Increased diagnostic accuracy of the labial gland biopsy in primary Sjögren's syndrome when multiple histopathological features are included

van Ginkel, Martha S; Nakshbandi, Uzma; Arends, Suzanne; Haacke, Erlin A; Liefers, Silvia C; Verstappen, Gwenny M; van Nimwegen, Jolien F; Brouwer, Elisabeth; Stel, Alja J; Spijkervet, Fred K L

Published in:
Arthritis & Rheumatology

DOI:
10.1002/art.42723

Publication date:
2024

Arthritis & Rheumatology
Vol. 76, No. 3, March 2024, pp 421–428
DOI 10.1002/art.42723

American College of Rheumatology
Empowering Rheumatology Professionals

Increased Diagnostic Accuracy of the Labial Gland Biopsy in Primary Sjögren Syndrome When Multiple Histopathological Features Are Included

Martha S. van Ginkel, Uzma Nakshbandi, Suzanne Arends, Erlin A. Haacke, Silvia C. Liefers, Gwenny M. Verstappen, Jolien F. van Nimwegen, Elisabeth Brouwer, Alja J. Stel, Fred K. L. Spijkervet, Arjan Vissink, Hendrika Bootsma, Bert van der Vegt, and Frans G. M. Kroese

Objective. The aim of this study was to evaluate the diagnostic accuracy of the labial salivary gland biopsy based on multiple histopathological features in patients with suspected primary Sjögren syndrome (pSS).

Methods. Patients from a diagnostic sicca cohort with clinically suspected pSS who underwent a labial gland biopsy were included. Patients were categorized as having pSS or non-Sjögren syndrome sicca (non-SS sicca) based on vignettes scored by an expert panel. Labial gland biopsies were analyzed for the presence of four histopathological features: focus score (FS) ≥1, prelymphoepithelial and lymphoepithelial lesions, immunoglobulin G plasma cell shift, and germinal centers. Sensitivity and specificity of histologic features were calculated, and the optimal cutoff value for the number of histopathological features needed to diagnose pSS was determined with receiver operating curve analysis.

Results. A total of 38 patients were categorized as having pSS and 65 as having non-SS sicca. In labial gland biopsies of patients with pSS, the prevalence of FS ≥1 was 82%, followed by 68% for pre-lymphoepithelial and lymphoepithelial lesions, 63% for plasma cell shift, and 24% for germinal centers. Although FS ≥1 showed the highest sensitivity for patients with pSS (82%), specificity was higher for the other three features (98%–100%). The presence of two or more (of four) histopathological features had almost comparable sensitivity to FS alone, but specificity increased with 12% to 100%. For fulfillment of American College of Rheumatology/EULAR criteria, specificity increased from 84% to 95% when an abnormal biopsy was defined by the presence of two or more histopathological features instead of FS ≥1 only.

Conclusion. The diagnostic accuracy of the labial gland biopsy increases when other histopathological features besides FS are taken into account, by reducing the number of false-positive biopsies.

INTRODUCTION

Primary Sjögren syndrome (pSS) is a chronic systemic autoimmune disease characterized by dry eyes, dry mouth and fatigue, various systemic manifestations, and serological abnormalities.[1] Due to the heterogeneity of the disease, classifying and diagnosing pSS can be challenging. In the past years, multiple classification criteria sets were developed for research purposes to allow selection of well-defined and homogenous populations of patients with pSS for clinical studies. Clinical diagnosis, on the other hand, is still based on expert opinion. Salivary gland biopsies play an important role in both classification and diagnosis of pSS. This is reflected by the prominent place of the salivary gland biopsy in the current American College of

Several authors of this publication are members of the European Reference Network ReCONNET.

Supported by the Jan-Kornelis de Cock Foundation (Groningen, The Netherlands) and in part by the Innovative Medicine Initiative-2 (NECESSITY) (grant 806975). This publication is part of project 09150162010166 of the Veni research program (to Dr. Verstappen), which is financed by the Dutch Research Council. There was no involvement of these funding sources in the study design, data collection, analysis, and interpretation of data, or writing of this report.

Martha S. van Ginkel, MD, Uzma Nakshbandi, MD, Suzanne Arends, PhD, Erlin A. Haacke, MD, PhD, Silvia C. Liefers, PhD, Gwenny M. Verstappen, PharmD, PhD, Jolien F. van Nimwegen, MD, PhD, Elisabeth Brouwer, MD, PhD, Alja J. Stel, MD, PhD, Fred K. L. Spijkervet, DMD, PhD, Arjan Vissink, DMD, PhD, Hendrika Bootsma, MD, PhD, Bert van der Vegt, MD, PhD, Frans G. M. Kroese, PhD: University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

Drs. van der Vegt and Kroese contributed equally to this work.

Author disclosures are available at https://onlinelibrary.wiley.com/doi/10.1002/art.42723.

Address correspondence via email to Martha S. van Ginkel, MD, at m.s.van.ginkel@umcg.nl.

Submitted for publication March 22, 2023; accepted in revised form September 21, 2023.

Rheumatology (ACR)/EULAR classification criteria for pSS.[2] These criteria consist of two major items (each 3 points), serology and salivary gland histopathology, and three minor items (each 1 point), two ocular function tests and measurement of salivary flow rate. Patients with a total score ≥4 points meet the criteria for pSS. Therefore, either the presence of serum anti-Ro/SSA antibodies or a salivary gland biopsy with ≥1 focus per 4 mm^2 salivary gland tissue is needed to classify a patient as having pSS.

