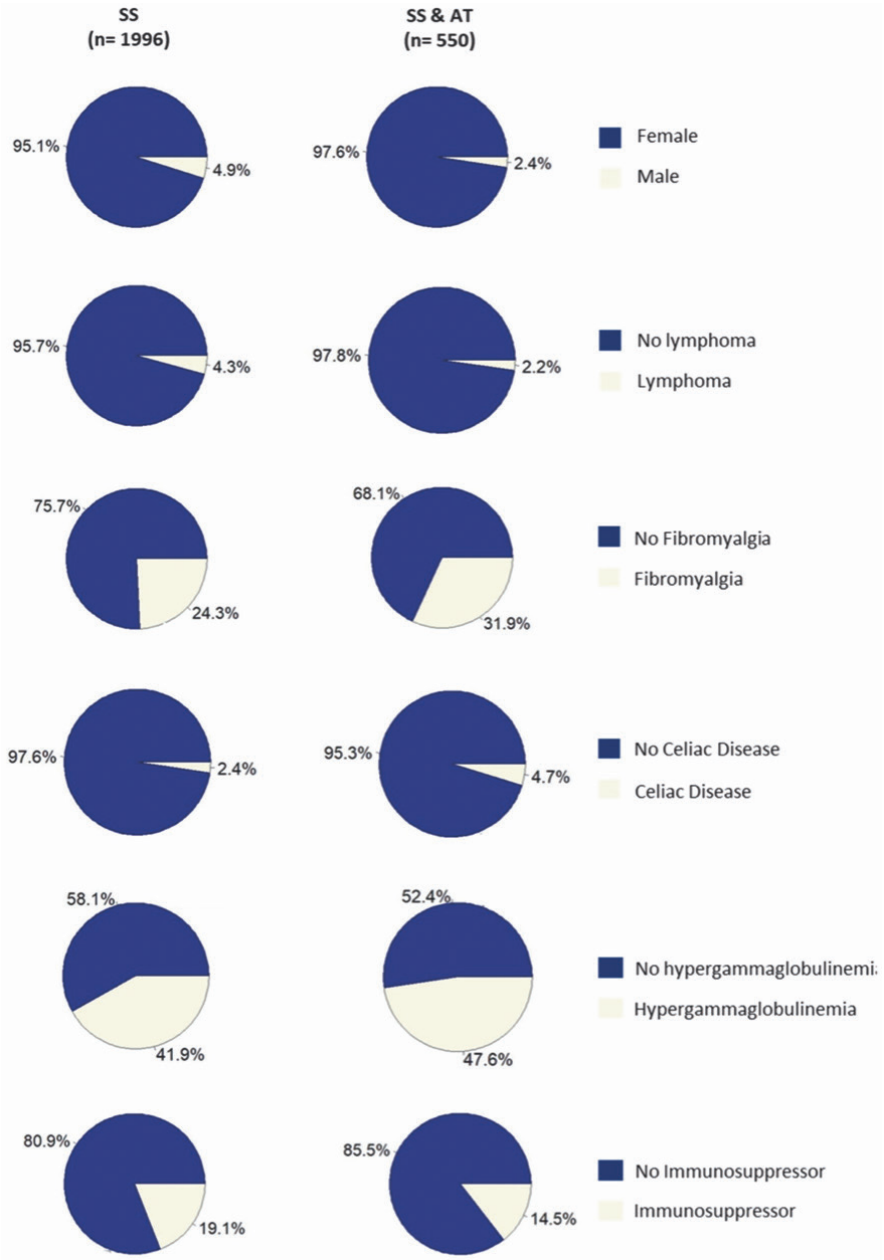
Supplementary material

Immunostaining (H&E and immunohistochemistry) on MSG

Paraffin embedded MSG were cut and stained in H&E in order to identify the presence of a Focal Lymphocytic Sialoadenitis (FLS), to identify the presence of foci (focus defined as a lymphocytic infiltrate composed by at least 50 cells), and to calculate the focus score (FS), the mean foci area and the percentage of infiltration. Immunohistochemistry (IHC) staining were performed with the purpose of detecting infiltrating T and B Lymphocytes as well as FDCs. The following antibodies were used: rabbit polyclonal antibody anti CD3 (DAKO); mouse monoclonal antibody anti-CD20 (DAKO), mouse monoclonal antibody anti-CD21 (DAKO)]. Paraffin embedded sections measuring 3µm in thickness (sequential to the H&E sections) were deparaffinised and rehydrated and following the antigen retrieval procedure (high temperature in citrate buffer), peroxidase and protein blocking was performed. For detection of CD3 and CD20 co-localization, the En Vision G/2 Doublestain System DAKO Kit was used. After 1 hour incubation with the primary antibody (anti-CD3), Dextran polymer conjugated with horseradish peroxidase and affinity isolated Immunoglobulins (Polymer/HRP) was incubated for 10 min. DAB + Cromogen was added for CD3+ cells detection. After 5 minutes of Doublestain Block the primary antibody (anti-CD20) was incubated for 1h. After washing, ten minutes of incubation with Dextran polymer coupled with the secondary antibody [Rabbit/Mouse (LINK] and ten minutes of incubation with Dextran polymer conjugated with alkaline phosphatase (Polymer/AP) were performed. Permanent Red Chromogen was used for CD20+ cells detection. For detection of CD21+ cells detection, single staining sequential to CD3/CD20 section was performed. After 1 hour incubation with the primary antibody (anti-CD21), Dextran polymer conjugated with horseradish peroxidase and affinity isolated Immunoglobulins (Polymer/HRP) was incubated for 10 min. DAB + Cromogen was added for CD21+ cells detection.



