



Clinical and laboratory features of patients with focal lymphocytic sialadenitis on minor salivary gland biopsy for sicca symptoms

A single-center experience

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Abstract

Minor salivary gland biopsy (MSGB) is often used in patients lacking specific autoantibodies (seronegative patients) to confirm the presence of focal lymphocytic sialadenitis (FLS), which would suggest a diagnosis of Sjogren syndrome. There are no current guidelines indicating when to refer patients for MSGB. The objective of our study was to ascertain distinguishing clinical and laboratory features among individuals with sicca symptoms based on their serologic and histopathologic status, and to identify factors associated with FLS.

Using a cross-sectional study design, patients ages 18 years or older with sicca symptoms who had MSGB performed at the University of Iowa from January 2000 to December 2016 were selected for chart reviews. The clinical and laboratory features of patients with and without FLS were analyzed using exact univariate and multivariable logistic regression, with Bonferroni correction for multiple comparisons.

We identified 177 patients who had MSGB performed and available clinical data. A total of 133 patients had FLS, 37 (27.8%) were seropositive (positive-anti-Sjogren syndrome type A [SSA] and/or anti-Sjogren syndrome type B) and 96 (72.2%) were seronegative. Dry eyes (unadjusted odds ratio [OR]: 5.17, 95% confidence interval [CI]: 1.16–26.30; adjusted odds ratio [aOR]: 12.58, 95% CI: 1.70–167.77) and the presence of anti-SSA (OR: 7.16, 95% CI: 1.70–64.24; aOR: 8.82, 95% CI: 1.73–93.93) were associated with FLS. Smoking (aOR 0.27, 95% CI: 0.11–0.63) and antihistamine use (aOR 0.23, 95% CI: 0.08–0.63) were associated with lower odds of FLS.

Our study suggests that dry eyes and anti-SSA positivity are associated with FLS. Smoking and antihistamine use were associated with lower odds of FLS. In the appropriate clinical context, seronegative patients with sicca symptoms and no smoking history could be considered for MSGB. A thorough medication and smoking history should be performed in all patients before referral for MSGB.

Abbreviations: ANA = antinuclear antibody, anti-CCP = anti-cyclic citrullinated peptides, aOR = adjusted odds ratio, CI = confidence interval, CNS = central nervous system, CRP = C-reactive protein, ESSDAI = European League Against Rheumatism Sjogren Syndrome Disease Activity Index, ESR = erythrocyte sedimentation rate, FLS = focal lymphocytic sialadenitis, MSGB = minor salivary gland biopsy, OR = odds ratio, RF = rheumatoid factor, SS = Sjogren syndrome, SSA = Sjogren syndrome type A, SSB = Sjogren syndrome type B, UIHC = University of Iowa Hospitals and Clinics.

Keywords: anti-Sjogren syndrome type A antibodies, anti-Sjogren syndrome type B antibodies, focal lymphocytic sialadenitis, minor salivary gland biopsy, sicca symptoms, Sjogren syndrome

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1. Introduction

Sjogren syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltrates in the salivary and lacrimal glands.^[1] The main clinical manifestations of SS include dry eyes and dry mouth (sicca symptoms), although extraglandular manifestations are also common.^[1–3] The classification criteria for SS have been developed to select well-defined and homogenous groups of patients for research studies.^[4–6] The newest criteria rely on the presence of anti-Sjogren syndrome type A (SSA; Ro) antibodies, objective evidence of lacrimal and salivary gland dysfunction, and focal lymphocytic sialadenitis (FLS) on minor salivary gland biopsy (MSGB), and are applicable to any patient with sicca symptoms or systemic features derived from the European League Against Rheumatism Sjogren Syndrome Disease Activity Index (ESSDAI).^[4] In the salivary gland histology, FLS is defined by the presence of 1 or more foci per 4 m² of tissue area (i.e., focus score ≥ 1), with a focus being an aggregate of 50 or more mononuclear cells.^[7,8]

Despite the classic diagnostic role of the anti-SSA and anti-Sjogren syndrome type B (SSB) autoantibodies in SS, there is a subset of patients with sicca symptoms and FLS on MSGB who are seronegative. To date, only a few studies have analyzed how these seronegative patients differ clinically from patients with positive anti-SSA and anti-SSB antibodies.^[9–11] The clinical applicability of the SS classification criteria for seronegative

patients is challenging, and a high index of clinical suspicion is necessary to diagnose this subset of patients. Moreover, it is unclear what factors are associated with a positive biopsy among seronegative patients with sicca symptoms. Accordingly, the objective of our study was to evaluate the clinical and laboratory features among individuals with sicca symptoms (i.e., dry eyes and/or dry mouth) based on autoantibody and histopathologic status, as well as to identify the main factors associated with FLS.

