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

Salam Abbara,1 Raphaele Seror,1,2 Julien Henry,1 Pascale Chretien,3 Aude Gleizes,3,4 Salima Hacein-Bey-Abina,3,5 Xavier Mariette,1,2 Gaetane Nocturne1,2

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-SS, 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 clinico-biologic 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.

INTRODUCTION

Primary Sjögren’s syndrome (pSS) is an autoimmune 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. Anti-SSA antibodies are the main antibodies in pSS. They are present in two-third of the patients, and among them, half have also anti-SSB antibodies. 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). Anti-Ku antibodies are associated with muscular involvement.

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). 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. 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. 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. Patients fulfilling Systemic Lupus International Collaborating Clinics (SLICC) criteria for SLE (positive if ≥1) were excluded. We compared these patients with all patients 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. For all patients, we assessed if they fulfilled the criteria of MCTD by Sharp et al, Alarcon-Segovia and Villareal and Kasukawa and Sharp.

Statistical analysis
Data were expressed as median (IQR) for continuous variables and number (%) for categorical variables. Comparisons were performed using the Mann-Whitney U test for continuous variables and χ² 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 0.1%, 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)                          | 296/444 (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
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 1

| 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.

### Table 2

| Positive ANA antibodies                   | Primary Sjögren with anti-RNP, n=21 | Primary Sjögren without anti-RNP (Paris-Sud cohort), n=446 | P value |
|------------------------------------------|-------------------------------------|-------------------------------------------------------------|---------|
| Positive RF                              | 21 (100.0)                          | 328/439 (74.7)                                              | 0.0035  |
| Anaemia*                                 | 10 (47.6)                           | 205/436 (47.0)                                              | 0.9570  |
| Thrombopenia*                            | 3 (14.3)                            | 63/430 (14.7)                                               | 1       |
| Neutropenia*                             | 0 (0.0)                             | 4/429 (0.9)                                                 | 1       |
| Lymphopenia*                             | 0 (0.0)                             | 3/433 (0.7)                                                 | 1       |
| 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 values: 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
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 none of the anti-RNP-positive patients 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.

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.

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.

Table 3

| 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.

1Normal CK value <170U/L, lymphocytes count <1.0G/L.
2C4 value<0.15g/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.

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. 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. 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.

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.

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. 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.

Author affiliations
1Department of Rheumatology, AP-HP, Paris-Sud University Hospitals, Le Kremlin-Bicêtre Hospital, Le Kremlin-Bicêtre, France
2INSERM U1184, Center for Immunology of Viral Infections and Autoimmune Diseases, Paris-Sud University, Le Kremlin-Bicêtre, France
3Department of Immunology, Hôpital Kremlin Bicêtre, Assistance Publique–Hôpitaux de Paris (AP-HP), Le Kremlin-Bicêtre, France
4Université Paris-Sud, INSERM UMR 996, Faculty of Pharmacy, Université Paris-Saclay, Châtenay-Malabry, France
5UTGBS, CNRS UMR 8258, INSERM U1022, Faculté de Pharmacie de Paris, Université Sorbonne-Paris-Cité, Université Paris-Descartes, Paris, France

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http://orcid.org/0000-0001-8486-433X

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