Ultra-high frequency ultrasonography of labial glands is a highly sensitive tool for the diagnosis of Sjögren's syndrome: a preliminary study

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ABSTRACT

Objective. *Ultra-high frequency ultrasonography (UHFUS) has been recently introduced in oral medicine due to its ability to image small anatomical structures including labial salivary glands (LSG). To date no ultrasonography morphological studies of labial salivary glands (LSG) have been carried out in SS. In this pilot study we aimed at analysing the distribution of UHFUS findings in LSG of patients with suspected SS, focusing in particular on the association with patients' oral dysfunction, antibody profiles and histopathology.*

Methods. *Consecutive patients undergoing a LSG biopsy for clinically suspected SS were included in this study between January 2018 and January 2020. Intraoral UHFUS scan of the lip mucosa was performed with Vevo MD equipment, using a 70 MHz probe with a standardised protocol. LSG were assessed by using a four-grade semiquantitative scoring system (0–3), similar to the OMERACT scoring system used for major salivary glands. The distribution of UHFUS grades was compared in patients stratified according their final diagnosis, patients antibody profiles and LSG histopathology.*

Results. *We included 128 patients with suspected SS: out of them, 54 (42.2%) received a final diagnosis of SS, made according to the ACR 2016 criteria and 74 (57.8%) were diagnosed as no-SS sicca controls. We found that LSG inhomogeneity was significantly greater in patients with SS than in no-SS subjects (p<0.0001). We also found that higher UHFUS pattern of inhomogeneity (i.e. grade 2 and 3) were significantly more frequent in both SSA^+/SSB^- and SSA^+/SSB^+ patients (p=0.001). A normal UHFUS pattern, by contrast, was significantly more common in SSA^-/SSB^- subjects (i.e. 15/83 (18.1%) vs. 1/33 (3%) vs. 0/12 (0%), p=0.001). Finally, LSG inhomogeneity was significantly associated with both the number of foci (p<0.001) and focus score (p<0.001). Particularly, we found that both the number of foci and the FS were significantly higher in patients presenting a UHFUS grading of 2 and 3 with respect to those presenting a UHFUS grading of 0 and 1 (p=0.01).*

Conclusion. *This preliminary study demonstrates the optimal feasibility of UHFUS and its high sensitivity in identifying negative patients on subsequent lip biopsy, thus avoiding invasive procedures in selected cases. Further studies are in progress to define the clinical and predictive role of the various patterns observed and their added value with respect to traditional salivary gland ultrasonography.*

Introduction

Sjögren's syndrome (SS) is a complex systemic disorder potentially affecting any organ and system, but particularly involving salivary and lachrymal glands (1, 2). The diagnosis of the disease requires objective tests able to quantify patients' ocular or oral dryness combined with serologic or histopathologic evidence of an underlying autoimmune basis for the exocrine glandular dysfunction (3-5). Indeed, the gold standard test for SS diagnosis remains the labial salivary glands (LSG) biopsy that, although invasively, allows to identify a typical focal lymphocytic sialadenitis (FLS) which is considered the hallmark of SS at tissue level (6).

Additionally, the inflammation and damage of the major salivary glands can be assessed non-invasively by ultrasonography (SGUS) or magnetic resonance (7-14). Lately, SGUS has

gained an increasing relevance in SS diagnosis and assessment; however, the involvement of major salivary glands corresponds only partially to the LSG infiltrate severity. From this perspective, parotid gland biopsies have been recently encouraged to improve SS patients' assessment (15). Moreover, the absence of pathological findings at SGUS does not completely exclude the presence of a FLS in the LSGs, retaining the indication for LSG biopsy in suspected cases (16).

Indeed, considering that LSG are 1-2 mm in diameter, contrary to major salivary glands, they cannot be visualised by conventional ultrasonography: therefore at present no ultrasonography morphological studies of LSG have been carried out in SS (17).