2. Methods

2.1. Study population and inclusion/exclusion criteria

The pathology database at the University of Iowa Hospitals and Clinics (UIHC) was queried to identify all adult patients with sicca symptoms who had an MSGB done between January 2000 and December 2016 (n = 230). Fifty-three patients were excluded due to incomplete data on clinical documentation or unavailable results for anti-SSA and anti-SSB antibodies (Fig. 1). A detailed medical record review was performed to extract relevant demographic and clinical information, including age, sex, presence of sicca symptoms, extraglandular manifestations, comorbidities, medication use (diuretics, antihistamines, antidepressants, muscle relaxants, and anxiolytics), laboratory values (complete blood cell counts, liver function test, renal function test, complement levels, gamma globulin levels, erythrocyte

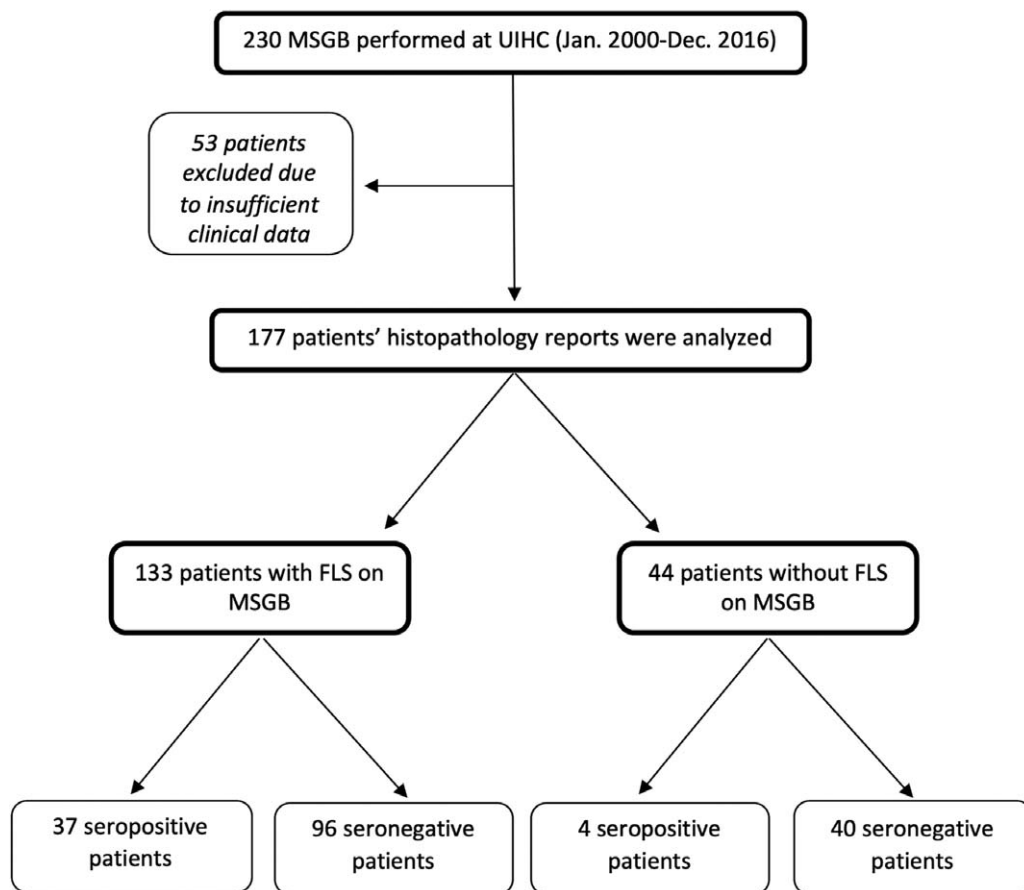


Figure 1. Flow diagram of study population and selection. Seropositive patients are those with anti-Sjogren syndrome type A (SSA) and/or anti-Sjogren syndrome type B (SSB) antibody positivity. Seronegative patients refer to those with negative anti-SSA and anti-SSB antibodies. FLS = focal lymphocytic sialadenitis, MSGB = minor salivary gland biopsy, UIHC = University of Iowa Hospitals and Clinics.

sedimentation rate [ESR], C-reactive protein [CRP], antinuclear antibodies [ANA], anti-SSA, anti-SSB, rheumatoid factor [RF], and anti-cyclic citrullinated peptide [anti-CCP] antibodies), and MSGB results. Herein, we defined seronegative patients as those having negative anti-SSA and anti-SSB antibodies, whereas seropositive patients as those with positive anti-SSA antibodies and/or anti-SSB antibodies. In the presence of multiple laboratory results, the values obtained around the time of MSGB were used. The biopsy slides were reviewed by a trained pathologist (DS) at UIHC and validated for the presence of FLS. FLS was defined as a focus score ≥ 1 on MSGB, as previously described.^[6,7] No patients with active hepatitis C infection, acquired immunodeficiency syndrome, graft versus host disease, previous head and neck radiation, or histologic findings on MSGB suggestive of sarcoidosis, amyloidosis, or IgG-4-related disease were included. The study was approved by the UIHC Institutional Review Board.

