Minor Salivary Gland Inflammatory Lesions in Sjögren Syndrome: Do They Evolve?

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ABSTRACT. Objective. The lymphocytic infiltrates of minor salivary gland (MSG) lesions of Sjögren syndrome (SS) vary in grade and composition and are generally thought to develop in stepwise manner. Their progression over time is not well defined.

Methods. We studied repetitive MSG biopsy specimens from 28 patients with primary SS.

Results. The infiltration grade and prevalence of the major infiltrating cell types (T and B cells, macrophages, dendritic cells, natural killer cells) remained largely unchanged during a median 55 month biopsy time interval followup (quartiles 42–81).

Conclusion. We found significant disease progression involving the development of mucosa-associated lymphoid tissue lymphoma in patients expressing adverse serologic prognostic factors, such as low serum C4 complement levels and cryoglobulinemia. (J Rheumatol First Release Aug 1 2013; doi:10.3899/jrheum.130256)

Key Indexing Terms:

SJÖGREN SYNDROME MINOR SALIVARY GLAND LESIONS AUTOIMMUNE DISEASES

Sjögren syndrome (SS) is a slowly progressive autoimmune disorder with a broad clinical spectrum that extends from exocrinopathy to diverse systemic manifestations. Evidence of the last 20 years revealed that the glandular and parenchymal extraglandular manifestations are driven by epithelial cells¹. Glandular dysfunction in SS is associated with the development of periductal inflammatory lesions of variable degree and composition². The composition of the minor salivary gland (MSG) lesions varies according to the infiltrate severity; T cells predominate in mild infiltrates, B cells in severe infiltrates, whereas the prevalence of

macrophages increases and interdigitating dendritic cells (iDC) decreases with lesion grade². Although MSG infiltrates are generally thought to develop in a stepwise manner, the progression and change of lymphocytic subpopulations over time have not been studied.

We investigated the progression of autoimmune MSG infiltrates over time, and particularly alterations of the grade and composition of lymphocytic infiltrates, as well as the progression of degenerative processes, such as fibrosis and fatty cell infiltration. Potential associations between histopathological measures of lesion progression and clinical or serological features of patients with SS were examined.

MATERIALS AND METHODS

Patients and MSG biopsies. Twenty-eight consecutive patients (27 women, 1 man) with primary SS³ who were diagnosed and followed in our department, and had been subjected to their first MSG biopsy at least 36 months previously, were studied. All patients signed a written informed consent. One patient refused to undergo a second biopsy. The study was approved by the Ethics Committee of the School of Medicine, National University of Athens (protocol no. 5107). The median interval between the 2 sequential biopsies was 55 months (quartiles 42–81). In all of the patients with SS, the biopsy focus score of the first MSG biopsy was ≥ 1 . In 24 patients, the first biopsy was performed at diagnosis.

The medical records of patients were evaluated for various clinical, laboratory, and therapeutic variables, as described². None of the patients with SS had evidence of B cell lymphoma, sarcoidosis, hepatitis B virus (HBV), hepatitis C virus (HCV), or HIV infection. Twenty-one patients at the time of the first biopsy had not received immune-modulating agents. None of the 28 patients had received B cell depletion therapy before or during followup. Characteristics of patients with SS and the therapeutic interventions are summarized in Table 1.

Evaluation of progression of autoimmune infiltrates in 2 sequential MSG biopsies. The histological evaluation of repetitive biopsies of at least 4 MSG, with sections stained using hematoxylin/eosin (H&E), was done

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Table 1. Demographic, clinical, and laboratory characteristics of the patients with SS.

