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Anti-RNP positivity in primary Sjögren's syndrome is associated with a more active disease and a more frequent muscular and pulmonary involvement

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ABSTRACT

Objectives To describe and compare the clinical and biological characteristics of subjects with primary Sjögren's syndrome (pSS) with and without anti-RNP antibodies.

Methods Patients fulfilling the American College of Rheumatology (ACR)/EULAR 2016 criteria for pSS and having anti-RNP antibodies, without other connective tissue disease diagnosed and no anti-dsDNA antibodies were retrieved from the database from our French National Reference Center. These patients were compared with all other patients with pSS with negative anti-Sm, anti-RNP and anti-dsDNA antibodies.

Results Overall, 21 patients with pSS positive for anti-RNP antibodies and 446 negative for anti-RNP antibodies were retrieved. Anti-RNP-positive patients had a lower median age at onset of pSS symptoms (41.0 vs 50.0 years, p=0.01), a higher median EULAR Sjögren's syndrome disease activity index at inclusion (8.0 vs 3.0, p<0.01), more frequently constitutional symptoms (14.3% vs 0.01%, p<0.01), myositis (19.0% vs 2.3%, p<0.01) and pulmonary (19.0% vs 5.7%, p=0.04) involvement. Moreover, anti-RNP-positive patients had higher median gammaglobulin levels (22.5 vs 13 g/L, p<0.01), more frequently anti-SSA antibodies (90.5% vs 67.1%, p=0.03), but less frequent lymphocytic sialadenitis with a focus score ≥1 (66.7% vs 85.5%, p=0.03). If the analysis is restricted to anti-SSA-positive patients, anti-RNP positivity is associated with the same clinicobiologic features except the pulmonary involvement.

Conclusion Patients with pSS with anti-RNP antibodies displayed a more active systemic disease, with more frequent muscular and pulmonary involvement, and increased gammaglobulin level, compared with anti-RNP-negative patients.

Key messages

What is already known about this subject?

- In primary Sjögren's syndrome (pSS), anti-RNP auto-antibodies can be positive without another defined connective disorder.

What does this study add?

- Anti-RNP-positive patients present more myositis (x10), pulmonary involvement (x4) and increased B cell biomarkers.

How might this impact on clinical practice?

- pSS with positive anti-RNP should be monitored for muscular and pulmonary involvement.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is an auto-immune disease that causes lymphocytic infiltration and dysfunction of salivary and lacrimal glands resulting in dryness. In addition to dryness, fatigue and pain are the classical symptoms of the disease. Primary Sjögren's syndrome is characterised by a huge heterogeneity, besides patients suffering only from these invalidating but benign symptoms, at least one-third to two-third of the patients will develop systemic multiorgan involvement associated with an increased morbidity.[1] Anti-SSA antibodies are the main antibodies in pSS.[2] They are present in two-third of the patients, and among them, half have also anti-SSB antibodies.[1] Patients with pSS may have number of other antibodies including rheumatoid factor (RF), anticyclic citrullinated proteins (CCP), anti-Ku, anti-Sm and anti-RNP antibodies. Associations between some of these antibodies and specific subsets of patients have been described. Anti-CCP antibodies have been shown to be associated with articular and pulmonary involvement and a risk of developing rheumatoid arthritis (RA).[3] Anti-Ku antibodies are associated with muscular involvement.[4]

Anti-RNP antibodies target proteins included in the U1 small nuclear

ribonucleoproteins (snRNP) complex; their presence was described to be specific (specificity ranging from 84% to 100%) of mixed connective tissue disease (MCTD).[5] Among undifferentiated connective tissue diseases, Sharp *et al* first described MCTD as a connective tissue disease that combines anti-RNP antibodies with selective features of systemic lupus erythematosus (SLE), RA, polymyositis and systemic sclerosis.[6] Other authors later described that anti-RNP antibodies may be present in defined CTD, and associated with particular clinical features, like scleroderma-like features in patients with SLE.[7] In patients with pSS, whether these auto-antibodies are associated with a specific phenotype or outcome is unknown. In this study, we wanted to describe the clinical and biological characteristics of patients displaying pSS with anti-RNP antibodies.

