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ORIGINAL ARTICLE

Diagnostic utility of submandibular and labial salivary gland biopsy in IgG4-related sialadenitis

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Abstract

Objective The diagnostic utility of labial salivary gland (LSG) biopsy for IgG4-related sialadenitis remains undetermined. The purpose of the present study was to determine whether submandibular gland biopsy could be replaced by LSG biopsy for diagnosing IgG4-RS.

Patients and methods Medical records of two groups of patients were reviewed. Group A contained 45 patients suspected to have IgG4-RS who underwent both SMG and LSG biopsies. Group B contained 25 patients who were clinically and pathologically diagnosed with Sjögren syndrome (SS). Biopsy samples were stained using hematoxylin and eosin (HE) and immunohistochemical techniques and observed under an optical microscope. Relevant data describing histopathological characteristics were collected and analyzed.

Results SMG of IgG4-RS patients presented typical histopathological characteristics of fibrosis and IgG4-positive plasmacytic infiltration, while LSG showed varied characteristics. The sensitivity and accuracy of SMG for diagnosing IgG4-RS were greater than those of LSG (100% and 100% versus 55.3% and 75.7%, respectively, $P < 0.05$).

Conclusions Biopsy of SMG showed greater sensitivity and specificity, whereas LSG biopsy showed varied histopathological and immunohistochemical characteristics; thus, SMG biopsy cannot be replaced by LSG biopsy for diagnosis of IgG4-RS.

Key Point
• *Biopsy of SMG showed greater sensitivity and specificity than LSG biopsy for diagnosis of IgG4-RS.*

Keywords Biopsy · Diagnosis · IgG4-related sialadenitis · Labial salivary gland · Sjögren syndrome · Submandibular gland

Introduction

IgG4-related sialadenitis (IgG4-RS) typically presents as swelling of single or multiple salivary glands and/or lacrimal glands along with increased IgG4 serum levels. Systemic symptoms can be associated. Complete evaluation of one's clinical history, physical examination, blood tests, and radiological studies is extremely important. However, biopsy results are usually sufficient for an accurate diagnosis [1]. Submandibular gland (SMG) biopsy is frequently used for diagnosis. However, although the position of SMG is relatively superficial, both physicians and patients still share concerns about bleeding, scars, and nerve damage. Recent studies of a patient group showed a decrease in the saliva flow rate of the labial salivary gland (LSG) in IgG4-RS patients, indicating LSG to be a potential target organ [2]. Thus, this raises concerns about the potential role of biopsy of a more superficial LSG in the diagnosis of IgG4-RS. In addition, although LSG biopsy definitely causes less damage, it is still debatable whether or not it can replace SMG biopsy for diagnosis [3–6].

On the other hand, according to the classification criteria for Sjögren syndrome (SS) in 2002, LSG biopsy showing focal lymphocytic sialadenitis with a focus score ≥ 1, defined

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as the number of lymphocytic foci (which are adjacent to normal-appearing mucosa acini and contain > 50 lymphocytes) per 4 mm^2 of glandular tissue, is the histopathological diagnosing criterion for SS [7]. Previous studies have reported that LSG of SS patients could be positive in IgG4 immunohistochemical staining, which indicates the possibility of a misdiagnosis [4, 8]. However, the differential diagnosis between IgG4-RS and SS in terms of histopathological characteristics of LSG warrants further investigation.

Accordingly, the objectives of the present study were (i) to investigate the diagnostic utility of SMG and LSG biopsy for IgG4-RS; (ii) to compare the differences in histopathological characteristics of LSG in SS and IgG4-RS; and (iii) to determine whether SMG biopsy could be replaced by LSG biopsy for diagnosing IgG4-RS.

Materials and methods

This study complies with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee for Human Experiments of the Peking University School of Stomatology. All patients included in the study provided written informed consent.

Patients

Group A contained patients with bilateral swelling of SMG and increased serum IgG4 levels who underwent both SMG and LSG biopsies between December 2014 and September 2018, at Peking University School of Stomatology. Group B contained patients who were diagnosed with SS according to classification criteria for SS in 2002 [7] and underwent LSG biopsy between January 2015 and September 2018, at Peking University School of Stomatology.

