The Roles of Anti-RNP Antibodies in Primary Sjögren's Syndrome Pathogenesis and Severity May Be Underestimated

wei lin
Hebei General Hospital https://orcid.org/0000-0001-5596-3494
Zhifei Xin
Hebei General Hospital
Kenan Peng
Hebei General Hospital
Xiaoran Ning
Hebei General Hospital
Shaoying Guo
Hebei Medical University
Liu Yang
Hebei Medical University
Yang Li
Hebei General Hospital
Yupeng Yang
Hebei General Hospital
Yixuan Liu
Hebei General Hospital
Xiuying Ren
Hebei General Hospital
Yashuang Su
Hebei General Hospital
Jingjing Cao
Hebei General Hospital
Meilu Liu
Hebei General Hospital
Lijun Sun
Hebei General Hospital
Fengxiao Zhang (zfx9911@163.com)
Department of Rheumatology and immunology, Hebei General Hospital, Shijiazhuang, Hebei, 050017, China;

Wen Zhang
Peking Union Medical College Hospital

Research article

Keywords: primary Sjögren's syndrome, anti-ribonucleoprotein antibodies, clinicopathologic features, disease severity, multivariate association

Posted Date: May 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-499695/v1

Abstract

Background Associations between anti-ribonucleoprotein (RNP) antibodies status and distinct clinical primary Sjögren's syndrome (pSS) subtypes have not yet been firmly established. The aim of our study i**s** to determine whether associations exist between RNP antibody status and the clinical manifestations, laboratory features, or disease activity in pSS. **Methods** A retrospective cohort of 39 anti-RNP antibody-positive and 294 anti-RNP antibody-negative pSS patients was assembled. Data regarding demographic information, glandular and extraglandular manifestations, laboratory findings, and disease activity (scored according to the European League Against Rheumatism SS disease activity index (ESSDAI)) were extracted from patient records. Univariate methods followed by multivariable logistic regression analysis were used to evaluate potential associations between anti-RNP antibody status and clinicopathologic features. **Results** Patients with anti-RNP antibody-positive pSS had a higher prevalence of Raynaud's phenomenon (RP) and hematological, pulmonary, lymphatic system, and mucocutaneous involvement; higher erythrocyte sedimentation rates and serum IgG levels; lower lymphocytes counts; and significantly higher ESSDAI scores (median (interquartile range): 13 (7–18) versus 7 (3–12), $p < 0.001$). No significant differences were observed between groups for C-reactive protein levels and rheumatoid factor or anti-Ro/SSA or -La/SSB antibody positivity. Multivariate analysis identified RP, interstitial lung disease (ILD), and lymphatic system involvement as independent predictors of anti-RNP antibody positivity in pSS patients. **Conclusions** In this cohort, anti-RNP antibodies were associated with several clinicopathologic features of severe pSS, such as RP and hematologic, lymphatic, and pulmonary disorders. Therefore, anti-RNP antibodies may play an important role in the pathogenesis and severity of pSS.

Highlights

- Significance of anti-RNP antibody in primary Sjögren's syndrome (pSS) is unclear.
- Anti-RNP antibody positivity is associated with extraglandular manifestations.
- RP, interstitial lung disease, and lymphatic involvement predict antibody positivity.
- Anti-RNP antibodies may play key roles in pSS pathogenesis and presentation.

1. Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration and dysfunction of lacrimal and salivary glands, resulting in xerophthalmia and xerostomia. However, the clinical features of pSS are heterogeneous and vary from localized sicca symptoms to systemic involvement. Extra-glandular manifestations occur in approximately one third of patients [1, 2]. In addition, the variety of autoantibody profiles observed in pSS patients is associated with differential clinical manifestations [1, 3]. Anti-Ro/SSA and -La/SSB antibodies are diagnostic hallmarks of pSS [4], but patients may also exhibit a number of other autoantibody types including rheumatoid factor (RF), anti-centromere antibodies (ACA), anti-Ro52 antibodies, and anti-ribonucleoprotein (RNP) antibodies. However, the existence of associations between different autoantibodies and particular clinical features

is controversial [5]. Nevertheless/For example, ACA antibodies are associated with a higher mean age at disease onset, and increased frequencies of Raynaud's phenomenon (RP), sclerodactyly, peripheral neuropathy, and concomitant autoimmune disorders (e.g., autoimmune thyroiditis and primary biliary cirrhosis) [6–9]. Similarly, RF is associated with more severe ocular symptoms; increased frequencies of articular manifestations, cutaneous vasculitis, salivary gland enlargement, cytopenias, RP, renal involvement, and central nervous system involvement; and higher European League Against Rheumatism SS disease activity index (ESSDAI) scores [10–12].