A focus is defined as a periductal infiltrate that consists of ≥50 lymphocytes. The focus score (FS) is calculated by the number of lymphocytic foci per 4 mm^2 of salivary gland tissue. Currently, biopsies with FS ≥1 are considered positive for pSS.[3,4] The sensitivity of the labial gland biopsy (with cutoff FS ≥1) to diagnose pSS varies between 64% and 94% and the specificity between 61% and 100%, dependent on the study.[5] In previous studies, the diagnosis of pSS often relied on the American–European Consensus Group classification criteria, in which the labial gland biopsy (FS ≥1) is also a major item.[5] As a consequence, the outcome of the biopsy has a major influence on positive or negative classification of pSS, leading to potential overestimation of sensitivity and specificity rates. Only a few studies (partly) eliminated circular reasoning, either applying the Japanese criteria without inclusion of the labial gland biopsy or by using the opinion of experienced clinicians as gold standard instead of criteria sets.[6,7] Lymphocytic foci in salivary glands are not restricted to patients with pSS. Lymphocytic infiltrates can also be found in salivary glands of healthy individuals and in patients with human immunodeficiency virus (HIV) and various autoimmune diseases other than pSS.[8–13] An FS >1 was seen in labial gland biopsies in up to 15% of healthy individuals without sicca symptoms.[8] Older age was associated with higher prevalence of more severe salivary gland lymphocytic infiltrates in a postmortem study of patients without autoimmune diseases and without known head/neck pathology.[14] These observations indicate that salivary gland biopsies may be false positive for pSS, especially in older patients. Vice versa, not all patients with pSS show a labial gland biopsy with FS ≥1; these patients are at risk of being falsely diagnosed with non–Sjögren syndrome (non-SS).[15,16] Previous studies tried to increase the diagnostic accuracy of the labial gland biopsy by analyzing FS in more than one section level or by adding immunohistochemical stainings to detect lymphocytic foci more precisely.[17,18] These approaches mainly affected the sensitivity of the labial gland biopsy but did not increase specificity.

Besides periductal infiltrates, other histopathological features that are specific for pSS can be found in salivary gland biopsies of patients with pSS: pre-lymphoepithelial and lymphoepithelial lesions (LELs), a relative increase in the number of immunoglobulin (Ig) G plasma cells (the so-called plasma cell shift), and the presence of germinal centers (GCs; Figure 1). LELs are defined as hyperplastic ductal epithelium with infiltrating lymphocytes.[19,20] As shown recently, they can relatively easy be

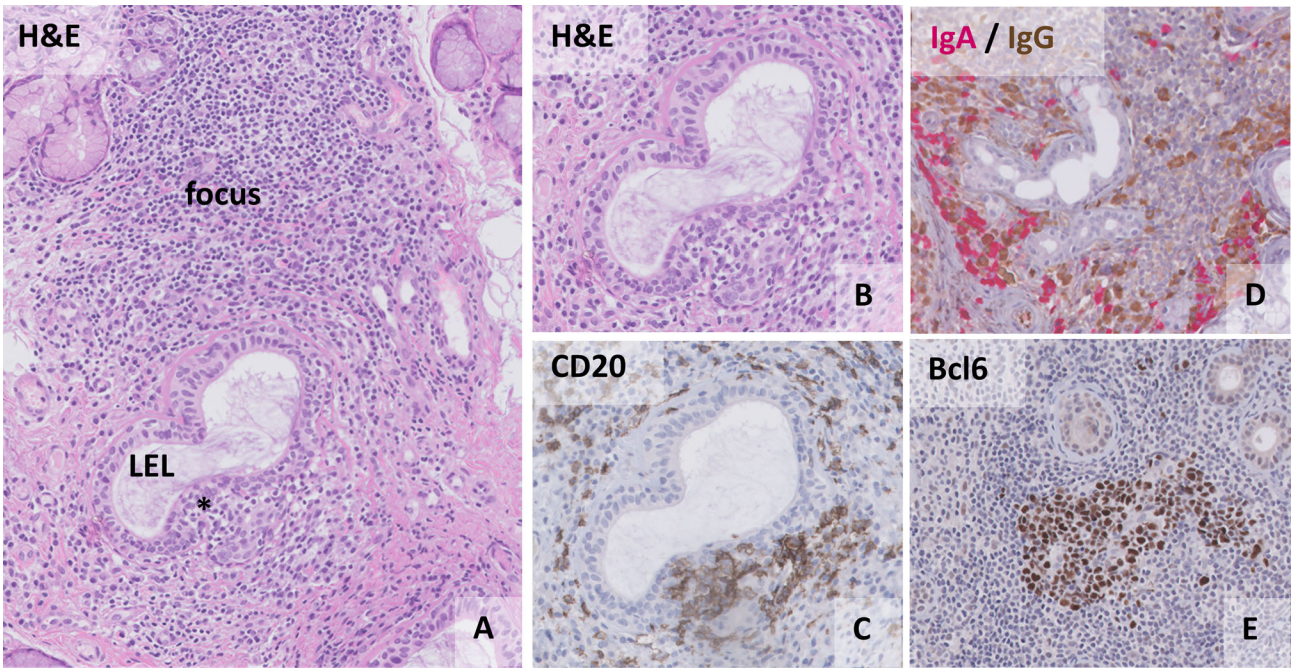

Figure 1. Histopathological features of primary Sjögren syndrome in labial gland biopsies. (A) The presence of a periductal infiltrate of ≥50 lymphocytes (focus) surrounding a striated duct with ductal hyperplasia is shown. (B) Shown is a high-resolution image of the same LEL with ductal hyperplasia in the area with the asterisk (*). (C) A consecutive CD20-stained slide shows the presence of intraepithelial $CD20^+$ B lymphocytes in the same LEL. (D) The presence of a plasma cell shift is shown by a relative increase in the number of IgG (brown) plasma cells compared to the IgA (pink) plasma cells. (E) The presence of a germinal center, defined as a cluster of five or more adjacent $Bcl6^+$ cells, is shown. H&E, hematoxylin and eosin; Ig, immunoglobulin; LEL, lymphoepithelial lesion.

