antibody. Interestingly, we also found CD16+CD56+ NK cells, innate lymphoid cells that exhibit a potential regulatory role in pSS disease (29, 30), were significantly increased in the ACA-positive group. Moreover, researchers confirmed the protective role of NK cells as negative regulators of autoantibodies producing B cells (31), which could give a reasonable explanation of our outcome.

Serum cytokines, which also participated in the immune system, were examined in our study. No significant differences were noted between pSS patients with and without ACA. Further investigations were needed to reveal whether the positivity of ACA in pSS impact serum cytokines.

The tendency of ACA-positive pSS patients evolve to definite SSc is debating since previous published studies on the follow-up of the disease have provided conflicting information (32, 33). Thus, it is necessary for us to follow up ACA-positive patients. In our current study, we did not observe those patients developing into SSc. Existing literature indicated the subset of ACA-positive pSS patients could be described as in-between pSS and SSc (13). Thus, further prospective study and more long-term observation is needed to pay attention to the possibility for development to CREST syndrome or SSc.

Previous published studies on the risk of lymphoma in patients with ACA-positive pSS have provided conflicting information (16, 20, 34). However, lower degree of chronic B-cell activation and reduced clinical predictors for the development of lymphoma (35) (lower age at diagnosis, positive RF, anti-SSA positivity and hyperglobulinemia) could suggest lower risks for the development of lymphoma in ACA-positive pSS patients. In our study, only one patient was diagnosed with lymphoma and there was no significant difference in lymphoma risk between two groups. It is necessary to monitor our patients for longer time.

There are some limitations in the current study. The retrospective observational design of the study could not determine the causative relationship well. Moreover, the study is conducted in a single centre, which may lead to selection bias. Finally, the lack of clinical and laboratory information of some patients may lead to unexpected various biases.

Conclusions

In conclusion, our study shows that ACA-positive patients with pSS have distinctive clinical manifestations and lower activation of the humoral immune system, present with less severe immunological features and lower disease activity, have decreased B cells and elevated circulating NK cells. Physicians should pay attention to RP, lung and digestive involvement in those patients.

Acknowledgements

The authors would like to thank Dr Donghui Zhang for the excellent flow cytometry work.

