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Parotid gland biopsy compared with labial biopsy in the diagnosis of patients with primary Sjögren's syndrome

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***Objective*. To assess the value of the parotid biopsy as a diagnostic tool for primary Sjögren's syndrome (pSS), and to compare the parotid biopsy and the labial biopsy with regard to diagnostic value and biopsy-related morbidity.**

***Methods*. In 15 consecutive patients with pSS and 20 controls, the parotid biopsy was assessed as a diagnostic tool based on the presence of lymphocytic foci, benign lymphoepithelial lesions and lymphoid follicles. These new histological criteria were compared with established diagnostic criteria for the labial biopsy in 35 consecutive patients suspected for pSS who underwent simultaneous biopsies from both sites. In addition, both biopsies were compared for morbidity.**

***Results*. The first analysis revealed a focus score of $\geq$1 or lymphocytic infiltrates (not fulfilling the criterion of a focus score of 1) combined with benign lymphoepithelial lesions as diagnostic criteria for pSS. When comparing the parotid biopsy with the labial biopsy sensitivity and specificity were comparable (sensitivity 78%, specificity 86%). Level of pain was comparable and no loss of motor function was observed. No permanent sensory loss was observed after parotid biopsy, while labial biopsy led to permanent sensory loss in 6% of the patients. Malignant lymphoma was detected in one parotid biopsy by chance, without involvement of the labial salivary gland.**

***Conclusion*. A parotid biopsy has a diagnostic potential comparable with that of a labial biopsy in the diagnosis of pSS, and may be associated with less morbidity.**

KEY WORDS: Sjögren's syndrome, Parotid biopsy, Labial biopsy, Diagnosis.

Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by chronic inflammation of salivary and lacrimal glands, frequently accompanied by systemic symptoms. In addition, 5% of the patients with SS develop B-cell lymphoma during follow-up, most frequently localized in the parotid gland [1].

Several sets of criteria for the diagnosis of SS have been proposed, the American–European (US–EU) classification criteria being the most recent and most commonly applied [2]. Although no single test can serve as a gold standard for diagnosing SS, histopathology of the labial salivary gland remains a key-feature in all sets of criteria [2–4]. A widely accepted criterion for histological confirmation of SS is focal lymphocytic sialoadenitis in the labial salivary gland [5]. However, biopsies of the labial salivary glands may have several disadvantages. The sensitivity and specificity of labial salivary gland biopsies vary in the literature. Data from different studies are often difficult to compare, because different sets of criteria for diagnosing SS have been used and the outcome of the labial biopsy is a strong determinant for the final diagnosis. In a normal population, the labial biopsy resulted in 6–9% false-positive diagnoses, and 18–40% of the patients with a clinical diagnosis of SS have a negative labial biopsy, resulting in a sensitivity of 60–82% and a specificity of 91–94% [6–11]. Moreover, it may be difficult to harvest a sufficient number of labial salivary glands in atrophic submucosa [9]. In addition, permanent sensory loss of the mucosa of the lower lip, occurring in 1–10% of the patients, is a known complication of a labial biopsy [6, 12, 13].

Incision biopsy of the parotid gland can probably overcome most of the disadvantages of the labial biopsy. Parotid gland tissue can be harvested easily, repeated biopsies from the same parotid gland are possible, and the histopathological results can be compared with other diagnostic results derived from the same gland (secretory function, sialographic appearance). In contrast to labial salivary glands, 'lymphoepithelial islands' or 'lymphoepithelial lesions' (LELs) are often observed in parotid gland tissue of SS patients. These LELs, a characteristic histological feature of the major salivary glands in SS [14], develop as a result of hyperplasia of ductal basal cells within a lymphocytic infiltrate. In addition, well-formed lymphoid follicles or germinal centres, often adjacent to ductal epithelium, can be found in the major salivary glands [15]. Since both LELs and reactive lymphoid follicles are also indicative of malignant lymphoma, benign LELs must be discriminated from (pre)malignant lesions, using strict criteria [16, 17].

Despite these aforementioned advantages, biopsies of the parotid gland have not become commonplace because of the fear of facial nerve damage, development of sialocèles and salivary fistulae. In addition, parotid gland biopsies are not part of the established criteria for diagnosing SS. As a result, validated histopathological criteria for diagnosing SS based on biopsy of the parotid gland are lacking.

The purposes of this study were to assess the value of the parotid biopsy as a tool for the diagnosis of primary SS (pSS), to determine whether the diagnostic value of the parotid biopsy is superior to that of the labial biopsy, and to assess morbidity (pain, sensory loss and motor function) associated with parotid and labial biopsies.