METHODS

Patient selection

Patients fulfilling the American College of Rheumatology (ACR)/EULAR 2016 criteria for pSS without other CTD diagnosis and having anti-RNP antibodies, without anti-DNA antibodies were searched in the database from the French National Reference Center for pSS in Paris-Sud University.[2] Patients fulfilling Systemic Lupus International Collaborating Clinics (SLICC) criteria for SLE (positive if ≥4) were excluded.[8] We compared these patients with all patients with pSS from the Paris-Sud cohort with negative anti-Sm, anti-RNP and anti-DNA antibodies. Paris-Sud cohort is a prospectively collected database of all patients participating in multidisciplinary sessions to assess a suspicion of pSS since 2000 in the Rheumatology Department of Paris-Sud University Hospital. All patients gave their informed consent to their data collection.

Data collection

We had access to the complete medical files of all patients. The following data were collected: age, sex, patient history, familial history, age at onset of pSS symptoms, EULAR Sjögren's syndrome disease activity index (ESSDAI) score at diagnosis, subjective symptoms of dry eyes and mouth, keratoconjunctivitis sicca (Schirmer's test ≤5 mm/5 min or van Bijsterveld score ≥4 or breakup time test <10 s), objective xerostomia (unstimulated salivary flow rate ≤0.1 mL/min), parotid gland enlargement, extraglandular involvement, treatment, duration between the diagnosis and the last follow-up. Biological and immunological features were collected: antinuclear antibodies (evaluated by indirect immunofluorescence on HEp2 cells), anti-dsDNA antibodies (ELISA), anti-ENA antibodies (multiplex technique Bioplex 2200, Bio-Rad; confirmed with an immunodot assay Euroline ANA Profile 3, Euroimmun) including anti-Ro/SSA and anti-La/SSB antibodies as well as anti-Sm and anti-RNP antibodies (multiplex: purified proteins for anti-Sm, anti-SSA and anti-SSB antibodies, and recombinant for anti-RNP antibodies; immunodot: all purified proteins), RF (nephelometry), myositis and scleroderma dot-blot assay (dot EUROLINE Systemic Sclerosis Profile, Euroimmun). Results of minor salivary gland biopsies were classified according to Chisholm and Mason and focus score (FS) and were considered positive if FS ≥1.[9] For all patients, we assessed if they fulfilled the criteria of MCTD by Sharp *et al*, Alarcon-Segovia and Villareal and Kasukawa and Sharp.[6 10 11]

Statistical analysis

Data were expressed as median (IQR) for continuous variables and number (%) for categorical variables. Comparisons were performed using the Mann-Withney U test for continuous variables and χ^2 test or Fisher's exact test for categorical variables, as appropriate. All p values were two-sided, p values <0.05 were considered as statistically significant. Statistical analyses were carried out using R software (V.3.3.2) and the online tool BiostaTGV.

RESULTS

Characteristics of anti-RNP antibody-positive patients with pSS

At the time of the first evaluation, 21 patients (18 (85.7%) women) were anti-RNP positive and 446 (426 (95.5%) women) were anti-RNP negative (table 1). All patients fulfilled the ACR/EULAR 2016 criteria for pSS and had negative anti-DNA antibodies. Among anti-RNP-positive patients, none had a diagnosis of lupus according to the SLICC criteria, and three fulfilled previously described criteria of MCTD by Kasukawa and Sharp (including two also fulfilling criteria by Alarcon-Segovia and Villareal).

Differences in clinical patterns among anti-RNP-positive and anti-RNP-negative patients with pSS

Anti-RNP-positive patients had a lower median age at onset of pSS symptoms of nearly 10 years (41.0 vs 50.0 years, p=0.01), a higher median ESSDAI at inclusion (8.0 vs 3.0, p<0.01), were more frequently anti-SSA positive (90.5% vs 67.1%, p=0.03), but had less frequently a lymphocytic sialadenitis with a focus score ≥1 (66.7% vs 85.5%, p=0.03) (table 1).

