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Anti-SSA/SSB-negative primary Sjögren's syndrome showing different clinical phenotypes: a retrospective study of 934 cases

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Abstract

Background Currently, only a few studies have described the general characteristics of patients with primary Sjögren's syndrome (pSS) who tested negatives for anti-SSA and anti-SSB antibodies. We aimed to further investigate the clinical characteristics of these patients in a large sample.

Methods Data from patients with pSS who were treated at a tertiary hospital in China between 2013 and 2022 were retrospectively analyzed. Clinical characteristics of the patients were compared between those with and without anti-SSA and anti-SSB antibody negativity. Factors associated with anti-SSA and anti-SSB negativity were identified by logistic regression analysis.

Results Overall, 934 patients with pSS were included in this study, among whom 299 (32.0%) tested negative for anti-SSA and anti-SSB antibodies. Compared with patients testing positive for anti-SSA or anti-SSB antibodies, that testing negative for the two antibodies had a lower proportion of females (75.3% vs. 90.6%, $p < 0.001$) and thrombocytopenia (6.7% vs. 13.6%, $p = 0.002$), but a higher proportion of abnormal Schirmer I tests (96.0% vs. 89.1%, $p = 0.001$) and interstitial lung disease (ILD) (59.2% vs. 28.8%, $p = 0.001$). Anti-SSA and anti-SSB negativity was positively associated with male sex (odds ratio [OR] = 1.86, 95% confidence interval [CI]: 1.05, 3.31), abnormal Schirmer I tests (OR = 2.85, 95% CI: 1.24, 6.53), and ILD (OR = 2.54, 95% CI: 1.67, 3.85). However, it was negatively related to thrombocytopenia (OR = 0.47, 95% CI: 0.24, 0.95).

Conclusion Approximately one third of pSS patients had anti-SSA and anti-SSB negativity. pSS patients testing negative for anti-SSA and anti-SSB showed a higher risk of abnormal Schirmer I tests and ILD, but a lower risk of thrombocytopenia.

Keywords Sjögren's syndrome, Anti-SSA, Anti-SSB, Clinical manifestation, Interstitial lung disease

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Introduction

Primary Sjögren's syndrome (pSS) is a chronic systemic autoimmune disease characterized by chronic lymphocytic infiltration of exocrine glands, that leads to dryness symptoms [1, 2]. Patients with pSS may have diverse manifestations ranging from mild dry mouth to severe extra-glandular involvement, including interstitial lung disease (ILD), interstitial nephritis, and thrombocytopenia [3]. Abnormal B-cell activity and differentiation produce large amounts of autoantibodies in patients with pSS [4, 5]. Of which, anti-SSA and anti-SSB antibodies are important markers for the diagnosis of pSS. Anti-SSA was included in the 2002 American-European Consensus group (AECG) classification criteria and the 2016 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for pSS [6, 7]; anti-SSB was included in the 2002 AECG classification criteria of pSS [6, 8]. These two antibodies often accompany each other and can be detected in 50–70% of pSS patients [9]. Combined with typical dry signs and positive proper tests, patients who test positive for anti-SSA and/or anti-SSB antibody can be classified as pSS. While, for suspected patients who test negative for anti-SSA antibody, only those with positive minor salivary gland biopsy (MSGB) can be classified as pSS [7].

It is generally accepted that positive antibody titers correlate with early onset of disease, more intense tissue infiltration, and a higher prevalence of extra-glandular manifestations [10]. However, the clinical characteristics of pSS with anti-SSA and anti-SSB antibody negativity have not been fully understood. Current studies on differences between pSS patients with and without antibody negativity have provided inconsistent results [11, 12]. The factors associated with anti-SSA and anti-SSB negativity in patients with pSS also remain unclear. We therefore conducted this retrospective study to further investigate the clinical characteristics of pSS patients with anti-SSA and anti-SSB antibody negativity; we also investigated relevant factors associated with negativity of the two antibodies in China to inform clinical decision making and future research.