Operative procedures

SMG biopsy Patients were placed in the supine position, disinfected, and administered 1% lidocaine local infiltration anesthesia. After making incision and protecting the marginal mandibular branch of facial nerve, SMG was exposed. If the gland was homogenized, a tissue section of approximately $0.8 \times 0.8 \times 0.5$ cm^3 was sectioned off the surface of the gland. If gland lobules remained, blunt separation was performed along the lobules to ensure that the tissue in the lobule was completely removed as a unit. Forced clamping of tissues should be avoided during the procedure. After closing the wound, the collected sample was sent for histopathological and immunohistochemical examination (Fig. 1).

LSG biopsy After local anesthesia, an approximately 0.8×0.5 cm^2 fusiform incision was performed in the lower lip mucosa. In addition, mucosa, submucosa, and acinar tissues were removed above the muscle surface. After closing the wound, the collected sample was sent for histological and immunohistochemical evaluation (Fig. 2).

Histological and immunohistochemical evaluation

All specimens were stained using hematoxylin and eosin (HE) and immunohistochemical techniques. The detailed procedures were described in the Supplementary Figure. Following characteristics were observed under an optical microscope: the range of inflammation, degree of acinus atrophy, degree of fibrosis around the duct and in the stroma, size of lymphoid follicles, and infiltration of eosinophils. In addition, while observing the immunohistochemically stained samples, three sights were selected and observed in the most severely inflamed area in a high-power field (HPF, 10×40), and the number of IgG- and IgG4-positive plasma cells was counted and averaged. Moreover, the average IgG4-/IgG-positive plasma cell count ratio was calculated as well.

Statistical analysis

The variables were expressed as mean and percentages. Correlation was analyzed using Pearson's chi-squared test. Differences among the three groups were analyzed using Kruskal–Wallis test. A P value of < 0.05 was considered statistically significant. All analyses were performed using SPSS version 20 (IBM, Chicago, IL, USA).

Results

Of all 45 patients (27 cases were male and 18 female, their age ranged from 22 to 76 years old, with a median age of 58 years) included in group A, 38 were diagnosed with IgG4-RS, six patient was diagnosed with bilateral chronic submandibular sialadenitis, and the other with mucosa-associated lymphoid tissue lymphoma (MALToma). All IgG4-RS patients had an evaluation of serum IgG4 concentration with the average of 765 mg/dL.

Group B contained 25 patients (all cases were female, their age ranged from 30 to 74 years old, with a median age of 58) who were both clinically and pathologically diagnosed with SS.

All SMG of IgG4-RS patients presented typical histopathological characteristics (Fig. 3), such as lymphocytic and plasmacytic infiltration along with the formation of lymphoid follicles containing map-shaped germinal centers, a large number of fibroblasts with storiform fibrosis in the stroma, and acinus with varying degrees of atrophy. Formation of a typical collagen sheath around the duct was observed in 10 (26.3%) cases. Twenty-five (65.8%) specimens revealed infiltration of

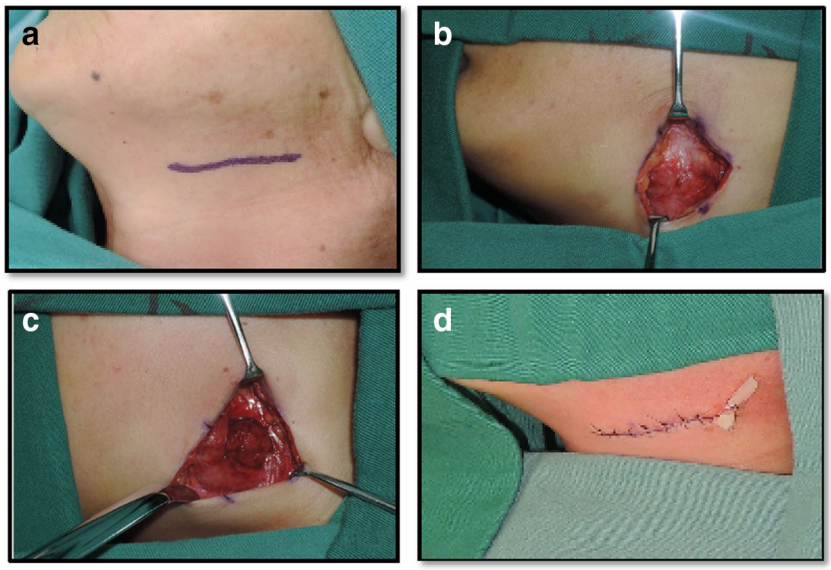

Fig. 1 SMG biopsy procedures. **a** Incision design, **b** SMG exposure, **c** SMG specimen removal, and **d** closing the wound

eosinophils. Thirteen specimens (34.2%) presented obliterative phlebitis.