detected by the presence of intraepithelial CD20$^+$ B lymphocytes.[21] B lymphocytes can also be present in striated ducts without hyperplasia of patients with pSS, and these ducts are hypothesized to represent an early stage of LEL formation (pre-LELs).[21] Both the presences of LELs and intraepithelial B lymphocytes are specific for pSS.[21,22] A relative increase in the number of IgG plasma cells, compared to the number of IgA plasma cells, appears to be more specific for pSS than FS alone.[23,24] The formation of GCs is another histopathological feature reflecting B cell hyperactivity in pSS and is present in around one-quarter of the labial glands of patients with pSS.[25,26] Although the diagnostic value of these three histopathological features in pSS was already shown separately, no study explored the added diagnostic value of these features together. Therefore, the aim of this study was to evaluate the diagnostic accuracy of the labial gland biopsy based on multiple histopathological features (FS, pre-LELs and LELs, IgG plasma cell shift, and GCs) in patients suspected of having pSS.

PATIENTS AND METHODS

Patients and inclusion. This prospective diagnostic sicca cohort consisted of consecutive patients with sicca who were suspected of having pSS, were aged ≥18 years, and who underwent a labial salivary gland biopsy during the diagnostic workup for pSS in the University Medical Center Groningen (UMCG), a tertiary referral center for patients with pSS.[27] Patients who were diagnosed with an associated autoimmune disease, positive hepatitis C serology, or (nonspecific) sclerotic sialadenitis within the labial gland biopsy were excluded.

Expert consensus. All patients were categorized as having pSS or non-SS sicca based on expert opinion of a panel of experienced rheumatologists (EB, AJS, and HB). The expert panel independently scored anonymized clinical vignettes as having pSS or non-SS sicca based on information about clinical history, physical examination, EULAR SS Disease Activity Index score, serological parameters (complete blood count, erythrocyte sedimentation rate, C-reactive protein, antinuclear antibodies, anti-SSA, anti–SSB, rheumatoid factor, IgG, C3 and C4, and cryoglobulinemia), results of the evaluation by an oral and maxillofacial surgeon (physical examination of the orofacial and neck area, analysis of unstimulated whole saliva [UWS] and stimulated whole saliva), results of ophthalmological evaluation (Schirmer's test, tear break-up time, and ocular staining score [OSS]), and FS of the labial and parotid gland biopsy (if present). In the UMCG, the parotid gland biopsy is part of the standard diagnostic workup of pSS, and most patients in this cohort underwent paired biopsies of the labial and parotid gland. Therefore, the parotid gland FS, if available, was also included in the vignettes for expert diagnosis. The vignettes did not include information about the other histopathological features (pre-LELs and LELs, IgG plasma cell shift, and GCs) or the diagnosis of the treating physician. HB scored all clinical vignettes, and AJS and EB each scored half of the clinical vignettes. In case of a discrepancy in categorization among the experts, the anonymized clinical vignette was discussed in a consensus meeting with all three experts to reach expert consensus.

Histochemical and immunohistochemical staining. Formalin-fixed and paraffin-embedded labial gland biopsies of 3-μm sections were stained with hematoxylin and eosin (H&E) and immunohistochemically for high MW cytokeratin (hmwCK; clone 34βE12) to detect epithelial cells, for CD20 (clone L-26) to detect B lymphocytes, and for Bcl-6 (clone GI19E/A8) to detect GCs. These immunohistochemical stainings were manually performed using a standardized procedure as previously described.[21,25] Immunohistochemical dual staining for IgA/IgG with polyclonal antibodies was performed on an automated staining platform (BenchMark XT, Ventana Medical Systems) following the manufacturer's protocols.

Histopathological analyses. All slides were digitized using a whole slide image scanner (Philips), and images were stored on a central image server. The total area of the biopsy was measured digitally on H&E-stained sections. The total number of foci was counted by an experienced pathologist, and FS was calculated. Because pre-LELs and LELs can be detected by assessing the presence of intraepithelial B lymphocytes within striated ducts, the presence of CD20$^+$ intraepithelial B lymphocytes was analyzed by using digital image analysis (DIA). Images from the CD20- and hmwCK-stained sections were loaded into the DIA platform Visiopharm Integrator System. After alignment of the consecutive hmwCK- and CD20-stained images, intraepithelial CD20$^+$ B lymphocytes were detected by a DIA algorithm as described before.[21] In case of difficulties with aligning the CD20 and hmwCK images, the presence of intraepithelial CD20$^+$ B lymphocytes was evaluated manually on the digitized slides. For the presence of a plasma cell shift, percentages of IgA$^+$ and IgG$^+$ plasma cells were manually evaluated on the digital slides. A relative decrease of IgA$^+$ plasma cells (≤70% of all IgA$^+$ and IgG$^+$ plasma cells) in the total parenchyma was considered as a plasma cell shift.[23] GCs, defined as a cluster of five or more adjacent Bcl-6$^+$ cells,[25] were manually detected and counted on digitized slides.

Statistical analyses. Patient characteristics were described as mean ± SD or number (%) as appropriate and were compared between patients with pSS and patients with non-SS sicca using the independent samples *t*-test and chi-square or Fisher's exact test. *P* values of less than 0.05 were considered statistically significant. The prevalence of histopathological features was calculated in patients with pSS and patients with non-SS sicca, and sensitivity and specificity

analyses were performed for all histopathological features separately. Receiver operating curve (ROC) analysis was performed to determine the optimal cutoff value for the number of histopathological features according to the highest Youden's index. The performance of the ACR/EULAR criteria after adjusting the biopsy item (two or more histopathological features instead of FS ≥1 only) to predict expert categorization was evaluated with the area under the ROC curve (AUC), which was interpreted as no discrimination (0–0.5), poor (0.5–0.7), fair (0.7–0.8), good (0.8–0.9), or excellent (0.9–1.0) accuracy. All analyses were performed in IBM SPSS Statistics, version 28.