High titers of anti-RNP antibodies are characteristically associated with mixed connective tissue disease (MCTD), originally described in 1972 [13, 14]. However, anti-RNP antibodies are also detectable across a broad spectrum of autoimmune diseases, including systemic lupus erythematosus (SLE), systemic sclerosis (SSc), myositis, rheumatoid arthritis (RA), and pSS [15–17]. Clinical significance of anti-RNP antibodies is a matter of debate. In the context of SLE, anti-RNP antibodies occur in 20–33 % of patients and are associated with specific clinical manifestations (e.g., scleroderma-like features) [18–20]. However, the utility of anti-RNP antibodies in classifying clinical pSS subgroups remains unknown. In the context of pSS, anti-RNP antibodies occur in 1.5–13.8 % of patients, depending on study population demographic characteristics [21, 22]. Despite this relatively high prevalence, anti-RNP antibody-positive pSS patients are poorly described, although anti-RNP antibodies may be associated with more active systemic disease, more frequent muscular and pulmonary involvement, and increased gammaglobulin levels [15].

Interpreting the clinical significance of anti-RNP antibody positivity in pSS patients is challenging, given the uncertain role of these autoantibodies in disease pathogenesis and their potential association with distinct clinicopathologic features. Moreover, these autoantibodies may have prognostic value in pSS patients. Finally, no study has yet examined anti-RNP antibody positivity in pSS patients of Asian descent. Because studies evaluating the potential association between anti-RNP antibody presence and clinical manifestations (including severity, organ involvement, and disease activity) are urgently required, the present study retrospectively assessed a large pSS patient cohort to determine whether associations exist between anti-RNP antibody status and clinicopathologic manifestations, including disease activity.

2. Patients And Methods

2.1 Patient selection

A retrospective medical record review of 333 pSS patients attending the Hebei General Hospital inpatient clinic between September 2016 and March 2019 was performed. Patients were eligible for inclusion if they met American-European Consensus Group 2002 classification criteria [4] for pSS, did not have other possible causes of sicca syndrome (infections or neoplasias), and did not have another concomitant systemic autoimmune disease. The study protocol was approved by the medical ethics committee of Hebei General Hospital (No.2016070) and written informed consent was obtained from all patients.

2.2 Data collection

Demographic, clinical, and laboratory data were recorded, including age, sex, medical history, disease duration, symptoms of keratoconjunctivitis sicca and xerostomia, extraglandular manifestations (including disease activity scored according to the 2010 ESSDAI [23]), treatment, white blood cell (WBC) count, lymphocyte count, neutrophil count, hemoglobin level, platelet count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, complement (C3 and C4) levels, immunoglobulin (IgG, IgA, and IgM) levels, RF titers, antinuclear antibody (ANA) titers, anti-dsDNA antibody titers, and anti-extractable nuclear antigen (ENA) antibody (including anti-Ro/SSA, anti-La/SSB, ACA, and anti-RNP antibody) titers.

2.3 Statistical analysis

All statistical analyses were performed using SPSS version 25.0 (SPSS Inc., IL, USA). Data were expressed as median (interquartile range) for continuous variables and number (%) for categorical variables. Anti-RNP antibody-positive and -negative patient groups were compared using the Mann-Whitney U test for continuous variables and the χ^2 test or Fisher's exact test for categorical variables. Differences were considered statistically significant at $p < 0.05$ (two-tailed). Variables identified as differing significantly between groups by univariate analysis were considered for multivariate logistic regression analysis. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated.