Immunohistochemical staining showed a large number of IgG- and IgG4-positive lymphocytic and plasmacytic infiltration; the average number of IgG4-positive plasma cells was 115.4/HPF, and the average IgG4-/IgG-positive plasma cell count ratio was > 40% (Fig. 4).

The histopathological and immunohistochemical characteristics of LSG in IgG4-RS patients showed wide variations (Fig. 5). Sixteen (42.1%) cases showed diffuse lymphocytic and plasmacytic infiltration, among which 10 cases showed formation of regular small lymphoid follicles. Three cases showed a mild degree of fibrosis caused by fibroblasts but no storiform fibrosis. Immunohistochemical staining showed extensive IgG- and IgG4-positive lymphocytic and plasmacytic infiltration; the average number of IgG4-positive plasma cells ranged 21–194/HPF (average, 86/HPF), and the average IgG4/IgG-positive plasma cell count ratio was from 56 to 93% (Fig. 5a).

Twelve (31.6%) cases presented a focus of lymphocytes and plasma cells with the center primarily composed of lymphocytes. Scattered plasma cells could be seen in the stroma. No lymph follicle formation or fibrosis was observed. Immunohistochemical staining revealed scattered IgG- and IgG4-positive lymphocytic and plasmacytic infiltration, and the average number of IgG4-positive plasma cells counted ranged from 10 to 76/HPF with an average of 26.4/HPF, and the average IgG4/IgG-positive plasma cell count ratio was from 10 to 78% (Fig. 5b).

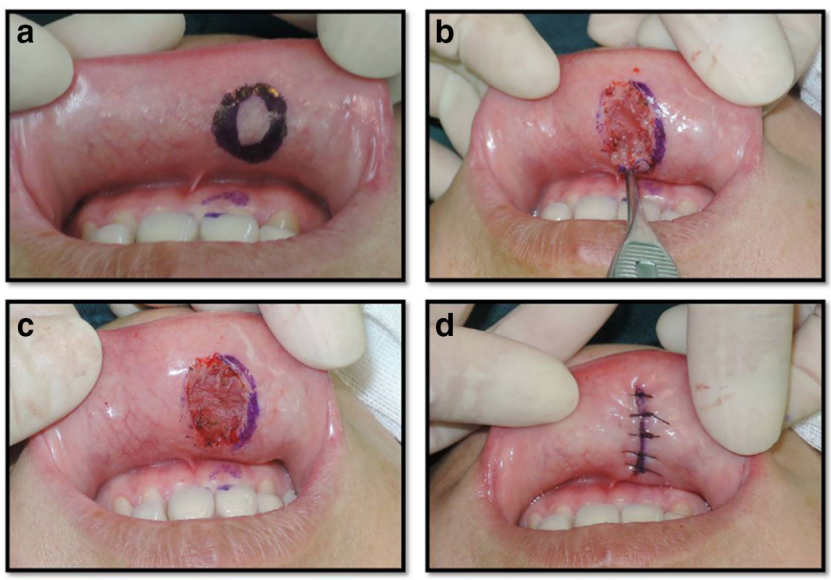

Fig. 2 LSG biopsy procedures. **a** Incision design, **b** LSG exposure, **c** LSG specimen removal, and **d** closing the wound