Supplementary Fig. S1. Main clinical and laboratory features differing between patients with isolated pSS and patients with pSS and associated AT.

MSG image analysis

Stained slides were scanned by Zeiss Axio Scan.

Histological parameters were calculated/detected as follows:

- Focus Score: [total number of foci/ total sg area (mm2)] * 4 mm2;
- GCs-like structures: lymphocyte aggregates identified by H&E with evidence of a "dark zone" and "light zone"; confirmed by CD3+ and CD20+ IHC staining with evidence of segregation in B and T cells areas and CD21+ staining.
- LEL: lymphocytes B (CD20+) infiltrating ductal epithelial cells.
- Fibrosis: collagen deposition with varying degrees of architectural distortion.

Supplementary Table S1. Demographic, clinical and laboratory features of patients with isolated pSS and patients with pSS and associated AT enrolled for the histological analysis.

	Isolated pSS n = 169	pSS & AT n = 54	*p* value
Female/Male (F%, M%)	161/8 (95.2%, 4.8%)	54/0 (100%, 0%)	0.103
Age (mean ± SD)	54.1 ± 10.1	53.5 ± 14.6	0.755
ANA (%)	118/169 (69.8)	31/54 (57.4)	0.091
Anti-Ro/SSA (%)	77/169 (54.5)	23/54 (42.5)	0.702
Anti-La/SSB (%)	37/169 (21.8)	11/54 (20.3)	0.812
Rheumatoid factor (%)	56/169 (33.1)	5/54 (9.2)	**0.006**
Hypergammaglobulinemia (%)	61/169 (36)	19/54 (35.1)	0.903
Hypocomplementemia (%)	28/169 (16.5)	8/54 (14.8)	0.760
Cryoglobulins (%)	1/169 (0.5)	0/54 (0)	0.571
Monoclonal component (%)	13/169 (7.6)	2/54 (3.7)	0.308
Leukopenia	35/169 (20.7)	9/54 (16.6)	0.515
NHL	3/169 (1.7)	0/54 (0)	0.324
ESSDAI at biopsy (mean ± SD)	1.7 ± 2.8	1.3 ± 2.2	0.755

ANA: anti-nuclear antibodies; Hypergammaglobulinemia: gammaglobulins >16 g/L; Hypocomplementemia: C3<80 and/or C4<15 mg/dl; Leukopenia: neutrophils <1500 mm^3/lymphocytes <1000 mm^3; RF: rheumatoid factor; SD: standard deviation; NHL: non-Hodgkin lymphoma.