They had more frequently constitutional symptoms (14.3% vs 0.01%, p<0.01), myositis (19.0% vs 2.3%, p<0.01) and pulmonary involvement (19.0% vs 5.7%, p=0.04) (table 1). Four patients had a myositis proven by either MRI (n=1) or histological typical pattern (n=3), with a negative myositis dot-blot assay (table 1). Four patients had an interstitial lung disease (ILD) defined by CT scan evaluation and pulmonary function testing (table 1). The two patients with non- specific interstitial pneumonia also had a myositis and positive criteria of MCTD by Kasukawa and Sharp, dryness and positive anti-SSA or a lymphocytic sialadenitis with a focus score ≥1; both did not present any sign of CTD apart from pSS with a follow-up of 4 and 6 years. The patient with usual interstitial pneumonia had a myositis but negative MCTD criteria. For the five patients who had myositis and/or

Table 1 Characteristics of the 21 patients with primary Sjögren's syndrome with anti-RNP antibodies at inclusion, as compared with other patients with primary Sjögren's syndrome from the Paris-Sud cohort

Characteristics	Primary Sjögren with anti-RNP n=21	Primary Sjögren without anti-RNP (Paris-Sud cohort), n=446	P value
Number of women/men (ratio)	18/3 (6)	426/20 (21.3)	0.0958
Classification			
Subjective xerostomia or xerophthalmia	21 (100.0)	442/446 (99.1)	1
Objective xerostomia or xerophthalmia	**15/16 (93.8)**	**418/446 (93.7)**	**1**
Lymphocytic sialadenitis (focus score ≥1)	**14 (66.7)**	**365/427 (85.5)**	**0.0293**
Median Chisholm score	4.0 (2.0–4.0)	3.0 (3.0–4.0)	0.8503
Positive anti-SSA antibodies	**19 (90.5)**	**298/444 (67.1)**	**0.0288**
Positive anti-SSB antibodies	5 (23.8)	166/442 (37.6)	0.2022
Positive ACR/EULAR 2016 SGS criteria	21 (100.0)	446 (100.0)	–
Positive anti-DNA antibodies	0 (0.0)	0 (0.0)	–
Positive anti-Sm antibodies	6 (28.6)	0 (0.0)	–
Positive anti-RNP antibodies	21 (100.0)	0 (0.0)	–
Positive MCTD criteria at inclusion	3 (14.3)	0 (0.0)	–
Sharp *et al*	0 (0.0)	0 (0.0)	–
Kasukawa and Sharp	3 (14.3)	0 (0.0)	–
Alarcon-Segovia and Villareal	2 (9.5)	0 (0.0)	–
Age at onset of Sjögren's symptoms	**41.0 (28.0–48.0)**	**500 (36.0–62.0)**	**0.0123**
ESSDAI at inclusion	**8.0 (5.0–17.0)**	**3.0 (1.0–6.0)***	**<0.0001**
Systemic manifestations			
Constitutional symptoms	**3 (14.3)**	**2/361 (0.01)**	**0.0013**
Parotid gland involvement	6 (28.6)	170/438 (38.8)	0.3457
Joint involvement	18 (85.7)	335/444 (75.4)	0.4329
Arthralgia	18 (85.7)	320/441 (72.6)	–
Non-erosive arthritis	4 (19.0)	48/438 (11.0)	–
Myalgia	5 (23.8)	132/438 (30.1)	0.5359
Myositis	**4 (19.0)**	**10/436 (2.3)**	**0.0025**
Pulmonary interstitial lung disease	**4 (19.0)**	**25/437 (5.7)**	**0.0366**
NSIP with altered lung diffusion, normal TLC	2 (9.5)	–	–
USIP with altered lung diffusion, decreased TLC	1 (4.8)	–	–
Ground glass opacities and bronchectasis	1 (4.8)	–	–
Cutaneous involvement	4 (19.0)	161/442 (36.4)	0.1042
Isolated sclerodactyly	1 (4.8)	NA	–
Vascular purpura	2 (9.5)	17/436 (3.9)	–
Histology showing leucocytic vasculitis	1 (4.8)	NA	–
Raynaud phenomenon	11 (52.4)	145/437 (33.2)	0.0698
Peripheral nervous system involvement	2 (9.5)	13/419 (3.1)	0.1567
Sensitive polyneuropathy—EMG confirmed	1 (4.8)	NA	–
Trigeminal neuralgia	1 (4.8)	NA	–
Central nervous system involvement	0 (0.0)	5/433 (1.2)	1
Renal involvement	0 (0.0)	1/124 (0.8)	1
Lymphoma	1 (4.8)	16/443 (3.6)	0.5514
Treatment			
Corticosteroids	11 (52.4)	149/438 (34.0)	0.0845
NSAIDs	4 (19.0)	167/432 (38.7)	0.1044
Hydroxychloroquine	11 (52.4)	142/436 (32.6)	0.0602
Methotrexate	3 (14.3)	28/434 (6.5)	0.1650
Cyclophosphamide	3 (14.3)	NA	–