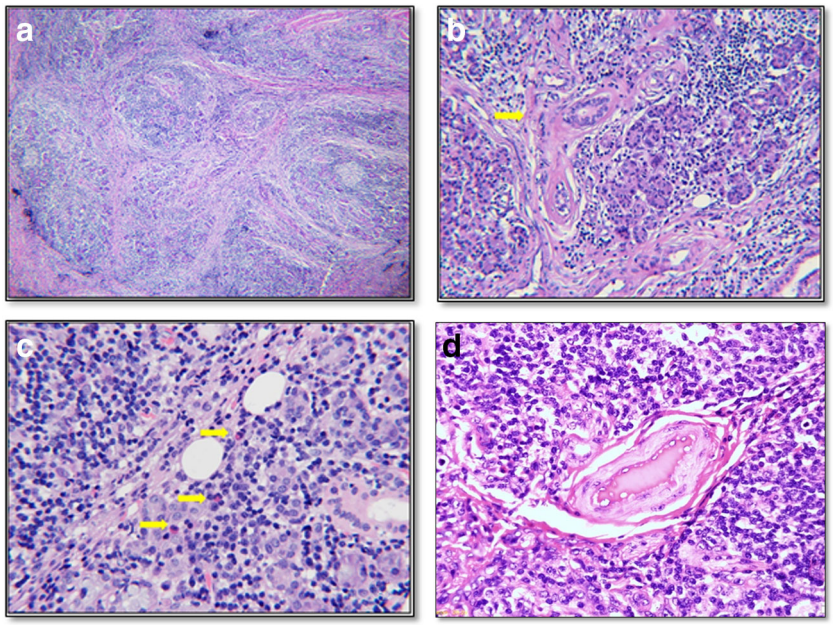

Fig. 3 Histopathological characteristics of SMG in IgG4-RS patients. **a** Lymphocytic and plasmacytic infiltration and storiform fibrosis, **b** lymphoid follicle formation, **c** duct collagen sheath, and **d** eosinophilic infiltration

Ten (26.3%) cases showed scattered lymphocytic and plasmacytic infiltration in the stroma, with no obvious inflammatory reaction. Few IgG- and IgG4-positive lymphocytes and plasma cells were observed, and the average number of IgG4-positive plasma cells counted was < 10/HPF, with an average of 3.6/HPF (Fig. 5c).

None of the LSG specimens showed any typical ductal collagen sheath formation, obliterating phlebitis, or eosinophilic infiltration.

Six patients were diagnosed with chronic submandibular sialadenitis. SMG biopsy showed obvious fibrosis and acinus atrophy. However, immunohistochemical staining showed IgG4-positive plasma cells of < 10/HPF. Results of LSG were negative for lymphocytic infiltration or IgG- and IgG4-positive plasma cells. Because of the increased IgG4 serum levels, a close follow-up was recommended to evaluate any disease progression.

One patient was diagnosed with MALToma based on a medical history of thymoma. SMG biopsy showed extensive lymphocytic and focal plasmacytic infiltration. Obvious glassy degeneration was observed in the stroma. No lymphoid follicles and anomalous cells were seen. On the other hand, LSG showed sporadic lymphocytes and plasma cells as well as acinus atrophy and glassy degeneration of the stroma. Immunohistochemical staining of both SMG and LSG showed scattered IgG- and IgG4-positive plasma cells.

HE staining of LSG biopsy of SS patients showed focal lymphocytic infiltration without lymphoid follicle formation. Plasma cells were hardly seen and primarily appeared near the edge of the lesion (Fig. 6a). Only one sample showed a very mild degree of fibrosis around the duct.

Immunohistochemical staining showed varying degrees of IgG-positive lymphocytic infiltration. The number of IgG-positive cells was counted, which ranged 25–121/HPF (average, > 40/HPF). A very few IgG4-positive plasma cells were observed. The average IgG4-/IgG-positive plasma cell count ratio was < 20%, with an average of 9.4% (Fig. 6b).

The sensitivity, specificity, and accuracy of SMG and LSG were 100%, 100%, and 100% versus 55.3%, 100%, and

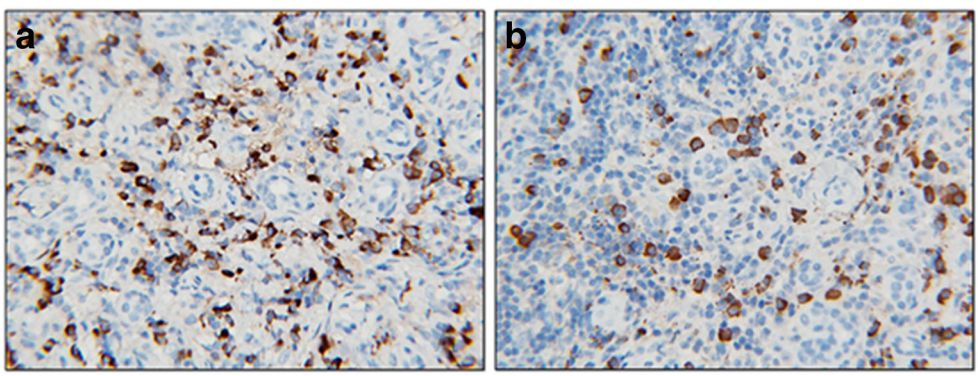

Fig. 4 Immunohistochemical staining of SMG of IgG4-RS patients showed a large number of **a** IgG- and **b** IgG4-positive plasma cells

