Review

Can salivary gland ultrasonography replace salivary gland biopsy in the diagnosis of Sjögren's syndrome?

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ABSTRACT

Ultrasound is a promising diagnostic method when it comes to assessing the involvement of major salivary glands in patients with primary Sjögren's syndrome (pSS). A matter of debate is whether ultrasound of the major salivary glands (SGUS) can replace a salivary gland biopsy in the diagnosis or classification of pSS. The intra- and inter-observer reliability of SGUS was found to be good, especially when focusing on hypoechogenic areas and homogeneity, and comparable to the reliability of histopathologic characteristics of salivary gland biopsies of pSS patients. However, replacing salivary gland biopsy by SGUS led to substantial decrease of the accuracy of the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria with clinical diagnosis as the gold standard. When SGUS was added as an additional item to the criteria, the accuracy of the criteria remained high, offering at the same time the clinicians a wider array of tools to assess patients. Combination of SGUS and anti-SSA antibodies was shown to be highly predictive of the classification of a patient suspected of pSS, making routine salivary gland biopsy debatable.

Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease, second to rheumatoid arthritis (RA), with a prevalence of 60.8 (95% CI: 43.7 to 77.9) cases per 100,000 inhabitants (1). pSS commonly affects the exocrine glands, in particular the salivary and lacrimal glands, resulting in a sensation of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia) (2). In search of widely accepted criteria, the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria have been developed (3). These criteria combine features of the previous ACR and AECG criteria, based on methodology consistent with current ACR and EULAR guidelines (3, 4). The 2016 ACR/EULAR classification criteria for pSS are based on five objective tests, *i.e.* anti-SSA(Ro) serology and salivary gland biopsy (each scoring 3) and Schirmer's test, ocular staining score and unstimulated whole saliva flow rate (each scoring 1). Patients with signs and/or symptoms suggestive of pSS who have a total score of ≥4 for the above-mentioned items are classified as pSS patients (3). The purpose of the classification criteria is to assist in defining homogeneous study groups for research. In daily clinical practice, however, these criteria are often helpful for diagnostic purposes, but expert opinion should be leading for the final clinical diagnosis.

Recent discussion has focused on the accuracy of ultrasonography to assess the involvement of the major salivary glands in pSS and eventually to classify or diagnose the disease or even to replace salivary gland biopsy. The aim of this focused review is to comprehensively present the current evidence regarding the possible replacement of the salivary gland biopsy by ultrasonography of the major salivary glands (SGUS) in patients suspected with pSS.

Salivary gland ultrasonography

SGUS is a popular diagnostic method when it comes to assessing the involvement of major salivary glands in pSS (Fig. 1) (5-8). SGUS is well tolerated, non-invasive, inexpensive, non-irradiating and widely available in the rheumatologic outpatient clinics.