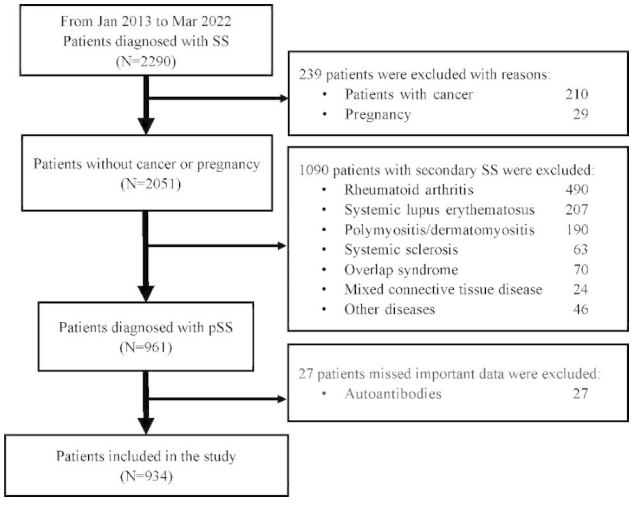

Fig. 1 Flow chart for patient selection

Materials and methods

Study design and participants

This retrospective study analyzed medical records of eligible patients with pSS, who were treated at the China-Japan Friendship Hospital between January 2013 and March 2022. All pSS patients (older than 18 years) were classified according to the 2002 AECG [6] and/or the 2016 ACR/EULAR classification criteria [7]. Patients with any of the following conditions were excluded: pregnancy; cancer; and diagnosed with other connective tissue diseases (CTD) including rheumatoid arthritis, systemic lupus erythematosus, idiopathic inflammatory myositis, systemic scleroderma, overlap syndrome, and mixed CTD, among others. Medical records without complete data on autoantibodies were also excluded (Fig. 1). This study was approved by the Clinical Research Ethics Committee of China-Japan Friendship Hospital (No: 2021-144-K102). The need for informed consent was waived because this was a retrospective study and the datasets were anonymized.

Data collection

Clinical data including age, sex, duration of disease, clinical symptoms, laboratory indicators, immunological characteristics, and MSGB findings were collected from medical records. If a patient was treated more than once, only data from the first record associated with pSS was included for analysis. ILD was detected by high-resolution computed tomography (HRCT), critically evaluated by two experienced thoracic radiologists, and finally diagnosed by clinicians. The following HRCT characteristics were particularly focused: reticular abnormalities, ground-glass opacities, nodules, consolidation, cysts, honeycombing and bronchiectasis [13]. MSGB procedures and histopathologic assessments were performed according to the protocol of the Sjögren's International Collaborative Clinical Alliance [14]. The MSGBs were performed by stomatologists and the pathological diagnoses were determined by pathologists. A lymphocyte infiltration focus was defined by more than 50 lymphocytes per 4 mm^2 of glandular tissue, located around blood vessels or ducts, with the surrounding acinar tissue appearing normal. The clinical symptoms included dry mouth, dry eyes, arthralgia, and palpable purpura, among others. Abnormal Schirmer I tests were defined by a result of ≤5 mm/5 min. Laboratory indicators included leucocyte, neutrophil, lymphocyte, and platelet counts and hemoglobin levels. Immunological characteristics

included the erythrocyte sedimentation rate (ESR) and levels of immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), complement 3 (C3), complement 4 (C4), and autoantibodies. The ANA titer was detected by indirect immunofluorescence assay on HEp2 cells; a titer of 1:320 was considered positive. Anti-SSA, anti-Ro52, anti-SSB, anti-centromere protein B (anti-CENP-B), anti-ribonucleoprotein (anti-RNP), and anti-mitochondrial M2 antibody (AMA-M2) antibodies were detected by using commercial immunoblot kits. The target antigen for detection of anti-SSA antibody is Ro60. RF was detected by immunoturbidimetric assay and positivity was defined by a level of over 20 IU/mL. All hematological indicators were evaluated at the China-Japan Friendship Hospital. All clinical data were obtained before treatment at the China-Japan Friendship Hospital.

Statistical analysis

All data analyses were performed using IBM-SPSS Statistics version 20 (IBM, Armonk, New York, USA). Data normality was detected by the Shapiro-Wilk test. Normally distributed continuous variables have been presented as the mean with standard deviations and non-normally distributed data have been presented as the median with interquartile ranges. Categorical variables are presented as numbers and percentages; some are presented as box plots, created using Tableau Public 2022.1 (Salesforce, San Francisco, California, USA). Comparisons were performed using the $\chi 2$, Mann-Whitney U, or Student's t-tests, as applicable. Multivariate logistic regression was used to further identify factors associated with anti-SSA and anti-SSB antibody negativity in patients with pSS, after adjusting for potential confounders (age, sex, disease duration, and nationality; nationality refers to Han Chinese versus other Chinese). Clinical characteristics were classified as categorical variables and univariate analyses were performed to screen potential related variables for further multivariate analyses. Variables including age, sex, disease duration, nationality, and those with a statistical p value of <0.1 on univariate analyses were included in multivariate regression analysis. The results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). A two-sided $p<0.05$ was considered significant.

