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ORIGINAL ARTICLE

Demographic, clinical, and laboratory features of Patients with Seronegative Primary Sjögren's Syndrome in Western Algeria

Les caractéristiques démographiques, clinico-biologiques et histopathologiques des patients atteints du Syndrome de Sjogren Primitif Séronégatif dans l'Ouest algérien

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KEYWORDS

Primary Sjogren's syndrome;
Seronegative;
Autoimmune thyroiditis;
Hypergammaglobulinemia;
Erythrocyte Sedimentation Rate;
Minor salivary gland biopsy

Abstract

Introduction: To investigate the clinical and immune features of patients with primary Sjögren's syndrome (pSS) negative for auto antibodies: anti-SSA/Ro, anti-SSB/La, Anti-Nuclear Antibodies (ANA) and Rheumatic Factor (RF) in a population of Western Algeria.

Studied population and Methods: 65 patients with pSS in western Algeria were retrospectively examined between 2021 and 2023. Patients with negative anti SSA/Ro, anti SSB/La, ANA, and RF antibodies comprised the seronegative group, while patients with at least one positive antibody were included in the seropositive group. Clinical manifestations, laboratory and histopathological findings were compared between the two groups.

Results: Among the 65 patients with pSS, 16 (24.6%) were seronegative. The most common clinical manifestations in the seronegative patient group are: xerostomia (87.5%), xerophthalmia (81.3%) and joint involvement (68.8%). All of these patients have positive salivary gland biopsies showing a focus score ≥ 1 (100%). Compared to seropositive pSS, seronegative pSS has a low proportion of female gender ($p = 0.029$). Among clinical manifestations, Endocrine involvement and Association with a specific auto immune disease exhibited a lower prevalence within the seronegative group ($p = 0.006$ and $p = 0.014$ respectively). Among biological and immunological characteristics, elevated sedimentation rate was more frequent in seronegative group ($p = 0.03$) while hypergammaglobulinemia was more frequent in seropositive group ($p = 0.025$).

MOTS CLÉS

Syndrome de Sjogren primitif ;
Séronégatif ;
Thyroïdite auto-immune ;
Hypergammaglobulinémie ;
Vitesse de sédimentation ;
Biopsie des glandes salivaires mineures

Conclusion: The seronegative pSS is distinct from seropositive pSS, indicating possible different underlying pathogenesis mechanisms. The absence of conventional serologic markers may reflect limitations in current tools, rather than true immunological silence. Novel (not part of diagnostic criteria) antibodies may play a role in identifying this group of pSS patients.

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Résumé

Introduction : Étudier les caractéristiques démographiques, clinico-biologiques, histopathologiques des patients atteints du syndrome de Sjögren primitif (SSp) négatifs aux autoanticorps : anti-SSA/Ro, anti-SSB/La, anticorps antinucléaires (ANA) et facteur rhumatoïde (FR) dans une population de l'Ouest algérien.

Population étudiée et Méthodes : 65 patients atteints de SSp dans l'Ouest algérien ont été examinés rétrospectivement entre 2021 et 2023. Les patients présentant des anticorps anti-SSA/Ro, anti-SSB/La, ANA et FR négatifs constituaient le groupe séronégatif, tandis que les patients présentant au moins l'un de ces autoanticorps positifs étaient inclus dans le groupe séropositif. Les données démographiques, les manifestations cliniques, les résultats biologiques et histopathologiques ont été comparés entre les deux groupes.

Résultats : Parmi les 65 patients atteints de SSp, 16 (24,6 %) étaient séronégatifs. Les manifestations cliniques les plus fréquentes chez les patients séronégatifs sont : la xérostomie (87,5 %), la xérophtalmie (81,3 %) et l'atteinte articulaire (68,8 %). Tous ces patients avaient une biopsie des glandes salivaires positive, montrant un score focus ≥ 1 (100 %). Comparativement au SSp séropositif, le groupe SSp séronégatif présente une faible proportion de femmes ($p = 0,029$). Parmi les manifestations cliniques, l'atteinte endocrinienne et l'association à une maladie auto-immune spécifique présentaient une prévalence plus faible au sein du groupe séronégatif ($p = 0,006$ et $p = 0,014$ respectivement). Parmi les caractéristiques biologiques et immunologiques, une vitesse de sédimentation élevée était plus fréquente dans le groupe séronégatif ($p = 0,03$) tandis que l'hypergammaglobulinémie était plus fréquente dans le groupe séropositif ($p = 0,025$).