RESULTS

Inclusion and patient characteristics. In total, 113 consecutive patients from a prospective diagnostic cohort suspected of having pSS, were evaluated. Ten patients were excluded from this study due to the presence of an associated autoimmune disease (n = 7), positive serology for hepatitis C (n = 2), or the presence of sclerotic sialadenitis in the labial gland biopsy (n = 1). Of the remaining 103 included patients, 38 patients (37%) were categorized as having pSS according to the expert panel, and 65 patients (63%) were categorized as having non-SS sicca. Patient characteristics of both patient groups are shown in Table 1.

High prevalence of histopathological features other than positive FS in patients with pSS. In the group categorized as having pSS, 31 of 38 patients (82%) had a positive FS (≥1). Furthermore, pre-LELs and LELs, assessed by the presence of intraepithelial B lymphocytes, were found in 68%, followed by the presence of a plasma cell shift in 63% and the presence of GCs in 24% of patients with pSS (Figure 2A; Table 2). In the subgroup of patients categorized as having pSS with an FS <1 (7 of 38), two patients showed presence of histopathological features associated with pSS other than a positive FS: One patient showed a presence of pre-LELs and LELs/intraepithelial B lymphocytes and a plasma cell shift, and the other patient showed a presence of pre-LELs and LELs/intraepithelial B lymphocytes only. The other five patients with pSS with an FS of <1 did not reveal any of the other three histopathological features (Figure 2C).

In the group categorized as having non-SS sicca by the experts, 8 of 65 patients (12%) had a positive FS. Importantly, the biopsies of these eight patients with non-SS did not exhibit other histopathological features besides the positive FS (Figure 2B and 2D). In only one additional patient with non-SS sicca with an FS <1.0 (FS = 0.6), pre-LELs and LELs/intraepithelial B lymphocytes were found. Plasma cell shifts and GCs were completely absent in patients with non-SS sicca, leading to a specificity of 100% for these two features (Figure 2D, Table 2).

Combination of histopathological features improves diagnostic specificity. ROC analysis for the number of positive histopathological features in the labial gland biopsy (calculated as the sum [score 0–4]) of all four features (ie, FS ≥ 1, presence of pre-LELs and LELs assessed by the presence of intraepithelial B lymphocytes, presence of a plasma cell shift, and presence of GCs) showed excellent accuracy for predicting expert categorization, with an AUC of 0.919 (95% confidence interval 0.850–0.998) and an optimal cutoff value of two or more histopathological features, including any combination of the four features. The presence of two or more features showed almost similar sensitivity compared to FS alone (79% for two or more features vs 82% for FS alone), whereas the specificity increased with 12% to 100% because none of the patients with non-SS sicca showed more than one histopathological feature (Figure 2D; Table 2). Also, the positive predictive value increased (100% for two or more features vs 80% for FS alone), and the negative predictive value remained 89% for both FS and two or more histologic features (Table 2). The majority of patients with pSS (29 of 38) showed a positive FS together with one or two of the other features, whereas one patient with pSS had a biopsy that showed two histopathological features without a positive FS (presence of pre-LELs and LELs/intraepithelial B lymphocytes and plasma cell shift; Figure 2C).

Table 1. Patient characteristics of included patients in the prospective diagnostic sicca cohort*

Characteristics	Patients with primary SS (by expert opinion), n = 38	Patients with non-SS sicca (by expert opinion), n = 65	*P* value
Female, n (%)	37 (94)	56 (86)	0.087
Age at time of biopsy, mean ± SD, y	52.6 ± 14.8	49.4 ± 12.7	0.243
Presence of anti-SSA, n (%)	31 (82)	7 (11)	<0.001[a]
Labial gland biopsy FS ≥ 1, n (%)	31 (82)	8 (12)	<0.001[a]
Parotid gland biopsy FS ≥ 1, n (%)[b]	22 (63)	1 (2)	<0.001[a]
Schirmer ≤ 5 mm/5 min, n (%)	31 (82)	38 (59)	0.016[a]
OSS ≥ 5, n (%)[b]	17 (47)	8 (12)	<0.001[a]
UWS ≤ 0.1 mL/min, n (%)	22 (58)	26 (40)	0.079

* FS, focus score; OSS, ocular staining score; SS, Sjögren syndrome; SSA, Sjögren-syndrome–related antigen A; UWS, unstimulated whole saliva.
[a] *p*-values < 0.05 were considered statistically significant.
[b] A total of 97 patients underwent paired labial and parotid gland biopsies in this cohort; OSS was available in 101 patients.