Results

Clinical characteristics of pSS patients testing negative for anti-SSA and anti-SSB antibodies

A total of 934 patients with pSS were included in this study (Fig. 1), among whom 800 (85.7%) were women. All included patients satisfied the 2002 AECG classification criteria and/or the 2016 ACR/ EULAR classification criteria of pSS. None of included pSS patients had other CTD. The median age of included patients was 58 (49–66) years, and the median disease duration was 36 (10–96) months (Table 1).

Overall, 299 (32.0%) patients demonstrated negativity for both anti-SSA and anti-SSB antibodies. A total of 635 (68.0%) patients demonstrated positivity for one or two of the antibodies; among them, 630 and 254 tested positive for anti-SSA and anti-SSB antibodies, respectively. Among patients with anti-SSA or anti-SSB antibody

Table 1 Clinical characteristics of pSS patients with and without anti-SSA and anti-SSB antibody negativity

Variables	Total N = 934	Patients with anti-SSA and anti-SSB negativity N = 299	Patients with anti-SSA and/or anti-SSB positivity N = 635	*p-value**
Age (years), Mean ± SD	57.0 ± 12.6	61.7 ± 10.2	54.8 ± 13.1	< 0.001
Sex (female), n (%)	800 (85.7)	225 (75.3)	575 (90.6)	< 0.001
Disease duration (months), M (IQR)	925/36 (10–96)	24 (6–60)	48 (12–108)	< 0.001
Nationality, Han Chinese, n (%)	883 (94.5)	283 (94.6)	600 (94.5)	0.920
Others Chinese, n (%)	51 (5.5)	16 (5.4)	35 (5.5)	
Dry mouth, n (%)	799 (85.5)	262 (87.6)	537 (84.6)	0.215
Dry eyes, n (%)	739 (79.1)	240 (80.3)	499 (78.6)	0.554
Fatigue, n (%)	466 (49.9)	148 (49.5)	318 (50.1)	0.869
Arthralgia, n (%)	361/925 (39.0)	102 (34.5)	259 (41.2)	0.051
Dental caries, n (%)	339/879 (38.6)	100 (36.1)	239 (39.7)	0.308
Palpable purpura, n (%)	60/926 (6.5)	14.12 (4.1)	48 (7.6)	0.045
Parotid enlargement, n (%)	131/932 (14.1)	35 (11.7)	96 (15.2)	0.156
Lymphadenopathy, n (%)	109/929 (11.7)	31 (10.5)	78 (12.3)	0.429
Schirmer I test ≤ 5 mm/5 min, n (%)	853 (91.3)	287 (96.0)	566 (89.1)	0.001
Interstitial lung disease, n (%)	360 (38.5)	177 (59.2)	183 (28.8)	< 0.001
Focus score on MSGB, M(IQR)	517/2 (1–4)	2 (1–4)	3 (1–4)	0.351

M (IQR): median with interquartile range; Mean ± SD: Mean with standard deviation; MSGB: Minor salivary gland biopsy

* Continuous variables are expressed as means ± standard deviation or medians [interquartile range (IQR)] and compared with Student's t-test or Wilcoxon's rank test; categorical variables are expressed as n (%) and compared with $\chi 2$ tests

positivity, 218 underwent MSGB; 194 (97.7%) demonstrated positive pathological findings. All of the 299 patients who tested negative for anti-SSA and anti-SSB antibodies had positive MSGB results. The other autoantibodies in the 934 patients were distributed as follows: 258 (27.6%) had an ANA titer of ≥1:320 and 533 (57.1%), 69 (7.4%), 66 (7.1%), 81 (8.7%), and 380/864 (44.0%) had anti-Ro52, anti-CENP-B, anti-RNP, AMA-M2, and RF positivity, respectively. The distribution of autoantibody positivity in the cohort is shown in Fig. 2.

As shown in Table 1, pSS patients with anti-SSA and anti-SSB antibody negativity were older (61.7±10.2 vs. 54.8±13.1 years, p<0.001), had shorter disease duration (24 [6–60] vs. 48 [12–108] months, p<0.001), and had a lower proportion of females (75.3% vs. 90.6%, p<0.001) than those with anti-SSA or anti-SSB positivity. Compared with patients having anti-SSA or anti-SSB positivity, those testing negative for these antibodies had a lower prevalence of palpable purpura (4.1% vs. 7.6%, p=0.045). However, compared with pSS patients having anti-SSA or anti-SSB positivity, those testing negative for these antibodies had a higher prevalence of abnormal Schirmer I tests (96.0% vs. 89.1%, p=0.001), and ILD (59.2% vs. 28.8%, p<0.001). No statistical differences were found between the two groups in terms of nationality, dry mouth, dry eyes, fatigue, arthralgia, dental caries, parotid enlargement, lymphadenopathy, and focus scores.