Continued

Table 1 Continued

Characteristics	Primary Sjögren with anti-RNP n=21	Primary Sjögren without anti-RNP (Paris-Sud cohort), n=446	P value
Rituximab	3 (14.3)	NA	–
Azathioprine	2 (9.5)	NA	–
Mycophenolate mofetil	1 (4.8)	NA	–

Results are presented as number (%), or median (IQR).
Bold values are statistically significant.
*Data available for 129 patients.
ACR, American College of Rheumatology; EMG, electromyogram; ESSDAI, EULAR Sjögren's syndrome disease activity index; MCTD, mixed connective tissue disease; NA, not available; NSAIDs, non-steroidal anti-inflammatory drugs; NSIP, non-specific interstitial pneumonia; TLC, total lung capacity; USIP, usual interstitial pneumonia.

ILD, pSS was diagnosed at the same time as anti-RNP positivity.

Another patient developed inflammatory polyarthralgia with a dry mouth at the age of 28 years; his pSS was diagnosed at age 44 years with positive anti-SSA and anti-RNP antibodies, an isolated mild sclerodactyly and a Mucosa Associated Lymphoid Tissue (MALT) lymphoma. The scleroderma dot-blot assay was negative. He was the third patient with positive criteria of MCTD by Kasukawa and Sharp, and the only patient with sclerodactyly; 9 years later, on the last follow-up, he showed no other sign of overlap with scleroderma. No patient presented an erosive arthritis.

Anti-RNP antibodies positivity was associated with increased B cell biomarkers (table 2), with higher median gammaglobulins (22.5 vs 13.0 g/L, p<0.01) and IgG levels (21.4 vs 13.1 g/L, p<0.01), systematic positive ANA (100.0% vs 74.7%, p<0.01), more frequent positive anti-SSA antibodies (90.5% vs 67.1%, p=0.03) and higher median beta-2 microglobulin levels (2.8 vs 2.2 g/L, p=0.03). The frequency of cryoglobulinemia was not significantly higher (4.8% vs 1.7%, p=0.32).

Regarding treatments, patients with anti-RNP antibodies tended to receive more frequently corticosteroids (52.4% vs 34.0%, p=0.08), hydroxychloroquine (52.4% vs 32.6%, p=0.06) and methotrexate (14.3% vs 6.5%, p=0.17), but less frequently non-steroidal anti-inflammatory drugs (19.0% vs 38.7%, p=0.10); none of these tendencies was statistically significant.

Table 2 Biological characteristics of patients with primary Sjogren's syndrome according to anti-RNP positivity

	Primary Sjögren with anti-RNP, n=21	Primary Sjögren without anti-RNP (Paris-Sud cohort), n=446	P value
Positive ANA antibodies	**21 (100.0)**	**328/439 (74.7)**	**0.0035**
Positive RF	10 (47.6)	205/436 (47.0)	0.9570
Anaemia*	3 (14.3)	63/430 (14.7)	1
Thrombopenia*	0 (0.0)	4/429 (0.9)	1
Neutropenia*	0 (0.0)	3/433 (0.7)	1
Lymphopenia*	5 (23.8)	57/433 (13.2)	0.1866
Gammaglobulins or IgG >16 g/L	**16 (76.2)**	**154/438 (35.2)**	**0.0001**
Cryoglobulinemia	1 (4.8)	7/423 (1.7)	0.3235
Low C4†	7 (33.3)	88/406 (21.7)	0.2781
Increased CK*	4/13 (30.8)	3/25 (12.0)	0.2025
Median ANA value	**1/1280**	**1/640**	**0.0369**
Median gammaglobulins, g/L	**22.5 (16.5–30.0)**	**13.0 (10.1–16.3)/422**	**<0.0001**
Median IgG, g/L	**21.4 (15.5–32.3)**	**13.1 (9.9–16.9)/409**	**0.0005**
Median beta-2 microglobulin, mg/L	**2.8 (2.3–4.3)**	**2.2 (1.7–2.7)/384**	**0.0321**
Median C4 value, g/L	0.18 (0.14–0.22)	0.21 (0.16–0.26)/406	0.1054
Median CK value, U/L	119.0 (80.0–508.0)	81.0 (57.0–125.0)/25	0.1029