Features of Patients with SS	1st MSG Biopsy	2nd MSG Biopsy
General		
Age, yrs; median (quartiles)	50.5 (36–55)	54.5 (42–59)
Duration of sicca symptoms, yrs; median (quartiles)	3 (1–7)	
Clinical		
Arthralgias, n (%)	11 (39.3)	11 (39.3)
Arthritis, n (%)	2 (7.1)	3 (10.7)
Salivary gland enlargement, n (%)	12 (42.9)	16 (57.1)
Raynaud's phenomenon, n (%)	9 (32.1)	9 (32.1)
Parenchymal organ involvement, n (%)	0 (0.0)	1 (3.6)
Lung involvement, n (%)	0 (0.0)	0 (0.0)
Renal involvement, n (%)	0 (0.0)	1 (3.6)
Liver involvement, n (%)	0 (0.0)	0 (0.0)
Indicative of vasculitic involvement, n (%)	4 (14.3)	6 (21.4)
Palpable purpura, n (%)	4 (14.3)	6 (21.4)
Vasculitis, n (%)	0 (0.0)	1 (3.6)
Peripheral neuropathy, n (%)	0 (0.0)	0 (0.0)
Lymphoma, n (%)	0 (0.0)	5 (17.9)
Laboratory		
Leukopenia, n (%)	5 (17.9)	5 (17.9)
Hyper-gammaglobulinemia, n (%)	9 (32.1)	9 (32.1)
C4 levels, median (quartiles)	18.7 (12.2–25.1)	21.8 (11.4–39.9)
Anti-Ro(SSA) and/or La(SSB) positive, n (%)	22 (78.6)	22 (78.6)
Anti-Ro(SSA) positive, n (%)	22 (78.6)	22 (78.6)
Anti-La(SSB) positive, n (%)	12 (42.9)	12 (42.9)
Cryoglobulinemia, n (%)	4 (14.3)	5 (17.9)
Therapy		
Pilocarpine-hydrochloride, n (%)	0 (0.0)	7 (25.0)
Hydroxychloroquine, n (%)	2 (7.1)	5 (17.9)
Pilocarpine-hydroxychloroquine, n (%)	1 (3.6)	3 (10.7)
Corticosteroids, n (%)	3 (10.7)	2 (7.1)
Pilocarpine-corticosteroids, n (%)	0 (0.0)	3 (10.7)
Hydroxychloroquine-corticosteroids, n (%)	1 (3.6)	0 (0.0)
Pilocarpine-corticosteroids-methotrexate, n (%)	0 (0.0)	1 (3.6)
B cell depletion	0 (0.0)	0 (0.0)
None, n (%)	21 (75.0)	7 (25.0)

blinded by EKK and AGT. To avoid inconsistency due to evaluation of sections at baseline and deeper edges of glands⁴, sections at the middle depth of tissue were routinely evaluated. Further, due to a reported change in the quantification of the focus score in a small percentage of samples with low scoring⁵, sections differing by roughly 200 μm were also evaluated in each sample. Similar focus scores were found in the 2 depths evaluated. Progression of the grade of inflammatory MSG lesions was evaluated by measuring the total number of infiltrating mononuclear cells (MNC)/mm² tissue, as well as by the standard MSG biopsy scores, according to Chisholm and Mason (biopsy focus score: number of lymphocytic foci/4 mm² tissue)⁶ and Tarpley, *et al*⁷. Although the Tarpley score is not adequate for MSG biopsy evaluation and diagnosis, it provides information on the infiltrate severity and its association with the destruction of glandular tissue. In a previous report, we classified patients with SS into 3 groups according to lesion severity². In this study, 4 patients with mild, 9 with intermediate, and 15 with severe lesions were included consecutively, suggesting a predisposition for patients with severe MSG inflammation, possibly due to their more frequent followup. None of the standard grading scores (alone or jointly) provides information about the size of the lymphocytic infiltrates, and thus they do not depict changes in the number of infiltrating cells. Therefore, evaluation of the progression of the lesion grade in this study was based primarily on the number of infiltrating MNC/tissue area. Tissue area and the number of infiltrating MNC in each biopsy

specimen were automatically estimated using ImageJ software on serial images of all H&E-stained sections². Neither of the scores applied was found to be significantly different between the biopsy sections at deeper levels. Degenerative changes, such as fibrosis and fatty-cell infiltration, were also analyzed⁸. The evaluation of germinal center (GC) formation was based on histopathologic criteria and the presence of follicular dendritic cell (fDC) networks².