3. Results

3.1 Anti-RNP antibody status, demographic characteristics, and disease duration

Of 333 reviewed pSS patients, 39 (11.71 %) were anti-RNP antibody-positive. The majority of patients (310; 93.09 %) were female, and patient age ranged from 18 to 89 years (mean age 53.92 ± 13.42 years) (Table 1). Anti-RNP antibody-positive patients were significantly younger than anti-RNP antibody-negative patients (48.49 ± 13.51 versus 54.65 ± 13.26 years, $p = 0.007$). Disease duration did not differ significantly between groups.

Table 1
Demographic and serological descriptors of pSS patients with and without anti-RNP antibody positive

	Anti-RNP (+) group (n = 39)	Anti-RNP (-) group (n = 294)	*P* value
Demographic features			
Gender(F/M)	37:2	373:21	1.0
Age at onset, year	48.49 ± 13.51	54.65 ± 13.26	0.007
Disease duration, months	48 [12–120]	48 [12–120]	0.74
Laboratory findings			
White Blood Cell counts ($\times 10^9$/L)	4.35 [3.53–6.49]	4.98 [4.03–6.25]	0.24
Neutrophils counts ($\times 10^9$/L)	2.66 [1.96–4.59]	2.95 [2.19–4.15]	0.66
Lymphocytes counts ($\times 10^9$/L)	1.38 [0.84–1.59]	1.53[1.12–1.89]	0.02
Hemoglobin (×g/L)	115.08 ± 17.85	121.13 ± 17.66	0.05
Platelets counts ($\times 10^9$/L)	200 [156–275]	226.5 [180-268.5]	0.24
ESR (mm/1h)	23 [17-42.75]	17 [8-35.5]	0.03
CRP (mg/L)	3.30 [0.92–15.69]	3.30 [1.12–4.26]	0.43
RF(IU/L)	32.20 [10.6-118.81]	15.30 [10.60–62.40]	0.07
IgG(g/L)	17.4 [14.6–24.70]	15.10 [12.05–19.45]	0.004
IgA(g/L)	2.53 [1.94–3.75]	2.77 [1.92–3.55]	0.92
IgM(g/L)	1.02 [0.74–1.43]	1.20 [0.83–1.63]	0.07
C3 (mg/L)	1.02 [0.87–1.16]	1.08 [0.92–1.21]	0.05
C4 (mg/L)	0.19 [0.16–0.23]	0.19 [0.15–0.24]	0.48
Elevated ESR (n, %)	20/36, 55.56	131/285, 45.96	0.28
Elevated CRP (n, %)	11/36, 30.56	52/278, 18.71	0.10
Low C3 (n, %)	11, 28.21	64, 21.77	0.37
Low C4 (n, %)	4, 10.26	25, 8.50	0.76
Elevated IgG (n, %)	19/39, 48.72	99/288, 34.38	0.08
RF (+) (n, %) *	22/35, 62.86	128/276, 46.38	0.07
ANA (+) (n, %) **	39, 100	224, 76.19	0.001

	Anti-RNP (+) group (n = 39)	Anti-RNP (-) group (n = 294)	*P* value
Anti-Ro52 (+) (n, %)	27, 69.23	173, 58.84	0.21
Anti-Ro/SSA (+) (n, %)	24, 61.54	163, 55.44	0.47
Anti-La/SSB(+)(n, %)	11, 28.21	68, 23.13	0.48
ACA (+) (n, %)	4, 10.26	41, 13.95	0.53
ESSDAI	13 [7–18]	7 [3–12]	< 0.001

pSS: primary Sjögren's syndrome; RF: rheumatoid factor; ANA: antinuclear antibodies; ACA: anti-centromere antibodies.

*positive RF > 20 IU/ml; **positive for ANA titres ≥ 1:320.