Ultra-high frequency ultrasonography (UHFUS) has been recently introduced in clinical medicine. By using frequencies up to 70 MHz, UHFUS allows a high-resolution image of tiny structures up to 30 μm opening new avenues for several clinical applications. In particular, intraoral use has been widely encouraged for the assessment of both normal anatomy and several oral lesions (18-21).

In this pilot study we report our preliminary experience on UHFUS of labial salivary glands of patients with suspected SS focusing in particular on the distribution of UHFUS findings in relation with patients' oral dysfunction, labial salivary glands histopathology and serology. Ultimately, we aimed at exploring the possibility of using this technique to refine the indication to LSG biopsy in patients with suspected SS, thus avoiding unnecessary invasive biopsy procedures.

Methods

Patients

Consecutive patients undergoing a LSG for clinically suspected SS were included in this study from January 2018 to January 2020. All the patients underwent a complete work-up in accordance with the ACR/EULAR 2016 classification criteria for the diagnosis of SS (3). The following information were recorded: patients' demographics, disease-related ocular and oral findings including unstimulated salivary flow (USFR), serological data including antinuclear antibodies, anti-Ro/SSA, anti-La/SSB, Rheumatoid factor, C3 and C4 levels and presence of hyper-gammaglobulinaemia. The study protocol was approved by local ethics committee, and all subjects gave written consent to undergo UHFUS examination.

UHFUS acquisition protocol, image post-processing and LSG biopsy

Vevo MD equipment (Visual Sonics) was employed for UHFUS scan performance. The study was preceded by a preparatory phase on healthy volunteers in which training and calibration were conducted in order to standardise UHFUS scan in terms of performance and image acquisition and obtain a good concordance among the examiners (Cohen's kappa value >0.70).

For each patient, a standardised intraoral UHFUS examination of the internal surface of the lower lip (central, left, right compartment) was carried out using a 70 MHz probe with the following characteristics: bandwidth 29-71 MHz, nominal frequency 52 MHz, axial resolution 30 μm, lateral resolution 65 μm, maximum depth 10.0 mm, maximum image windth 9.7 mm, maximum image depth 10.0 mm, focal depth 5 mm.

For each compartment, axial and longitudinal B-mode acquisitions a were obtained. The UHFUS scans were performed using a standardised preset, and keeping gain, time gain compensation, dynamic range, mechanical index, and thermal index constant. Scan depth and focus position were adjusted to optimise the scan. The scans were saved as DICOM format images and were processed using Horos software (https://horosproject.org).

LSG were assessed by using a four-grade semiquantitative scoring system, similar to the OMERACT scoring system used for major salivary glands (22). Namely, grade=0 indicated normal glandular parenchyma; grade=1: the presence of mild glandular alteration, with fine echogenicity in absence of clear alterations, or slight, diffuse glandular hypoechogenicity; grade=2: a moderate glandular alteration, with the presence of focal hypoechoic areas, but partial conservation of normal glandular parenchyma, and finally, grade=3: a severe glandular alteration, with diffuse presence of hypoechoic areas in absence of normal glandular parenchyma, or presence of glandular fibrosis (Fig. 1).