Conclusion : Le SSp séronégatif se distingue du SSp séropositif, ce qui suggère des mécanismes pathogéniques sous-jacents différents. L'absence de marqueurs sérologiques conventionnels pourrait refléter les limites en outils d'exploration actuels, plutôt qu'un véritable silence immunologique. De nouveaux anticorps (ne faisant pas partie des critères diagnostiques) pourraient jouer un rôle dans l'identification de ce groupe de patients atteints de SSp.

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Introduction

Primary Sjögren's syndrome (pSS) is a systemic rheumatism autoimmune disorder that predominantly affects middle-aged women [1]. Patients with SS present have a spectrum of clinical manifestations from sicca symptoms (eye and/or mouth dryness), constitutional symptoms (fatigue, myalgia, and arthralgia) to potentially severe extra glandular and/or systemic features, such as interstitial pneumonia, neurological dysfunction, renal disease, autoimmune cytopenia, cutaneous vasculitis, synovitis, myositis and lymphoma as a rare and severe complication [1,2,3].

The typical cases of pSS have emphasized the presence of dry eyes and mouth, the presence of anti-SSA/Ro antibodies and/or lymphocytic infiltration of salivary gland as main criteria to support diagnosis. Because of its nonspecific symptoms, diversity of clinical manifestations and lack of standardized diagnostic methods, the most appropriate diagnostic criteria are still being debated, and misdiagnosis rates are high in the early stages of the disease. Therefore, many patients with SS are not accurately diagnosed until the onset of more serious complications [4].

The hallmark of pSS pathogenesis is B cell hyperactivity and consequently, autoantibodies production such as antinuclear antibodies (ANA; including anti-Ro60/ Ro52/La) and rheumatoid factor (RF). Patients fulfilling pSS criteria who do not express classic serum antibodies are called patients with seronegative pSS [5].

In line with the possible relationship between the presence of autoantibodies and the clinical and laboratory findings, the question arose whether seronegative pSS patients may have different characteristics. This study was designed to examine whether disparities exist in the demographic, clinical, and laboratory features of patients with pSS who exhibit quadruple (ANA, anti-SSA/Ro, anti-SSB/La, and RF) antibody negativity.

On a research level, understanding why a subset of patients with pSS do not produce serum autoantibodies would be instrumental in understanding its immunopathology and developing targeted therapeutic strategies.

Studied population and Methods

Studied Population

Our study was conducted at the University Hospital Center (EHU) 1st November 1954 in Oran, from 2021 to 2023. This study included 65 patients diagnosed with primary Sjögren's syndrome (pSS) according to the 2016 ACR/EULAR classification criteria.

pSS Patients who presented with an organ-specific autoimmune disease were included in the study.

Patients with secondary Sjögren's syndrome or those with another systemic autoimmune disease were excluded.

Epidemiological, clinical, and histopathological data were collected using a questionnaire filled out by clinicians from the relevant departments, including internal medicine, nephrology, and rheumatology.

Methods

The immunological workup of the patients was carried out in the Immunology Department. The detection of antinuclear antibodies (ANA), anti-SSA/Ro and anti-SSB/La antibodies, as well as the rheumatoid factor (RF), was performed respectively by indirect immunofluorescence on Hep-2 cells, immunodot assay, and latex agglutination.

Serum protein electrophoresis was performed for all patients. Cryoglobulins were only searched for, quantified, and typed in patients with pSS presenting symptoms suggestive of cryoglobulinemic disease.