Results are presented as number (%), or median (IQR).
Bold values are statistically significant.
*Normal CK value <170 U/L, lymphocytes count <1.0 G/L, Hb level <12 g/dL, neutrophils count <1.0 G/L, platelet count <100 G/L.
†C4 value≤0.15 g/L.
ANA, antinuclear antibodies; CK, creatine kinase; EMG, electromyogram; Hb, haemoglobin; RF, rheumatoid factor.

Table 3 Characteristics of the 19 patients with primary Sjögren's syndrome with anti-RNP and anti-SSA antibodies at inclusion, as compared with others patients with primary Sjögren's syndrome from the Paris-Sud cohort with anti-SSA antibodies

Characteristics	Primary Sjögren with anti-RNP and anti-SSA, n=19	Primary Sjögren without anti-RNP (Paris-Sud cohort), n=297	P value
Number of women/men (ratio)	16/3 (5.3)	284/13 (21.8)	0.0627
Classification			
Subjective xerostomia or xerophthalmia	19 (100.0)	293/297 (98.7)	1
Objective xerostomia or xerophthalmia	**13/14 (92.9)**	**260/286 (90.9)**	**1**
Lymphocytic sialadenitis (focus score ≥1)	12 (63.2)	222/279 (79.6)	0.1429
Median Chisholm score	3.5 (2.0–4.0)	3.0 (3.0–4.0)	0.6078
Positive anti-SSA antibodies	19 (100.0)	298/298 (100.0)	–
Positive anti-SSB antibodies	**5 (26.3)**	**161/295 (54.6)**	**0.0186**
Positive anti-Sm antibodies	5 (26.3)	0 (0.0)	–
Positive anti-RNP antibodies	19 (100.0)	0 (0.0)	–
Positive MCTD criteria at inclusion	2 (10.5)	0 (0.0)	–
Sharp *et al*	0 (0.0)	0 (0.0)	–
Kasukawa and Sharp	2 (10.5)	0 (0.0)	–
Alarcon-Segovia and Villareal	2 (10.5)	0 (0.0)	–
Age at onset of Sjögren's symptoms	**41.0 (27.5–47.5)**	**49.0 (36.5–61.0)**	**0.0173**
ESSDAI at inclusion	**8.0 (5.5–16.5)**	**3.0 (1.0–7.0)/89**	**<0.0001**
Systemic manifestations			
Constitutional symptoms	**3 (15.8)**	**1/278 (0.004)**	**0.0009**
Parotid gland involvement	6 (31.6)	136/293 (46.4)	0.2082
Joint involvement	17 (89.5)	211/293 (72.0)	0.1142
Myalgia	4 (21.1)	91/290 (31.4)	0.4464
Myositis	**3 (15.8)**	**8/288 (2.8)**	**0.0243**
Myalgia, 1N<CK<6N	1 (5.3)	NA	–
Myalgia, CK≥6N	2 (10.5)	NA	–
Myositis on the MRI	1 (5.3)	NA	–
Histological confirmation	2 (10.5)	NA	–
Pulmonary interstitial lung disease	3 (15.8)	20/290 (6.9)	0.1589
Cutaneous involvement	4 (21.1)	28/288 (9.7)	0.1224
Raynaud phenomenon	9 (47.4)	98/291 (33.7)	0.2239
Peripheral nervous system involvement	2 (10.5)	8/283 (2.8)	0.1248
Lymphoma	1 (5.3)	13/294 (4.4)	0.5918
Treatment			
Corticosteroids	**9 (47.4)**	**45/288 (15.6)**	**0.0019**
NSAIDs	4 (21.1)	59/286 (20.6)	1
Hydroxychloroquine	**11 (57.9)**	**62/288 (21.5)**	**0.0010**
Methotrexate	2 (10.5)	9/288 (3.1)	0.1429
Biology			
Positive ANA antibodies	19 (100.0)	250/292 (85.6)	0.0877
Positive RF	10 (52.6)	177/290 (61.0)	0.4679
Lymphopenia *	4 (21.1)	38/287 (13.2)	0.3098
Gammaglobulins or IgG >16 g/L	**15 (78.9)**	**152/291 (52.2)**	**0.0310**
Cryoglobulinemia	1 (4.8)	4/281 (1.4)	0.2807
Low C4†	6 (31.6)	69/269 (2.6)	0.5916
Increased CK*	3/13 (23.1)	2/15 (13.3)	0.6389
Median ANA value	1/1280	1/1280	0.5304
Median gammaglobulins, g/L	**24.0 (16.5–30.95)**	**14.0 (12.75–16.25)/40**	**0.0013**