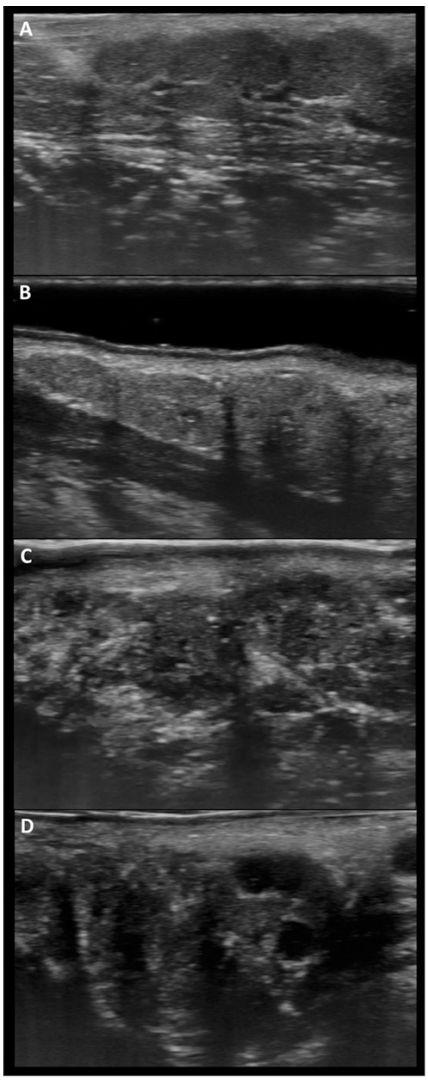

Fig. 1. shows the four-grade semiquantitative UHFUS scoring system utilised to assess LSG
A: grade=0: normal glandular parenchyma;
B: grade=1: mild glandular alteration, with fine echogenicity in absence of clear alterations, or slight, diffuse glandular hypoechogenicity;
C: grade=2: moderate glandular alteration, with the presence of focal hypoechoic areas, but partial conservation of normal glandular parenchyma;
D: grade=3: severe glandular alteration, with diffuse presence of hypoechoic areas in absence of normal glandular parenchyma, or presence of glandular fibrosis.

UHFUS imaging was used to help locate the LSG for the US-guided biopsy: after completing the UHFUS scoring, we selected and marked the gland with the highest grade by using a surgical

marking pen and biopsy was then performed under local anesthesia immediately after (19). An expert pathology assessed the number of foci and the focus score according to the literature recommendations.

Results

We included a total of 128 patients with suspected SS: out of them, 54 (42.2%) received a final diagnosis of SS made according to the ACR 2016 criteria (3) and 74 (57.8%) were diagnosed as no-SS sicca controls. The two groups did not differ in their demographic features. Table I summarises the features of the patients' cohort.

UHFUS and differential diagnosis

We compared LSG inhomogeneity in SS patients with respect to no-SS controls. Out of 128 subjects, 16/128 (12.5%) presented a normal UHFUS pattern; 55/128 (43%) a mild glandular alteration, (*i.e.* grade 1); 51/128 (39.5%) a moderate glandular alteration (*i.e.* grade 2) and finally 6/128 (4.7%) a severe glandular alteration (grade 3). As shown in Figure 2, the distribution of the UHFUS four-grade semiquantitative scoring was significantly different in SS patients and in no-SS controls (p<0.001). More specifically, a normal pattern was almost exclusively observed in no-SS controls whereas the most severe pattern with diffuse hypoechoic areas and/or glandular fibrosis was detected almost exclusively in SS patients. The only no-SS subject receiving a grade 3 at the UHFUS examination actually presented a focal sialadenitis in her LSG biopsy with a focus score of 1.34 but she did not satisfy the ACR/EULAR criteria (3) for SS because she did not present nor a positivity for anti-Ro/SSA nor clearly pathological findings in her oral and ocular tests. Fine echogenicity in absence of clear alterations, or slight, diffuse glandular hypo-echogenicity (*i.e.* grade 1) was the most common pattern detect in no-SS controls but was also observed in one third of SS patients whereas focal hypoechoic areas (*i.e.* grade 2) was the most frequently observed pattern in SS patients. The mean (S.D.) unstimulated salivary flow rate was 2.47 (2.37) ml/15'. No correlation was found between the UHFUS scoring and patients unstimulated salivary flow (p= n.s.).

Table I. Study population.

	SS (54)	No-SS (74)	*p*-value
Age, mean (SD)	55 (14)	56 (15)	ns
Gender (female)	52/54 (96%)	66/74 (89%)	ns
Anti-Ro/SSA, n (%)	39/54 (72%)	6/74 (8%)	0.0001
Anti-La/SSB, n (%)	12/54 (24%)	none	0.0001
RF, n (%)	17/54 (31%)	6/74 (8%)	0.003
Hyper-gammaglobulinaemia, n (%)	13/54 (24%)	5/74 (7%)	0.05
Schirmer's test, mean (SD)	6 (5)	7 (6)	ns
OSS>5, n (%)	4/54 (7%)	5/74 (7%)	ns
USFR/15', mean (SD)	3 (2.8)	2.6 (1.9)	ns
FS, mean (SD)	1.6 (1)	0.2 (0.3)	0.0001
N° foci, n (%)	3.3 (2.4)	0.4 (0.7)	0.0001
N° of ELS, n (%)	1.2 (1.3)	none	0.0001