Hematological characteristics of pSS patients testing negative for anti-SSA and anti-SSB antibodies

As shown in Table 2, patients testing negative for anti-SSA and anti-SSB antibodies demonstrated lower ANA positivity than those testing positive for anti-SSA or anti-SSB antibodies (18.7% vs. 31.8%, p<0.001), anti-Ro52 antibodies (23.7% vs. 72.8%, p<0.001), and RF (22.2% vs. 54.4%, p<0.001); however, they demonstrated higher positivity for anti-CENP-B (12.7% vs. 4.9%, p<0.001). Compared with patients testing positive for anti-SSA or anti-SSB antibodies, patients testing negative for these antibodies demonstrated a lower prevalence of leucopenia (13.4% vs. 32.4%, p<0.001), neutropenia (3.7% vs. 10.2%, p=0.001), anemia (10.7% vs. 23.5%, p<0.001), and thrombocytopenia (6.7% vs. 13.6%, p=0.002); the findings were similar for hyper-IgA (20.9% vs. 29.8%, p=0.004), hyper-IgG (28.3% vs. 53.5%, p<0.001), low C3 (15.9% vs. 24.1%, p=0.005), low C4 (25.4% vs. 38.2%, p<0.001), and elevated ESR (39.5% vs. 54.4%, p<0.001).

As shown in Fig. 3, compared with patients having anti-SSA or anti-SSB antibody positivity, those testing negative had higher counts of leucocytes (5.76 [4.58, 7.05] vs. 4.76 [3.71, 6.31], p<0.001), neutrophils (3.42 [2.56, 4.46] vs. 2.74 [1.97, 4.02], p<0.001), lymphocytes (1.69±0.66 vs. 1.50±0.61, p<0.001), and platelets (207 [161, 251] vs. 186 [145, 234], p=0.001) and higher hemoglobin levels (129 [120, 139] vs. 122 [110, 132], p<0.001); the findings for C3 (0.89 [0.78, 0.99] vs. 0.82 [0.71, 0.96], p<0.001) and C4 (0.18 [0.14, 0.22] vs. 0.20 [0.16, 0.24], p<0.001) levels were similar. Compared with patients testing positive for anti-SSA or anti-SSB antibodies, those testing negative had lower levels of IgA (2.68 [1.83, 3.57] vs. 2.95 [2.16, 3.98], p=0.001) and IgG (13.5 [11.15, 17.00] vs. 16.90 [13.13, 21.40], p<0.001). No statistical differences were found in terms of IgM levels.