3.2 Immunological parameters

Anti-RNP antibody-positive patients exhibited significantly lower lymphocyte counts than anti-RNP antibody-negative patients (1.38 (0.84–1.59) versus 1.53 (1.12–1.89) × 10^9/L, $p = 0.02$); in contrast, anti-RNP antibody-positive patients exhibited significantly higher ESR (23 (17–42.75) versus 17 (8–35.5) mm/h, $p = 0.03$) and serum IgG levels (17.4 (14.6–24.70) vs. 15.10 (12.05–19.45) g/L, $p = 0.004$) (Table 1). Other immunological parameters (WBC, neutrophil count, platelet count, CRP level, IgA level, IgM level, and complement levels) did not differ significantly between groups. In addition, there was no difference between groups in the proportion of patients exhibiting low complement levels or elevated IgG levels. Regarding autoantibodies, significantly more anti-RNP antibody-positive patients were ANA positive (100 % versus 76.19 %, $p = 0.001$). Status of other autoantibodies (anti-Ro/SSA, anti-La/SSB, ACA, and anti-Ro52) did not differ significantly between groups. Furthermore, only 24 patients (7.21 %) exhibited both anti-Ro/SSA and anti-RNP antibodies, while 131 (39.34 %) exhibited neither anti-Ro/SSA nor anti-RNP antibodies.

3.3 Glandular manifestations

Anti-RNP antibody-positive patients exhibited a significantly lower rate of Schirmer's test positivity (67.65 % versus 84.00 %, $p = 0.02$) (Table 2). Other glandular manifestations (keratoconjunctivitis sicca, xerostomia, tear film break-up time, corneal fluorescein staining, parotid gland enlargement, salivary scan positivity, dental caries, and minor salivary gland positive focus scores) did not differ significantly between groups.

Table 2
Glandular manifestations at the baseline in 333 patients with pSS

Variables (n, %)	Anti-RNP (+) group (n = 39)	Anti-RNP (-) group (n = 294)	*P* value
Ocular symptom	29, 74.36	217, 73.81	0.94
Oral symptom	33, 84.62	257, 87.41	0.62
Corneal fluorescein staining positive	20/34, 58.82	158/253, 62.45	0.68
Positive Schirmer's test	23/34, 67.65	210/250, 84	0.02
BUT positive rates	32/34, 94.12	238/251, 94.82	0.70
Salivary gland enlargement	8, 20.51	30, 10.20	0.06
Positive salivary scan	30/32, 93.75	222/228, 97.37	0.26
Rampant dental caries	17, 43.59	107, 36.39	0.38
Pathological MSG with focus score ≥ 1 (n, %)	37/39, 92.68	272/284, 92.83	0.68

Groups were compared using chi-square test for categorical data. BUT, tear film break-up time. MSG: minor salivary gland.

3.4 Extraglandular organ involvement

Anti-RNP antibody-positive patients exhibited a significantly higher frequency of hematological involvement ($p = 0.001$), including leukocytopenia ($p = 0.005$), lymphopenia ($p = 0.01$), and thrombocytopenia ($p = 0.02$), and of RP (61.54 % versus 4.76 %, $p < 0.001$) (Table 2). Anti-RNP antibody-positive patients also exhibited a significantly higher frequency of pulmonary involvement (53.85 % versus 18.03%, $p < 0.001$) (including interstitial pneumonia, pleuritis, and bronchiolitis), lymphatic system involvement (28.21 % versus 7.48 %, $p < 0.001$), and mucocutaneous involvement (66.67 % versus 20.07 %, $p < 0.001$). Other extraglandular features (including arthritis, renal involvement, digestive system involvement, and nervous system involvement) did not differ significantly between groups. Accordingly, anti-RNP antibody-positive patients exhibited significantly higher ESSDAI scores (13 (7–18) versus 7 (3–12), $p < 0.001$).(Fig. 1 and Table sup1)

3.5 Multivariate analysis to identify risk factors associated with anti-RNP antibody positivity

Multivariate analysis identified three variables as being independently and significantly associated with anti-RNP antibody positivity: interstitial lung disease (ILD) (OR 3.41, 95 % CI [1.16, 10.04]), RP (OR 24.08, 95 % CI [8.09, 71.69]), and lymphatic system involvement (OR5.54, 95 % CI [1.71, 18.01]) (Fig. 2).

4. Discussion

Findings of the present study suggest that anti-RNP antibody-positive pSS - despite sharing many common (especially pathological) features with anti-RNP antibody-negative pSS - constitutes a clinical subgroup distinct from other well-known pSS subgroups.Specifically, anti-RNP antibody-positive patients demonstrated significantly higher frequencies of extra-glandular manifestations more commonly associated with MCTD and SLE, including RP, and pulmonary, lymphatic system, mucocutaneous, and hematological involvement. Additionally, anti-RNP antibody-positive patients exhibited significantly higher ESSDAI scores.