The change of composition of the MSG infiltrates was assessed by calculating the percentage of each infiltrating cell type [total T cells and their subpopulations CD4+ T cell, CD8+ T cell, and Treg cells; B cells; macrophages; iDC; fDC; and natural killer (NK) cells] to total infiltrating MNC in serial sections of the sequential biopsies². MNC types were identified by immunohistochemistry and counted using ImageJ software in all serial sections of paraffin-embedded MSG biopsy specimens, whereas the number of total infiltrating MNC was automatically estimated in each section by the ITCN plug-in (ImageJ software), as described². In preliminary experiments, the percentages of each infiltrating MNC type were found not to differ significantly between sections at deeper levels; however, in all cases, evaluation of the sections at the edges of glands was avoided².

MSG tissues with GC formation or severe infiltration were routinely evaluated for the development of lymphoma by a specialist hemopathologist (AT). The diagnosis and characterization of lymphoma in MSG tissues were assessed by immunohistochemical identification and/or

molecular detection of B or T cell monoclonality. Immunohistochemical detection was performed with antibodies against specific markers, such as CD20, CD3, CD4, CD8, CD45, CD43, cytoplasmic immunoglobulin (CIG), CD5, CD10, CD15, CD21, CD23, CD35, CD79a, CD30, Bcl-2, Bcl-6, cyclinD1, DBA44, and MIB. Molecular evaluation involved detection of clonal IGH gene rearrangements by PCR using specific primer sets, such as (1) FR3A, L1H, VLGH, and (2) FR2A, L1H, VLGH. Diagnosis of mucosa-associated lymphoid tissue (MALT) lymphoma required the predominance of κ to λ chains (at least 7 to 1 ratio) and/or detection of monoclonal IGH gene rearrangements.

Statistical analyses. Continuous variables are presented as mean $\pm$ SD (if normally distributed), otherwise as median (quartiles). The significance of the changes observed between the 2 sequential biopsies was analyzed using general linear models for repeated measures (RM ANOVA), adjusted for biopsy time interval. Chi-square test was used to evaluate associations between categorical variables. The limit for considering a change of infiltrating MNC/mm² tissue to be significant was arbitrarily set at 60%. Wilcoxon-ranked test was applied to compare patients with SS that presented progression of the MSG infiltrates and those without. Significance was defined as $p < 0.05$. Analyses were done using SPSS version 17.0 and GraphPad-Prism version 5.

RESULTS

Progression of grade of autoimmune MSG lesions of patients with SS. Data analysis revealed that neither the number of infiltrating MNC/tissue area nor the MSG grading scores (biopsy focus and Tarpley scores) changed significantly during the median biopsy time interval of 55 months' followup (quartiles 42-81; Table 2). Similarly, the

other histological variables studied, including GC formation and indicators of tissue degeneration, such as fibrosis and fat infiltration, did not vary significantly in the repetitive MSG biopsy specimens (Table 2). None of the variables was found to be affected by the biopsy time interval or to correlate with demographic, clinical, laboratory, and therapeutic features of patients with SS.

Generally, the severity of the lesion grade remained similar during followup and even in samples with $\geq 60\%$ change of the infiltrating MNC number/mm² tissue between the 2 biopsies; it was not readily evident at microscopic evaluation (data not shown). Six patients presented $\geq 60\%$ fluctuation of the infiltrating MNC number/mm² tissue (increase in 4 patients, decrease in 2; Table 3). In 3 patients, this was accompanied by a change of the biopsy focus and Tarpley scores (Table 3). Except for one patient (Patient 17), these changes were not followed by a change in lesion severity, as assessed by Tarpley score²; mild, intermediate, or severe lesions remained the same (Table 3). These alterations were not affected by the biopsy time interval or any of the other histological, demographic, clinical, or therapeutic variables studied. Neo-organization of GC was observed in 4 patients (Patients 1, 13, 15, and 18), but it was not statistically significant. It was not associated with infiltration increase, biopsy time interval, or other histological, demographic, clinical or therapeutic variables (data not

Table 2. Histological features of the successive minor salivary gland (MSG) biopsy specimens of the SS patients. Statistical significance was evaluated by general linear model for repeated measures adjusted for the biopsy time interval.