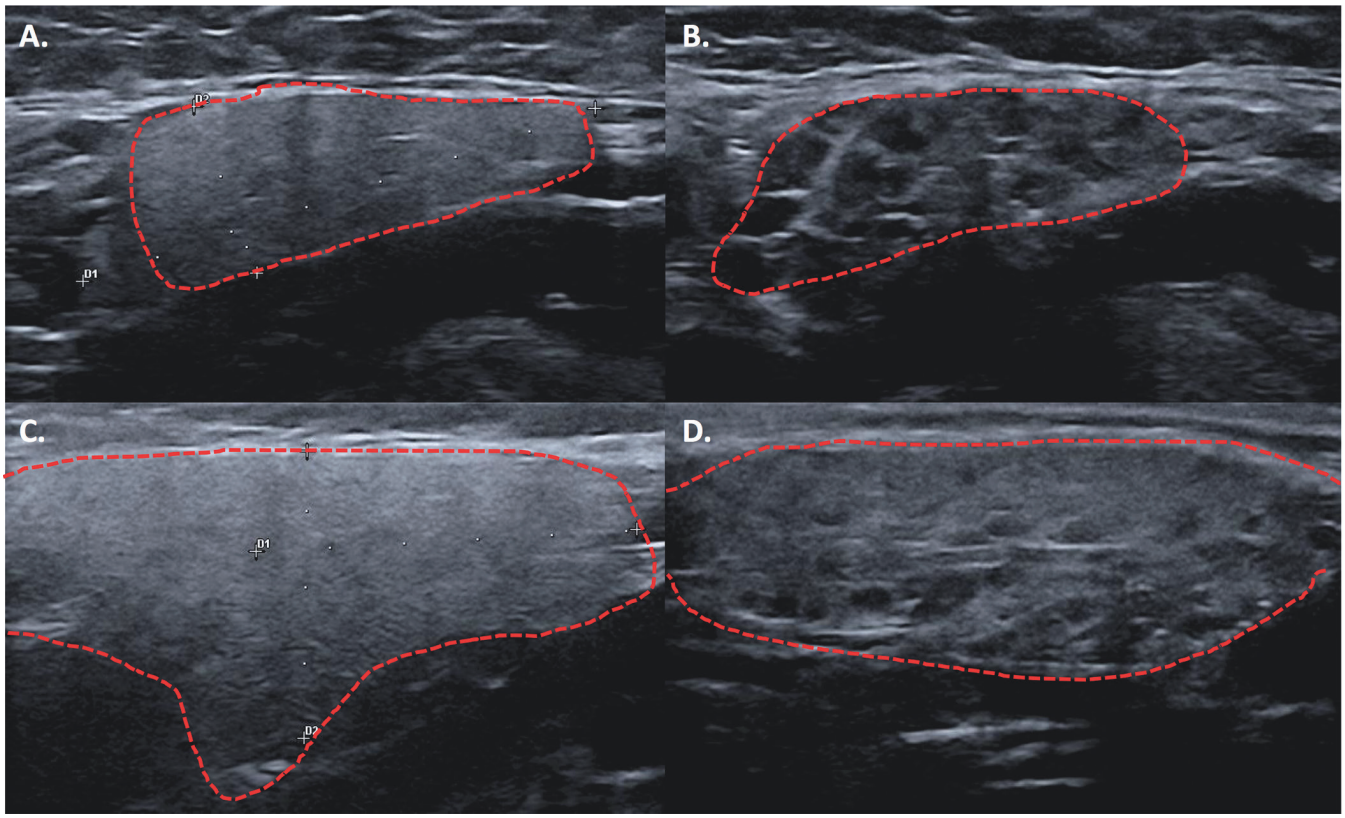

Fig. 1. Ultrasonographic image of: (**A**) normal submandibular gland; (**B**) submandibular gland suggestive of primary Sjögren's syndrome; (**C**) normal parotid gland and (**D**) parotid gland suggestive of primary Sjögren's syndrome.

For optimal results, patients are examined with an ultrasonographic scanner equipped with a high-resolution linear scanner (4–13MHz) and are lying in supine position with their neck slightly extended and turned away from the examined side (9, 10). Usually, the parotid and the submandibular salivary glands are examined bilaterally, nevertheless, ultra-high frequency ultrasonography (UHFUS) has also been recently used to assess labial glands (11).

Assessing the ultrasonographic images obtained was, however, challenging in the past. A meta-analysis has identified 33 different scoring systems used to evaluate the major salivary glands in patients with SS, with most of them evaluating the following ultrasonographic characteristics: (I) parenchymal echogenicity; (II) homogeneity; (III) presence of hypoechogenic areas; (IV) hyperechogenic reflections; and (V) clearness of the salivary gland border (5). In an attempt to increase the feasibility of SGUS, Mossel *et al*. suggested that examination of parotid and submandibular glands on one side and scoring only hypoechogenic areas is also sufficient to predict classification of patients according to the ACR-EULAR criteria (12).