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Patients and metrods

The study protocol was approved by the Ethics Committee of the University Medical Center Groningen. All patients and controls provided written informed consent according to the Declaration of Helsinki and were at least 18 yrs of age.

Assessment of the diagnostic value of the parotid biopsy

In order to assess the value of the incisional parotid biopsy as a criterion for the diagnosis of SS, 15 consecutive patients with a diagnosis of pSS were studied. The diagnosis of pSS was based on the US–EU criteria, leaving out the histological criterion [2]. This meant that patients were positive for anti-Ro/SSA or anti-La/SSB antibodies and positive for at least three of the other four criteria (ocular symptoms, oral symptoms, ocular signs and/or salivary gland involvement), or they were positive for the three objective criteria items left (i.e., ocular signs, salivary gland involvement and serology). Patients with any other connective tissue disease were excluded.

Control parotid biopsies were obtained from 20 age- and sex-matched patients with malignancies in the region of the head and neck without involvement of the parotid gland, and undergoing a neck dissection as part of the surgical treatment of their malignancy. All patients had squamous cell carcinoma of the oral cavity or oropharynx. These patients did not have subjective mouth or eye dryness, and no signs of lacrimal or salivary gland dysfunction. Parotid tissue was removed from the dorsal caudal lobe during the neck dissection procedure.

Sensitivity, specificity, positive predictive value and negative predictive value were calculated for various histological criteria in the parotid gland. The histological criteria with the highest diagnostic value were then used in the second part of the study (diagnostic value).

Diagnostic value of parotid and labial biopsy

The potential of the parotid biopsy as a diagnostic tool in 35 consecutive patients suspected of pSS was compared with the diagnostic potential of the labial biopsy in a prospective single-centre study. All patients were referred to the department of Oral and Maxillofacial Surgery of the University Medical Center Groningen, The Netherlands, because of suspicion of SS. The diagnostic work-up carried out in all patients included: subjective complaints of ocular and oral dryness, eye tests (rose bengal staining and Schirmer's tear test), measuring unstimulated whole salivary flow, parotid sialography and serology (anti-SSA/B-antibodies). These patients were subjected to both a parotid and labial biopsy at the same point of time (double biopsy group). All biopsies were performed by the same surgeon (F.K.L.S.). A diagnosis of pSS was made according to the US–EU criteria (including labial histopathology) [2]. The histological criteria for the parotid biopsy, derived from the assessment study (see 'Results' section), were compared with the established criteria for the labial biopsies [5]. In addition, both types of biopsies were analysed for morbidity associated with the two biopsy techniques.

Biopsy technique

All labial biopsies were performed under local anaesthesia. The labial biopsy was accomplished according to the guidelines of Greenspan *et al.* [5]. A lower lip mucosal incision of ~3 cm was made and at least seven individual labial glands were harvested (Fig. 1A). Parotid biopsies from (suspected) pSS patients were taken under local anaesthesia according to the technique described by Kraaijenhagen [18]. In short, a 1 cm skin incision was performed around the lower earlobe (Fig. 1B and C). After blunt dissection to the parotid gland, an incisional biopsy was taken. The wound was closed in layers. No post-operative drape was applied. Control parotid biopsies were obtained under general anaesthesia (during neck dissection).