Patients were classified as having seropositive or seronegative primary Sjögren's syndrome (pSS) based on autoantibodies results. Seropositive pSS was defined by a positive result for at least one of the four autoantibodies (anti-Ro, anti-La, ANA, and RF). Seronegative pSS was defined by a negative result for all four autoantibodies.

Statistical Analysis

All statistical analyses were performed using SPSS software, version 26. Categorical data were expressed as counts and percentages (%), while continuous variables were expressed as means $\pm$ standard deviation (SD).

For comparisons between the two groups—seronegative and seropositive—the Chi-square test (χ^2) (2x2 contingency tables) was used. The classical evaluation of χ^2 is valid when expected frequencies exceed 5. Otherwise, a corrected χ^2 must be applied, using either Yates' correction (if expected frequency is <5) or Fisher's exact test (if <3).

Odds ratios (OR) were reported with 95% confidence intervals (CI). A two-tailed p-value < 0.05 was considered statistically significant.

Results

Descriptive analysis of studied population

Among the 65 pSS patients included in this study, 49 (75.4%) were seropositive and 16 (24.6%) were seronegative (Figure 01).

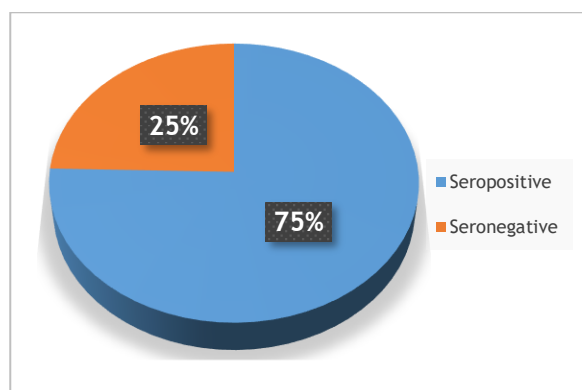


Figure 1: Distribution of patients according to autoantibody status

The most frequent clinical manifestations in the seropositive group were: xerostomia (87.2%), xerophthalmia (85.1%), articular involvement (76.6%) and endocrine involvement (43.2%).

In this group, at least one of the following autoantibodies was present: antinuclear antibodies (ANA), anti-SSA/Ro, anti-SSB/La or rheumatoid factor (RF). The most prevalent autoantibodies are: ANA (93.2%), Anti-SSA/Ro (91.8%), followed by: Anti-SSB/La (37.8%) and RF (66.7%). Cryoglobulins were found in 3 out of 15 tested patients (20%) (Figure 02).

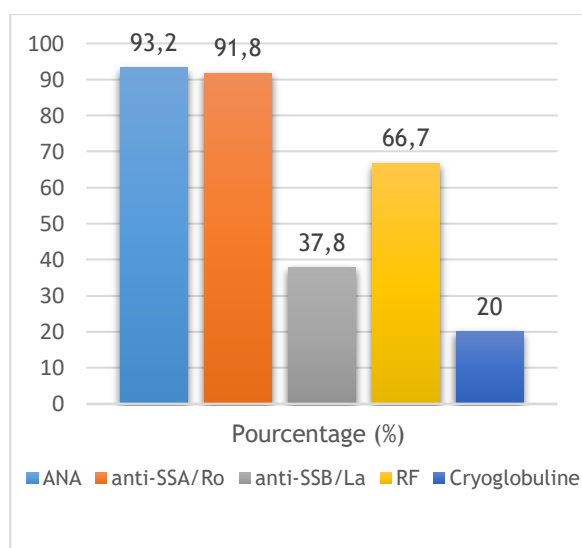


Figure 2: Autoantibody distribution among seropositive patients

88.8% of these patients had a positive salivary gland biopsy, with 34.4% at stage III and 53.1% at stage IV according to Chisholm and Mason score. In the seronegative group, the most frequent clinical manifestations were: xerostomia (87.5%), xerophthalmia (81.3%) and articular involvement (68.8%). All seronegative patients had a positive salivary gland biopsy with a focus score ≥ 1 (100%).