In the present cohort, anti-RNP antibody positivity prevalence was approximately 12 %, which is consistent with that reported in a previous study. In an Israeli pSS cohort (N = 82), 13.41 % of patients exhibited anti-RNP antibody positivity [24]. However, in a pSS cohort of Hispanic descent (N = 402), anti-RNP antibody positivity prevalence was only 2.49 % [25], and a French pSS cohort (N = 467) exhibited 4.50 % anti-RNP antibody positivity prevalence [15]. This relatively broad range of prevalence is likely attributable to ethnicity-related genetic factors.

Although the presence of anti-RNP antibodies is not included in American-European Consensus Group 2002 classification criteria for pSS [4], they do seem to be associated with a specific subset of system involvements and disease activity. Notably, the present study demonstrated that such patients exhibited a higher frequency of RP, and that RP was independently predictive for anti-RNP antibody positivity. This is consistent with anti-RNP antibody positivity association with RP across a broad spectrum of connective tissue disorders (e.g., MCTD, SSc, SLE, and RA) [26, 27]. This is interesting considering that ANA, ACA, anti-RNP antibody, anti-Sm antibody, and anti-Scl-70 antibody status is apparently not of utility in elucidating the pathology underlying RP [28]. However, our previous research also suggested that pSS patients with RP represent a distinct subset characterized by more frequent presence of ACA or anti-RNP antibodies, and more frequent pulmonary involvement. Furthermore, it has been hypothesized that RP may be associated with antibodies targeting oxidized U1-70kDa (a common target of anti-RNP antibody-positive autoimmune sera), which may be generated due to the formation of reactive oxygen species during ischemia-reperfusion [29, 30].

Regarding pulmonary manifestations of pSS, anti-RNP antibody positivity is known to be associated with pulmonary hypertension and ILD. Pulmonary fibrosis is more prevalent in anti-RNP antibody-positive than in anti-RNP antibody-negative pSS (51.28 % versus 15.65%). Moreover, we previously demonstrated that pSS patients with ILD exhibited a significantly higher frequency of anti-RNP antibody positivity than those without ILD (i.e., that the presence of anti-RNP antibodies was significantly associated with the risk of ILD). This is consistent with other studies reporting a higher incidence of ILD in the anti-RNP antibody-positive pSS patient cluster. Within such a patient cohort (N = 188), 26 % who underwent a computed tomography (CT) scan of the chest had ILD [31]. Interestingly, ILD (one of the most common extramuscular features of myositis) occurs in 45 % of anti-U1-RNP antibody-positive myositis patients, but in significantly lower proportions of DM patients (13 %) and IMNM patients (6 %) (all: $p < 0.01$) [32]. It was hypothesized that anti-RNP antibodies may contribute to connective tissue disorder-associated ILD by recognizing pulmonary endothelial antigens and stimulating inflammatory cytokine production [33–

36]. In contrast, a meta-analysis revisiting antisynthetase syndrome patients demonstrated a lower frequency of ILD in patients with anti-U1-RNP autoantibodies [37]. It is possible that anti-U1-RNP antibodies contribute to disease pathogenesis via distinct mechanisms in different types of connective tissue disorder [34, 38]. Considering the seriousness of pulmonary fibrosis (which is generally asymptomatic during its early stages), screening by means of pulmonary CT may be prudent in anti-RNP antibody-positive pSS. In contrast, the present study found no significant difference in pulmonary hypertension frequency (12.82 % versus 5.78 %, $p = 0.16$) on the basis of anti-RNP antibody status.

Anti-RNP antibody positivity also correlated with hematological involvement in the present study: the proportion of patients exhibiting leukocytopenia, lymphopenia, and thrombocytopenia was significantly higher in the anti-RNP antibody-positive group. It has previously been suggested that anti-Sm/RNP antibodies (but not anti-Sm antibodies) are strong correlates of hematological disorders in SLE patients of European-American and African-American (but not Hispanic) descent. Relatedly, autoantibodies targeting RNP (but not Sm) were independently enriched in SLE patients of Hispanic descent exhibiting lymphopenia [39]. There may be an inter-individual difference in mechanisms by which anti-RNP antibodies contribute to pSS pathology. However, mechanisms underlying the above associations require further elucidation. Regarding muscular involvement, anti-RNP antibody positivity is associated with more frequent muscular involvement in pSS patients [15]. However, the present study did not confirm the association between anti-RNP antibody positivity and myositis.