2.2. Statistical methods and analysis

Categorical variables were reported as the number and proportion of patients (%). Means \pm standard deviations were used for continuous variables. A complete case analysis approach was used to address missing data (i.e., participants with information missing for the variable of interest were excluded from the analysis). Exact univariate logistic regression analysis was used to compare the clinical and laboratory features between patients with FLS and those without FLS on MSGB. Adjusted odds ratios (ORs) were calculated using multivariable logistic regression (only variables with a *P* value < 0.05 in the univariate analysis were included; due to limited sample size, multivariable analyses were considered exploratory). Exact univariate logistic regression analyses were also used to compare seropositive and seronegative groups among patients with FLS, as well as seronegative patients with and without FLS on MSGB. To account for multiple comparisons, *P* values were adjusted using the Bonferroni correction. All analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC).

3. Results

3.1. Patients' demographic and clinical characteristics

A total of 177 patients who underwent MSGB were included in the study (Fig. 1). FLS was identified in 133 patients, of which 27% ($n=37$) were seropositive and 72% ($n=96$) were seronegative. The mean age at the time of MSGB was 52 years. Most patients in the study were white, not Hispanic (92.7%), and female (88.1%). The most common clinical manifestations were dry mouth (95.5%), dry eyes (94.3%), fatigue (72.9%), arthralgia (54.2%), and peripheral nervous system manifestations (26.7%). Other comorbidities included depression (30.5%) and fibromyalgia (29.4%). Inflammatory markers, including elevated ESR or CRP (60%), ANA positivity (60.5%), and anemia (20.4%) were the most common laboratory abnormalities in these patients. Antidepressant and anxiolytic medication use at the time of MSGB was seen in 41.8% and 23.2% of patients, respectively. Additional demographic and clinical characteristics of the study population are shown in Table 1.

3.2. Comparison between patients with and without FLS on MSGB

When examining the clinical and laboratory features associated with FLS among patients who underwent MSGB for sicca

Table 1

Demographic and clinical characteristics of the study population (n = 177).

	N (%)
Demographics	
Race/ethnicity	
White, not Hispanic	164 (92.7)
Hispanic	2 (1.1)
African American	6 (3.4)
Asian	1 (0.6)
Native American	1 (0.6)
Multi-racial or unknown	3 (1.7)
Age at the time of MSGB (y) [*]	52.0 \pm 12.2
Sex	
Male	21 (11.9)
Female	156 (88.1)
Clinical features	
Dry eyes [†]	166/176 (94.3)
Dry mouth	169 (95.5)
Fatigue	129 (72.9)
Salivary gland swelling	35 (19.8)
Central nervous system manifestations	15 (8.5)
Peripheral nervous system [†] manifestations	47/176 (26.7)
Interstitial lung disease	8 (4.5)
Pulmonary hypertension	9 (5.1)
Hypothyroidism	
No disease	129 (72.9)
Present	35 (19.8)
Present with autoimmune thyroiditis	13 (7.3)
Raynaud phenomenon	25 (14.1)
Pancreatitis	3 (1.7)
Purpura	5 (2.8)
Lymphoma	2 (1.1)
Inflammatory arthritis	19 (10.7)
Myalgia	16 (9.0)
Arthralgia	96 (54.2)
Depression	54 (30.5)
Fibromyalgia	52 (29.4)
Labs	
Antinuclear antibody positive [†]	104/172 (60.5)
Rheumatoid factor positive [†]	24/126 (19.1)
Anti-SSA positive	36 (20.3)
Anti-SSB positive	25 (14.1)
Low complement, C3 [†]	6/105 (5.7)
Low complement, C4 [†]	10/105 (9.5)
Anemia [†]	34/167 (20.4)
Leukopenia ^{†,‡}	5/167 (3.0)
Hypergammaglobulinemia ^{†,§}	13/89 (14.6)
Elevated ESR and/or CRP [†]	99/165 (60.0)
Medication use	
Diuretic	34 (19.2)
Antihistamine	31 (17.5)
Antidepressant	74 (41.8)
Muscle relaxant	34 (19.2)
Anxiolytic	41 (23.2)

Values are expressed as N (%) for categorical variables and mean \pm standard deviation (SD) for continuous variables.

CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, MSGB = minor salivary gland biopsy, SSA = Sjogren syndrome type A, SSB = Sjogren syndrome type B.

^{*} Data are expressed as mean \pm SD.

[†] Variables contain missing data. The denominator is shown.

[‡] Leukopenia was defined as having a leukocyte count $< 3000/\text{mm}^3$.

[§] Hypergammaglobulinemia was defined as having levels $> 1.6\text{g/L}$.

symptoms, we found significant associations with dry eyes (unadjusted OR 5.17, 95% confidence interval [CI]: 1.16–26.30; adjusted OR [aOR] 12.58, 95% CI: 1.70–167.77) and positive

Table 2
Clinical and laboratory features of patients with focal lymphocytic sialadenitis on minor salivary gland biopsy compared to those with a negative minor salivary gland biopsy.

	– FLS n (%)	+ FLS n (%)	OR (95% CI)	P
Sample size	44 (24.9)	133 (75.1)		
Demographics				
Race/ethnicity				
White, not Hispanic	39 (88.6)	125 (94.0)	REF	REF
Hispanic	0 (0)	2 (1.5)	–	–
African American	2 (4.6)	4 (3.0)	0.63 (0.09–7.17)	.893
Asian	0 (0)	1 (0.8)	–	–
Native American	0 (0)	1 (0.8)	–	–
Multiracial or unknown	3 (6.8)	0 (0)	–	–
Age at the time of MSGB (y) [*]	49.9±10.5	52.7±12.7	1.02 (0.99–1.05)	.192
Sex				
Male	5 (11.4)	16 (12.0)	REF	REF
Female	39 (88.6)	117 (88.0)	0.94 (0.25–2.91)	1.000
Clinical features				
Dry eyes [†]	37/43 (86.1)	129 (97.0)	5.17 (1.16–26.30)	.030 [‡]
Dry mouth	41 (93.2)	128 (96.2)	1.87 (0.28–10.07)	.632
Fatigue	31 (70.5)	98 (73.7)	1.17 (0.50–2.63)	.813
Salivary gland swelling	11 (25.0)	24 (18.1)	0.66 (0.28–1.66)	.427
Central nervous system manifestations	2 (4.6)	13 (9.8)	2.27 (0.48–21.52)	.456
Peripheral nervous system manifestations [†]	15/43 (34.9)	32 (24.1)	0.59 (0.27–1.35)	.234
Interstitial lung disease	0 (0)	8 (6.0)	–	–
Pulmonary hypertension	1 (2.3)	8 (6.0)	2.74 (0.35–124.91)	.594
Hypothyroidism				
No disease	36 (81.8)	93 (69.9)	REF	REF
Present	5 (11.4)	30 (22.6)	2.31 (0.80–8.23)	.145
Present with autoimmune thyroiditis	3 (6.8)	10 (7.5)	1.29 (0.31–7.70)	.997
Raynaud phenomenon	7 (15.9)	18 (13.5)	0.83 (0.30–2.54)	.863
Pancreatitis	1 (2.3)	2 (1.5)	0.66 (0.03–39.62)	1.000
Purpura	0 (0)	5 (3.8)	–	–
Lymphoma	0 (0)	2 (1.5)	–	–
Inflammatory arthritis	7 (15.9)	12 (9.0)	0.53 (0.18–1.70)	.317
Myalgia	3 (6.8)	13 (9.8)	1.48 (0.38–8.48)	.804
Arthralgia	29 (65.9)	67 (50.4)	0.53 (0.24–1.12)	.104
Depression	12 (27.3)	42 (31.6)	1.23 (0.55–2.89)	.736
Smoking	27 (61.4)	37 (27.8)	0.25 (0.11–0.53)	.0002 [§]
Fibromyalgia	17 (38.6)	35 (26.3)	0.57 (0.26–1.25)	.175
Laboratory results				
Antinuclear antibody positive [†]	22/42 (52.4)	82/130 (63.1)	1.55 (0.72–3.32)	.293
Rheumatoid factor positive [†]	5/29 (17.2)	19/97 (19.6)	1.17 (0.37–4.43)	1.000
Anti-SSA positive	2 (4.6)	34 (25.6)	7.16 (1.70–64.24)	.002 [‡]
Anti-SSB positive	4 (9.1)	21 (15.8)	1.87 (0.58–7.95)	.397
Low Complement, C3 [†]	0/22 (0)	6/83 (7.2)	–	–
Low Complement, C4 [†]	2/22 (9.1)	8/83 (9.6)	1.07 (0.19–11.08)	1.000
Anemia [†]	7/40 (17.5)	27/127 (21.3)	1.27 (0.48–3.78)	.789
Leukopenia ^{†,}	0/40 (0)	5/127 (4.0)	–	–
Hypergammaglobulinemia ^{†,¶}	2/23 (8.7)	11/66 (16.7)	2.09 (0.40–20.93)	.577
Elevated ESR and/or CRP [†]	28/40 (70.0)	71/125 (56.8)	0.57 (0.24–1.27)	.192
Medication use				
Diuretic	10 (22.7)	24 (18.1)	0.75 (0.31–1.94)	.631
Antihistamine	16 (36.4)	15 (11.3)	0.23 (0.09–0.55)	.0007 [§]
Antidepressant	24 (54.6)	50 (37.6)	0.50 (0.24–1.06)	.073
Muscle relaxant	9 (20.5)	25 (18.8)	0.90 (0.36–2.41)	.965
Anxiolytic	10 (22.7)	31 (23.3)	1.03 (0.44–2.62)	1.000