Histological Features of SS Patients	1st MSG Biopsy	2nd MSG Biopsy	p
No. infiltrating MNC/mm ² of tissue, mean $\pm$ SD	2774 $\pm$ 2081	3035 $\pm$ 2403	0.67
Glandular area (mm ²) examined, mean $\pm$ SD	17.0 $\pm$ 7.4	19.4 $\pm$ 5.4	0.08
Biopsy focus score (no. lymphocytic foci/4mm ²), median (quartiles)	4.3 (2.3–5.8)	4.4 (2.5–5.8)	0.72
Tarpley biopsy score, median (quartiles)	3 (2–3)	2.5 (2–3)	0.98
Fibrosis, median (quartiles)	1 (1–1.8)	1 (1–2)	0.64
Fatty cell infiltration, median (quartiles)	1 (1–2.8)	2 (1–3)	0.49
Presence of ectopic germinal center (GC), n (%)	7 (25)	11 (39)	0.97
Prevalence of infiltrating cell types (mean percentage of total infiltrating MNC or MNC types $\pm$ SD)			
CD3+ T cells	45.8 $\pm$ 9.8	47.2 $\pm$ 12.3	0.12
CD4+ T cells	30.8 $\pm$ 7.9	26.9 $\pm$ 10.2	0.02
CD4+/CD3+ T cells	67.1 $\pm$ 9.0	54.6 $\pm$ 13.0	0.02
CD8+ T cells	15.1 $\pm$ 5.2	21.9 $\pm$ 8.3	0.25
CD8+/CD3+ T cells	32.9 $\pm$ 9.0	45.4 $\pm$ 13.0	0.02
CD4+/CD8+ T cell ratio	2.2 $\pm$ 1.0	1.4 $\pm$ 0.9	0.09
FOXP3+ Treg cells	1.4 $\pm$ 1.1	2.0 $\pm$ 1.7	0.49
FOXP3+ Treg/CD3+ T cells	3.0 $\pm$ 2.5	4.4 $\pm$ 3.3	0.44
CD20+ B cells	46.7 $\pm$ 11.8	44.5 $\pm$ 13.1	0.45
CD3+ T/CD20+ B cell ratio	1.1 $\pm$ 0.7	1.3 $\pm$ 1.0	0.16
CD68+ macrophages	4.5 $\pm$ 3.6	4.7 $\pm$ 3.9	0.33
Fascin+ fDC	1.5 $\pm$ 1.2	1.9 $\pm$ 1.4	0.57
S100+ iDC	0.9 $\pm$ 0.8	0.7 $\pm$ 0.5	0.45
CD56+ NK cells	0.2 $\pm$ 0.5	0.6 $\pm$ 0.6	0.96

MNC: mononuclear cells; fDC: follicular dendritic cell; iDC: interdigitating dendritic cells; NK: natural killer cell.

Table 3. Progression of grade of MSG autoimmune lesions during followup.