Several potential limitations of the present study are acknowledged. First, the retrospective nature of the study precluded inclusion of comprehensive patient clinical data. For example, electromyography and muscle biopsy findings were not available for all patients, thereby preventing conclusions regarding the true prevalence of myositis in this population. A longitudinal prospective study would address such shortcomings. Second, the proportion of anti-RNP antibody-positive pSS patients was small relative to that of anti-RNP antibody-negative pSS patients. However, it was large enough to facilitate conclusions regarding statistical significance of between-group differences. Third, an insufficient number of anti-RNP antibody-positive patients had long-term follow-up data available. Finally, we did not evaluate associations between the presence of anti-RNP antibodies and other autoantibodies commonly found in pSS patients. Larger, multicenter, prospective studies (including systematic evaluation of autoantibody profiles) are recommended to elucidate the mechanistic and clinical significance of autoantibodies in pSS.

5. Conclusions

Taking together, our findings suggest that anti-RNP antibodies, long underestimated, may play key roles in the pathogenesis of pSS, resulting in distinct clinical and immunological characteristics of the anti-RNP antibody-positive patient group. In particular, RP, thrombocytopenia, and lymphatic system involvement are independently associated with anti-RNP antibody positivity. These observations highlight the potential mechanistic and clinical significance of anti-RNP antibody positivity, and suggest that knowledge of anti-RNP antibody status may have utility in risk stratification, diagnosis, and

prognostication (e.g., enhancing early diagnosis of severe organ dysfunction, such as pulmonary fibrosis). To the best of our knowledge, this is the first study exploring the association between anti-RNP antibody status and clinicopathologic features in pSS patients of Asian descent, and the first study to demonstrate that RP, thrombocytopenia, and lymphatic system involvement are independently associated with anti-RNP antibody positivity.

Abbreviations

ACA: anti-centromere antibodies; ANA: antinuclear antibody; ORs: Odds ratios; Cis: confidence intervals; CRP:C-reactive protein; CT: computed tomography; ENA: extractable nuclear antigen; ESR: erythrocyte sedimentation rate; ESSDAI: European League Against Rheumatism SS disease activity index; ILD: interstitial lung disease; MCTD: mixed connective tissue disease; MSG: minor salivary gland; pSS: primary Sjögren's syndrome; RA: rheumatoid arthritis; RF: rheumatoid factor; RNP: ribonucleoprotein; RP: Raynaud's phenomenon; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; WBC: white blood cell

Declarations

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee of the Hebei General Hospital (NO.2016070).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the grant from the National Nature Science Foundation of China (No. 81801630) and the National Nature Science Foundation of Hebei Province (No. H2018307047).

Authors' contributions

Lin Wei, Xin Zhifei, and Ning Xiaoran were involved in drafting the manuscript. All authors participated in the study conception and design, participated in the acquisition of data, and analysis and interpretation of data. All authors were involved in revising it critically for important intellectual content, and revising the final version to be submitted for publication.

Acknowledgements

This work was funded by the grant from the National Nature Science Foundation of China (No. 81801630) and the National Nature Science Foundation of Hebei Province (No. H2018307047).