Values are expressed as N (%) for categorical variables and mean ± standard deviation (SD) for continuous variables. Odds ratios were calculated using exact univariate logistic regression. Variables may contain missing data.

CI = confidence interval, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, FLS = focal lymphocytic sialadenitis, MSGB = minor salivary gland biopsy, OR = odds ratio, SSA = Sjogren syndrome type A, SSB = Sjogren syndrome type B.

^{*} Data are expressed as mean ± SD.

[†] Variables contain missing data. The denominator is shown.

[‡] Marginally significant ($P < .05$).

[§] Significant (Bonferroni corrected $P < .002$); –, not calculated when $n = 0$.

^{||} Leukopenia was defined as having a leukocyte count $< 3000/\text{mm}^3$.

[¶] Hypergammaglobulinemia was defined as having levels $> 1.6\text{g/L}$.

anti-SSA (OR 7.16, 95% CI: 1.70–64.24; aOR 8.82, 95% CI: 1.73–93.93) (Table 2). Smoking (OR 0.25, 95% CI: 0.11–0.53; aOR 0.27, 95% CI: 0.11–0.63) and antihistamine medication use (OR 0.23, 95% CI: 0.09–0.55; aOR 0.23, 95% CI: 0.08–0.63) were associated with decreased odds of FLS on MSGB.

3.3. Comparison between seropositive and seronegative patients with FLS

Among patients with FLS on MSGB, seronegative patients had a significantly higher proportion of dry mouth compared to seropositive patients (100% vs 86.5%, respectively, $P < .002$). Dry eyes (100% vs 89.2%), fibromyalgia (31.3% vs 13.5%), depression (38.5% vs 13.5%), and antidepressant use (43.8% vs 21.6%) were also more common in seronegative than in seropositive patients, but the statistical association was only marginally significant ($P < .05$). In contrast, RF positivity was more frequently found in seropositive patients with FLS (40% in seropositive patients vs 10.5% in the seronegative group, $P = .002$). There were 12 patients with FLS who had inflammatory arthritis without positive RF, but one of them had positive anti-CCP antibodies. Central nervous system (CNS) manifestations, myalgia, anemia, and hypergammaglobulinemia also tended to be increased among seropositive patients compared to seronegative individuals.

3.4. Comparison between seronegative patients with and without FLS

A comparison of seronegative patients with and without FLS on MSGB is shown in Table 3. Seronegative patients with FLS tended to be older (53.9 ± 11.8 vs 49.0 ± 10.6 , $P = .026$), and more likely to have dry eyes (100% vs 84.6%, $P = .0004$) and dry mouth (100% vs 92.5%, $P = .024$) compared to patients without FLS. Smokers were less likely to have an FLS than nonsmokers (28.1% vs 62.5%, $P = .0002$). Similarly, FLS was less frequently observed in MSGB from patients using antihistamine medications (12.5% vs 35.0% in patient without FLS, $P = .004$).

4. Discussion

The classification criteria for SS are based on a combination of clinical, serological, and histological findings that have been continuously evolving.^[4–6] The hallmark of the disease is exocrinopathy, which often translates into sicca symptoms, fatigue, and arthralgias. A formal diagnosis of SS is, however, usually made in the presence of classic anti-SSA antibodies, unless the patient has undergone MSGB. Therefore, diagnosing SS in seronegative patients remains a challenge, particularly when they present with only vague, nonspecific manifestations. Our study provides factors that are positively and negatively associated with FLS, which can be taken into consideration when contemplating a referral for MSGB. In addition, we provide a comparison between seropositive and seronegative patients, among those with FLS on MSGB.

