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Minor salivary gland evaluation: Sjögren's syndrome

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Background/aim: We aimed to analyze the value of 3 serial sections, spaced 200 µm apart, for quantification of lymphocyte and plasma cell foci in minor salivary gland biopsy (MSGB).

Materials and methods: Labial MSGBs from 69 patients with Sjögren's syndrome (SS) and scleroderma were used for this study. Each sample was prepared as 3 serial sections spaced 200 µm apart. Lymphocytic and plasma cell focus score (LFS, PFS) were determined for each section, and the diagnostic results were compared to those obtained from a single section.

Results: For 22 of the 69 patients, all 3 sections were scored at <1 and interpreted as inconclusive for the presence of SS. For 20 cases, all 3 sections were scored at ≥1 and interpreted as diagnostic for SS. In the remaining 27 cases, the score was found to vary between sections. Plasma cell foci were observed in 11 cases, with 5 cases exhibiting a PFS of ≥1. Of those 5 cases, 4 also had a LFS of ≥1.

Conclusion: Assessment of 3 serial sections in MSGB has the potential to improve accuracy of SS diagnosis by detecting specific features that may not have been detected in a single section. We concluded that data about the PFS require further evaluation.

Key words: Sjögren's syndrome, focus score, multilevel examination

1. Introduction

Sjögren's syndrome (SS) is a chronic inflammatory disorder of exocrine glands that clinically presents as 'sicca syndrome'. Inflammation with specific features observed on labial minor salivary gland biopsy (MSGB) is considered to be one of the 'gold-standard' criteria for the diagnosis of SS (1). However, the distribution of the inflammatory cells in the gland may be uneven. It has previously been suggested that, considering this uneven distribution, a single tissue section may result in underdiagnosis. While other investigators have attempted to address this possibility, a conclusive answer has not been found, and the topic remains a source of some controversy (2). In this series, we aimed to analyze the diagnostic impact of examination of MSGB at 3 serial levels and compare that to the diagnostic impact of examination at a single level.

2. Materials and methods

MSGBs of 69 patients with a documented history of xerostomia and/or xerophthalmia complaints, as well as clinical suspicion of SS, were evaluated retrospectively. All patients had presented to a rheumatology clinic for follow-up appointments. Biopsies were performed in order to determine if these patients met the criteria for SS diagnosis. The evaluation was performed using only biopsy results, with no consideration of American-European Consensus Group (AECG) criteria or serological markers.

For routine histopathological evaluation, MSGB sections were fixed in 10% formalin and embedded in paraffin. Tissue was sectioned at 3 levels spaced 200 µm apart. All samples were stained with hematoxylin and eosin.

The tissue sections were reevaluated separately in order to detect both lymphocyte and plasma cell foci. A lymphocytic focus was defined as an accumulation of ≥50 lymphocytes (3). The focus score (FS) was classified as: FS 0, no lymphocytic infiltration; FS 1, slight lymphocytic infiltrate; FS 2, less than 1 lymphocytic foci per 4 mm^2; FS 3, 1 lymphocytic foci per 4 mm^2; FS 4, more than 1 lymphocytic foci per 4 mm^2 (4). Plasma cell focus was defined as the accumulation of ≥20 plasma cells; plasma cell FS was determined similarly to the lymphocytic FS. The lymphocytic and plasmacytic foci were counted under light microscopy. The surface area of the MSGB section was determined by point-counting with a grid overlying the tissue section.

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Focus scores for plasma cell and lymphocytic foci were determined for each section. A focus score of ≥1 was accepted as diagnostic for SS. Once focus scores had been determined for all sections, the 3 focus scores for each patient were compared to each other to detect variation between sections.

3. Results

The mean age of the patients was 51.9 years (min: 22, max: 79), including 60 (87%) females and 9 (13%) males. Of 69 patients, 35 (50.72%) had primary SS, 22 (31.88%) had scleroderma + SS, and 12 (17.39%) had scleroderma. Distributions of sex and final diagnosis are shown in the Table. If the highest FS among the 3 sections is considered, FS was less than 1 in 37 cases (53.6%) and higher than 1 in 32 cases (46.4%).

For 22 of the 69 patients, all 3 sections were scored at <1, interpreted as inconclusive for the presence of SS. For 20 cases, all 3 sections were scored at ≥1, interpreted as diagnostic for SS. In the remaining 27 cases, the score was found to vary between sections. Had only a single section been used for evaluation, these 27 cases (39.1% of the total) may have erroneously been found inconclusive for a diagnosis of SS (Figure 1).

Plasma cell foci were observed in 11 cases, and 5 (7.2%) cases had plasma cell FS of ≥1. Four of these 5 cases were also found to have a lymphocytic FS higher than 1 (Figure 2).

4. Discussion

SS is characterized by diffuse chronic inflammation of the exocrine glands. The disease most frequently affects middle-aged women with a sex ratio of 10:1 (1). The diagnosis is based on the evaluation of multiple clinical, serological, functional, and morphological parameters (5).

Characteristic patterns of inflammation observed on labial MSGB is considered one of the 'gold-standard' criteria for diagnosis of SS (1). Salivary gland inflammation is assessed by scoring the degree of infiltration. The histopathological diagnostic criterion for SS is >50 lymphocytes per 4 mm^2 of minor salivary gland tissue (FS ≥ 1) (4,6).

As inflammatory cells in the gland may be unevenly distributed, the examination of a single tissue section may underestimate the FS (2). While increasing the number of sections has the potential to reduce this problem, the optimal number of sections has yet to be determined (8). Studies dealing with this issue are still limited, and what results do exist remain controversial.