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Figures

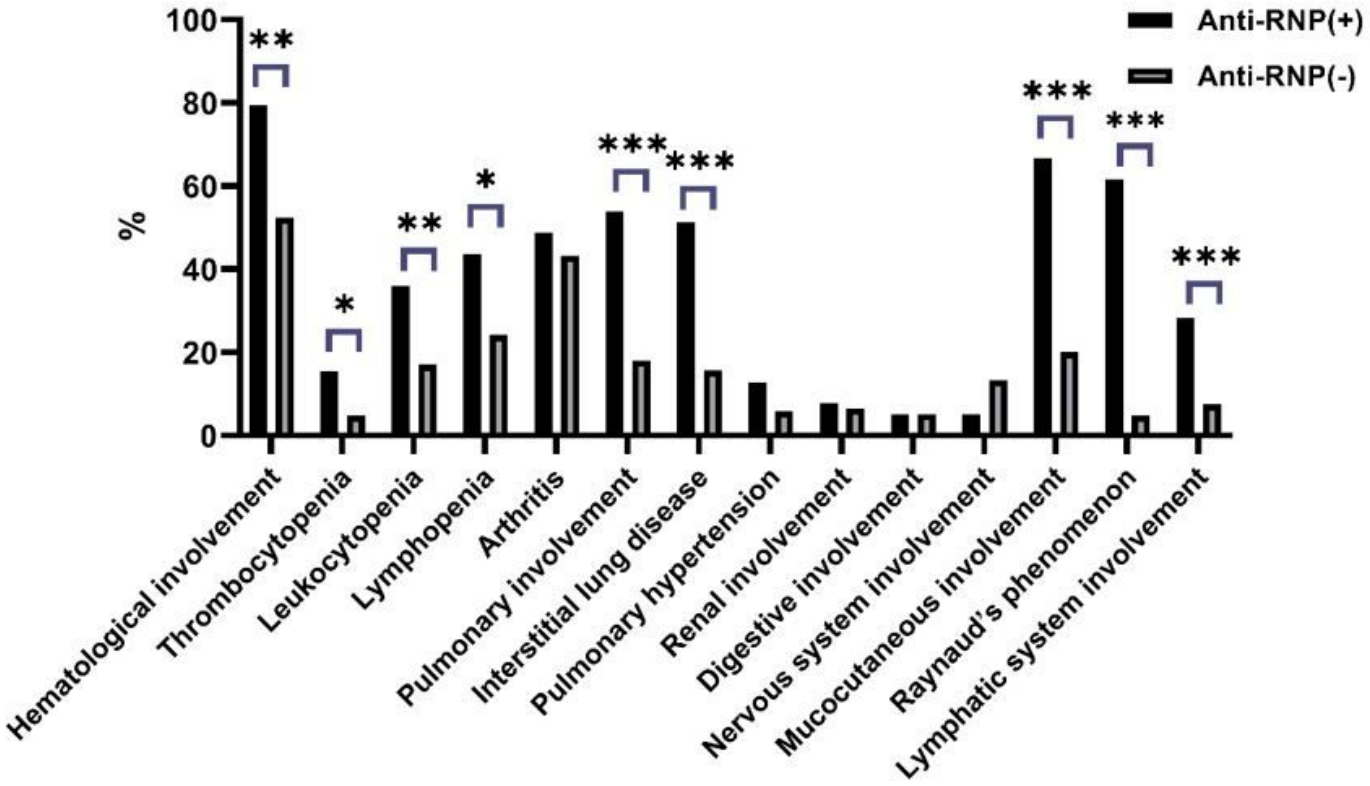
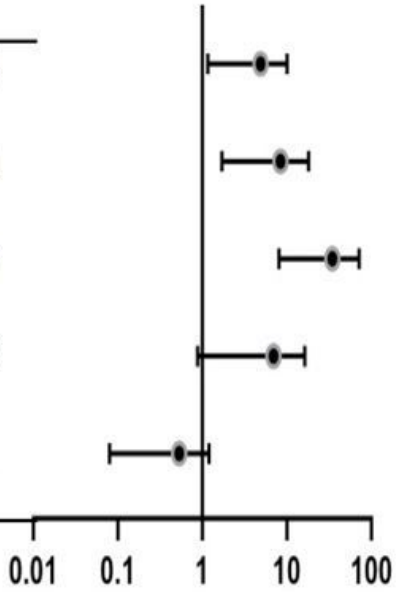


Figure 1

Extraglandular involvements in pSS patients with anti-RNP positive and negative group

	p value	OR	95% CI
Interstitial lung disease	0.03	3.41	1.16-10.04
Lymphatic system involvement	0.04	5.54	1.71-18.01
Raynaud's phenomenon	< 0.001	24.08	8.09-71.69
Thrombocytopenia	0.07	3.78	0.88-16.26
ACA	0.09	0.31	0.08-1.20



CI: indicates confidence interval; and OR, odds ratio.

Figure 2

Multivariate Analysis of features predicting anti-RNP positive in pSS patients.