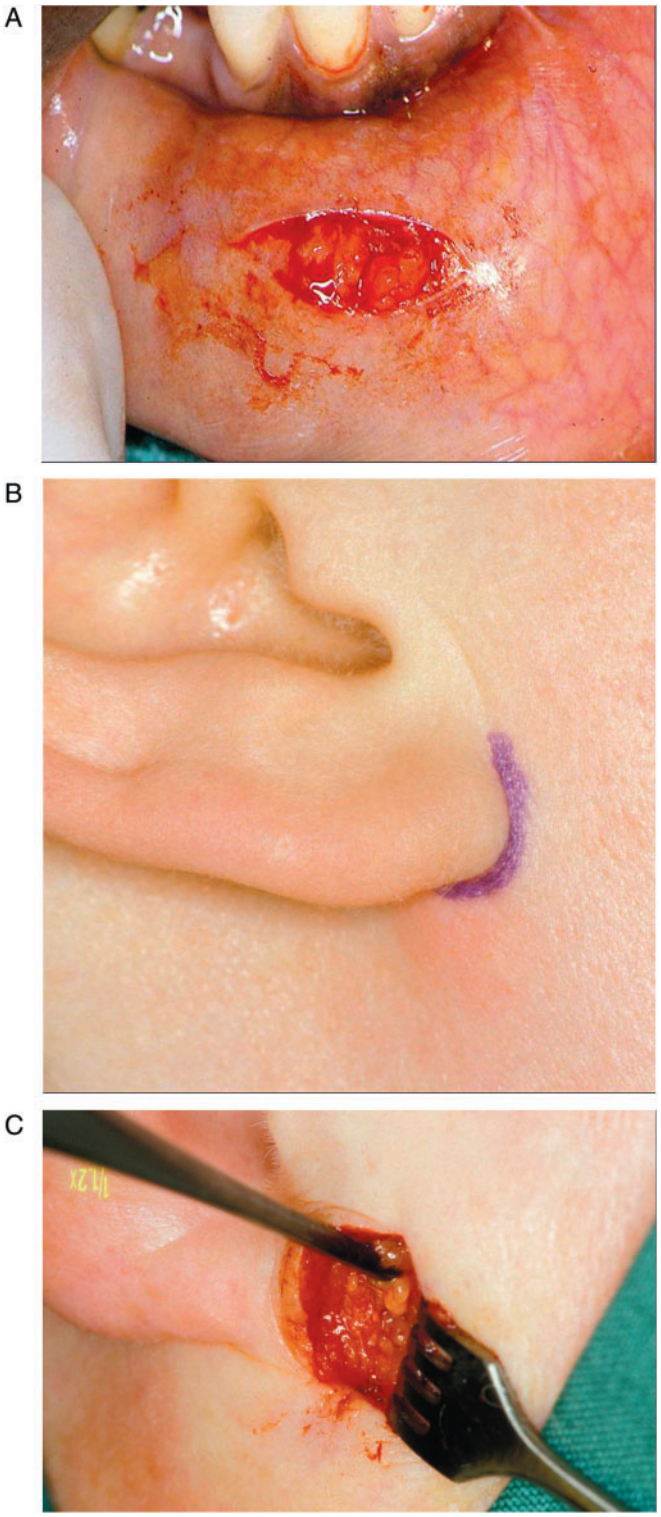

FIG. 1. Biopsy techniques. (A) Horizontal incision of the lower lip exposing salivary glands. (B) Placement of the incision in the ear lobe for the parotid biopsy. (C) Parotid gland tissue at sight.

Histopathological evaluation

Parotid and labial biopsies were fixed in 4% phosphate-buffered formalin, embedded in paraffin blocks, and cut at a thickness of 3 μm. The slides were stained with haematoxylin and eosin.

Since parotid tissue is more or less replaced by fat tissue [19], histomorphometrical analysis was performed to calculate the amount of salivary gland tissue and fat tissue (in square millimetres) in the parotid biopsies, using computer-assisted image-analysis at a magnification of 20× (RVC, Research Assistant 5, Soest, The Netherlands; www.rvc.software.com). All available parotid gland tissues were analysed. The number of lymphocytic foci was counted to determine a focus score, which provides a semiquantitative assessment of inflammation. In accordance with the focus score in labial biopsies, this parotid focus score was based on the number of focal inflammatory cell aggregates containing 50 or more lymphocytes per 4 mm^2 salivary gland. Areas with prominent duct dilatation and/or parenchymal atrophy were excluded from scoring, as were areas of a gland showing extravascular polymorphonuclear leucocytes. The total area of parotid gland tissue was analysed in order to calculate a mean parotid focus score. A minimum of 4 mm^2 of salivary gland was required (in the parotid gland, this included areas with fat deposition). The biopsies were further analysed for the presence of benign LELs [14], confluence of the infiltrate, lymphoid follicles/germinal centres, atrophy, fibrosis and malignant lymphoma, according to the classification of lymphoid neoplasms [20].

A fully benign lymphoid infiltrate was defined by the following criteria: the lobular architecture of the gland must be preserved, monocytoid and/or centrocyte-like lymphocytes are restricted to the LELs (so-called benign LELs), and reactive follicles have no expansion of the marginal zone. Non-confluent centrocyte-like cell halos surrounding the LELs and broad inter-connecting strands of centrocyte-like cells between LELs were considered indicative of malignancy [16, 17].

The labial biopsies were evaluated according to established criteria [5], as well as for the presence of LELs, confluence, germinal centres, atrophy, fibrosis and malignant lymphoma. Histopathological evaluation of each specimen (parotid, labial) was performed independently and in a blinded fashion by two investigators (J.P., J.E.vdW.). Any discrepancies were resolved by consensus.

Morbidity

Morbidity of the biopsy technique was evaluated prospectively in the double biopsy-group at pre-biopsy (baseline value), 1 week, and 6 and 12 months after the biopsy by means of a standardized questionnaire, scored at a 100 mm visual analogue scale (VAS) for pain, and physical examination including sensibility and motor function. Morbidity was evaluated by one of the physicians (J.P., W.W.I.K.) other than the surgeon (F.K.L.S.). Sensibility was tested in the pre-auricular region and ear lobe and at the lower lip near the biopsy sites (two-point discrimination test). Motor function was tested by asking the patients to perform voluntary movements associated with facial nerve innervation.