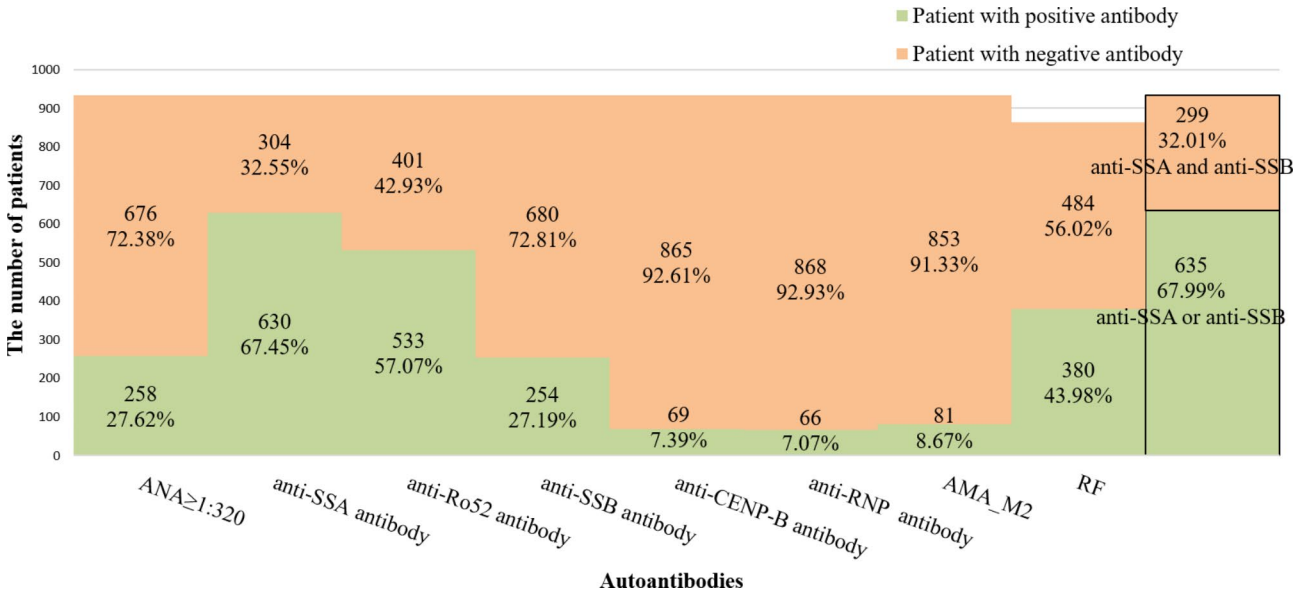

Fig. 2 Distribution of autoantibodies in patients with primary Sjögren's syndrome. Data are expressed as n (%). ANA, antinuclear antibodies; anti-CENP-B, anti-centromere protein B; anti-RNP, anti-ribonucleoprotein; AMA-M2, anti-mitochondrial M2 antibody; RF, rheumatoid factor

Table 2 Imunologic and hematologic characteristics of pSS patients with and without anti-SSA and anti-SSB antibody negativity

Variables	Total N = 934	Patients with anti-SSA and anti-SSB negativity N = 299	Patients with anti-SSA and/or anti-SSB positivity N = 635	*p-value**
Autoantibodies, n (%)				
ANA titres ≥ 1:320	258(27.6)	56 (18.7)	202 (31.8)	< 0.001
Positive anti-Ro52	533 (57.1)	71 (23.7)	462 (72.8)	< 0.001
Positive anti-CENP-B	69 (7.4)	38 (12.7)	31 (4.9)	< 0.001
Positive anti-RNP	66 (7.1)	20 (6.7)	46 (7.2)	0.757
Positive AMA-M2	81 (8.7)	29 (9.7)	52 (8.2)	0.444
Positive RF (> 20 IU/mL)	380/864(44.0)	62 (22.2)	318 (54.4)	< 0.001
Hematological characteristics, n (%)				
Leucopenia ($< 4 \times 10^9$/L)	243/924 (26.3)	40 (13.4)	203 (32.4)	< 0.001
Neutropenia ($< 1.5 \times 10^9$/L)	75/924 (8.1)	11 (3.7)	64 (10.2)	0.001
Lymphopenia ($< 0.8 \times 10^9$/L)	71/924 (7.7)	17 (5.7)	54 (8.6)	0.119
Anemia (< 110 g/L)	179/924 (19.4)	32 (10.7)	147 (23.5)	< 0.001
Thrombocytopenia (100×10^9/L)	105/924 (11.4)	20 (6.7)	85 (13.6)	0.002
Hyper-IgA (> 3.78 g/L)	247/917 (26.9)	62 (20.9)	185 (29.8)	0.004
Hyper-IgG (> 16.2 g/L)	416/917 (45.4)	84 (28.3)	332 (53.5)	< 0.001
Hyper-IgM (> 2.63 g/L)	72/917 (7.9)	29 (9.8)	43 (6.9)	0.136
Low C3 (< 0.7 g/L)	196/912 (21.5)	47 (15.9)	149 (24.1)	0.005
Low C4 (< 0.16 g/L)	311/912 (34.1)	75 (25.4)	236 (38.2)	< 0.001
Elevated ESR (> 20 mm/h)	430/868 (49.5)	113 (39.5)	317 (54.4)	< 0.001

* Categorical variables are expressed as n (%) and compared with $\chi 2$ tests

ANA, antinuclear antibodies; anti-CENP-B, anti-centromere protein B; anti-RNP, anti-ribonucleoprotein; AMA-M2, anti-mitochondrial M2 antibody; RF, rheumatoid factor; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; C3, complement 3; C4, complement 4; ESR, erythrocyte sedimentation rate