Continued

Table 3 Continued

Characteristics	Primary Sjögren with anti-RNP and anti-SSA, n=19	Primary Sjögren without anti-RNP (Paris-Sud cohort), n=297	P value
Median IgG, g/L	**21.4 (16.5–32.6)**	**14.8 (11.7–18.9)/172**	**0.0057**
Median beta-2 microglobulin, mg/L	2.9 (2.2–4.4)	2.4 (1.9–3.0)/259	0.1377
Median C4 value, g/L	0.18 (0.14–0.22)	0.20 (0.15–0.25)/269	0.2230
Median CK value, U/L	103.0 (77.5–272.5)	72.0 (50.0–84.8)/16	0.0992

Results are presented as number (%), or median (IQR).
Bold values are statistically significant.
*Normal CK value <170 U/L, lymphocytes count <1.0 G/L.
†C4 value≤0.15 g/L.
ANA, antinuclear antibodies; CK, creatine kinase; EMG, electromyogram;ESSDAI, EULAR Sjögren's syndrome disease activity index; MCTD, mixed connective tissue disease; NA, not available;NSAIDs, non-steroidal anti-inflammatory drugs;RF, rheumatoid factor.

When focusing only on patients with anti-SSA+ and comparing patients with and without anti-RNP, we confirmed that anti-RNP positivity in patients with pSS was associated with younger age, a more active disease as assessed by ESSDAI, higher frequency of constitutional symptoms, muscular involvement and higher level of gammaglobulins (table 3). However, the higher frequency of pulmonary involvement was still observed numerically (n=3/19 (15.8%) vs n=20/290 (6.9%)) but was no more statistically significant (p=0.16).

Outcome and follow-up

After a median follow-up of 5 years (IQR 1–15 years), none of the anti-RNP-positive patients developed a defined CTD. Moreover, the 18 patients with negative MCTD criteria but positive anti-RNP antibodies did not present positive criteria of MCTD during follow-up.

DISCUSSION

Our study is the first to focus on the specific characteristics of patients with defined pSS associated with anti-RNP antibodies. When comparing 21 patients with pSS with anti-RNP with 446 patients with pSS without anti-RNP antibodies, we found that the positivity of anti-RNP antibodies was associated with a more active systemic disease as assessed by ESSDAI, particularly with a higher prevalence of some specific organ involvements including myositis (10 times more) and pulmonary involvement (4 times more), and an increase in B cell biomarker levels

Of note, patients with anti-RNP antibodies were also more frequently anti-SSA positive, a feature that has previously been shown to be associated with a higher prevalence of systemic manifestations.[12] However, in our study, when focusing only on patients with pSS with anti-SSA+ only, anti-RNP positivity was still associated with a more active disease and the same specific phenotype demonstrating that anti-RNP and not a higher frequency of anti-SSA was associated with this specific phenotype.