Recently, the Outcome Measures in Rheumatology (OMERACT) group has proposed a consensus-based scoring system to harmonise and standardise the analysis of SGUS for the assessment of pSS (13). This OMERACT SGUS scoring system includes a semi-quantitative evaluation of the parenchymal heterogeneity of the four major salivary glands, *i.e*. both parotid and submandibular ones, with a scoring ranging from 0 (normal parenchyma) to 3 (pathological parenchyma without area of normal parenchyma). A score ≥2 when assessing parotid and submandibular glands is interpreted as a SGUS compatible with the diagnosis of SS. In order to assess pathologic salivary gland vascularisation in patients with pSS, the same group introduced a consensus-based semiquantitative scoring system for colour Doppler findings, where: grade 0 represents no visible vascular signals; grade 1 represents focal, dispersed vascular signals; grade 2 diffuse vascular signals detected in <50% of the gland; grade 3, diffuse vascular signals in >50% of the gland. In static images, both the intra- and inter-observer reliability were excellent, but the exact role of colour Doppler in the diagnosis and follow-up of patients with pSS needs to be further elucidated (14).

Salivary gland biopsy

Salivary gland biopsy is a diagnostic method broadly applied for the diagnosis of pSS, as well as for the detection of salivary gland lymphoma associated with pSS, sarcoidosis, amyloidosis and other connective tissue disorders (15). In contrast to salivary glands of healthy individuals (Fig. 2A), salivary glands of patients with pSS have characteristic microscopic findings, involving lymphocytic infiltration of B- and T-lymphocytes surrounding the excretory ducts in combination with a decline in salivary gland parenchyma (Fig. 2B) (16). In affected glands, striated ducts may become infiltrated by B-lympho-

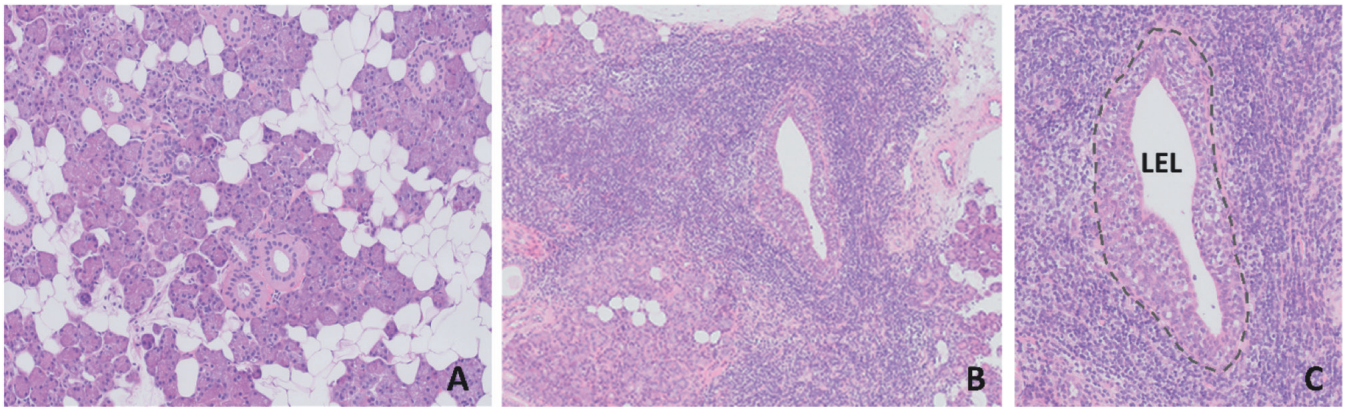

Fig. 2. (**A**) Parotid salivary gland biopsy of a non-SS patient; (**B**) Parotid salivary gland biopsy of a pSS patient showing a periductal lymphocytic infiltrate around a lympoepithelial lesion (LEL) and (**C**) Same lymphoepithelial lesion, showing epithelial hyperplasia and intraepithelial lymphocytes. Grey line indicates the ductal border.

cytes resulting in hyperplasia of the epithelial cells, so-called lymphoepithelial lesions (LELs) (Fig. 2C) (17, 18). Another characteristic feature is the presence of IgG plasma cells. A shift towards >30% IgG plasma cells and <70% IgA plasma cells is associated with pSS (19). Biopsy of the labial salivary glands is considered as the cornerstone of the pSS diagnostics and historically has been always part of the most widely used classification criteria for pSS (3, 20-25). The parotid gland biopsy has been shown as a viable alternative for the labial salivary gland biopsy with similar sensitivity and specificity. Additionally, parotid gland biopsies allow the clinician to monitor the disease progression and to assess the effect of an intervention treatment in pSS as the parotid tissue can be harvested easily, the same gland can be repeated biopsied, and the histopathological results can be compared with other diagnostic results derived from the same gland (*e.g.* secretory function, sialographic appearance, and ultrasound) (26, 27). Furthermore, by performing parotid biopsies as a routine diagnostic procedure for SS, developing lymphomas can be identified early (28, 29).