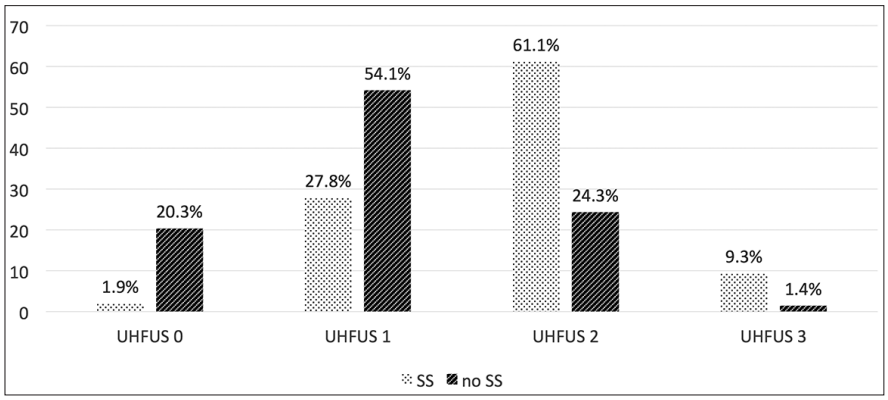

Fig. 2. Distribution of the UHFUS four-grade semiquantitative scoring in the patient cohort.
Grade=0 was found in 1/54 (1.9%) SS and in 15/74 (20.3%) no-SS controls; grade=1 in 15/54 (27.8%) and in 40/74 (54.1%); grade =2 in 33/54 (61.1%) and in 18/74 (24.3%); grade =3 in 5/54 (9.3%) and in 1/74 (1.4%) no-SS controls (p<0.001).

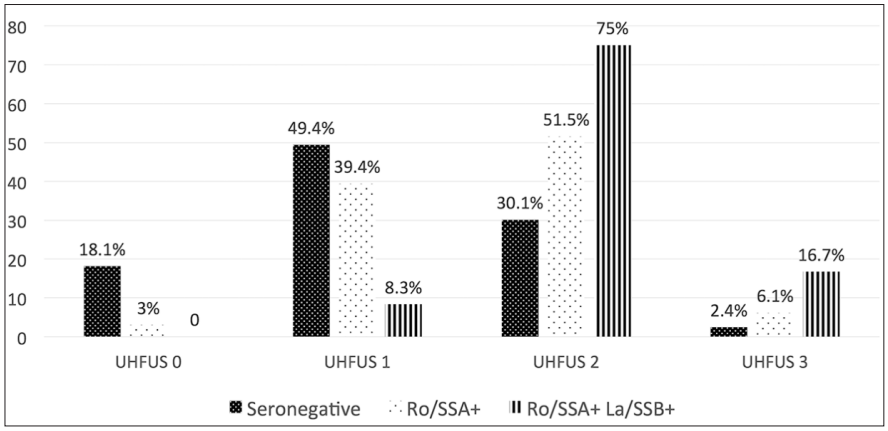

Fig. 3. Distribution of the UHFUS four-grade semiquantitative scoring according to patients' autoantibody profiles.
Grade=0 was found in 15/83 (18.1%) SSA-/SSB- patients *vs*. 1/33 (3%) SSA+/SSB- patients and 0/12 SSA+/SSB+ patients; grade=1 in 41/83 (49.4%) SSA-/SSB- patients *vs*. 13/33 (39.4.%) SSA+/SSB- patients and 1/12 (8.3%) SSA+/SSB+ patients; grade=2 in 25/83 (30.1%) SSA-/SSB-patients *vs*. 17/33 (51.5%) SSA+/SSB- patients and 9/12 (75%) SSA+/SSB+ patients; grade=3 in 2/83 (2.4%) SSA-/SSB- patients *vs*. 2/33 (6.1%) SSA+/SSB- patients and 2/12 (16.7%) SSA+/SSB+ patients.