Statistical analysis

Data are presented as number of patients and as mean ± S.D. To define new diagnostic criteria, parotid biopsies from 15 pSS patients and control parotid biopsies from 20 volunteers were analysed. Starting from the null-hypothesis that labial and parotid biopsies performed equally well for diagnosis of SS, assessment of labial and parotid biopsies in 35 patients was necessary to show a relevant statistical difference between the two biopsies, assuming a power of 90% and an α of 5%. This power-analysis was based on the average predictive value of the labial biopsy as reported in the literature [6, 11, 12, 21–24], and an assumption of 95% for the predictive value of the parotid biopsy. The degree of agreement with respect to the assessment of inflammation before the consensus meeting of the two observers was expressed as weighted Cohen's κ. Statistical analysis of data was performed using the statistical package SPSS for Windows, release 12.0.1. A P-value >0.05 was considered significant.

Results

Assessment of the diagnostic value of the parotid biopsy

To assess the usefulness of the parotid biopsy as a criterion in the diagnosis of SS, parotid tissue of 15 patients with SS and 20 control patients was evaluated. A diagnosis of SS was made according to the US–EU criteria, without histology [2]. As a consequence, all 15 patients had to be positive for anti-Ro/SSA or anti-La/SSB antibodies. Furthermore, these patients were not clinically or serologically suspected of malignant lymphoma, i.e. they had no persistent parotid gland swelling, mixed monoclonal cryoglobulinaemia or monoclonal protein. All SS and control parotid biopsies offered sufficient salivary gland tissue (>4 mm^2) for analysis. Inter-observer agreement on the assessment of inflammation was high (Cohen's κ 0.87). Disagreements were easily resolved by consensus between observers. Benign LELs, without halos or monocytoid B cells, were present in 12 of the 13 parotid biopsies of SS patients with a focus score of ≥1 (92%), and in one of the two biopsies of SS patients with a focus score <1 (Table 1). Six of the 12 parotid biopsies with LELs also showed lymphoid follicles.

Of the 20 control parotid biopsies, one biopsy had a confluent focus with a solitary LEL (focus score of ≥1). This patient was neither clinically nor serologically suspected of SS.

Using a focus score of ≥1 or the presence of small lymphocytic infiltrates (not fulfilling the criteria of a focus score of 1) in combination with benign LELs as criteria for diagnosing SS resulted in a sensitivity of 93% and a specificity of 95%. The positive predictive and negative predictive values were 93 and 95%, respectively.

From the results of this study, we composed the following criteria for the parotid biopsy as histopathological criterion for SS:

(i) a focus score of ≥1, defined as the number of lymphocytic foci (which are adjacent to normal-appearing acini and contain >50 lymphocytes) (Fig. 2A) per 4 mm^2 of glandular parotid tissue (including fat tissue), irrespective of the presence of benign LELs (Fig. 2B).
(ii) small lymphocytic infiltrates, not fulfilling the criterion of a focus score of >1, in combination with the presence of benign LELs.

The parotid biopsy was considered positive if criterion (i) or (ii) was fulfilled.

Diagnostic value of labial and parotid biopsies

A total of 35 consecutive patients, who were referred to our department because of pSS suspicion, were included.

TABLE 1. Focus score of <1 and ≥1 in parotid biopsies of SS patients and controls

Parotid biopsy	Controls (head/neck cancer patients)	Sjögren's syndrome
Focus score <1	19	2 (LEL present in one patient)
Focus score ≥1	1 (LEL present)	13 (LEL present in 12 patients)
Total	20	15

LEL, lymphoepithelial lesion.