Comparative analysis between seropositive and seronegative groups

Compared to seropositive pSS, seronegative pSS had a lower proportion of female patients ($p = 0.029$) (Table 01).

Regarding clinical manifestations, endocrine involvement was more frequent in the seropositive group (43.8%) than seronegative group (6.3%) with an Odds ratio of 7 (CI=1.02-48). This difference is statistically significant ($p = 0.006$). Seronegative patients are less susceptible to develop a specific autoimmune disease than seropositive patients (0% vs 28.9% respectively). This difference is statistically significant ($p = 0.014$). All other clinical characteristics are not statistically different between the two groups (Table 01).

For biological and immunological parameters, elevated erythrocyte sedimentation rate (ESR) was more common in the seronegative group (46.2%) than seropositive group (16.2%) with a statistically significant difference ($p = 0.03$, OR= 1.56 (CI=0.92-2.63)). However, hypergammaglobulinemia was more frequent in the seropositive group (55.1%) compared to the seronegative one (18.8%). The difference is statistically significant and is multiplied by 2.9 (CI=1.03-8.4). All other laboratory findings are not statistically different between the two groups (Table 01).

The frequency of seronegative pSS patients with a lymphocytic focus score of ≥ 1 in the minor salivary gland biopsies (100%) was higher than that of seropositive pSS (88.9%) patients but without significant difference ($p = 0.3$) (Table 01).

Discussion

Patients with autoimmune diseases (AID) may not show any autoantibody, a condition that is referred to as seronegative AID. Although relatively rare, seronegative AID poses a peculiar challenge in clinical practice and may lead to false or delayed diagnoses. In case of seronegativity, the diagnostic approach is usually based, when feasible, on histological evidence of tissue damage related to immune cell infiltration, or may rely on other clinical, laboratory, and instrumental criteria. In some cases, seronegative AID is a mere diagnosis of exclusion [6].

There is no universally accepted definition of which autoantibodies should define seropositive from seronegative pSS [5]. While certain studies exclusively focused on the absence of anti-SSA/Ro and anti-SSB/La antibodies [7, 8, 9]. Few other studies exploring the concept of quadruple negativity [10, 11, 12, 13, 14].

Proportion of seronegative pSS patients

The proportion of seronegative patients in our study is 24.6%. This proportion is elevated than the proportion found in Yalya et al (10.9%), Yaziris et

al (15.5%), Karahan and Karabulut (16.5%) and Chatsiz et al (4.6%) studies. It is included in rang of 8% to 37% reported in literature. This rang include all studies on seronegative pSS no matter how it is defined [5].

Demographic features

In term of demographic findings, feminine gender is statistically less frequent in seronegative group in our study ($p=0.029$). Chen et al were reported a similar result in their study of comparison between double seronegative and seropositive pSS patients (anti-SSA/Ro and anti-SSB/La) (75.3% vs. 90.6% respectively, $p < 0.001$) [7]. The pSS is generally a disease with feminine predominance. The lower proportion of women in the seronegative group may reflect a different clinical phenotype of pSS, possibly less influenced by hormonal factors.

No difference in age of onset of symptoms or diagnostic has been reported in our study. Contrary to the study of Karahan and Karabulut, which reported that patients with seronegative pSS were diagnosed later ($p=0.026$) [11]. The same result has been reported in Bodakci's study, the age at diagnosis of quadruple-seropositive pSS patients was significantly younger than that quadruple-seronegative patients. The interval between the onset of sicca symptoms and diagnosis was the shortest in quadruple-seropositive patients, averaging 23 months, whereas it was the longest in quadruple-seronegative patients, averaging 60 months ($p < 0.0001$). This shows that the presence of autoantibodies in patients with pSS aids early diagnosis [14].