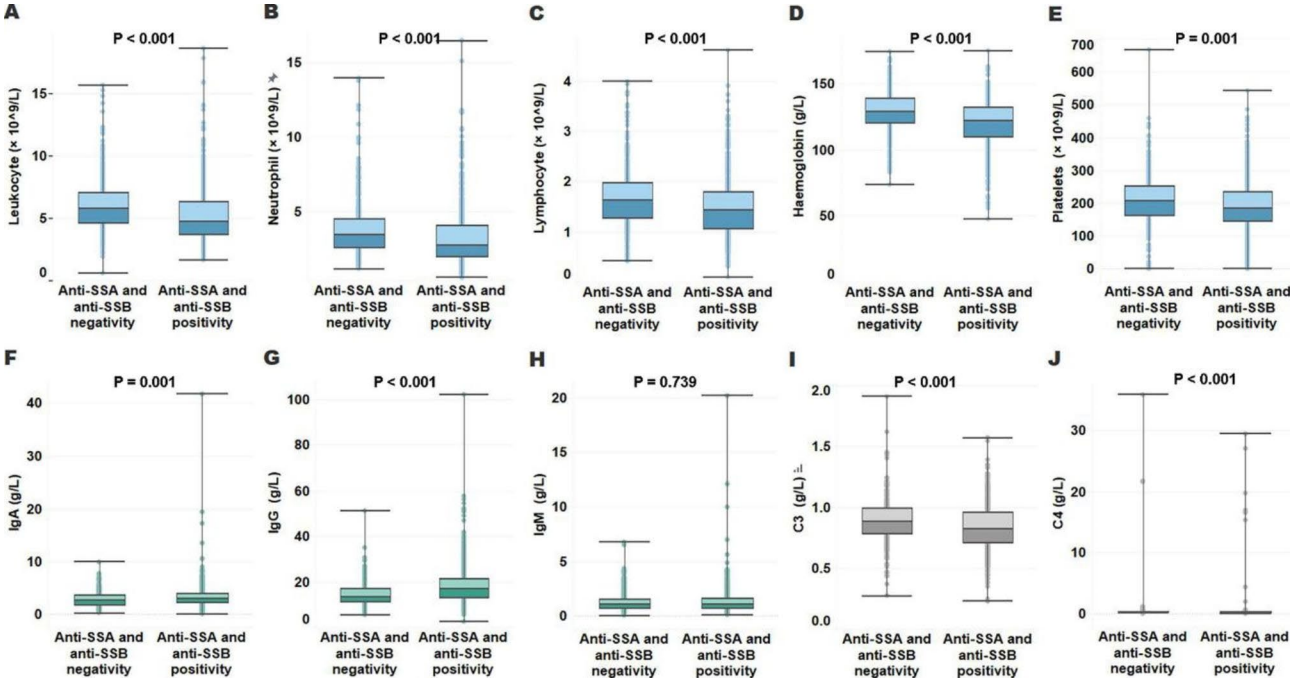

Fig. 3 Imunologic and hematologic distribution of patients with and without anti-SSA and anti-SSB antibody negativity. The uppermost and lowermost lines represent the maximum and minimum values of the data, respectively; the upper-line and lower-line of the boxplot represent the third and first quartiles, respectively; the thick line segment in the middle of the box plot represents the median of the data. IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; C3, complement 3; C4, complement 4

Factors associated with anti-SSA and anti-SSB antibody negativity in patients with pSS

In multivariate regression analysis, the following variables were included: age, sex, disease duration, nationality, arthralgia, palpable purpura, Schirmer I test results, ANA titers, anti-Ro52 positivity, anti-CENP-B positivity, RF positivity, leucopenia, neutropenia, anemia, thrombocytopenia, levels of IgA, IgG, C3, and C4, ESR, and ILD. No multicollinearity was found between the independent variables after the covariance diagnosis.

As shown in Table 3, anti-SSA and anti-SSB antibody negativity in patients with pSS was positively associated with age (OR=1.03, 95% CI: 1.01, 1.05), male sex (OR=1.86, 95% CI: 1.05, 3.31), ILD (OR=2.54, 95% CI: 1.67, 3.85), anti-CENP-B positivity (OR=4.17, 95% CI: 2.04, 8.53), and abnormal Schirmer I test results (OR=2.85, 95% CI: 1.24, 6.53). Anti-SSA and anti-SSB antibody negativity was negatively associated with disease duration (OR=1.00, 95% CI: 0.99, 1.00), anti-Ro52 positivity (OR=0.13, 95% CI: 0.09, 0.19), RF positivity (OR=0.40, 95% CI: 0.25, 0.63), and thrombocytopenia (OR=0.47, 95% CI: 0.24, 0.96).

Table 3 Factors associated with anti-SSA and anti-SSB antibody negativity in patients with pSS.