In addition to SS, there are multiple other causes of sicca symptoms, including medications such as anxiolytics, antidepressants, muscle relaxants, and diuretics, chronic rhinosinusitis, obstructive sleep apnea, and smoking.^[12–14] In these cases, the symptoms usually resolve after treating the underlying condition or holding the culprit agent, and FLS is not often identified on histology in these patients.^[13–16] Even though the MSGB is a

valuable tool in the diagnosis of SS, it can potentially lead to postprocedural complications, including local swelling, wound infection, and, more commonly, local paresthesias.^[17] In addition, false-positive results can occur. FLS has been found in patients with conditions other than SS, as well as healthy individuals.^[18] On the contrary, prior reports have shown a decreased risk of FLS in current and former smokers.^[19–21] Therefore, the interpretation of FLS must be carefully considered in the context of other clinical findings, which may be particularly difficult in patients who lack specific autoantibodies for SS.

At present, no clear guidelines exist as to which patients should be referred for MSGB to evaluate for FLS, as an attempt to fulfill criteria for SS or to rule out the disease. Very few studies have looked at the predictors of FLS on MSGB, and results to date are controversial. For example, a study from the Netherlands evaluating 94 patients that underwent MSGB for suspected SS showed that the yield of the salivary biopsy was significantly higher in patients in whom the biopsy was performed or requested by internists or rheumatologists, compared to other departments, presumably due to a stronger suspicion for the disease.^[22] It has also been reported that patients with a focus score of >1 compared to those with a focus score ≤ 1 have increased frequency of salivary gland swelling (25% vs 9%), ANA $>1:100$ (68% vs 32%), RF $>1:160$ (63% vs 22%), anti-SSA (46% vs 9%), and anti-SSB (32% vs 4%). In the same study, it was also shown that abnormal biopsies were more frequent in those patients biopsied for serologic abnormalities (53%) than for sicca symptoms (33%) or systemic illness (29%).^[23] In agreement with these observations, we found that patients with positive anti-SSA antibodies were more likely than not to have FLS on MSGB. A small study of 47 patients referred for MSGB to evaluate for SS in the United States showed that neither positive serology nor the presence of sicca symptoms predicted a positive biopsy (likelihood ratio = 0.95 and 0.96, respectively), but it is possible that this study had insufficient power to detect a difference.^[24] We did not identify a significant association between the presence of anti-SSB (in the presence or absence of anti-SSA) antibodies and FLS. Interestingly, a large cohort study from the Big Data Sjogren Project Consortium suggested that patients with isolated anti-SSB antibodies exhibit a different phenotype compared to seronegative patients, with higher systemic activity as measured by the ESSDAI.^[25] Our limited sample size of patients with isolated anti-SSB antibodies, however, did not allow for separate analysis of this subgroup.

Our data suggest that the presence of dry eyes was strongly associated with FLS in all patients referred for biopsy, and specifically in the seronegative group. This finding may indicate that patients with FLS could also have lacrimal gland involvement as part of a generalized process causing exocrine dysfunction. As objective tests to confirm decreased lacrimal gland function were, however, not routinely performed in most of these patients, we are unable to confirm this hypothesis. Smokers were less likely to have FLS on biopsy when analyzed in all patients referred for MSGB, and specifically in the seronegative group. A potential explanation for the negative association between smoking and FLS in our study is direct mucosal irritation without the presence of an inflammatory disorder, as smoking is known to be associated with decreased salivary flow and tear production.^[15,16] Therefore, sicca symptoms secondary to smoking could have prompted a referral for MSGB in these patients. Previous reports have, however, suggested that tobacco smoking is negatively associated with SS, and specifically with a

Table 3

Clinical and laboratory features of seronegative patients with focal lymphocytic sialadenitis on minor salivary gland biopsy compared to seronegative patients with no focal lymphocytic sialadenitis on minor salivary gland biopsy.