Patient	Biopsy Interval (mo)	Infiltrating MNC/mm ² of Tissue			MSG Biopsy Focus Score		
		1st Biopsy	2nd Biopsy	Change, %	1st Biopsy	2nd Biopsy	Change, %
1	77.5	668	703	2.2	1.5	1.6	6.5
2	43.0	579	530	-8.5	1.0	1.00	0.00
3	36.0	146	154	5.5	1.3	1.0	-23.1
4	42.0	748	560	-25.1	2.3	2.6	14.5
5	36.0	1001	386	-61.4 [†]	1.9	1.5	-20.9
6	36.0	888	831	-6.4	2.3	2.3	-2.2
7	110.0	749	1105	47.5	2.7	3.3	24.7
8	36	431	1225	184.2 [†]	2.2	2.5	12.4
9	66.0	1369	1221	-10.8	3.1	3.0	-4.2
10	56.0	1827	1824	-0.2	4.6	5.0	9.4
11	52.0	908	1713	88.7 [†]	3.6	3.4	-5.3
12	48.0	1198	1648	37.6	3.4	3.2	-4.7
13*	54.0	715	1214	69.8 [†]	1.8	3.1	71.1 [†]
14*	42.0	4781	4817	0.8	5.6	6.9	24.1
15	109.0	2098	6383	204.2 [†]	4.0	11.6	190.0 [†]
16	58.0	3575	3318	-7.2	5.8	4.8	-17.5
17	89.0	2954	943	-68.1 [†]	6.9	2.0	-71.1 [†]
18*	41.0	3500	3522	0.6	5.9	5.8	-1.4
19*	36.0	6159	5737	-6.9	5.2	5.3	2.5
20*	64.0	5809	6027	3.8	6.0	6.1	2.3
21	82.0	5110	5856	14.6	6.3	6.4	1.6
22	77.0	3868	3949	2.1	6.0	5.8	-3.3
23	107.5	4522	4556	0.8	5.0	5.1	2.0
24	82.0	2479	2667	7.6	4.0	4.0	0.0
25	42.0	7019	9604	36.8	8.2	11.4	39.0
26	54.0	4654	4739	1.8	5.3	5.8	8.4
27	89.0	4711	4462	-5.3	5.7	5.5	-3.0
28	64.0	5197	5220	0.4	4.8	4.9	2.1

[†] Changes over 60% or change in Tarpley score. * Patients diagnosed with MSG-associated MALT lymphoma at the second biopsy. MSG biopsy focus score: number of lymphocytic foci per 4 mm² of tissue.

shown), including the expression of autoantibodies against Ro/SSA and/or La/SSB ribonucleoproteins (1 out of 4 patients with GC neo-organization was negative). This can be attributed to the fact that in our sample, GC formation was found not to correlate significantly with the presence of autoantibodies — 9/11 (81.8%) GC-positive patients versus 13/17 (76.5%) GC-negative patients were positive for these autoantibodies ($p = 0.07$).

Progression of composition of inflammatory MSG lesions of patients with SS. In accord with the poor progression of the infiltration grade, data analysis revealed that the prevalence of the majority of the MSG infiltrating immune cells, including total T cells and Tregs, B cells, macrophages, DC (interdigitating and follicular), and NK cells, was not significantly changed in repetitive biopsies. Only the CD4+/CD3+ T cell and CD8+/CD3+ T cell percentages were significantly decreased and increased, respectively, during followup. These changes were consistent in all samples, but they were not accompanied by a significant alteration of the CD4+/CD8+ T cell ratio (Table 2). The infiltration by the MNC types was not affected by biopsy time interval, histological, demographic, clinical, or therapeutic measures.

Development of MALT lymphoma at the MSG lesions of patients with SS over time. Five patients (Patients 13, 14, 18, 19, and 20) developed MALT lymphoma during followup, as attested by the immunohistochemical and/or molecular detection of B cell monoclonality at the second MSG biopsy. In all 5 patients, lymphoma was restricted to MSG. The development of lymphoma was not associated with expansion of B cells, since their prevalence remained unchanged between the 2 repetitive biopsies (mean percentage of B cells/total infiltrating MNC in the first vs the second biopsy: $40.7 \pm \text{SD } 15.6$ vs $42.3 \pm \text{SD } 16.6$; $p = 0.6$). Further, it was not associated with a fluctuation in the lesion size, since the grade of infiltration remained unaltered in 4 out of 5 patients (Patients 14, 18, 19, and 20; Table 2), nor with severe infiltration or lymphoid organization to GC. MALT lymphoma development was not correlated with biopsy time interval, biopsy scores, number of infiltrating MNC/mm² tissue, or the other histological variables tested (data not shown). It correlated with the 2 previously identified clinical adverse prognostic factors⁹, low serum C4 levels ($p = 0.05$) and cryoglobulinemia ($p = 0.03$), which were evident at the time of the first biopsy.