Clinical features

In our study, mouth, eye and cutaneous dryness are distributed with the same frequency between the two groups. However, urogenital dryness and parotid swelling are most prevalent in seronegative pSS without significant difference. This result is discordant with result reported by Yayla et al, in which dry mouth was found to be more prevalent among seronegative patients ($p = 0.003$) [12] and Chatsiz et al, in which quadruple seronegative patients presented a high frequency of dry eyes (98.6% vs. 87.5% $p=0.01$) [13]. This suggests that seronegative patients had a limited glandular symptomatology.

In our study, the most clinical manifestations had not a different distribution between the two groups. This is similar to results of Karahan and Karabulut [11] and Chatsiz et al [13] studies. Autoantibody status is considered as the main factor driving the phenotypic expression of pSS and may help identify subgroups of patients with poor prognosis. Autoantibody-related and circulating immune complexes mechanisms play a major role in organ damage such as vasculitis, Raynaud's phenomenon, and cryoglobulinemia. This suggests that seropositive had more systemic

manifestations than seronegative patients [14]. This suggestion is discordant with our finding and findings of another studies (no significant difference were found in extra glandular manifestations between the two groups). It may be explained by the fact that the definition of seronegative group is not correct. Another autoantibodies not researched in routine may explain the similarity in frequency of systemic manifestations between seronegative and seropositive groups [6, 15]. In addition, the clinical expression of the disease should not be interpreted only from the side of the effector arm (e.g. autoantibodies) but the counter immunoregulation should be also considered.

In our study, the only extra glandular clinical manifestations which demonstrated a statistically significant difference between the two groups are endocrinal involvement ($p=0.006$) and association with specific autoimmune diseases (autoimmune thyroiditis was the most prevalent) ($p=0.014$). In the study of Karahan and Karabulut, Thyroid dysfunction is more frequent in seronegative group (26.7% vs 17.5%) but without significant difference ($p=0.154$) [11]. Similar to our result, Quadruple seronegative patients presented a high frequency of autoimmune thyroiditis (44.1% vs. 17.1% $p=0.02$) in Chatsiz et al study [13].

Sjögren's syndrome is a complex and heterogeneous autoimmune disease that frequently co-occurs with organ-specific autoimmune diseases. Autoimmune thyroid disease and SS can be considered as pathogenetically correlated. First, these two disorders are characterized by similar histological features with a tissue infiltrate that consists primarily of CD4+ T lymphocytes and the possible formation of germinal center-like structures unrevealing B cell activation [8]. Second, the two conditions present a similar genetic background with thyroid and epithelial cells expressing the same HLA molecules class II: HLA-B8 and HLA-DR3. Another point reinforcing the pathogenetical link between this two diseases is represented by the crucial role of the epithelial cells in orchestrating the tissue inflammation [16]. The finding that seronegative patients presented less thyroid dysfunction and association with autoimmune thyroiditis might unleash strong local and systemic immunoregulatory mechanisms, capable of restricting the autoimmune response confined to the epithelial structures of exocrine glands avoiding in this way wide-spread immune responses against another self-antigens.

Laboratory features

In terms of biological findings, hypergammaglobulinemia was identified more frequently in the seropositive group (55.1% vs 18.8% respectively, $p=0.025$). This result is

concordant with Yazisiz et al ($p=0.05$) [10], Yalya et al ($p=0.004$) [12], Bodakci (<0.0001) [15] but different from the finding of Karahan and Karabulut ($p=0.76$) [11]. While classic pSS involves B-cell hyperactivity and autoantibody production, seronegative patients may exhibit more subtle T-cell-driven inflammation, local cytokine activation and neuro immune dysregulation. Histologic evidence of focal lymphocytic sialadenitis supports an immune-mediated process, even in the absence of detectable autoantibodies. Non-immune mechanisms such as autonomic dysfunction or acinar cell apoptosis may predominate [17].

Another reasons which may explain the difference between seronegative and seropositive patients is underlying etiology as genetics and epigenetics. Several variants in the HLA region were found to be unique to patients with seropositive pSS [18]. Some observations on epigenetic regulation have also reported the upregulated expression of IFN-induced genes, for example, the IFN signature, was mainly seen in patients with seropositive pSS [18,19]. The HLA class II and interferon may participate in the initiation and perpetuation of an autoimmune response respectively in anti-SSA/Ro positive pSS patients.