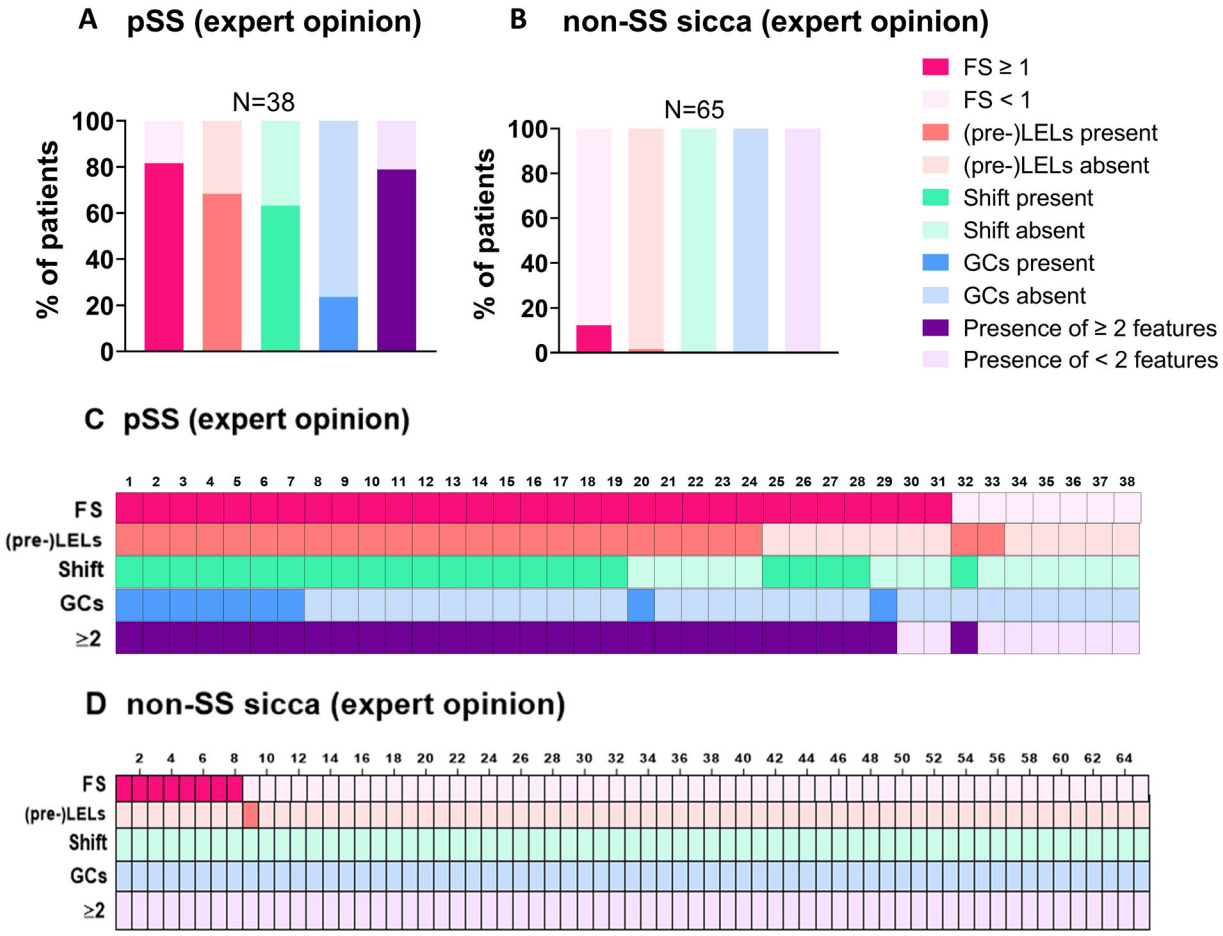

Figure 2. Presence of histopathological features in labial gland biopsies of patients with pSS and patients with non-SS sicca. (A,B) Shown are percentages of (A) patients with pSS and (B) patients with non-SS sicca with the presence of the following histopathological features within labial gland biopsies: a positive FS (≥1), pre-LELs and LELs/intraepithelial B lymphocytes, plasma cell shift (shift), GCs, and two or more of these four histopathological features. (C,D) Shown is the presence of the four histopathological features presented for each individual (C) patient with pSS and (D) patient with non-SS sicca separately. FS, focus score; GC, germinal center; LEL, lymphoepithelial lesion; pSS, primary Sjögren syndrome; SS, Sjögren syndrome.

Combination of histopathological features improves performance of ACR/EULAR classification. Of the patients categorized as having non-SS sicca by the experts with an FS ≥1.0 (n = 8), seven were classified as having pSS according to the ACR/EULAR criteria (Figure 3A). These seven patients had relatively low ACR/EULAR scores (4–6), indicating that these patients would not fulfill the ACR/EULAR criteria without a positive labial gland biopsy (Figure 3B). Furthermore, these patients had lower OSS and higher Schirmer's test results and UWS rates compared to patients categorized as having

Table 2. Sensitivity, specificity, and positive and negative predictive values of histopathological features for the diagnosis of pSS*

Characteristics	Total	Sensitivity, % (fraction)	Specificity, % (fraction)	PPV, % (fraction)	NPV, % (fraction)
Focus score ≥ 1	103	82 (31/38)	88 (57/65)	80 (31/39)	89 (57/64)
(Pre-)LELs/intraepithelial B lymphocytes	101	68 (26/38)	98 (62/63)	96 (26/27)	84 (62/74)
Plasma cell shift	103	63 (24/38)	100 (65/65)	100 (24/24)	82 (65/79)
Germinal centers	102	24 (9/38)	100 (64/64)	100 (9/9)	69 (64/93)
Two or more histopathological features (any combination)	102	79 (30/38)	100 (64/64)	100 (30/30)	89 (64/72)

* LEL, lymphoepithelial lesion; NPV, negative predictive value; PPV, positive predictive value; pSS, primary Sjögren syndrome.