Whether the association between pSS with anti-RNP antibodies and a more active disease is supported by physiopathology or is linked to a differential response to treatments deserves further studies. Implication of toll-like receptor (TLR) 7 has been suggested for patients with pSS, SLE and MCTD, suggesting that they could share a common pathway.[13–15] Actually, nucleic acid contained in RNP can link to TLRs and stimulate their signalling. The involvement of TLR 8 and 9, which is observed in patients with MCTD, is still debated in patients with pSS.[14 15]

Among our anti-RNP-positive patients, 28.6% had anti-Sm antibodies but none fulfilled criteria for SLE or developed an SLE with a median follow-up of 5 years. Anti-Sm antibodies target proteins that bind the small uridine-rich nuclear ribonucleic acids U1, U2, U4 and U5 in the cytoplasm to form snRNP in the nucleus, which explains why they are found in association with anti-RNP.[5]

Despite having positive anti-RNP antibodies, all patients fulfilled criteria for pSS with authentic typical Sjögren's symptoms associated with typical features and no diagnosis of another CTD with a median follow-up of 5 years (IQR 1–15 years). A minority of patients fulfilled criteria for MCTD (n=3, 14.3%), but none presented enough clinical and biological features to suggest an overlap with another CTD with a median follow-up of 5 years.

Overall, our results confirm that the presence of anti-RNP antibodies is associated with certain clinical associations. In patients with pSS, these results support the need for a specific monitoring of muscular and pulmonary involvement in case of anti-RNP positivity in patients with pSS.

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REFERENCES

1. Ramos-Casals M, Brito-Zerón P, Seror R, *et al*. Characterization of systemic disease in primary Sjögren's syndrome: EULAR-SS Task force recommendations for articular, cutaneous, pulmonary and renal involvements. *Rheumatology* 2015;54:30–8.
2. 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;69:35–45.
3. Payet J, Belkhir R, Gottenberg JE, *et al*. Acpa-Positive primary Sjögren's syndrome: true primary or rheumatoid arthritis-associated Sjögren's syndrome? *RMD Open* 2015;1:e000066.
4. Cavazzana I, Ceribelli A, Quinzanini M, *et al*. Prevalence and clinical associations of anti-Ku antibodies in systemic autoimmune diseases. *Lupus* 2008;17:727–32.
5. Benito-Garcia E, Schur PH, Lahita R, *et al*. Guidelines for immunologic laboratory testing in the rheumatic diseases: anti-Sm and anti-RNP antibody tests. *Arthritis Care Res* 2004;51:1030–44.
6. Sharp GC, Irvin WS, Tan EM, *et al*. Mixed connective tissue disease--an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med* 1972;52:148–59.
7. ter Borg EJ, Groen H, Horst G, *et al*. Clinical associations of antiribonucleoprotein antibodies in patients with systemic lupus erythematosus. *Semin Arthritis Rheum* 1990;20:164–73.
8. Petri M, Orbai A-M, Alarcón GS, *et al*. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–86.
9. Chisholm DM, Mason DK. Labial salivary gland biopsy in Sjögren's disease. *J Clin Pathol* 1968;21:656–60.
10. Alarcon-Segovia D, Villareal M. Classification and diagnosis criteria for mixed connective tissue disease. In: Kasukawa R, sharp G, eds. *Mixed connective tissue disease and antinuclear antibodies*. Amsterdam: Elsevier Science, 1987: 33–40.
11. Kasukawa R, Sharp G. *Mixed connective tissue disease and antinuclear antibodies*. 357. Amsterdam: Elsevier Science, 1987.
12. Bournia V-K, Vlachoyiannopoulos PG. Subgroups of Sjögren syndrome patients according to serological profiles. *J Autoimmun* 2012;39:15–26.
13. Paradowska-Gorycka A. Review papers U1-RNP and Toll-like receptors in the pathogenesis of mixed connective tissue disease Part II. endosomal TLRs and their biological significance in the pathogenesis of mixed connective tissue disease. *Reumatologia/Rheumatology* 2015;3:143–51.
14. Karlsen M, Jakobsen K, Jonsson R, *et al*. Expression of Toll-like receptors in peripheral blood mononuclear cells of patients with primary Sjögren's syndrome. *Scand J Immunol* 2017;85:220–6.
15. Goules AV, Kapsogeorgou EK, Tzioufas AG. Insight into pathogenesis of Sjögren's syndrome: dissection on autoimmune infiltrates and epithelial cells. *Clin Immunol* 2017;182:30–40.