	Seronegative		P
	– FLS n (%)	+ FLS n (%)	
Sample size	40 (29.4)	96 (70.6)	
Demographics			
Race/ethnicity			.027*
White, not Hispanic	35 (87.5)	93 (96.9)	
Hispanic	0 (0)	0 (0)	
African American	2 (5.0)	1 (1.0)	
Asian	0 (0)	1 (1.0)	
Native American	0 (0)	1 (1.0)	
Multiracial or unknown	3 (7.5)	0 (0)	
Age at the time of MSGB (y) [†]	49.0 ± 10.6	53.9 ± 11.8	.026*
Sex			1.000
Male	5 (12.5)	12 (12.5)	
Female	35 (87.5)	84 (87.5)	
Clinical features			
Dry eyes [‡]	33/39 (84.6)	96 (100.0)	.0004 [§]
Dry mouth	37 (92.5)	96 (100.0)	.024*
Fatigue	27 (67.5)	74 (77.1)	.284
Salivary gland swelling	11 (27.5)	20 (20.8)	.501
Central nervous system manifestations	2 (5.0)	5 (5.2)	1.000
Peripheral nervous system manifestations [‡]	13/39 (33.3)	25 (26.0)	.405
Interstitial lung disease	0 (0)	4 (4.2)	.320
Pulmonary hypertension	1 (2.5)	4 (4.2)	1.000
Hypothyroidism			.093
No disease	33 (82.5)	63 (65.6)	
Present	4 (10.0)	25 (26.0)	
Present with autoimmune thyroiditis	3 (7.5)	8 (8.3)	
Raynaud phenomenon	7 (17.5)	12 (12.5)	.430
Pancreatitis	1 (2.5)	1 (1.0)	.503
Purpura	0 (0)	2 (2.1)	1.000
Lymphoma	0 (0)	1 (1.0)	1.000
Inflammatory arthritis	7 (17.5)	9 (9.4)	.242
Myalgia	3 (7.5)	5 (5.2)	.693
Arthralgia	26 (65.0)	50 (52.1)	.188
Depression	11 (27.5)	37 (38.5)	.243
Smoking	25 (62.5)	27 (28.1)	.0002 [§]
Fibromyalgia	15 (37.5)	30 (31.3)	.550
Laboratory results			
Antinuclear antibody positive [‡]	20/38 (52.6)	58/93 (62.4)	.331
Rheumatoid factor positive [‡]	4/25 (16.0)	7/67 (10.5)	.482
Low Complement, C3 [‡]	0/20 (0)	5/59 (8.5)	.322
Low Complement, C4 [‡]	2/20 (10.0)	4/59 (6.8)	.640
Anemia [‡]	5/36 (13.9)	13/90 (14.4)	1.000
Leukopenia ^{‡,}	0/36 (0)	2/87 (2.3)	1.000
Hypergammaglobulinemia ^{‡,¶}	1/21 (4.8)	4/45 (8.9)	1.000
Inflammatory markers [‡]	24/36 (66.7)	46/89 (51.7)	.164
Medication use			
Diuretic	10 (25.0)	18 (18.8)	.486
Antihistamine	14 (35.0)	12 (12.5)	.004*
Antidepressant	21 (52.5)	42 (43.8)	.451
Muscle relaxant	7 (17.5)	18 (18.8)	1.000
Anxiolytic	10 (25.0)	25 (26.0)	1.000

Values are expressed as N (%) for categorical variables and mean ± standard deviation (SD) for continuous variables. P values were calculated using exact univariate logistic regression. Variables may contain missing data.

FLS = focal lymphocytic sialadenitis, MSGB = minor salivary gland biopsy.

* Marginally significant ($P < .05$).

[†] Data are expressed as mean ± SD.

[‡] Variables contain missing data. The denominator is shown.

[§] Significant (Bonferroni corrected $P < .002$).

^{||} Leukopenia was defined as having a leukocyte count $< 3000/\text{mm}^3$.

[¶] Hypergammaglobulinemia was defined as having levels $> 1.6 \text{ g/L}$.

lower risk of FLS.^[20,21] Although the exact mechanism responsible for this finding remains to be fully elucidated, smoking has been linked to a dose-dependent decrease in the number of circulating CD4+ T cells and decreased T cell proliferation in response to mitogens.^[21,26] The chronic exposure to benzo[a]pyrene, a component of cigarette smoke, results in a decreased mass and cellularity of lymphoid tissues.^[19] The aforementioned mechanisms raise the question of whether a protective effect of smoking on lymphocytic infiltration of the salivary glands could at least partially explain the negative association between smoking and FLS on MSGB that we identified in our study. Consequently, a smoking history should be considered when deciding which patients to refer for MSGB as part of the evaluation for suspected SS, particularly in the absence of anti-SSA antibodies.

Medications are a well-established cause of sicca symptoms.^[12] Our study showed that antihistamine use was negatively associated with FLS. A population-based study showed patients on antihistamines had an OR of 1.67 for having dry eyes or dry mouth, without evidence of an autoimmune-mediated process like SS.^[12] Therefore, the use of antihistamines may have directly caused the sicca symptoms, which prompted referral for MSGB for some patients in our study. As chronic rhinosinusitis is both associated with sicca symptoms and antihistamine use, this is also a potential explanation for our findings. Therefore, a thorough medication history, including over-the-counter agents, should be part of the routine evaluation of patients with sicca symptoms before referral for MSGB.