Variables	OR	95%CI	*P-value**
Age (years)	1.03	1.01, 1.05	0.002
Sex (male)	1.86	1.05, 3.31	0.034
Disease duration (months)	1.00	0.99, 1.00	0.018
Nationality (Han)	1.18	0.47, 2.98	0.727
Arthralgia	1.37	0.90, 2.10	0.143
Palpable purpura	0.53	0.22, 1.28	0.160
Schirmer I test ≤ 5 mm/5 min	2.85	1.24, 6.53	0.013
ANA titers ≥ 1:320	0.76	0.46, 1.25	0.278
Positive anti-Ro52	0.13	0.09, 0.19	< 0.001
Positive anti-CENP-B	4.17	2.04, 8.53	< 0.001
Positive RF (> 20 IU/mL)	0.40	0.25, 0.63	< 0.001
Leucopenia (< 4×10^9/L)	0.98	0.45, 2.14	0.953
Neutropenia (< 1.5×10^9/L)	0.61	0.25, 1.48	0.274
Anemia (< 110 g/L)	0.68	0.38, 1.19	0.171
Thrombocytopenia (100×10^9/L)	0.47	0.24, 0.96	0.037
Hyper-IgA (> 3.78 g/L)	0.71	0.43, 1.17	0.181
Hyper-IgG (> 16.2 g/L)	0.78	0.49, 1.26	0.315
Low C3 (< 0.7 g/L)	1.02	0.60, 1.75	0.935
Low C4 (< 0.16 g/L)	0.83	0.53, 1.30	0.408
Elevated ESR (> 20 mm/h)	0.87	0.54, 1.41	0.564
Interstitial lung disease	2.54	1.67, 3.85	< 0.001

* Multivariate logistic regression analysis

ANA, antinuclear antibodies; anti-CENP-B, anti-centromere protein B; RF, rheumatoid factor; IgA, immunoglobulin A; IgG, immunoglobulin G; C3, complement 3; C4, complement 4. ESR, erythrocyte sedimentation rate

Discussion

pSS is a heterogeneous disease that usually presents with mucosal dryness. However, it may also be associated with systemic involvement and occasionally with aggressive conditions, such as ILD and lymphoma. Autoantibodies and MSGB play important roles in the diagnosis of pSS. This is the first retrospective study with a large sample size to explore the clinical characteristics of pSS patients testing negative for anti-SSA and anti-SSB antibodies in Chinese population. We found that 32.0% of patients tested negative for anti-SSA and anti-SSB antibodies. Patients with anti-SSA and anti-SSB antibody negativity had older age, shorter disease duration, and higher proportions of male individuals and abnormal Schirmer I tests; additionally, they demonstrated a lower prevalence of anti-Ro52 and RF positivity than those with anti-SSA or anti-SSB positivity. We also found that patients with anti-SSA and anti-SSB negativity had a higher prevalence of ILD and a lower prevalence of thrombocytopenia; these findings are particularly meaningful from the clinical perspective.

In this study, the distribution of anti-SSA (67.5%), anti-Ro-52 (57.1%), anti-SSB (27.2%), anti-CENP-B (7.4%), anti-RNP (7.1%), AMA-M2 (8.7%), and RF (44.0%) positivity was similar to that of some previous studies [9, 15, 16]. The prevalence of ANA titers of ≥1:320 was 27.6% in our data, which was lower than that of two previous studies (45.5-60%) [16, 17]; this may be attributed to the differences in the study populations. Our results showed that patients with anti-SSA and anti-SSB antibody negativity had a lower prevalence of ANA and RF positivity; these findings are similar to studies in other ethnicities including Italy, Sweden and Poland [11, 18, 19]. Several studies have shown that RF positivity is strongly associated with anti-SSA positivity; however, no correlation was found with anti-SSB positivity [20, 21]. Our results also showed that patients with anti-SSA and anti-SSB antibody negativity had a lower prevalence of anti-Ro52 positivity. Natural purified SSA shows only Ro60, and anti-SSA and anti-Ro52 are two independent antibody systems [22]. It has been reported that anti-Ro52 antibody alone is not a specific diagnostic antibody for pSS [1]. We also found that patients testing negative for anti-SSA and anti-SSB antibodies had a high proportion of anti-CENP-B positivity, and anti-SSA and anti-SSB antibody negativity was positively associated with anti-CENP-B positivity. Anti-CENP-B antibody is the major anti-centromere antibody (ACA), which is thought to be frequently associated with scleroderma and can also be seen in pSS [15]. In this study, we explicitly excluded patients with other CTD including scleroderma at the time of inclusion. We found a rate of 7.4% for anti-CENP-B positively in pSS, confirming previous studies reporting a range of 3.7–10% for ACA in pSS [15, 23, 24]. Positive

ACA in pSS is associated with more severe exocrine gland dysfunction [25]. Moreover, pSS patients with ACA positivity have a higher prevalence of Raynaud's phenomenon and sclerodactyly [23]. Some scholars have suggested that the ACA-positive group in patients with pSS may represent a special subtype of pSS, and these patients require to monitor the occurrence of systemic sclerosis. Previous studies have shown that pSS patients with ACA positivity have a lower prevalence of anti-SSA and anti-SSB, similar to the result of this study [23–26]. Further studies are needed to explore the role of ACA in the pathogenesis of pSS.