The first grading system for salivary gland biopsies was employed by Chisholm and Mason in an attempt to standardise the examined area and to record the degree of histopathological change (30). At present, according to the 2016 ACR-EULAR criteria, a labial salivary gland biopsy is considered positive if minor salivary glands (obtained through normal appearing mucosa) demonstrate focal lymphocytic sialadenitis, evaluated by an expert histopathologist, with a focus score (FS) ≥1. The FS is defined as the number of lymphocytic foci, containing more than 50 lymphocytes, per 4 mm^2 of glandular tissue. Similarly, Pijpe *et al.* have shown that in parotid gland biopsies the FS should also be based on the number of focal lymphocytic cell clusters containing ≥50 lymphocytes per 4 mm^2 salivary gland tissue. Areas with prominent duct dilatation and/or parenchymal atrophy should be excluded from scoring. A minimum of 4 mm^2 of parotid salivary gland tissue is required, including areas with fat deposition, if present (31).

Salivary gland ultrasonography *vs.* salivary gland biopsy

According to OMERACT filter, truth (validity), discrimination (reliability) and feasibility are essential requirements for implementation of ultrasound in daily clinical practice, or even to replace salivary gland biopsy with SGUS (32). Validity determines whether the instrument truly measures that which it was intended to measure, while reliability shows the extent to which the results can be reproduced when the research is repeated under the same conditions (33).

Validity

Mossel *et al.* have demonstrated that the accuracy of SGUS to predict a labial gland biopsy outcome is very high, with an area under the curve (AUC) of 0.824 (95% CI 0.714–0.934). The absolute agreement between SGUS outcome and labial gland biopsy was 79%, with a sensitivity of 72%, specificity of 85%, PPV of 84% and NPV of 74% (34). The results presented by Baldini *et al.* pointed at the same direction and showed that SGUS compared to labial gland biopsy has a sensitivity of 92%, specificity of 98%, PPV 98% and NPV 93% (35). Al Tabaa *et al.* recently conveyed that the concordance between a positive SGUS score and positive FS in labial gland biopsy was 83% with a Cohen's kappa agreement of 0.48 (36). The accuracy of SGUS to predict a parotid gland biopsy outcome was slightly better, with an AUC of 0.849 (95% CI 0.746–0.952). The absolute agreement between SGUS outcome and parotid gland biopsy was 83%, with a sensitivity of 75% specificity of 88%, PPV of 78% and NPV of 86% (34). To supplement the abovementioned results, Mossel *et al.* showed that the correlation between SGUS and labial gland focus score was 0.41 (37), while Cornec *et al.* (2016) reported a correlation of ϱ=0.61 between SGUS and focus score in labial salivary glands of participants in the TEARS trial (38).

Mossel *et al.* (2017) showed that the SGUS accuracy compared to parotid gland biopsy is better than SGUS accuracy compared to labial gland biopsy (34). The reason for this is that the parotid glands were assessed during the SGUS examination and included in the SGUS scoring, whereas the labial

glands were not. Furthermore, the labial gland biopsy is positive in 6–15% of the general population, while the parotid gland biopsy is positive in only 5% of the general population (39-41). This suggests that when biopsies of parotid and labial glands are taken from a patient at the same time, at least up to 10% of the biopsies may be discordant with each other (37).