Previous studies have assessed the clinical differences between seropositive and seronegative SS patients.^[9–11,27,28] In agreement with our observations, Quartuccio et al^[10] identified seronegative patients with primary SS had a lower prevalence of hypergammaglobulinemia and positive RF compared to seropositive patients. In contrast to their findings, we did not find glandular swelling, purpura, hypocomplementemia, ANA positivity, and lymphoma were more common in the seropositive group. Similarly, Brito-Zerón et al^[11] also identified a higher frequency of extraglandular manifestations in seropositive patients with both anti-SSA and anti-SSB, compared to patients with either of these antibodies.

Our data suggest that CNS manifestations were more common in the seropositive group, as previously reported.^[29,30] Prior studies have shown more severe neuropathic pain and a higher prevalence of peripheral neuropathy in seronegative SS patients.^[31,32] We, however, did not find an association between the presence of peripheral nervous system manifestations and autoantibody status among patients with FLS. As not all patients with neuropathic symptoms had undergone nerve conduction studies or biopsy to evaluate for small fiber neuropathy, it is possible that neuropathy was underreported or underrecognized in our study. Fibromyalgia and depression are prevalent in SS patients and can affect their quality of life^[29,30,32]; we found these conditions to be more frequent in the seronegative patients.

Inflammatory arthritis is associated with SS and manifests as mild symmetric nonerosive synovitis involving predominantly small joints. RF positivity may not necessarily be associated with inflammatory arthritis in patients with SS.^[33–35] In agreement with these observations, we identified 12 patients with FLS, elevated inflammatory markers, and negative RF who had nonerosive inflammatory arthritis. There were also 19 patients with FLS and positive RF, who did not have inflammatory arthritis.^[30–32] Conversely, one of the patients with FLS who had

positive anti-CCP antibodies also had inflammatory arthritis. Although only present in 5% to 10% of patients with SS, the presence of anti-CCP antibodies has been reported as a predictor of future progression to inflammatory arthritis.^[33,34]

The prevalence of positive RF is approximately 50% to 60% in patients with SS.^[36,37] Similarly, we found that a positive RF was more likely to be present in seropositive patients with FLS (40% in this subgroup), which is consistent with the literature compiling anti-SSA, anti-SSB, and RF positivity to an active immunologic profile and more systemic complications.^[38,39] Other associations with this phenotype include hypergammaglobulinemia and anemia, which were also more frequently identified in the seropositive patients with FLS in our study.

The limitations of our study include the cross-sectional design and the use of chart reviews to obtain the clinical data, which limited our ability to assess causality, evolution, fulfillment of SS criteria, and severity of the disease by a standardized scoring system such as the ESSDAI. Similarly, we were unable to evaluate the natural history of the seronegative patients. Hence, it is unclear if a subset of these patients would evolve into a seropositive phenotype with more systemic manifestations in the long term. The results of a recent large study, however, suggest stability of phenotypic features and SS status over a 2- to 3-year period.^[40] It should also be noted that the proportion of patients referred for MSGB who were seropositive was lower than previously reported in the literature, presumably as seropositive patients may be less likely to be referred for MSGB by their treating rheumatologists. Despite the suspicion for SS, most patients did not have documented salivary and lacrimal gland functional testing, such as Schirmer test and whole saliva flow rate determination; therefore, we were unable to confirm the fulfillment of classification criteria for SS. Most patients were women and White, which may decrease the generalizability to other racial/ethnic groups; nonetheless, SS has been shown to be more common in this population.^[41] Finally, our study may not have been powered to detect differences in less common clinical manifestations such as interstitial lung disease, tubulointerstitial renal disease, and lymphoma.

The strengths of the study include the detailed manual chart reviews to extract the clinical and laboratory features among a large cohort of patients with sicca symptoms who underwent MSGB. In addition, the biopsies were all evaluated by members of the pathology department at a single tertiary academic center, and findings were confirmed by a single pathologist (DS), to ensure the use of standardized methods to process and examine the tissue.

5. Conclusion

In summary, our findings indicate that the presence of dry eyes and positive anti-SSA antibodies are the main factors associated with FLS in patients referred for MSGB. Conversely, smoking was associated with lower odds of FLS. Seronegative patients with FLS had a higher rate of dry eyes. Hence, seronegative patients with sicca symptoms, particularly dry eyes, and no smoking history could be considered for MSGB for further diagnostic workup. A thorough medication and smoking history should be performed in all patients before referral for MSGB. Further prospective studies are needed to evaluate seronegative patients with sicca symptoms and their disease evolution, as well as other features and biomarkers that can facilitate the diagnosis of SS in this group.

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