UHFUS and autoantibody profiles

We analysed the distribution of the UHFUS four-grade semiquantitative scoring in relation with patients' autoantibody profiles. Out of 128, 83 (64.8%) patients were seronegative (SSA^-/SSB^-), 33 (25.8%) were anti-Ro/SSA positive (SSA^+/SSB^-) and 12 (9.4%) were

Table II. Distribution of the UHFUS four-grade semiquantitative scoring according to patients' autoantibody profiles.

		UHFUS				
		grade 0	grade 1	grade 2	grade 3	*p*-value
SSA/SSB	SSA^-/SSB^-	15/83 (18.1%)	41/83 (49.4%)	25/83 (30.1%)	2/83 (2.4%)	0.001
	SSA^+/SSB^-	1/33 (3%)	13/33 (39.4%)	17/33 (51.5%)	2/33 (6.1%)	
	SSA^+/SSB^+	none	1/12 (8.3%)	9/12 (75%)	2/12 (16.7%)	
RF	RF+	3/23 (13%)	3/23 (13%)	13/23 (57%)	4/23 (17%)	0.008
	RF-	14/105 (13%)	52/105 50%)	37/105 (35%)	2/105 (2%)	

anti-Ro/SSA and anti-La/SSB positive (SSA^+/SSB^+). We found that higher UHFUS pattern of inhomogeneity (*i.e.* grade 2 and 3) were significantly more frequent in both SSA^+/SSB^- and SSA^+/SSB^+ patients (p=0.001). A normal UHFUS pattern, by contrast, was significantly more common in SSA^-/SSB^- subjects *(i.e.* 15/83 (18.1%) *vs.* 1/33 (3%) *vs.* 0/12 (0%), p=0.001). Figure 3 and Table II show the distribution of the UHFUS four-grade semiquantitative scoring according to the autoantibody profiles. LSG inhomogeneity was also significantly greater in patients with a positivity for rheumatoid factor (p=0.008) (Table II).

Notably, when we limited the analysis to patients satisfying the ACR/EULAR criteria, no significant differences were observed in LSG inhomogeneity between SSA^-/SSB^- and seropositive SS patients; however, despite not significant, we still observed a trend in the distribution of the highest UHFUS grade in SSA^+/SSB^+ patients (*i.e.* UHFUS grade 3: 1/15 (6.7%). SSA^-/SSB^- *vs.* 2/27(7.4%) SSA^+/SSB^- *vs.* 2/12 (16.7%) SSA^+/SSB^+, p-value= n.s.).

UHFUS and histology

LSG biopsies were all performed by using UHFUS to locate and select the glands. The mean area (S.D.) of the specimens was 8.081 (3.253) mm^2. Out of the 128 LSG biopsies, 57/128 (44.5%) were characterised by a non-specific chronic sialadenitis whereas a focal lymphocytic sialadenitis (FLS) was described in the remaining 71/128 (55.5%). In samples with FLS the number of foci ranged from 1 to 10, with a mean (S.D.) number of foci of 2.94 (2.16), whereas the FS varied from 0.26 to 5.30, with a mean (S.D.) of 1.41

