Editorial

With Minor Salivary Gland Biopsy in Sjögren Syndrome, Is a Negative Result Possible?

In this issue of *The Journal*, Sharma, *et al*[1] report on the differences among 3 groups of patients with Sjögren syndrome (SS). The first had a focus score (FS) of < 1 in the minor salivary gland (MSG) biopsy, another had an FS ≥ 1, and the other had an FS of zero. Patients without focal lymphocytic infiltration (i.e., FS = 0) exhibited a low frequency of anti-La antibodies, corneal compromise, and hypergammaglobulinemia, and had no elevated expression of interferon-regulated genes, but did have systemic disease. However, all these patients showed positive anti-Ro antibodies. These results indicate that anti-Ro antibodies may be a key factor influencing the development of the disease, and that the MSG biopsy may be negative in patients with SS.

SS is a chronic autoimmune disease characterized by a progressive lymphocytic and plasma cell infiltration that mainly affects the salivary and lachrymal glands and leads to xerostomia and keratoconjunctivitis sicca (sicca symptoms). The diagnosis of SS is based on the combination of symptoms (sicca symptoms) and the presence of autoimmune characteristics: activation of B cells (i.e., presence of autoantibodies) and/or T cells (i.e., positive MSG biopsy)[2]. The classification of the disease is currently based on the American-European consensus group classification criteria [American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR)], which include serological tests, clinical findings, and histological examination[3]. The presence of autoantibodies or a positive MSG biopsy is mandatory[3].

MSG biopsy plays an essential role in the diagnosis, stratification, and prognosis of SS as well as in the differential diagnosis of the disease (i.e., sarcoidosis, amyloidosis, etc.)[4,5]. In our view, for diagnostic purposes the MSG biopsy is preferred to parotid and lacrimal gland biopsies because it is less invasive and safer while offering similar pathological information. Histologically, MSG can have several changes throughout the disease[6,7]. In the early stages, there are intralobular and interlobular chronic inflammatory infiltrates with or without loss of glandular architecture. Thereafter, there is the formation of small focal mononuclear (mostly lymphocytes) infiltrates around epithelial ducts. Finally, infiltrating cells spread into the parenchyma, causing the formation of a large diffuse infiltrate with glandular destruction, loss of acini architecture, and alteration of the physiological functions[6,7]. The magnitude of the infiltrate (i.e., FS) increases with the duration of disease but does not correlate with salivary secretion[8]. The aggregates of mononuclear cells are formed preferentially in periductal areas. In contrast to the focal sialadenitis of the MSG, lymphocytes infiltrating the major salivary glands often form secondary lymph follicles with, in some instances, clonally expanded B cells, thus rendering them prone to transformation into lymphoma[9]. The current prevalence of lymphoma in SS is < 5% and is the lowest in Latin Americans[2].

The existence of focal lymphocytic sialadenitis (FLS), defined as one or more dense aggregates consisting of at least 50 mononuclear cells per 4 mm^2 (1 focus) located in perivascular and periductal areas, is one of the 5 classification criteria for SS established by the ACR/EULAR, with a sensitivity and specificity > 80%[3]. To correctly evaluate the presence of FLS, the following requirements must be met: examination of 3–5 glands or a minimum glandular surface area of 8 mm^2, and a foci count adjacent to normal-appearing acini in lobules preferably without duct dilation or interstitial fibrosis[10,11].

In 1974 Greenspan, *et al*[12] introduced the concept of FS as an expansion of the Chisholm and Mason classification. According to their definition, the FS is calculated by measuring the entire surface area of lobules in a tissue section through a microscope with a calibrated eyepiece graticule. Afterward, it is necessary to quantify the number of periductal or perivascular aggregates (foci) adjacent to normal acini. The total number of aggregates is divided by the total area of salivary gland lobules to obtain the number of foci per mm^2. Finally, the number obtained is multiplied by 4 to get the FS. An MSG biopsy with FS ≥ 1 is considered positive and correlates with glandular damage, diagnosis, and severity of SS[11].