Another biological result which is found significantly different between the two groups is the elevated sedimentation rate (seronegative 46.2% vs seropositive 16.2%, $p=0.03$) with an odds ratio of 1.56 (CI=0.92-2.63). In Literature, the difference was statistically significant but in the inverse sense (seronegative 16.5% vs seropositive 33%, $p=0.044$) [8], (seronegative 39.5% vs seropositive 54.4%, $p<0.001$) [7].

To explain this discrepancy, the seronegative group may be subdivided into subtypes, some are marked by pronounced inflammatory response and others with a modest one. Although seropositivity is generally associated with systemic manifestations, the elevated erythrocyte sedimentation rate in some seronegative patients may suggest alternative inflammatory pathways unrelated to classical autoantibody mediated mechanisms such as cytokine production and epithelial cells activation. Implication of innate immunity in seronegative pSS is proven in Han's study [9].

In pathological examination, our seronegative group had a high frequency of score focus ≥ 1 compared with seropositive group (100% vs 89% respectively) but without significant difference ($p=0.3$). This finding is different from Karahan and Karabulut's study, in which the Focus score ≥ 1 was statistically more prevalent in seronegative group (100% vs 68.9% respectively, $p=0.005$) [11] and Yazisiz's study (96.6% vs 68.4% respectively, $p<0.001$) [10]. This discordance may be explained by the fact that autoantibodies and/or focal lymphocytic infiltration are the two main criteria for pSS diagnosis. When autoantibodies are present

(seropositive group), histopathological examination is no longer needed. However, in absence of classical autoantibodies, especially anti-SSA/Ro (seronegative group), the diagnosis of pSS can't be established without positive histological result. This may create a selection bias. This discordance may be caused by the heterogeneity of the definition of a positive result, the collection conditions and interpretation expertise.

Conclusion

Seronegative and seropositive patients with SS appear to display some different demographic (low female prevalence), clinical features (the low rate of association with autoimmune thyroiditis), laboratory features (normal gammaglobulinemia, elevated sedimentation rate). It may be explained by different immunoregulatory, underlying pathogenesis pathways or etiology.

There were several limitations in our study. Firstly, this study was performed in Western Algeria and the sample size included was relatively small, especially the seronegative group of patients pSS. More multi-center studies are needed to validate our results and clarify the features of this distinct subtype.

Secondly, limited by retrospective nature of this study, our results could have been compromised by the bias due to the missing of some clinical and laboratory information.

Thirdly, the retrospective nature of this study and the short median follow-up period of the patients prevented an investigation into the development and frequency of malignancy. Therefore, the effect of seronegativity on lymphoma development could not be explored.

Fourth, we defined our seronegative patients by the absence of the four autoantibodies, so, our discussion was restricted to studies which used the same definition.

In term of perspectives, unknown antibodies may play a role in autoimmune diseases included pSS. Consequently, it can be speculated that the prevalence of seronegative pSS will diminish in time paralleling the increase in the knowledge of autoantibodies associated [6]. Antibodies Anti-Carbonic Anhydrase 6 (CA6), Anti Parotid Secretory Protein (PSP) and Anti-Salivary Gland Protein (SP1) have been identified in a mouse model and confirmed in SS patients and in patients with idiopathic sicca syndrome [15]. Another autoantibodies (anti-BTBD7, anti-CCL4, anti-M5, anti-HNRNPA1, anti-KDM6B, anti-TMPO, anti-TONSL and anti-OAS3) are found in 53% of anti-SSA/Ro negative patients in the recent study of Engelke et al [20]. This autoantibodies may serve as immunological biomarkers to help in identifying and diagnosing pSS seronegative patients.

Although anti-SSA antibodies are a major component of conventional serology, other

immune dysregulations, both humoral and cellular, may also be at play. Finding new genetic biomarkers that could aid in the stratification of seronegative patients and offer insight into possible disease mechanisms is in perspective.