DISCUSSION

Our findings suggest that the major progression of MSG inflammatory lesions involves the B cell malignant transformation and not a change in the grade and the prevalence of the major infiltrating MNC types. Indeed, even in cases accumulating a 10-year followup, the intensity of the lesions did not change; mild, intermediate, or severe lesions remained the same. This was also observed in specimens that presented a notable fluctuation of the infiltrating MNC number/tissue area or the biopsy focus score during followup. This fluctuation is in agreement with previous data, presenting an alteration of biopsy focus score in 67%¹⁰ and 33%¹¹ of patients with primary SS. The differences in the percentage of patients with SS whose biopsy focus score changed over time among the 3 groups can be attributed to the lack of a cutoff value for considering an alteration as significant in the 2 previous studies^{10,11}, or to the predominance of patients with severe lesions in our study. Changes in the degree of infiltration were detected only in patients with SS with intermediate and severe lesions, without affecting lesion severity. They were not found to correlate with any histological or clinical factors studied, including the biopsy time interval and the therapeutic interventions applied. This is in accord with previous data showing that treatment with corticosteroids or disease-modifying antirheumatic drugs (DMARD) does not modify the salivary flow rate of patients with SS¹². The change of infiltrating CD4+ and CD8+ T cell subpopulations was rather mild, since it did not affect the CD4+/CD8+ T cell ratio. Histological indicators of tissue degeneration, such as fibrosis and fatty cell infiltration, remained unchanged over time, a fact also observed in heavily infiltrated tissues, suggesting that the chronic inflammation in SS does not necessarily lead to degeneration of the glandular tissue and replacement by fibrosis and fatty cells, as assumed¹³. These findings, along with previous data showing insignificant histological deterioration of the liver lesions of SS patients with primary biliary cirrhosis¹⁴, indicate that the SS autoimmune lesions are slowly progressing. Further, the histological findings are consistent with previous assessments of salivary function showing that the unstimulated saliva flow rates do not change over time^{10,12}; only the stimulated ones worsen¹² in patients with SS.

In 24 out of the 28 patients with SS, the first MSG biopsy was done at diagnosis and remained generally unaltered thereafter, suggesting that fully developed MSG lesions occur at diagnosis. The abiding histopathologic features of the MSG inflammatory lesions over time imply the operation of homeostatic mechanisms that contribute to survival of the inflamed epithelia and tightly regulate the size and composition of the invariable inflammatory infiltrates¹³. Epithelial cells, which are major regulators of local immune responses¹⁵, may determine the extent of inflammation. The fully developed MSG lesions at diagnosis and

their minor modification thereafter suggest that the lesion course follows the clinical picture of the disease. In SS, several clinical and laboratory indices, including adverse prognostic factors for lymphoma development, such as salivary gland (SG) enlargement, palpable purpura, C3 or C4 hypocomplementemia, and cryoglobulinemia, are evident at diagnosis and do not change significantly thereafter^{1,9,13}.

One of the noteworthy findings in our study was the development of MALT lymphoma in 5 patients during followup. The elevated percentage of patients that developed B cell transformation possibly relates to the high number of patients with severe inflammation that were included in our study. However, this transformation was found not to associate with any histological factors, including GC formation¹³, but only to previously identified laboratory measures such as low serum C4 levels and cryoglobulinemia. Although our study did not reveal any correlation with histological variables and the offending factors for this transformation remain unknown, our findings suggest that in high-risk patients, an MSG biopsy must be repeated in order to diagnose development of lymphoma.