Recently, Mossel *et al*. confirmed that moderate to good associations exist between histopathological parameters (*e.g*., FS, percentage of $CD45^+$ infiltrate) and parotid gland ultrasound scores in a more in-depth comparison of parotid gland ultrasound and histopathology. Furthermore, patients with presence of lymphoepithelial lesions and patients with presence of germinal centres in the parotid gland had significantly higher parotid ultrasound scores compared with patients without these features. However, they also showed that a FS ≥1 is not always reflected by the presence of hypoechogenic areas in the parotid gland, and the presence of hypoechogenic areas is not always reflected by a positive FS, indicating that parotid gland histopathology and parotid gland ultrasonography assess only partly related constructs in pSS. (42). Similar correlations between FS and ultrasound scores were found in a study comparing labial gland ultrasound with histopathology of the labial gland (43).

Reliability

Ultrasound in general is considered as an operator dependent diagnostic method. As a result, concerns were raised in the past regarding the reliability of SGUS. On the other hand, the histopathologic evaluation of minor salivary glands has been always thought as highly reproducible and thus as a key in the diagnostics of pSS, but is also dependent on expert pathologists.

Delli *et al*. showed that for the Hocevar scoring system (10) the intra-observer reliability of the SGUS was excellent, with an intraclass correlation (ICC) ranging from 0.89 to 0.96. The inter-observer reliability was also good to excellent, with ICCs of 0.84 and 0.76 for the total ultrasound score in the two sessions. The kappa value ranged from 0.60 to 0.83. Hypoechogenic areas and homogeneity of parotid glands showed the highest interobserver reliability (45). These results are in agreement with those of the study of Jousse-Joulin *et al*. in which was reported that intra-observer reliability for detecting and scoring ultrasonographic abnormalities was excellent (Cohen's kappa 0.81) and inter-observer reliability was good (Light's kappa 0.66) (13). Similarly, Zabotti *et al*. showed in a recent European multicentre reliability exercise that the inter-rater reliability for the OMERACT score was substantial with Light's Kappa of and 0.77 as well as no significant difference was noticed among sonographers with different levels of experience (45).

When it comes to assessing the histopathologic characteristics of labial gland biopsies, studies indicate that the reliability is similar and not superior to SGUS. Specifically, regarding the intra-observer agreement, the intraclass correlation coefficient (ICC) for the FS was found to be 0.76 (95% CI 0.66, 0.84) in a multicentre study of Costa *et al*. (46). Agreement is substantial for focal lymphocytic sialadenitis (FLS), non-specific chronic sialadenitis (NSCS) and germinal centre (κ=0.71, κ=0.64 and κ=0.67, respectively); moderate for fibrosis and duct dilatation. Inter-observer agreement between pathologists was substantial for dichotomous FS and dichotomous Chisholm-Mason (κ=0.71 and κ=0.64, respectively). The ICC for FS was 0.66 (95% CI 0.49, 0.78) (46).

To further assist in the challenging diagnostic process of pSS and to reduce inter-observer variability, researchers are now focusing in the digital analysis of salivary gland biopsies (47, 48) and ultrasonographic images (49).

Salivary gland ultrasonography and the 2016 ACR-EULAR classification criteria

Salivary gland ultrasonography vs. the 2016 ACR-EULAR classification criteria

According to Mossel *et al*., the accuracy of SGUS to predict the 2016 ACR-EULAR classification was good, with an AUC of 0.802 (95% CI 0.710–0.894). The absolute agreement between SGUS outcome and ACR-EULAR classification was 80%, with a sensitivity of 67%, specificity of 94%, PPV of 92 and NPV of 72%. The ultrasound score was significantly higher in pSS versus non-pSS according to the classification criteria (p<0.001) (34).

The abovementioned results are in line with the study of Al Tabaa *et al*., who reported that SGUS showed a high specificity and NPV compared to the 2016 ACR-EULAR classification criteria (36). Specifically, they detected a sensitivity of 49–57%, a specificity of 92–97%, a PPV of 72–87% and NPV 81–82%.

Salivary gland ultrasonography as an item of the 2016 ACR-EULAR classification criteria

Le Goff *et al*. supported that including SGUS among the 2016 ACR/EULAR criteria increased their sensitivity from 87.4% to 91.1% when physician's diagnosis was the reference standard (50). Similarly, Geng *et al*. showed that when *adding* SGUS to 2016 ACR/EULAR criteria, it showed better performance by improving the sensitivity (90.8% *vs*. 85.6%), while not losing the specificity (83.7% *vs*. 82.2%) (51). However, when *replacing* labial biopsy by SGUS in 2016 ACR/EULAR criteria, both sensitivity and specificity decreased slightly (85.0% *vs*. 85.6% and 79.8% *vs*. 82.2%).