References

1. BRITO-ZERÓN P, BALDINI C, BOOTSMA H *et al*.: Sjögren syndrome. *Nat Rev Dis Primers* 2016; 2: 16047. https://doi.org/10.1038/nrdp.2016.47
2. CAFARO G, BURSI R, CHATZIS LG *et al*.: One year in review 2021: Sjögren's syndrome. *Clin Exp Rheumatol* 2021; 39 (Suppl. 133): S3-13. https://doi.org/10.55563/clinexprheumatol/eojaol
3. IBRAHEM HM: B cell dysregulation in primary Sjögren's syndrome: A review. *Jpn Dent Sci Rev* 2019; 55(1): 139-44. https://doi.org/10.1016/j.jdsr.2019.09.006
4. SHEN L, GAO C, SURESH L *et al*.: Central role for marginal zone B cells in an animal model of Sjögren's syndrome. *Clin Immunol* 2016; 168: 30-6. https://doi.org/10.1016/j.clim.2016.04.008
5. NOCTURNE G, MARIETTE X: B cells in the pathogenesis of primary Sjögren syndrome. *Nature Rev Rheumato* 2018; 14(3): 133-45. https://doi.org/10.1038/nrrheum.2018.1
6. DU W, HAN M, ZHU X *et al*.: The multiple roles of B cells in the pathogenesis of Sjögren's syndrome. *Front Immunol* 2021; 12: 684999. https://doi.org/10.3389/fimmu.2021.684999
7. VITALI C, BOMBARDIERI S, JONSSON R *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(6): 554-8. https://doi.org/10.1136/ard.61.6.554
8. SHIBOSKI CH, SHIBOSKI SC, SEROR R *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. *Arthritis Rheumatol* 2017; 69(1): 35-45. https://doi.org/10.1002/art.39859
9. ROUTSIAS JG, TZIOUFAS AG: Sjögren's syndrome--study of autoantigens and autoantibodies. *Clin Rev Allergy Immunol* 2007; 32(3): 238-51. https://doi.org/10.1007/s12016-007-8003-8
10. TZIOUFAS AG, TATOULI IP, MOUTSOPOULOS HM: Autoantibodies in Sjögren's syndrome: clinical presentation and regulatory mechanisms. *Presse Med* 2012; 41(9 Pt 2): e451-60. https://doi.org/10.1016/j.lpm.2012.05.022
11. BOURNIA VK, VLACHOYIANNOPOULOS PG: Subgroups of Sjögren syndrome patients according to serological profiles. *J Autoimmun* 2012; 39(1-2): 15-26. https://doi.org/10.1016/j.jaut.2012.03.001
12. SHEN L, SURESH L: Autoantibodies, detection methods and panels for diagnosis of Sjögren's syndrome. *Clin Immunol* 2017; 182: 24-9. https://doi.org/10.1016/j.clim.2017.03.017
13. BOURNIA VK, DIAMANTI KD, VLACHOYIANNOPOULOS PG, MOUTSOPOULOS HM: Anticentromere antibody positive Sjögren's Syndrome: a retrospective descriptive analysis. *Arthritis Res Ther* 2010; 12(2): R47. https://doi.org/10.1186/ar2958
14. SALLIOT C, GOTTENBERG JE, BENGOUFA D, DESMOULINS F, MICELI-RICHARD C, MARIETTE X: Anticentromere antibodies identify patients with Sjögren's syndrome and autoimmune overlap syndrome. *J Rheumatol* 2007; 34(11): 2253-8.
15. PARK Y, LEE J, KOH JH *et al*.: Clinical influences of anticentromere antibody on primary Sjögren's syndrome in a prospective Korean cohort. *Korean J Intern Med* 2021; 36(6): 1492-503. https://doi.org/10.3904/kjim.2020.146
16. NOTARSTEFANO C, CROIA C, PONTARINI E *et al*.: A clinical and histopathological analysis of the anti-centromere antibody positive subset of primary Sjögren's syndrome. *Clin Exp Rheumatol* 2018; 36 (Suppl 112): S145-9.
17. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69(6): 1103-9. https://doi.org/10.1136/ard.2009.110619
18. SEROR R, BOWMAN SJ, BRITO-ZERON P *et al*.: EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. *RMD Open* 2015; 1(1): e000022. https://doi.org/10.1136/rmdopen-2014-000022
19. TAN EM, RODNAN GP, GARCIA I, MOROI Y, FRITZLER MJ, PEEBLES C: Diversity of antinuclear antibodies in progressive systemic sclerosis. Anti-centromere antibody and its relationship to CREST syndrome. *Arthritis Rheum* 1980; 23(6): 617-25. https://doi.org/10.1002/art.1780230602