Lymphoma development is the most serious complication of pSS. The progression towards B-cell lymphoma is a multistep process related to local chronic antigenic stimulation of B cells. These neoplastic B cells in SS frequently derived from autoreactive clones, most commonly RF-producing B cells, which undergo uncontrolled proliferation and malignant escape [21]. Autoantibodies in pSS are considered as predictive factors of the development of lymphoma [22]. A comparative study must be conducted in a population of lymphoma associated pSS patients to investigate prevalence of lymphoma and/or its pathogenesis pathways in seronegative pSS patients.

Declaration of Interest

The authors declare that they have no competing interest.

Acknowledgments

Special thanks to the receptionists and technicians of the Immunology Department at EHU Oran for their assistance with patient recruitment and to the residents of the Internal Medicine Department and the patients themselves for their assistance with data collection.

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Table 01: Comparison of demographic, clinical and laboratory features between seronegative and seropositive pSS patients

	Seropositive (n=49)	Seronegative (n=16)	p value
Demographic features			
Age	49.3 ans +/- 11.8 [26-82]	48.1 ans +/- 15.6 [22-74]	p= 0.47
Age of onset of symptoms	45.8+/- 11.9	45.5 +/- 15.3	p= 0.3
Sex (Women/Men)	47W/2M 95.9% des femmes	12W/4M 75% des femmes	p= 0.029
Clinical features			
Xerostomia (mouth dryness)	41/47 (87.2)	14/16 (87.5)	pF=1
Xerophthalmia (eye dryness)	40/47 (85.1)	13/16 (81.3)	pY=1
Cutaneous dryness	20/47 (42.6)	7/16 (43.8)	p=0.93
Urogenital dryness	15/47 (31.9)	7/16 (43.8)	p=0.39
Parotid swelling	9/47 (19.1)	4/16 (25)	p=0.62
Articular involvement	36/47 (76.6)	11/16 (68.8)	p=0.53
Kidney involvement	7/48 (14.6)	1/16 (6.3)	pF=0.67
Lung involvement	6/47 (12.8)	3/16 (18.8)	pY=0.86
Endocrine involvement	21/48 (43.8)	1/16 (6.3)	pF=0.006 OR=7 (1.02-48)
Muscular involvement	18/47 (38.3)	6/16 (37.5)	p=0.96
Raynaud phenomenon	13/48 (27.1)	5/16 (31.3)	p=0.75
Sensitive Neuropathy	7/47 (14.9)	3/16 (18.8)	pY= 1
Vasculitis	3/47 (6.4)	1/16 (6.3)	pF=1
Purpura	6/47 (12.8)	4/16 (25)	pY=0.45
pSS association with specific autoimmune diseases	13/45 (28.9)	0/16 (0)	pF=0.014
Biological features			
Hematological involvement	21/39 (53.9)	4/13 (30.8)	pY=0.26
Anemia	14/39 (35.9)	4/13 (30.8)	pY=1
Thrombopenia	2/39 (5.1)	0/13 (0)	pF=1
Lymphopenia	5/39 (12.8)	0/13 (0)	pF=0.31
Elevated sedimentation rate	6/37 (16.2)	6/13 (46.2)	p=0.03 OR=1.56 (0.92-2.63)
Hypergammaglobulinemia	27/49 (55.1)	3/16 (18.8)	pY=0.025 OR=2.9 (1.03-8.4)
Histopathological features			
Positive MSGB	32/36 (88.9)	16/16 (100)	pF=0.3
MSGB stage III	11/32 (34.4)	5/16 (31.2)	p=0.83
MSGB stage IV	17/32 (53.1)	11/16 (68.8)	p=0.3

OR: Odds Ratio, **PF:** Fischer adjusted p value, **PY:** Yates adjusted p value, **CI:** Confidence Interval, **MSBG:** Minor Salivary Gland Biopsy, **W:** Women, **M:** Men