The lack of progression of histological features of MSG biopsy over time does not signify simply its diagnostic value, but suggests that it might be useful for patient classification. The fixed T or B cell predominance suggests that patients with SS can be classified into distinct subgroups according to the dominating immune responses. This is of major significance for the selection of appropriate targeted therapeutic approaches, e.g., B cell depletion therapy in patients with SS with predominating B cell responses at the inflammatory lesions.

Our findings indicate that in the majority of patients with SS, progression of MSG autoimmune lesions is rather slow. However, in a subgroup of SS patients expressing adverse serological prognostic factors, the MSG infiltrates progress and MALT lymphoma develops. This progression is not accompanied by changes in the grade and incidence of the major infiltrating MNC types. These findings suggest that, except in cases of suspected development of lymphoma, repeated evaluation of MSG biopsies is unnecessary and underline the value of MSG biopsy for the selection of an appropriate therapy.

REFERENCES

1. Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjogren's syndrome: What we know and what we should learn. *J Autoimmun* 2012;39:4-8.
2. Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjogren's syndrome. *J Autoimmun* 2010;34:400-7.
3. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: A revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*

- 2002;61:554-8.
4. Al-Hashimi I, Wright JM, Cooley CA, Nunn ME. Reproducibility of biopsy grade in Sjogren's syndrome. *J Oral Pathol Med* 2001;30:408-12.
 5. Morbini P, Manzo A, Caporali R, Epis O, Villa C, Tinelli C, et al. Multilevel examination of minor salivary gland biopsy for Sjogren's syndrome significantly improves diagnostic performance of AECG classification criteria. *Arthritis Res Ther* 2005;7:R343-8.
 6. Chisholm DM, Mason DK. Labial salivary gland biopsy in Sjogren's disease. *J Clin Pathol* 1968;21:656-60.
 7. Tarpley TM Jr, Anderson LG, White CL. Minor salivary gland involvement in Sjogren's syndrome. *Oral Surg Oral Med Oral Pathol* 1974;37:64-74.
 8. Skopouli FN, Li L, Boumba D, Stefanaki S, Hanel K, Moutsopoulos HM, et al. Association of mast cells with fibrosis and fatty infiltration in the minor salivary glands of patients with Sjogren's syndrome. *Clin Exp Rheumatol* 1998;16:63-5.
 9. Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjogren's syndrome. *Nat Rev Rheumatol* 2010;6:529-37.
 10. Jonsson R, Kroneld U, Backman K, Magnusson B, Tarkowski A. Progression of sialadenitis in Sjogren's syndrome. *Br J Rheumatol* 1993;32:578-81.
 11. Leroy JP, Pennec YL, Soulier C, Berthelot JM, Letoux G, Youinou P. Follow up study of labial salivary gland lesions in primary Sjogren's syndrome. *Ann Rheum Dis* 1992;51:777-80.
 12. Pijpe J, Kalk WW, Bootsma H, Spijkervet FK, Kallenberg CG, Vissink A. Progression of salivary gland dysfunction in patients with Sjogren's syndrome. *Ann Rheum Dis* 2007;66:107-12.
 13. Jonsson R, Vogelsang P, Volchenkov R, Espinosa A, Wahren-Herlenius M, Appel S. The complexity of Sjogren's syndrome: Novel aspects on pathogenesis. *Immunol Lett* 2011;141:1-9.
 14. Hatzis GS, Fragoulis GE, Karatzaferis A, Delladetsima I, Barbatis C, Moutsopoulos HM. Prevalence and longterm course of primary biliary cirrhosis in primary Sjogren's syndrome. *J Rheumatol* 2008;35:2012-6.
 15. Manoussakis MN, Kapsogeorgou EK. The role of intrinsic epithelial activation in the pathogenesis of Sjogren's syndrome. *J Autoimmun* 2010;35:219-24.