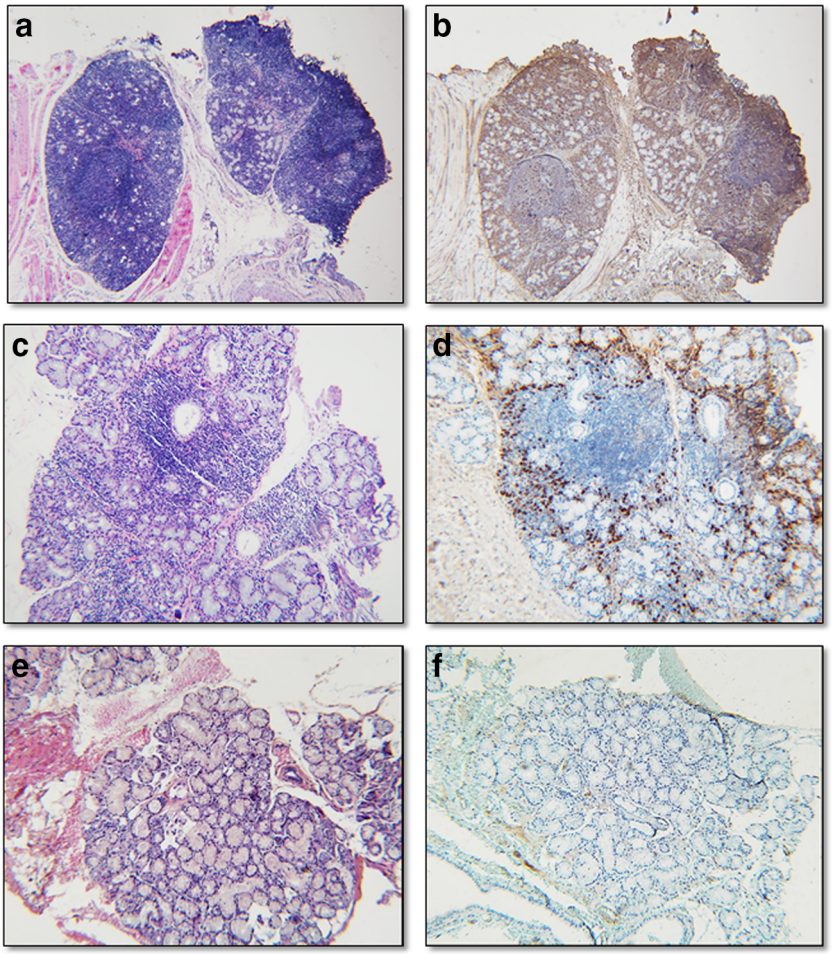

Fig. 5 Histopathological and immunohistochemical characteristics of LSG in IgG4-RS patients. **a**, **b** Diffuse lymphocytic and plasmacytic infiltration with a large number of IgG- and IgG4-positive cells; **c**, **d** Focuses of lymphocytic and plasmacytic infiltration with scattered IgG- and IgG4-positive cells; **e**, **f** No obvious inflammatory reaction with negative results for IgG4-positive cells

75.7%, respectively (Table 1). The difference of sensitivity and accuracy between the two biopsy techniques was statistically significant ($P < 0.05$).

Discussion

Among all the diagnostic criteria of IgG4-RS, the results of histological and immunohistochemical evaluation are of great value [1, 9]. It is vital to rule out neoplastic disease. Because IgG4-positive plasma cells may be observed as well in certain neoplastic diseases such as lymphoma, pathological diagnosis should rely more on histopathological features [10]. SMG is one of the most common target organs of IgG4-RS, and SMG biopsy is often the first choice when selecting biopsy sites [11, 12].