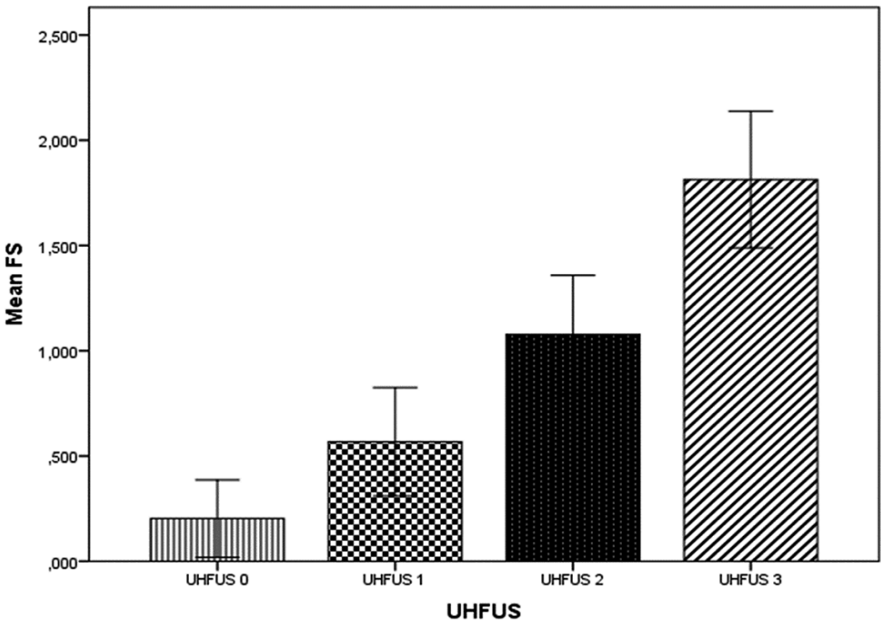

Fig. 4. Distribution of the UHFUS four-grade semiquantitative scoring according to the histology results. LSG inhomogeneity was significantly associated with LSG focus grade.

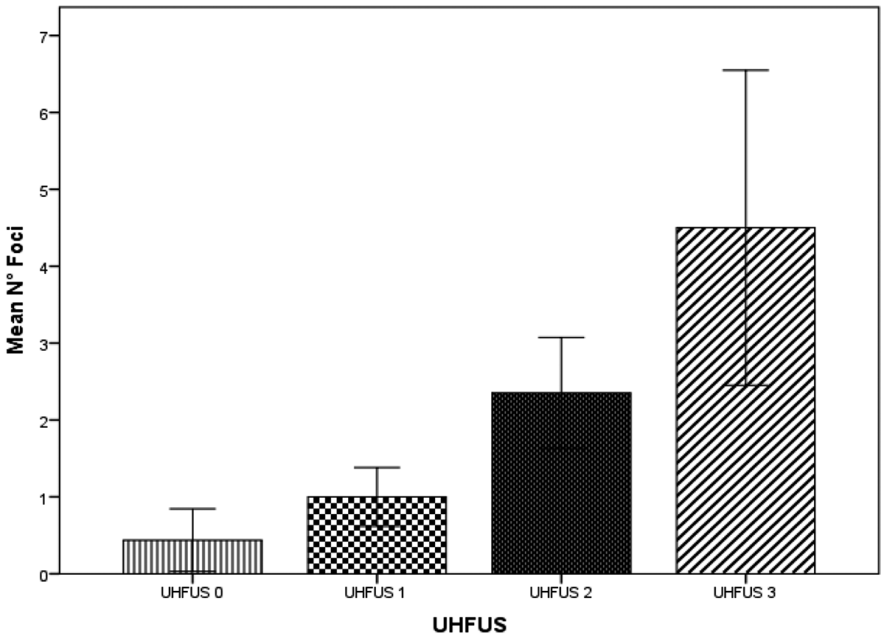

Fig. 5. Distribution of the UHFUS four-grade semiquantitative scoring according to the histology results. LSG inhomogeneity was significantly associated with LSG number of foci.

Table III. Distribution of the UHFUS four-grade semiquantitative scoring according to the histology results.