Difficulties in MSG biopsy interpretation may arise,

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because in addition to the FLS, other conditions may also be observed, such as nonspecific chronic sialadenitis (NSCS; i.e., focal or scattered infiltrate of lymphocytes with mild to moderate structure alteration in lobules), chronic sclerosing sialadenitis (i.e., advanced stage of NSCS), granulomatous inflammation, germinal center (GC) formation, acinar atrophy, interstitial fibrosis, and ductal dilation. These findings are relatively common and increase with age[13]. If FLS is identified despite these alterations, all foci should be counted to calculate the FS, including foci adjacent to abnormal acini. All the alterations should be stated in the pathology report[14].

Pathophysiology of glandular damage is mainly attributed to a cytotoxic response. The majority of the infiltrating cells are T cells (> 75%) and within these, CD4+ T cells are the most prevalent[15]. The composition of the inflammatory cell infiltration varies with the severity of the lesion[13]. Christodoulou, *et al*[7] confirmed through immunohistochemistry (IHC) that mononuclear infiltrates mainly consist of T and B cells, whereas macrophages and dendritic cells (DC) were observed in heavy infiltrates linked to advanced lesions organized in GC. Moreover, they showed that CD4+ T cells, Treg, B cells, macrophages, and interdigitating DC percentages were significantly different depending on whether the autoimmune lesions were mild, intermediate, or severe. However, CD8+ T cells, follicular DC, and natural killer cells did not have a significant percentage variation based on lesion severity. T cells predominate in mild lesions, whereas B cells predominate in advanced ones[7]. Further, other factors beyond inflammatory infiltration have been implicated in the development of SS. Several studies have demonstrated that homeostasis alteration in epithelial cells (target of the disease) plays an important role in the beginning and progression of SS. Morphological and molecular changes in acinar and ductal cells (principally acinar cell polarity modification by tight junctions, hemidesmosomes, alterations of polarity complexes, and changes in mucin quality and quantity) alter the secretory machinery[16]. These alterations are found mainly in MSG biopsies with little inflammation occurring regardless of the amount of or proximity to inflammatory foci[6].

Although the Sjögren's International Clinical Collaborative Alliance released a protocol for sample preparation and determination of FS in patients suspected of having SS[11], confirming oral compromise of SS remains difficult because of the poor reproducibility of the MSG biopsy[14,17]. Fisher, *et al*[5] highlighted the need to standardize histopathological interpretation from the acquisition and processing of the MSG to the interpretation of the local aggregates. Owing to the dispersed character of foci infiltration, reading an inadequate glandular area may cause an over- or underestimation of the FS. To improve the reliability of the reading, it is necessary to evaluate multiple tissue levels, particularly in MSG biopsies with low FS and few ducts. After the standardization of MSG histopathology done by Fisher, *et al*[10], it has been recommended that at least 4 glands be examined, although the minimum of 8 mm^2 surface area suggested may be obtained with 2 to 3 glands. However, because some glands may be damaged during the biopsy process or found to be atrophied, the more glands obtained, the better. Morbini, *et al*[18] demonstrated that multilevel examination of the MSG biopsy improves its diagnostic performance. The authors suggested reading a minimum of 3 different section levels, assuming that a 200-μm length is sufficient to detect independent foci while decreasing the probability of losing the smaller ones. Additionally, another study showed that the difference between deeper intervals was enough to change the MSG biopsy result from positive to negative or vice versa[19]. Up to this point, there is no consensus regarding the optimal intervals to be used.

The MSG biopsy is a major variable in the diagnosis of SS, as long as it is done correctly (Figure 1). Although the majority of the histological studies for the diagnosis of SS are based on H&E staining, IHC procedures are currently carried out to characterize mononuclear infiltration and obtain additional information about proliferation, migration, antibody secretion, and possible formation of GC. However, in some cases, the MSG biopsy can be negative. Under such circumstances and if SS is suspected, anti-Ro antibodies should be present[1,20].