20. LEE KE, KANG JH, LEE JW *et al*.: Anti-centromere antibody-positive Sjögren's syndrome: a distinct clinical subgroup? *Int J Rheum Dis* 2015; 18(7): 776-82. https://doi.org/10.1111/1756-185X.12684
21. YAN SM, ZENG XF, ZHAO Y, DONG Y: A clinical analysis of primary Sjögren's syndrome with anticentromere antibodies. *Zhonghua Nei Ke Za Zhi* 2008; 47(4): 296-9. https://doi.org/10.3321/j.issn:0578-1426
22. 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(10): 2238-44.
23. BAER AN, MEDRANO L, MCADAMS-DEMARCO M, GNIADEK TJ: Association of anticentromere antibodies with more severe exocrine glandular dysfunction in Sjögren's syndrome: Analysis of the Sjögren's International Collaborative Clinical Alliance Cohort. *Arthritis Care Res* (Hoboken) 2016; 68(10): 1554-9. https://doi.org/10.1002/acr.22859
24. LI Y, BOOKMAN AAM: Comparison of effect on sicca symptoms of anticentromere antibody-positive Sjögren syndrome and primary Sjögren syndrome alone. *J Rheumatol* 2020; 47(6): 876-80. https://doi.org/10.3899/jrheum.190462
25. LIN W, XIN Z, NING X *et al*.: Clinical features and risk factors of Raynaud's phenomenon in primary Sjögren's syndrome. *Clin Rheumatol* 2021; 40(10): 4081-7. https://doi.org/10.1007/s10067-021-05749-w
26. QUARTUCCIO L, BALDINI C, BARTOLONI E *et al*.: Anti-SSA/SSB-negative Sjögren's syndrome shows a lower prevalence of lymphoproliferative manifestations, and a lower risk of lymphoma evolution. *Autoimmun Rev* 2015; 14(11): 1019-22. https://doi.org/10.1016/j.autrev.2015.07.002
27. TZIOUFAS AG, WASSMUTH R, DAFNI UG *et al*.: Clinical, immunological, and immunogenetic aspects of autoantibody production against Ro/SSA, La/SSB and their linear epitopes in primary Sjögren's syndrome (pSS): a European multicentre study. *Ann Rheum Dis* 2002; 61(5): 398-404. https://doi.org/10.1136/ard.61.5.398
28. SUZUKI Y, FUJII H, NOMURA H *et al*.: 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. *Mod Rheumatol* 2018; 28(5): 872-8. https://doi.org/10.1080/14397595.2017.1418164
29. IZUMI Y, IDA H, HUANG M *et al*.: Characterization of peripheral natural killer cells in primary Sjögren's syndrome: impaired NK cell activity and low NK cell number. *J Lab Clin Med* 2006; 147(5): 242-9. https://doi.org/10.1016/j.lab.2006.01.001
30. MING B, WU T, CAI S *et al*.: The increased ratio of blood CD56(bright) NK to CD56(dim) NK is a distinguishing feature of primary Sjögren's syndrome. *J Immunol Res* 2020; 2020: 7523914. https://doi.org/10.1155/2020/7523914
31. RIZZO C, LA BARBERA L, LO PIZZO M, CICCIA F, SIRECI G, GUGGINO G: Invariant NKT cells and rheumatic disease: focus on primary Sjögren syndrome. *Int J Mol Sci* 2019; 20(21): 5435. https://doi.org/10.3390/ijms20215435
32. TANAKA N, MURO Y, SUZUKI Y *et al*.: Anticentromere antibody-positive primary Sjögren's syndrome: epitope analysis of a subset of anticentromere antibody-positive patients. *Mod Rheumatol* 2017; 27(1): 115-21. https://doi.org/10.1080/14397595.2016.1176327
33. MIYAWAKI S, ASANUMA H, NISHIYAMA S, YOSHINAGA Y: Clinical and serological heterogeneity in patients with anticentromere antibodies. *J Rheumatol* 2005; 32(8): 1488-94.
34. BALDINI C, MOSCA M, DELLA ROSSA A *et al*.: Overlap of ACA-positive systemic sclerosis and Sjögren's syndrome: a distinct clinical entity with mild organ involvement but at high risk of lymphoma. *Clin Exp Rheumatol* 2013; 31(2): 272-80.
35. SOLANS-LAQUÉ R, LÓPEZ-HERNANDEZ A, BOSCH-GIL JA, PALACIOS A, CAMPILLO M, VILARDELL-TARRES M: Risk, predictors, and clinical characteristics of lymphoma development in primary Sjögren's syndrome. Semin *Arthritis Rheum* 2011; 41(3): 415-23. https://doi.org/10.1016/j.semarthrit.2011.04.006