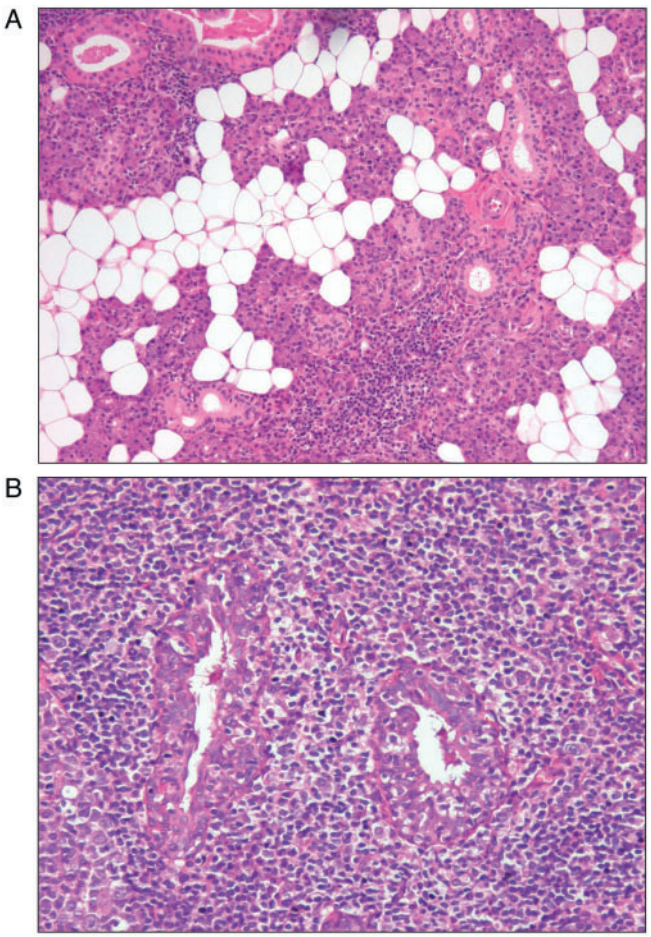

FIG. 2. Histopathogical analysis (haemoglobin and eosin staining). (A) Parotid gland tissue showing a periductular lymphocytic infiltrate and deposition of fat between the serous acini (magnification 10×). (B) Lymphoepithelial lesions surrounded by a lymphocytic infiltrate (magnification 20×).

Sufficient salivary gland tissue both in the parotid and labial biopsy for histopathological analysis was available in 30/35 patients (Table 2); a mean of $10.5 \pm 4.0\,mm^2$ labial salivary gland tissue and a mean of $10.1 \pm 4.0\,mm^2$ parotid gland tissue was available (excluding areas with parenchymal atrophy, nerves and fibrosis). Insufficient salivary gland tissue was observed in two labial biopsies ($<4\,mm^2$) and three parotid biopsies (no salivary gland tissue). Out of these patients 23 fulfilled the criteria for pSS, while seven did not fulfill the criteria for SS (Table 2). The latter patients were clinically diagnosed as having sialoadenosis ($n=4$), medication-induced xerostomia ($n=2$), or as having no disease directly related to salivary gland pathology ($n=1$). Of the five patients with insufficient salivary gland tissue, three met the other criteria for a diagnosis of pSS, while the remaining two could not be diagnosed as pSS.

A parotid biopsy positive for the aforementioned criteria resulted in a sensitivity of 78%, a specificity of 86%, a positive predictive value of 95% and a negative predictive value of 55%. In comparison, a focus score of ≥ 1 in the labial biopsy resulted in exactly the same level of sensitivity, specificity and positive and negative predictive value. In nine patients, the outcome of the labial biopsy determined whether they were diagnosed with pSS (patients 2, 4, 8, 13, 14, 15, 18, 19 and 20; Table 2). In eight of these patients, the parotid biopsy was also considered positive, based on the criteria mentioned earlier. In the remaining 14 pSS patients, the labial biopsy did not influence the definite diagnosis (i.e. these patients had positive autoantibodies and were positive for at least three of the other four criteria (ocular symptoms, oral symptoms, ocular signs and/or salivary gland involvement; Table 2)). The parotid biopsy was positive in nine of these patients, while the labial biopsy was positive in eight. If the parotid biopsy was substituted for the labial biopsy in the diagnostic criteria, only two diagnoses would change. Patient 19 would become negative, while patient 26 would be diagnosed with SS (Table 2). So, as comparable with the labial biopsy, in nine patients the parotid biopsy would determine the definite diagnosis, while in the remaining 14 pSS patients the parotid biopsy would not influence the diagnosis.

Histopathological comparison of labial and parotid biopsies

Histopathological characteristics of the 30 labial and parotid biopsies are described in Table 3. The presence of foci, confluence of the infiltrate and fibrosis was comparable in both major and minor salivary glands. Germinal centres were present in four patients, in both labial and parotid biopsies. LELs were only present in parotid gland tissue, while labial salivary gland tissue showed more atrophy (parotid gland tissue is more characterized by fat deposition [19]).

Screening for malignant lymphoma

All parotid and labial biopsies were screened for malignant lymphoma [20]. A marginal zone B-cell lymphoma was found in the parotid biopsy of one patient, without lymphoma localization in the labial gland (patient 23, Table 3). This patient had no parotid gland swelling, monoclonal protein, cryoglobulins, weight loss or night sweats. Full staging, including CT-scans of thorax, abdomen, pelvis and bone marrow, showed no dissemination. The patient participated in a prospective clinical trial and was treated with four infusions of rituximab ($375\,mg/m^2$). A repeated parotid biopsy was performed after 3 months, which showed stable disease. The patient is alive without progression with a follow-up for more than 2 yrs [25].