An incisional biopsy of SMG is reliable and can provide an abundant amount of tissue. The surgery is performed under local anesthesia, and only a small piece of tissue is removed from the surface of the gland, which would hardly affect gland function. As reported in our previous ultrasound study on diagnosis of IgG4-RS [13], the superficial region of SMG is

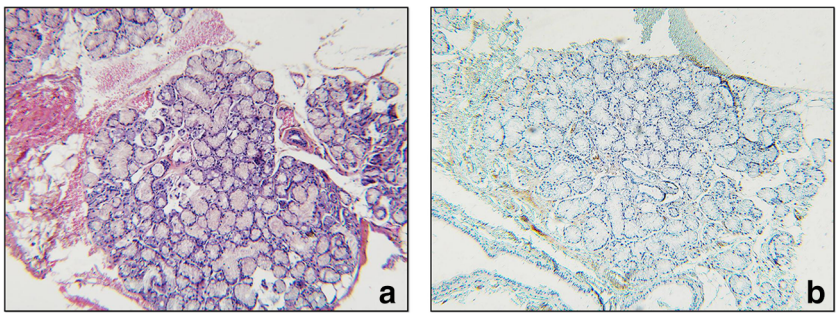

Fig. 6 Histopathological and immunohistochemical characteristics in LSG of SS patients. **a** Focal lymphocytic infiltration in HE staining and **b** negative results for IgG4-positive plasmacytic infiltration

Table 1 Diagnostic utility of SMG and LSG biopsies

Definitive diagnosis	Accord with histopathological and immunopathological diagnosing criteria of IgG4-RS		Total
	Yes	No	
SMG biopsy			
IgG4-RS	38	0	38
Non-IgG4-RS	0	7	7
Total	38	7	45
Sensitivity 100%, specificity 100%, and accuracy 100%			
LSG biopsy			
IgG4-RS	21	17	38
Non-IgG4-RS	0	32	32
Total	21	45	70
Sensitivity 55.3%, specificity 100%, and accuracy 75.7%			

usually the most representative region of the lesion, which is suitable for incisional biopsy. Moreover, direct observation of shape, texture, and existence of glandular structures during biopsy facilitates accurate evaluation of disease severity. However, the operative procedure would definitely leave a scar and possibly hurt the marginal mandibular branch of the facial nerve. Therefore, it is necessary to be cautious in order to avoid complications. In our study, the procedure of incisional biopsy was standardized and no complications were occurred.

On the other hand, core needle biopsy has been used to diagnose autoimmune pancreatitis and IgG4-related disease (IgG4-RD) of the breast [14–16]. To our knowledge, the utility of core needle biopsy in diagnosis of IgG4-RS has not been evaluated yet. Although it definitely causes less damage, it is limited by inaccessibility to reach the typical lesion site and achieve enough samples. Further research is warranted to confirm if specimens achieved from core needle biopsy can reveal typical histological and immunohistochemical characteristics of the disease.

A previous study reported that LSG biopsy could contribute toward accurate diagnosis of SS [7]. However, studies have reported lower accuracy and sensitivity of LGS biopsy in the diagnosis of IgG4-RD. [3, 5, 6, 17, 18] The findings of our study were consistent with these observations.

According to the consensus statement regarding the pathology of IgG4-RD in 2012, its diagnosis is primarily based on histopathological characteristics of the tissue, while IgG4-positive cell counts and IgG4-/IgG-positive cell ratios are secondary factors; the three primary pathological characteristics are lymphocytic and plasmacytic infiltration, storiform fibrosis, and obliterative phlebitis [19]. Unlike SMG, LSG does not present the typical features such as storiform fibrosis and obliterative phlebitis. In a previous study, Takano reported that 27.3% of SMG specimens in IgG4-RD patients presented obliterative phlebitis, but this was absent in all LSG specimens [18]. In the present study, 34.2% of SMG specimens in the IgG4-RS group showed obliterative phlebitis, while no obliterative phlebitis was observed in the LSG specimens, and only 6.7% of specimens showed non-typical fibrosis. The extent of inflammatory infiltration varies in three types of presentation. The diffuse infiltration type presents lymphocytic and plasmacytic infiltration along with a large number of IgG- and IgG4-positive plasma cells; combined with clinical symptoms and serological examinations, this type may most accurately indicate IgG4-RS. On the other hand, the focal infiltration type is difficult to differentiate from SS because of the similar presentation of lymphocytic infiltration; this type provides very limited information for accurate diagnosis of IgG4-RS. No infiltration type can rarely provide any definite evidence for diagnosis. Therefore, negative results of LSG biopsy do not necessarily exclude the possibility of IgG4-RS.