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REFERENCES

1. Sharma R, Chaudhari KS, Kurien BT, Grundahl K, Radfar L, Lewis DM, et al. Sjögren syndrome without focal lymphocytic infiltration of the salivary glands. J Rheumatol 2020;47:394-9.
2. Restrepo-Jiménez P, Molano-González N, Anaya JM. Geoepidemiology of Sjögren's syndrome in Latin America. Joint Bone Spine 2019 Feb 16 (E-pub ahead of print).
3. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome. Ann Rheum Dis 2017;76:9-16.
4. Guellec D, Cornec D, Jousse-Joulin S, Marhadour T, Marcorelles P, Pers JO, et al. Diagnostic value of labial minor salivary gland biopsy for Sjögren's syndrome: a systematic review. Autoimmun Rev 2013;12:416-20.
5. Fisher BA, Brown RM, Bowman SJ, Barone F. A review of salivary gland histopathology in primary Sjögren's syndrome with a focus on its potential as a clinical trials biomarker. Ann Rheum Dis 2015;74:1645-50.

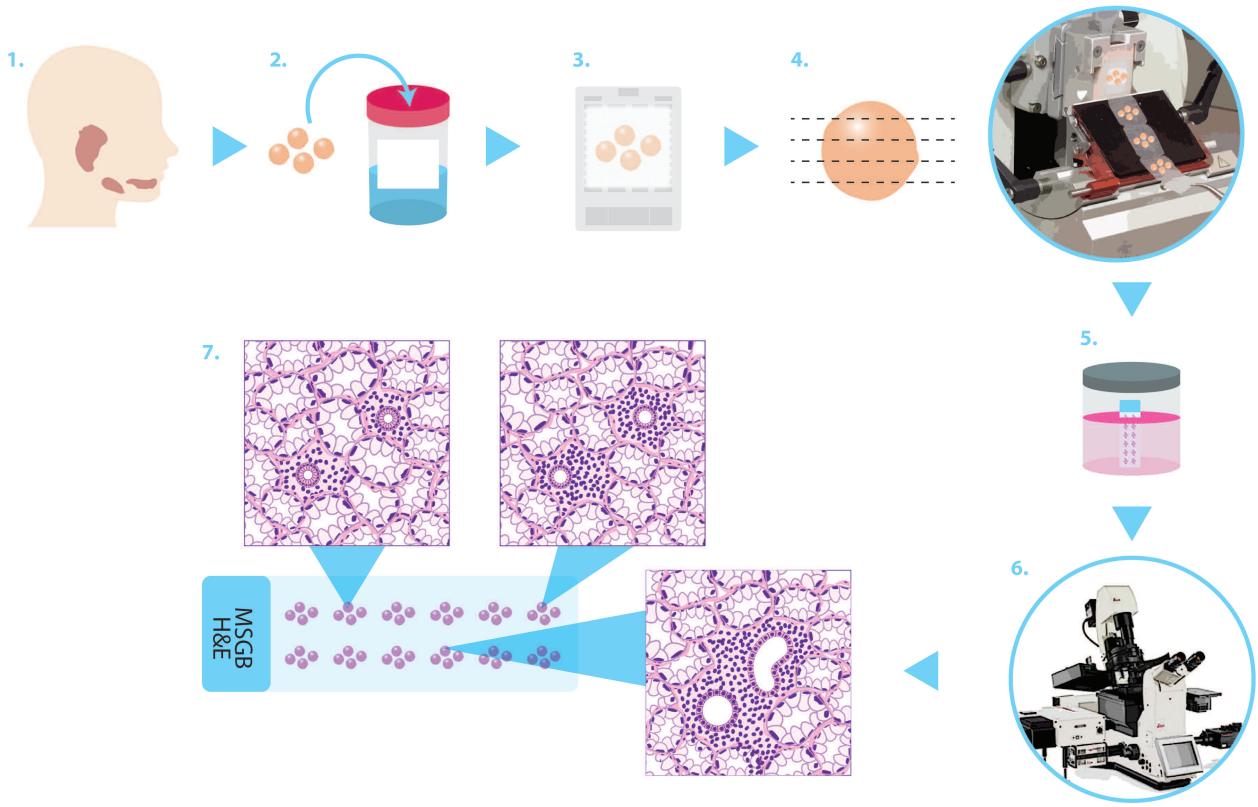

Figure 1. Minor salivary gland biopsy (MSGB) procedure. The following steps should be carried out: (1) incision of 1–2 mm from clinically normal-appearing mucosa of the lower lip between the midline and commissure (at least 4 MSG should be included); (2) fixation in formalin; (3) inclusion in paraffin; (4) taking multiple tissue sections of 4-μm thickness with 200-μm intervals; (5) H&E staining; (6) evaluation of 4 glands or a minimum 8 mm^2 of normal glandular surface in search of focal lymphocytic infiltration; and (7) reading different cutting levels, thereby ensuring the detection of foci on each section. The pathological report should describe number and integrity of MSG (i.e., normal-appearing acini, presence or absence of fibrosis, acinar atrophy, and duct dilation, among others) as well as the presence and location of scattered or focal infiltrates. If these latter are present, FS should be calculated. Preferably, 2 different observers should perform the reading. FS: focus score.

6. González S, Aguilera S, Alliende C, Urzúa U, Quest AF, Herrera L, et al. Alterations in type I hemidesmosome components suggestive of epigenetic control in the salivary glands of patients with Sjögren's syndrome. Arthritis Rheum 2011;63:1106-15.
7. Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. J Autoimmun 2010;34:400-7.