Morbidity

Morbidity was evaluated using VAS-scores in the 35 patients in whom both a labial and a parotid biopsy were performed (Table 4). Overall subjective judgement on a scale from 0 to 10 for the labial biopsy was 7.5 (0 = very bad, 10 = no problem) and 7.9 for the parotid biopsy (not significant). At baseline, no sensory disturbances were observed. Nine patients (26%) experienced a subjective temporary change in sensation in the area of the pre-auricular incision, and four patients (11%) at the incision site in the lower lip. Two of the 35 patients (6%) had persistent subjective hypoaesthesia in the lower lip for more than 12 months, while the pre-auricular hypoaesthesia had disappeared in all cases.

The labial biopsies were performed in 9 ± 2 min, the parotid biopsies in 13 ± 3 min. No significant post-operative complications were observed. No sialocèles, fistulae or facial pareses were observed. Eleven patients (31%) experienced some pain after the labial biopsy and 16 patients after the parotid biopsy (46%). The pain following labial and parotid biopsies was comparable in severity and had disappeared in almost all patients within 1 month. Fourteen patients (40%) reported the labial biopsy to cause more complaints, and 15 patients (43%) the parotid biopsy to be more unpleasant. The remaining six patients reported no difference.

TABLE 2. Simultaneous labial and parotid biopsies in 30 patients suspected of Sjögren's syndrome (in five patients insufficient tissue for analysis was available of either the labial or parotid gland). See [2] for criteria for ocular and oral symptoms, ocular signs and salivary gland involvement

	Diagnosis	Ocular symptoms	Oral symptoms	Ocular signs	SS-A/B	Salivary gland involvement	Focus score labial biopsy ≥1	Focus score parotid biopsy ≥1
1	SS	Yes	Yes	Pos	Pos	Pos	Neg	Pos
2	SS[a]	Yes	Yes	Pos	Neg	Pos	Pos	Pos
3	SS	Yes	Yes	Pos	Pos	Pos	Pos	Pos
4	SS[a]	Yes	Yes	Pos	Neg	Pos	Pos	Pos
5	SS	Yes	Yes	Pos	Pos	Pos	Pos	Pos
6	SS	Yes	Yes	Pos	Pos	Pos	Neg	Pos
7	SS	Yes	Yes	Neg	Pos	Pos	Neg	Neg
8	SS[a]	Yes	Yes	Pos	Neg	Pos	Pos	Pos
9	SS	Yes	Yes	Neg	Pos	Pos	Neg	Pos
10	SS	Yes	Yes	Neg	Pos	Pos	Neg	Neg
11	SS	Yes	Yes	Pos	Pos	Pos	Pos	Pos
12	SS	No	Yes	Pos	Pos	Pos	Pos	Neg
13	SS[a]	Yes	Yes	Pos	Neg	Pos	Pos	Pos
14	SS[a]	Yes	Yes	Pos	Neg	Pos	Pos	Pos
15	SS[a]	Yes	Yes	Pos	Neg	Pos	Pos	Pos[b]
16	SS	Yes	No	Pos	Pos	Pos	Pos	Pos
17	SS	No	No	Pos	Pos	Pos	Pos	Pos
18	SS[a]	Yes	Yes	Pos	Neg	Pos	Pos	Pos[b]
19	SS[a]	Yes	No	Pos	Neg	Pos	Pos	Neg
20	SS[a]	No	Yes	Pos	Neg	Pos	Pos	Pos
21	SS	Yes	Yes	Pos	Pos	Pos	Pos	Neg
22	SS	Yes	Yes	Pos	Pos	Pos	Pos	Pos
23	SS	Yes	Yes	Pos	Pos	Pos	Pos	Pos
24	Non-SS	Yes	Yes	Neg	Neg	Pos	Neg	Neg
25	Non-SS	No	Yes	Neg	Neg	Pos	Pos	Neg
26	Non-SS	Yes	Yes	Pos	Neg	Pos	Neg	Pos
27	Non-SS	Yes	Yes	Pos	Neg	Neg	Neg	Neg
28	Non-SS	No	Yes	Neg	Pos	Pos	Neg	Neg
29	Non-SS	Yes	No	Pos	Neg	Pos	Neg	Neg
30	Non-SS	No	Yes	Neg	Neg	Pos	Neg	Neg

SS, Sjögren's syndrome; non-SS, negative for SS (diagnosis according to US–EU criteria [2]); SS-A/B, anti-SSA or SSB antibodies; Pos, positive; Neg, negative; [a]positive outcome of labial biopsy determined the definite diagnosis of pSS; [b]parotid biopsy with focus score <1, but with the presence of LELs.