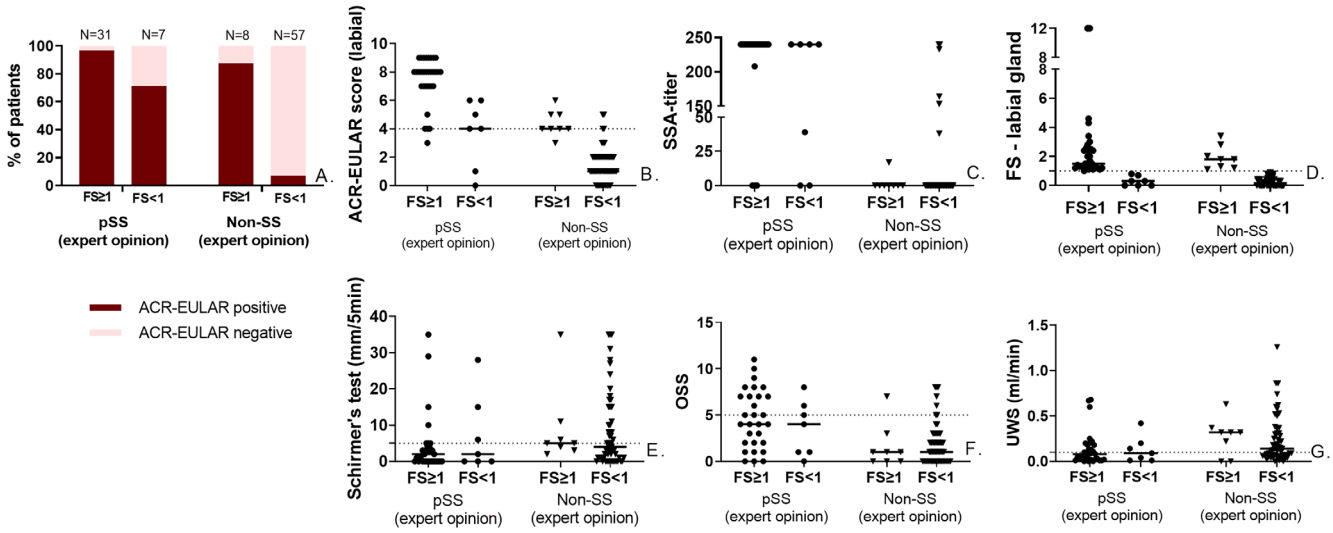

Figure 3. Fulfillment of ACR/EULAR criteria and clinical characteristics of patients divided into four groups based on expert classification and FS. (A) Fulfillment of the ACR-EUALR criteria and (B) the ACR/EULAR scores (range 0–9) is presented in four groups based on expert classification (pSS vs non-SS sicca) and the FSs (positive vs negative). (C) FSs in the labial biopsy, and (D) SSA titers, (E) OSSs, (F) Schirmer's test scores, and (G) UWS, all presented for the same four groups based on expert classification (pSS vs non-SS sicca) and positivity of the FS. ACR, American College of Rheumatology; FS, focus score; OSS, ocular staining score; pSS, primary Sjögren syndrome; SS, Sjögren syndrome; SSA, Sjögren-syndrome–related antigen A; UWS, unstimulated whole saliva. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.42723/abstract.

pSS by the experts (Figure 3C–G). Also, most of these patients were anti-SSA negative (seven of eight), except for one patient with a low anti-SSA titer of <25 (Figure 3C). The patients categorized as having non-SS sicca by the experts with a positive FS were not older compared to the other groups, and no differences were found in smoking behavior among the groups.

The sensitivity and the specificity of the original ACR/EULAR criteria (FS ≥ 1) were 92% and 84% in this cohort, respectively. When the original biopsy item was replaced for the biopsy item presence of two or more histopathological features, sensitivity decreased with 2% to 90%, but, importantly, the specificity increased by 11%, resulting in a specificity of 95%. The optimal cutoff value of the ACR/EULAR criteria remained 4 points (Table 3). The slight decrease in sensitivity is explained by one patient who changed from being diagnosed with pSS (original ACR/EULAR criteria) to non-SS (adjusted ACR/EULAR criteria). This patient had an FS of 2.6 without other histopathological features, was SSA negative, and was categorized as having pSS by the experts (Table 3). Importantly, the increase in specificity is explained by seven patients changing from being diagnosed with pSS (original ACR/EULAR criteria) to non-SS (adjusted ACR/EULAR criteria). All these seven patients had a positive FS in their labial gland biopsy only without any of the other three histopathological features, and these patients were all categorized as having non-SS sicca by the experts.

DISCUSSION

In a diagnostic cohort of patients with sicca clinically suspected of having pSS, we showed here that the specificity and the positive predictive value of the labial gland biopsy both increase to 100% when, besides FS ≥1, the following histopathological features are taken into account: presence of pre-LELs and LELs (assessed by the presence of intraepithelial B lymphocytes), a plasma cell shift, and GCs. The presence of two or more histopathological features in the labial gland biopsy performed was best to diagnose pSS according to expert opinion. When we replaced the biopsy item (FS ≥ 1) in the current ACR/EULAR criteria with the biopsy item "presence of two or more histopathological features," the specificity of the ACR/EULAR criteria increased by >10%. Thus, the diagnostic accuracy of the labial gland biopsy as well as the performance of the ACR/EULAR criteria increases when other histopathological features besides FS are taken into account. This leads to a lower number of false-positive labial gland biopsies and thereby a lower number of patients with misclassified pSS.

In this cohort, patients who solely had a positive FS in the labial gland biopsy without one of the other three histopathological features were mostly categorized as having non-SS sicca by the experts. This indicates that patients are at risk of having a false-positive biopsy for pSS when the histopathological criteria for pSS solely rely on the FS. Seven of the eight patients with a positive biopsy based on FS ≥1 who were categorized as having non-SS by the experts, however, did fulfill the ACR/EULAR criteria for having pSS (including the labial gland biopsy result). For these patients, expert categorization as having non-SS sicca was based on a combination of clinical characteristics, serology, and results of ocular and oral tests. These patients with non-SS sicca were mostly SSA negative and had higher Schirmer scores, higher UWS, and lower OSS (Figure 3). Also, in most (six of seven)

Table 3. Analysis of the cutoff value of the adjusted ACR/EULAR score when FS ≥1 is replaced by the presence of two or more histologic features*

Characteristics	Cutoff value	Sensitivity, %	Specificity, %
Original ACR/EULAR score (FS ≥ 1)	3	94.7	78.1
	4	92.1	84.4
	5	78.9	93.8
Adjusted ACR/EULAR score (two or more histopathological features)[a]	3	92.1	89.1
	4	89.5	95.3
	5	81.6	98.4

* ACR, American College of Rheumatology; FS, focus score; LEL, lymphoepithelial lesion.
[a] Two or more histopathological features include the four primary Sjögren syndrome–specific features: FS ≥1, presence of pre-LELs and LELs/intraepithelial B lymphocytes, presence of a plasma cell shift, and presence of germinal centers.