Moriyama et al. believed that LSG biopsy alone may not be sufficient enough to diagnose IgG4-RD. [4] In that study, IgG4-positive lymphocytic and plasmacytic infiltration was evident in LSG biopsy in 8 out of 20 IgG4-RD patients without any salivary gland involvement, and the mean number of affected organs and serum IgG4 levels were significantly higher in the positive cases than in the negative cases. Such findings may indicate that as the disease develops, salivary gland tissues in IgG4-RD patients might show histopathological changes before clinical symptoms appear.

To conclude, SMG biopsy showed higher sensitivity and accuracy, whereas LSG biopsy showed a variety of histopathological and immunohistochemical characteristics and lower sensitivity and accuracy; thus, SMG biopsy is recommended as the first choice for diagnosis of IgG4-RS and cannot be replaced by LSG biopsy.

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Compliance with ethical standards

This study complies with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee for Human Experiments of the Peking University School of Stomatology.

Disclosures None.

References

1. Khosroshahi A, Wallace ZS, Crowe JL et al (2015) International consensus guidance statement on the management and treatment of IgG4-related disease. Arthritis Rheumatol 67:1688–1699
2. Wang Z, Li W, Hong X et al (2016) Minor salivary glands function is decreased in hyposalivation-related diseases. Arch Oral Biol 69: 63–70
3. Akiyama M, Kaneko Y, Hayashi Y, Takeuchi T (2016) IgG4-related disease involving vital organs diagnosed with lip biopsy: a case report and literature review. Medicine (Baltimore) 95:e3970
4. Moriyama M, Ohta M, Furukawa S et al (2016) The diagnostic utility of labial salivary gland biopsy in IgG4-related disease. Mod Rheumatol 1–5
5. Ogawa Y, Nakagawa M, Ishii W, Ikeda S (2013) Lip biopsy in Mikulicz's disease phenotype IgG4-related disease. Intern Med 52:1007–1008
6. Takano K, Keira Y, Seki N et al (2014) Evaluation of submandibular versus labial salivary gland fibrosis in IgG4-related disease. Mod Rheumatol 24:1023–1025
7. Vitali C, Bombardieri S, Jonsson R et al (2002) Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 61:554–558
8. Hermet M, Andre M, Kemeny JL et al (2012) Is IgG4-related disease a cause of xerostomia? A cohort study of 60 patients. Int J Rheumatol 2012:303506
9. Kamisawa T, Zen Y, Pillai S, Stone JH (2015) IgG4-related disease. Lancet 385:1460–1471
10. Strehl JD, Hartmann A, Agaimy A (2011) Numerous IgG4-positive plasma cells are ubiquitous in diverse localised non-specific chronic inflammatory conditions and need to be distinguished from IgG4-related systemic disorders. J Clin Pathol 64:237–243
11. Lin W, Lu S, Chen H et al (2015) Clinical characteristics of immunoglobulin G4-related disease: a prospective study of 118 Chinese patients. Rheumatology (Oxford) 54:1982–1990
12. Li W, Chen Y, Sun ZP et al (2015) Clinicopathological characteristics of immunoglobulin G4-related sialadenitis. Arthritis Res Ther 17:186
13. Li W, Xie XY, Su JZ et al (2016) Ultrasonographic features of immunoglobulin G4-related sialadenitis. Ultrasound Med Biol 42: 167–175
14. Detlefsen S, Mortensen MB, Pless TK, Cribe AS, de Muckadell OB (2015) Laparoscopic and percutaneous core needle biopsy plays a central role for the diagnosis of autoimmune pancreatitis in a single-center study from Denmark. Pancreas 44:845–858
15. Ogiya A, Tanaka K, Tadokoro Y et al (2014) IgG4-related sclerosing disease of the breast successfully treated by steroid therapy. Breast Cancer 21:231–235
16. Hirano K, Fukushima N, Tada M et al (2009) Diagnostic utility of biopsy specimens for autoimmune pancreatitis. J Gastroenterol 44: 765–773
17. Moriyama M, Furukawa S, Kawano S et al (2014) The diagnostic utility of biopsies from the submandibular and labial salivary glands in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease. Int J Oral Maxillofac Surg 43:1276–1281
18. Takano K, Nomura K, Abe A et al (2016) Clinicopathological analysis of salivary gland tissue from patients with IgG4-related disease. Acta Otolaryngol 136:717–721
19. Deshpande V, Zen Y, Chan JK et al (2012) Consensus statement on the pathology of IgG4-related disease. Mod Pathol 25:1181–1192