Al-Hashimi et al. (1) reported that the measured FS can change significantly at different tissue depths within the minor salivary glands. They analyzed 38 MSGBs, which were examined at 6 different tissue depths (6 µm, 50 µm, 100 µm, 150 µm, 200 µm, 250 µm). In their study, the majority of the biopsies were found to exhibit substantial variability at all depths. The differences observed were sufficient to affect the diagnosis in approximately 60% of the biopsies.

Table. Distribution of sex and final diagnosis of patients.

Patients	n (%)	Primary SS (%)	Scleroderma + SS (%)	Scleroderma (%)
Female	60 (87%)	31 (51.7%)	19 (31.7%)	10 (16.7%)
Male	9 (13%)	4 (44.4%)	3 (33.3%)	2 (22.2)

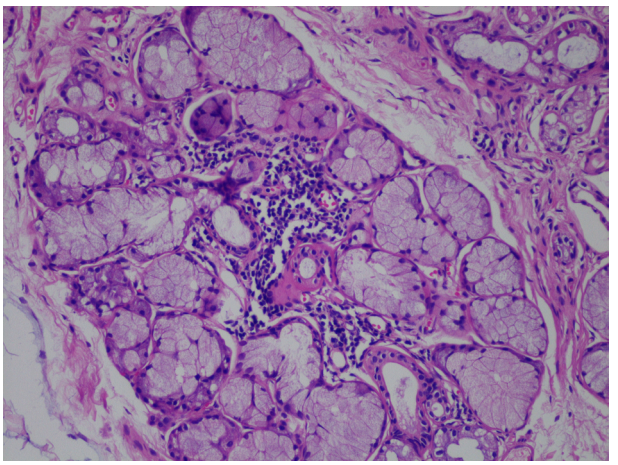

Figure 1. Lymphocytic foci around salivary duct (H&E, 200×).

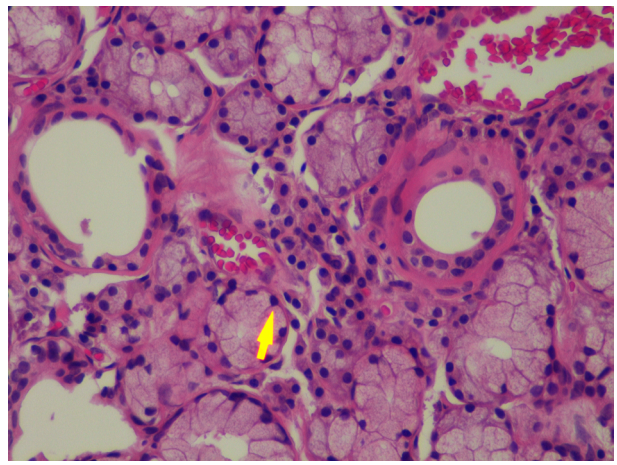

Figure 2. Plasma cell foci at the salivary gland stroma (H&E, 200×).

Morbini et al. (2) also analyzed 120 MSGBs, cut into 3 sections at 200-µm intervals. When multiple sections were taken, the diagnostic classification changed in 6% of the cases. They subsequently divided the cases into 2 groups: cases with a FS of ≥1 and <2, and cases with FS of ≥2. Taking multiple sections was also shown to increase the specificity of MSGB for the first group, whereas the increase was minimal in the second group (2). When patients were classified according to the AECG criteria, the specificity of MSGB evaluation increased by 9.8%, with a statistically significant improvement observed in the diagnostic performance of MSGB.

Contrary to these results, Scardina et al. (8) analyzed 24 labial MSGBs, also sectioned at 3 levels 200 µm apart. In their study, they observed no significant variation in FS distribution between the 3 serial sections.

In our study, a category discrepancy between different sections was observed in 27 (39.1%) cases. These cases represent a significant portion of all the cases examined and could have been misdiagnosed if only a single section was examined.

Although plasma cells may be found in salivary glands, there is insufficient data to determine the diagnostic significance of an elevated number of plasma cells in MSGB. Antibodies secreted by plasma cells can mediate protection against many microorganisms, as well as contribute to the pathogenesis of autoimmune diseases (9). Autoantibodies can persist for years in patients with autoimmune diseases such as SS. The aim of the treatment of autoimmune conditions should be to prevent the initiation of immune responses without affecting noncycling memory and the long-lived plasma cells formed long before treatment (10). Recently, Szyszko et al. (11) evaluated the presence of plasma cells residing in the salivary glands of primary SS patients with high FS and found that these cells showed phenotypic characteristics of the long-lived plasma cell subtype. However, they did not evaluate the diagnostic value of plasma cell foci in MSGB.

In our study, plasma cell FS was defined as the accumulation of 20 plasma cells, and this was equal to plasma cell FS of 1 if only one focus was observed in 4 mm^2 of tissue. We observed ≥1 plasma cell FS in 5 (7.2%) cases; of these, 4 cases had a lymphocytic FS higher than 1, sufficient to diagnose SS without any consideration of plasma cells. Considering that only one case was found to have a plasma cell FS of ≥1 with a lymphocytic FS of <1, it is difficult to evaluate the utility of plasma cell foci in diagnosing SS. A larger study with more patients may allow for a more thorough evaluation.

In conclusion, our study suggests that the assessment of an FS obtained for each of the 3 serial sections at a distance of 200 µm increases the diagnostic usefulness of labial MSGBs. We concur with the previous recommendations of Al-Hashimi et al. (1) and Morbini et al. (2), who suggested that multiple sections for labial MSGB should be used in daily practice. We also provided data that suggest that the diagnostic utility of plasma cell foci in labial MSGB requires further evaluation.

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