of these patients, the FS of the parotid gland was <1. Together, this indicates that these patients may represent a subgroup of patients with sicca complaints but without specific clinical characteristics of pSS except for a (false) positive labial gland biopsy. It is known that salivary gland biopsies can be positive for pSS in healthy individuals without sicca symptoms (≤15%).[8] Lymphocytic infiltrates in the salivary glands are found in patients with diseases other than pSS, such as patients with myasthenia gravis, bone marrow transplant recipients, patients with HIV, and patients with hepatitis C.[11–13,28] The reason for the presence of lymphocytic infiltrates in the glandular tissue of patients with non-SS sicca potentially causing false-positive biopsies for pSS is not completely understood. These infiltrates might be caused by local injury, such as chewing or biting or subclinical infections.[8]

Classification criteria for pSS are developed and widely used for the selection of patients for clinical studies and trials.[2,29] The pathophysiology of sicca symptoms in the subgroup of misclassified patients with pSS with a false-positive biopsy could be different from patients with pSS. Therefore, this could influence studies that investigate pSS pathogenesis or treatment efficacy. Preferably, the misclassified patients should not be included in these studies. In contrast to the FS, the presence of pre-LELs and LELs/intraepithelial B lymphocytes, a plasma cell shift, and GCs were highly specific for pSS in the presented cohort of patients with sicca. These three features are all signs of hyperactivity of B lymphocytes, which is a hallmark of pSS, and seem to better reflect the severity of the lymphocytic infiltrate than FS alone. Adjusting the biopsy item to two or more histopathological features in the ACR/EULAR criteria resulted in a higher specificity and a lower number of patients with misclassified (false-positive) pSS. Sensitivity, however, slightly decreased in the adjusted ACR/EULAR criteria. This decrease in sensitivity was caused by only one patient with pSS who was reclassified from having pSS (original criteria with FS only) to non-SS (adjusted criteria with two or more histopathological features). In comparison, previous studies showed that adding salivary gland ultrasonography to the ACR/EULAR criteria only slightly changed the performance but increased feasibility of the criteria.[30,31] By taking the four histopathological criteria into account instead of FS alone, the performance of the ACR/EULAR criteria clearly improved. Regarding feasibility, the immunohistochemistry to detect pre-LELs and LELs/intraepithelial B lymphocytes, a plasma cell shift, and GCs can be performed in most diagnostic pathology laboratories. Detection of intraepithelial B lymphocytes can also be performed manually in case of an absence of DIA software.[19] Before adjusting the biopsy item in the ACR/EULAR criteria, our findings should be validated in another diagnostic cohort. However, the current results already show that for diagnosis, clinicians (rheumatologists and pathologists) should be cautious when interpreting the results of biopsies with only a positive FS (and no other pSS-related histopathological features) because this may lead to a false-positive diagnosis of pSS in the absence of serum anti-SSA antibodies.

A strength of this study is the use of expert opinion as the gold standard. Although the FS was included in the clinical vignettes, the experts were blinded for the other three histopathological features and the diagnosis of the treating physician. Blinding the experts for the additional histopathological features is essential to evaluate its diagnostic accuracy. Blinding for FS would have resulted in categorization difficulties and misclassified patients because FS plays an important role in diagnosing pSS. Furthermore, the use of an expert panel and vignettes with extensive clinical information minimized the impact of circular reasoning compared to using the classification criteria as the gold standard.[5] Because parotid gland biopsies are part of the standard diagnostic workup in our center, the experts also had access to the parotid gland FS in most patients (Table 1), which could have influenced expert opinion. However, by including both the labial and parotid FSs in the vignettes, they stayed as close as possible to the normal diagnostic workup. Besides labial and parotid FSs, the experts took the combination of all clinical characteristics, serological results, and oral and ocular examination into account, thereby limiting the impact of the parotid FS on expert categorization. This is also illustrated by the fact that some patients in this cohort had a positive labial gland biopsy FS and a corresponding negative parotid gland biopsy FS but were categorized as having SS by the experts (10 of 97). Of these 10 patients, 4 were SSA negative, and these patients were categorized as having pSS on the combination of clinical features included in the vignettes (even without SSA antibodies and with a negative parotid FS). Importantly, our analysis showed that three of these four patients did have a presence of other histopathological features besides FS in their labial gland biopsy, further underlining the increase in specificity that these features give.

In conclusion, these data show that the diagnostic accuracy of the labial gland biopsy to diagnose pSS increases when the histopathological features pre-LELs and LELs, assessed by the presence of intraepithelial B lymphocytes, a plasma cell shift, and GCs are taken into account besides the FS, leading to a lower number of false-positive labial gland biopsies in patients clinically suspected of having pSS.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. van Ginkel had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. van Ginkel, Nakshbandi, Arends, Haacke, Verstappen, van Nimwegen, Spijkervet, Vissink, Bootsma, van der Vegt, Kroese.

Acquisition of data. van Ginkel, Nakshbandi, Liefers, Brouwer, Stel, Bootsma, van der Vegt.

Analysis and interpretation of data. van Ginkel, Nakshbandi, Arends, van der Vegt, Kroese.