Discussion

In order to evaluate the diagnostic potential of the parotid incisional biopsy for the diagnosis of SS, criteria for histological grading were developed. We defined a focus score of ≥1 or the presence of lymphocytic infiltrates less than required for a focus score of 1 in combination with benign lymphoepithelial lesions in the parotid biopsy as a criterion for the diagnosis of SS. Using this criterion, we found that the sensitivity and specificity of the parotid biopsy was comparable with that of the labial biopsy in the diagnostic work-up of SS. Standardized criteria for the labial salivary glands have been described by Chisholm *et al.* [26] and Greenspan *et al.* [5] A focus score of ≥1 is required for the diagnosis of SS by the latest US–EU criteria [2]. In the majority of our pSS patients, both the labial and the parotid biopsy had a focus score of ≥1. In addition to the focus score, benign LELs in the parotid gland can be used as an additional aid in diagnosis. This contrasts with the labial biopsy, where LELs do not arise. Furthermore, a lymphoma of the mucosa-associated lymphoid tissue (MALT)-type was detected in the parotid biopsy of one patient, without manifestation in a labial salivary gland.

In the first part of the study, potential histopathological criteria for the parotid biopsy were assessed. In order to evaluate the contribution of histopathology without bias, the histopathological criterion was not included (i.e. results of labial biopsies were not taken into account). Therefore, patients had to be positive for anti-Ro/SSA or anti-La/SSB antibodies in all cases, so introducing a selection bias towards a possible subgroup of patients. This might, in part, explain the high sensitivity and specificity of the parotid biopsy as a criterion for SS found in this study.

In the second part of the study, the parotid biopsy was compared with the labial biopsy as a diagnostic tool for SS in 35 patients suspected of pSS. In these patients, a full diagnostic work-up was performed, including labial histopathology. So, patients were diagnosed with pSS when at least either histopathology or serology was positive. Only patients with a strong suspicion of pSS were included, explaining the relatively high sensitivity and specificity (78 and 86%, respectively) of the histopathology of the labial biopsy. The sensitivity and specificity of the parotid biopsy was comparable with that of the labial biopsy. In the majority of the patients diagnosed with pSS (14 of 23 patients; 61%), the outcome of the salivary gland biopsy did not influence the definite diagnosis. These patients were positive for anti-Ro/SSA or anti-La/SB autoantibodies and for the other (subjective or objective) criteria. In such cases, a salivary gland biopsy (either a labial or parotid biopsy) is not mandatory in the clinical setting to make a diagnosis of SS [2].

Two other studies have compared labial and parotid biopsies, showing that an incisional parotid gland biopsy was superior to or at least comparable with a labial biopsy in the diagnostic work-up of SS [12, 24]. Unfortunately, no firm histological criteria were provided for the parotid biopsies in these two studies, rendering the studies irreproducible. Parotid tissue was only rated as normal or as showing diffuse or focal inflammatory changes.

Insufficient salivary gland tissue (<4 mm^2) was encountered in two labial biopsies and three parotid biopsies. There seems to be no difference in the amount of tissue obtained by either technique. In SS patients, it has been reported that salivary gland tissue is replaced by fat in the parotid gland [19], so the fatty tissue must be included when calculating the focus score (Fig. 2). The fat content

TABLE 3. Histopathological comparison of labial (Lbx) and parotid biopsies (Pbx) in the 30 patients in whom both a labial and a parotid biopsy was performed