As we found in this study, pSS patients testing negative for anti-SSA and anti-SSB have distinct clinical phenotypes to those with positive antibodies. The underlying pathological mechanisms may somewhat different [27]. ILD is considered the most frequent and serious pulmonary complication of pSS [13, 28]. The prevalence of ILD in the present study was 36.14%; this is similar to that of other Chinese studies (30.1-42.6%) [29–32]. However, the prevalence of pSS-ILD seems lower (12.1-20.0%) in the European population [33, 34], which need to be verified in future studies. Numerous studies have explored the risk factors of ILD in pSS. Anti-Ro52 positivity, male sex, and older age are considered as risk factors for the development of ILD in pSS [13]; an abnormal Schirmer I test is also considered a risk factor for the development of ILD in non-sicca onset pSS [35]. We found that pSS patients with anti-SSA and anti-SSB antibody negativity were more likely to be male, older, and have abnormal Schirmer I test results. Several studies have shown that ILD is more common in patients with elderly-onset pSS [29, 36, 37], consistent with results of the present study; pSS patients with different age of onset have different organ specificity seemingly with different pathogenesis [36]. This difference is also similar in sex [38]. Older and male patients with suspected pSS who test negative for anti-SSA and anti-SSB antibodies also need to undergo evaluation for pulmonary involvement after confirming a diagnosis of pSS by MSGB. However, the mechanisms underlying the increased prevalence of ILD in pSS patients with anti-SSA and anti-SSB antibody negativity remain unclear; further investigation is warranted in the future.

To the best of our knowledge, this is the first report on the association of anti-SSA and anti-SSB antibodies with thrombocytopenia in pSS patients. Although the pathophysiology of thrombocytopenia in these patients is not fully understood, reports indicate that autoantibodies including antiplatelet antibody, P-selectin autoantibodies, ANA, and anti-SSB, are related to the development of thrombocytopenia [39–41]. We found that anti-SSA and anti-SSB antibody negativity was negatively associated with thrombocytopenia. Patients testing positive for anti-SSA and anti-SSB antibodies were more likely to have thrombocytopenia; this suggests that platelet counts need to be regularly reviewed in pSS patients with anti-SSA and anti-SSB antibody positivity. A 10-year Chinese cohort study showed that shorter disease duration, male sex, older age at onset, ILD and thrombocytopenia were independent predictors for the mortality of pSS [42], and some of these factors were also positively associated with anti-SSA and anti-SSB negativity. Thus, pSS patients testing negative for anti-SSA and anti-SSB antibodies may have poor prognosis who deserve closer follow-up and regular monitoring.

This study has certain limitations. First, this was a single-center retrospective study; although we adjusted for main factors during data analyses, some potential confounders may be unknown. The study provides a deeper understanding on clinical characteristics of Chinese pSS patients with anti-SSA and anti-SSB antibody negativity; however, the results may not be generalized to all ethnicities. Second, we were not able to analyze and report the outcomes in pSS patients with anti-SSA and anti-SSB antibody negativity, because we only retrospectively analyzed the clinical characteristics at one time-point. Third, we can't analyse all clinical characteristics of pSS such as Raynaud's phenomenon and disease activity because relevant data was missing in the medical records for most patients. Future multi-center prospective studies with long term follow-up are needed to confirm our findings and explore the prognosis of pSS patients with anti-SSA and anti-SSB antibody negativity.

Conclusions

In conclusion, approximately one third of patients in this cohort tested negative for anti-SSA and anti-SSB antibodies. We found that pSS patients testing negative for anti-SSA and anti-SSB antibodies were likely to have abnormal Schirmer I test and ILD, but less likely to have thrombocytopenia. The findings indicate that pSS patients with anti-SSA and anti-SSB antibody negativity have distinct clinical manifestations compared to those testing positive for these antibodies. More high-quality prospective studies are needed to confirm these findings.

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Author contributors

Conception and design of the work—JL and QWT; Data acquisition, analysis and interpretation—JQC, QH, JYY, ZHW, ZWH, YZ, JHL, LNZ, XBY and CHY; Original draft preparation—JQC; Critical revision of the draft—JL, QWT, JQC, QH, JYY, ZHW, ZWH, YZ, JHL, LNZ, XBY and CHY. All authors give approval of

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Data availability
The data for the analyses in this study are available on reasonable request.

Declarations

Ethics approval and consent participate
This study was approved by the Clinical Research Ethics Committee of the China-Japan Friendship Hospital (approval number: 2021-144-K102). The need for informed consent was waived, because the datasets were anonymized.

Informed consent
was waived because this is a retrospective study and the datasets are devoid of personally identifiable information.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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