REFERENCES

1. Brito-Zerón P, Baldini C, Bootsma H, et al. Sjögren syndrome [review]. Nat Rev Dis Primers 2016;2:16047.
2. Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. Arthritis Rheumatol 2017;69:35–45.
3. Greenspan JS, Daniels TE, Talal N, et al. The histopathology of Sjögren's syndrome in labial salivary gland biopsies [review]. Oral Surg Oral Med Oral Pathol 1974;37:217–229.
4. Daniels TE, Cox D, Shiboski CH, et al; Sjögren's International Collaborative Clinical Alliance Research Groups. Associations between salivary gland histopathologic diagnoses and phenotypic features of Sjögren's syndrome among 1,726 registry participants. Arthritis Rheum 2011;63:2021–2030.
5. Guellec D, Cornec D, Jousse-Joulin S, et al. Diagnostic value of labial minor salivary gland biopsy for Sjögren's syndrome: a systematic review [review]. Autoimmun Rev 2013;12:416–420.
6. Morbini P, Manzo A, Caporali R, et al. Multilevel examination of minor salivary gland biopsy for Sjögren's syndrome significantly improves diagnostic performance of AECG classification criteria. Arthritis Res Ther 2005;7:R343–R348.
7. Obinata K, Sato T, Ohmori K, et al. A comparison of diagnostic tools for Sjögren syndrome, with emphasis on sialography, histopathology, and ultrasonography. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:129–134.
8. Radfar L, Kleiner D, Fox P, et al. Prevalence and clinical significance of lymphocytic foci in minor salivary glands of healthy volunteers. Arthritis Rheum 2002;47:520–524.
9. Segerberg-Konttinen M, Konttinen YT, Bergroth V. Focus score in the diagnosis of Sjögren's syndrome. Scand J Rheumatol Suppl 1986; 61:47–51.
10. Drosos A, Andonopoulos A, Costopoulos J, et al. Prevalence of primary Sjögren's syndrome in an elderly population. Br J Rheumatol 1988;27:123–127.
11. Lindahl G, Hedfors H. Focal lymphocytic infiltrates of salivary glands are not confined to Sjögren's syndrome. Scand J Rheumatol Suppl 1986;61:52–55.
12. Lindahl G, Lönnquist B, Hedfors E. Lymphocytic infiltration and HLA-DR expression of salivary glands in bone marrow transplant recipients: a prospective study. Clin Exp Immunol 1988;72:267–273.
13. Ihrler S, Zietz C, Sendelhofert A, et al. Differential diagnosis of lymphoepithelial lesions of salivary glands. With particular reference to characteristic duct lesions [review]. Pathologe 2000;21:424–432.
14. Takeda Y, Komori A. Focal lymphocytic infiltration in the human labial salivary glands: a postmortem study. J Oral Pathol 1986;15:83–86.
15. Sharma R, Chaudhari KS, Kurien BT, et al. Sjögren syndrome without focal lymphocytic infiltration of the salivary glands. J Rheumatol 2020; 47:394–399.
16. Monsalve DM, Anaya J-M. With minor salivary gland biopsy in Sjögren syndrome, is a negative result possible? J Rheumatol 2020;47: 310–312.
17. Trivedi A, Cornejo KM, O'Donnell P, et al. Employing immunohistochemical staining to labial minor salivary gland biopsies from patients with Sjogren's syndrome increases diagnostic certainty. J Oral Pathol Med 2021;50:98–102.
18. Bredahl SK, Reibel J, Pedersen AM. Value of multilevel sectioning of labial salivary gland biopsies in the diagnosis of Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131: 85–91.
19. van Ginkel MS, Haacke EA, Bootsma H, et al. Presence of intraepithelial B-lymphocytes is associated with the formation of lymphoepithelial lesions in salivary glands of primary Sjögren's syndrome patients. Clin Exp Rheumatol 2019;37(Suppl 118): 42–48.
20. Ihrler S, Zietz C, Sendelhofert A, et al. Lymphoepithelial duct lesions in Sjogren-type sialadenitis. Virchows Archiv 1999;434: 315–323.
21. van Ginkel MS, van der Sluis T, Bulthuis ML, et al. Digital image analysis of intraepithelial B-lymphocytes to assess lymphoepithelial lesions in salivary glands of Sjögren's syndrome patients. Rheumatology (Oxford) 2022;62:428–438.
22. Leroy JP, Pennec YL, Letoux G, et al. Lymphocytic infiltration of salivary ducts: a histopathologic lesion specific for primary Sjögren's syndrome? Arthritis Rheum 1992;35:481–482.
23. Zandbelt MM, Wentink JR, de Wilde PC, et al. The synergistic value of focus score and IgA% score of sublabial salivary gland biopsy for the accuracy of the diagnosis of Sjögren's syndrome: a 10-year comparison. Rheumatology (Oxford) 2002;41:819–823.
24. Bodeutsch C, de Wilde PC, Kater L, et al. Quantitative immunohistologic criteria are superior to the lymphocytic focus score criterion for the diagnosis of Sjögren's syndrome. Arthritis Rheum 1992;35: 1075–1087.
25. Nakshbandi U, Haacke EA, Bootsma H, et al. Bcl6 for identification of germinal centres in salivary gland biopsies in primary Sjögren's syndrome. Oral Dis 2020;26:707–710.
26. Kroese FG, Haacke EA, Bombardieri M. The role of salivary gland histopathology in primary Sjögren's syndrome: promises and pitfalls [review]. Clin Exp Rheumatol 2018;36(Suppl 112):222–233.
27. van Nimwegen JF, van Ginkel MS, Arends S, et al. Validation of the ACR-EULAR criteria for primary Sjögren's syndrome in a Dutch prospective diagnostic cohort. Rheumatology (Oxford) 2018;57: 818–825.
28. Maldonado JO, Beach ME, Wang Y, et al. HCV infection alters salivary gland histology and saliva composition. J Dent Res 2022;101: 534–541.
29. Arends S, de Wolff L, Deroo L, et al. Selection of study endpoints and patients for clinical trials in primary Sjögren's syndrome. Clin Exp Rheumatol 2022;40:2225–2232.
30. van Nimwegen JF, Mossel E, Delli K, et al. Incorporation of salivary gland ultrasonography into the ACR-EULAR criteria for primary Sjögren's syndrome. Arthritis Care Res (Hoboken) 2020;72: 583–590.
31. Jousse-Joulin S, Gatineau F, Baldini C, et al. Weight of salivary gland ultrasonography compared to other items of the 2016 ACR/EULAR classification criteria for primary Sjögren's syndrome. J Intern Med 2020;287:180–188.