	Lbx focus score	Pbx focus score	Lbx LEL	Pbx LEL	Lbx Confl	Pbx Confl	Lbx GC	Pbx GC	Lbx Atrophy	Pbx Atrophy	Lbx Fibrosis	Pbx Fibrosis
1	0	1	–	–	–	–	–	–	Yes	–	Yes	Yes
2	1	2	–	Yes	Yes	Yes	–	–	Yes	–	Yes	–
3	3	0	–	Yes	Yes	Yes	Yes	Yes	Yes	–	–	Yes
4	4	3	–	Yes	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes
5	3	2	–	Yes	–	–	–	–	–	–	–	–
6	0	2	–	Yes	–	Yes	Yes	Yes	Yes	–	Yes	Yes
7	0	0	–	–	–	–	–	–	–	Yes	–	Yes
8	2	1	–	Yes	–	Yes	–	–	Yes	–	Yes	Yes
9	0	1	–	–	–	Yes	–	–	–	–	Yes	Yes
10	0	0	–	–	–	–	–	–	–	–	–	–
11	2	2	–	Yes	Yes	Yes	–	–	Yes	Yes	Yes	–
12	4	0	–	–	Yes	–	–	–	Yes	–	Yes	–
13	4	1	–	Yes	Yes	Yes	–	–	Yes	Yes	Yes	Yes
14	1	2	–	Yes	Yes	Yes	–	–	Yes	Yes	Yes	–
15	2	0	–	Yes	Yes	–	–	–	–	–	–	–
16	2	2	–	Yes	Yes	Yes	–	–	Yes	–	Yes	–
17	3	1	–	Yes	Yes	Yes	–	–	Yes	–	Yes	–
18	4	0	–	Yes	Yes	–	–	–	Yes	Yes	Yes	Yes
19	3	0	–	–	Yes	–	–	–	Yes	–	Yes	Yes
20	2	1	–	Yes	–	Yes	–	–	–	–	–	Yes
21	5	0	–	–	Yes	–	–	–	Yes	–	Yes	–
22	3	2	–	–	Yes	Yes	–	–	Yes	Yes	–	Yes
23	4	3	–	Yes	Yes	Yes	Yes	Yes	–	–	–	–
24	0	0	–	–	–	–	–	–	–	–	–	–
25	2	0	–	–	–	–	–	–	Yes	–	Yes	Yes
26	0	2	–	–	–	Yes	–	–	Yes	–	–	–
27	0	0	–	–	–	–	–	–	–	–	–	–
28	0	0	–	–	–	–	–	–	–	–	Yes	–
29	0	0	–	–	–	–	–	–	-	–	–	Yes
30	0	0	–	–	Yes	Yes	–	–	Yes	–	Yes	Yes

LEL, lymphoepithelial lesion; Confl, confluence of the infiltrate, GC, germinal centre/lymphoid follicles.

TABLE 4. Morbidity of labial and parotid biopsies in all 35 patients

	Labial biopsy			Parotid biopsy		
	1 week	6 months	12 months	1 week	6 months	12 months
Pain (range) (mean VAS score, mm)	34 (15–65)	0	0	34 (5–65)	0	0
Paraesthesia (number of patients,%)	4 (11%)	2 (6%)	2 (6%)	9 (26%)	0	0
Motor function (number of patients)	0	0	0	0	0	0

VAS, visual analogue scale for pain (0 = no pain, 100 = extreme pain); physical examination including sensibility and motor function.

may vary widely, but tends to increase with age in healthy subjects and patients [27]. In inexperienced hands, the risk of harvesting only fatty tissue is even greater. Due to the close resemblance of subcutaneous fat and parotid gland tissue, adequate training is necessary for recognition of these structures. A labial biopsy does not demand such specific surgical expertise, and can therefore be more easily performed in most hospitals.

In this study, a marginal zone B-cell lymphoma was accidentally found in a parotid gland biopsy, without clinical suspicion of a lymphoma. The clinical relevance of early detection of MALT lymphoma, without complaints, is not known. The observed incidence of 3% in the investigated population in this study is compatible with the figures mentioned in the literature [1]. Lymphomas associated with SS often arise in the parotid gland [1], although localization in the labial glands has been described incidentally [28]. It is important to differentiate between benign lesions, borderline lesions and definitely malignant lesions. LELs and reactive lymphoid follicles are prominent in all lesions, but halos and interconnecting strands of centrocyte-like cells are suggestive of malignancy [16].

Both procedures are comparable with respect to burden and acceptance, but up to 6% of the patients may suffer from permanent sensory loss in a part of the lower lip after a labial biopsy. Many patients experience transient hypoaesthesia of the pre-auricular region after a parotid biopsy, probably because the terminal branches of the great auricular nerve are cut by the skin incision. This recovered in all patients within 6 months. Conversely, damage to branches of the mental nerve, caused by a labial incision, is often permanent. Biasi *et al.* [21] already demonstrated that a parotid gland biopsy is a safe procedure in the diagnosis of SS. This is in full agreement with the current study showing only temporarily hypoaesthesia in the pre-auricular region. In experienced hands, no sialocèles or facial nerve damage was observed, so this seems to be an unfounded fear.

In conclusion, a focus score of $\geq$1, or the presence of smaller lymphocytic infiltrates in combination with benign LELs in the parotid gland, is highly suggestive of SS, with a diagnostic sensitivity and specificity comparable with that of a labial biopsy. The histopathological conditions of the minor and major salivary glands in SS seem to be comparable. This study shows that

an incisional biopsy of the parotid gland is a safe and effective procedure in the diagnostic work-up of SS. It may even be considered superior to a labial biopsy as it may cause less long-term morbidity, and gives the possibility for repeated biopsies of the same gland [29]. Therefore, histopathology of the parotid gland should also be included in the classification criteria for SS as an alternative for labial gland biopsy.

Rheumatology	Key messages
	• Parotid biopsy is comparable with labial biopsy in the diagnosis of primary Sjögren's syndrome. • Parotid biopsy might be associated with less morbidity.

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