8. Jonsson R, Kroneld U, Bäckman K, Magnusson B, Tarkowski A. Progression of sialadenitis in Sjögren's syndrome. Br J Rheumatol 1993;32:578-81.
9. Dong L, Chen Y, Masaki Y, Okazaki T, Umehara H. Possible mechanisms of lymphoma development in Sjogren's syndrome. Curr Immunol Rev 2013;9:13-22.
10. Fisher BA, Jonsson R, Daniels T, Bombardieri M, Brown RM, Morgan P, et al. Standardisation of labial salivary gland histopathology in clinical trials in primary Sjögren's syndrome. Ann Rheum Dis 2017;76:1161-8.
11. Daniels TE, Cox D, Shiboski CH, Schiødt M, Wu A, Lanfranchi H, et al. Associations between salivary gland histopathologic diagnoses and phenotypic features of Sjögren's syndrome among 1,726 registry participants. Arthritis Rheum 2011;63:2021-30.
12. Greenspan JS, Daniels TE, Talal N, Sylvester RA. The histopathology of Sjögren's syndrome in labial salivary gland biopsies. Oral Surg Oral Med Oral Pathol 1974;37:217-29.
13. Daniels T, Aufdemorte T, Greenspan J. Histopathology of Sjögren's syndrome. In: Talal N, Moutsopoulos HM, Kassan SS, editors. Sjögren's syndrome: clinical and immunological aspects. Berlin: Springer-Verlag; 1987:41-52.
14. Wicheta S, Van der Groen T, Faquin WC, August M. Discrepancies in interpretation of the minor salivary gland biopsy in the diagnosis of Sjögren syndrome. J Oral Maxillofac Surg 2019;77:1628-35.
15. Fox RI, Adamson TC 3rd, Fong S, Young C, Howell FV. Characterization of the phenotype and function of lymphocytes infiltrating the salivary gland in patients with primary Sjogren syndrome. Diagn Immunol 1983;1:233-9.
16. Barrera MJ, Bahamondes V, Sepúlveda D, Quest AF, Castro I, Cortés J, et al. Sjögren's syndrome and the epithelial target: a comprehensive review. J Autoimmun 2013;42:7-18.
17. Fox RI. Standardisation of labial salivary gland biopsies in Sjogren's syndrome: importance for the practicing rheumatologist. Ann Rheum Dis 2017;76:1159-60.
18. 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.
19. Al-Hashimi I, Wright JM, Cooley CA, Nunn ME. Reproducibility of biopsy grade in Sjögren's syndrome. J Oral Pathol Med 2001;30:408-12.
20. Pijpe J, Kalk WW, van der Wal JE, Vissink A, Kluin PM, Roodenburg JL, et al. Parotid gland biopsy compared with labial biopsy in the diagnosis of patients with primary Sjogren's syndrome. Rheumatology 2007;46:335-41.

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