Jousse-Joulin *et al*. investigated the weight of SGUS compared to other items of the 2016 ACR/EULAR classification criteria for pSS. They concluded that SGUS should have a similar weight compared to minor items, *i.e*. equal to 1. Also, they showed that adding SGUS to the criteria improves their sensitivity from 90.2% to 95.6% while specificity slightly changed from 84.1% to 82.6% (52).

These results are in agreement with the results of the study of Nimwegen *et al*. in which it was confirmed that the optimal weight for SGUS positivity in the 2016 ACR-EULAR criteria should be 1 and that the cut-off for 2016 ACR/EULAR fulfilment should remain ≥4 (53). Also, van Nimwegen *et al*. showed that the addition of SGUS to the criteria resulted in an AUC of

0.966, a sensitivity of 97.3% and specificity of 90.2%. Consequently, they concluded that the addition of SGUS to the 2016 ACR-EULAR criteria offers the clinicians a larger array of tests to evaluate the patients. Nevertheless, they reported that the sensitivity of the criteria decreased substantially when SGUS replaced salivary gland biopsy or anti-SSA antibodies. Specifically, when SGUS replaced the labial gland biopsy the AUC decreased from 0.965 to 0.903, the sensitivity decreased from 95.9 to 82.2 while the specificity increased from 92.2 to 94.1 (53).

Combination of salivary gland ultrasonography and serology

Mossel *et al*. have shown that in patients with positive SGUS combined with anti-SSA/Ro antibodies, 78% had a positive parotid gland biopsy and 94% had a positive labial gland biopsy (34). In patients with negative SGUS combined with absence of anti-SSA/Ro antibodies, 93% had a negative parotid gland biopsy and 77% had a negative labial gland biopsy. In patients with positive SGUS as well as presence of anti-SSA/Ro antibodies, 97% fulfilled the ACR-EULAR criteria. In patients with negative SGUS as well as absence of anti-SSA/Ro antibodies, 89-98% did not fulfil the ACR-EULAR criteria (34). The aforementioned results were recently confirmed by Al Tabaa *et al*., who showed that in anti-SSA-negative patients, SGUS exhibited a high specificity and NPV of 91% and 92%, respectively. They concluded that this strategy could avoid two-thirds of labial gland biopsies in a population of patients suspected of pSS (35). This means that when both SGUS and anti-SSA/Ro antibodies are compatible with pSS or when both SGUS and anti-SSA/Ro antibodies are not compatible with pSS, a salivary gland biopsy is not mandatory and the suspected patient could be safely classified respectively as a pSS patient or not.

Implications for monitoring the disease and assessing treatment efficacy

The possible role of SGUS, however, should not be restricted to the diagnostic work-up of pSS. Studies also have shown that SGUS and specifically ultrasound-guided core needle biopsy are useful in evaluating pSS patients suspected for salivary gland lymphoma (54-56). Furthermore, studies have shown that SGUS could have a role in assessing the efficacy of systemic treatment in pSS (57-61). For the time being, studies assessing the role of SGUS in the long-term monitoring of patients with pSS are missing and thus the results of, *e.g*. the REgistry of Sjögren Syndrome LongiTudinal (RESULT) cohort, are eagerly awaited (62).

Conclusion

Currently, available data suggest that replacing a salivary gland biopsy by SGUS decreases substantially the accuracy of the 2016 ACR-EULAR classification criteria. When SGUS is added as an additional item to the criteria, the accuracy of the criteria remained high, offering at the same time the clinicians a wider array of tools to assess patients. The combination of SGUS and anti-SSA antibodies was shown to be highly predictive of the classification of a patient suspected of pSS, making routine salivary gland biopsy more debatable. There is growing evidence to add SGUS to the 2016 ACR-EULAR classification criteria for pSS as an additional item.

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