	UHFUS				
	grade 0	grade 1	grade 2	grade 3	*p*-value
N° Foci, mean (SD)	0.44 (0.81)	1.00 (1.41)	2.35 (2.58)	4.50 (2.51)	0.0001
FS, mean (SD)	0.20 (0.37)	0.57 (0.96)	1.08 (1.00)	1.81 (0.40)	0.0001

(0.91). When we compared the number of foci and the FS in patients stratified according to their UHFUS four-grade scoring, we found that LSG inhomogeneity was significantly associated with both the number of foci ($p<0.001$) and focus score ($p<0.001$). Particularly, we found that both the number of foci and the FS were significantly higher in patients presenting a UHFUS grading of 2 and 3 with respect to those presenting a UHFUS grading of 0 and 1 ($p=0.01$) (Fig. 4 and 5 and Table III). Moreover, the number of foci and FS were not significantly different in LSG biopsies characterised at UHFUS by a normal pattern (*i.e.* UHFUS grade 0) with respect to those presenting a mild inhomogeneity (*i.e.*, UHFUS grade 1); similarly, no differences was observed in number of foci or FS between LSG presenting a moderate inhomogeneity (*i.e.* UHFUS grade 2) and those with severe inhomogeneity (*i.e.* UHFUS grade 3).

Discussion

In this pilot study, firstly we observed that the UHFUS four-grade semiquantitative scoring differed significantly between SS patients and no-SS controls; second, we found that the UHFUS abnormalities were more frequent in seropositive patients. Finally, we pinpointed a good correlation between the UHFUS grading and both the FS and the number of foci in the LSGs.

To our knowledge this is the first study exploring the application of UHFUS to the study of LSG in SS.

Indeed, the diagnostic accuracy of traditional SGUS has been largely investigated and a recent systematic review and meta-analysis found that SGUS pooled sensitivity was 0.69 (95% CI: 0.67-0.71), and specificity of 0.92 (95% CI: 0.91-0.93) (23). When compared to traditional SGUS our preliminary results, seem to indicate that UHFUS may have a higher sensitivity than traditional SGUS. Notably, we found very few false negative when we compared the UHFUS scores and the LSG biopsy histopathological findings of our patients. Particularly, a normal pattern at the UHFUS examination was assigned only to one patient fulfilling the criteria for SS. Therefore, we may speculate that normal LSG-UHFUS pattern might unlikely correspond to a positive LSG biopsy (*i.e.* FS≥1). In other terms, we may hypothesise that LSG biopsies could be reasonably avoided in subjects with suspected SS if their LSG-UHFUS showed a normal pattern.

Regarding the specificity of this novel technique, we found that both the FS and the number of foci in the LSG biopsies were significantly higher in patients presenting a UHFUS grade≥2 with respect to those presenting a lower UHFUS grade (*i.e.* UHFUS grade=0 and grade=1). We may therefore speculate that severe UHFUS inhomogeneity might be associated with very few false positive cases with a high probability of detecting LSG focal sialadenitis at the biopsy. However, a greater uncertainty remains for intermediate UHFUS grade, and particularly for patterns characterised by mild inhomogeneity that might indicate either a non-specific chronic sialadenitis or with a focal sialadenitis. Therefore, considering the variability in the UHFUS patterns, further studies are necessary to better define the optimal cut-off to be used for UHFUS clinical application.

We clearly observed that the UHFUS abnormalities were more frequent in subjects showing a positivity for anti-Ro/SSA isolated or associated with anti-La/SSB than in seronegative patients. The association between salivary gland inhomogeneity and SS antibody profiling has been extensively reported for major salivary glands (10, 12, 23-26). The observations that UHFUS scoring was also higher in seropositive than in seronegative patients further support the work hypothesis that likely SGUS, the inhomogeneity of LSG might correlate with SS clinical and serological features.

The possibility of using UHFUS as a complementary tool in SS diagnostic is still in its infancy. We are aware that several issues should be addressed before this tool could be translated in clinical research.

First of all in order to promote the applicability of this tool, a standardisation of the UHFUS acquisition protocol, image post-processing and scoring system is highly required. Moreover, efforts should be made in order to assess the intra and inter- readers reliability to foster the generalisability of the results.

Our next step is now to compare head to head LSG UHFUS findings with SGUS (particularly submandibular glands) in our patients to better define the diagnostic accuracy of UHFUS and its role in the diagnostic algorithm of SS. Despite all the open issues, however, our pilot study has paved the way for fueling the use of UHFUS to improve the morphological assessment of LSG in SS. In the future, hopefully, LSG UHFUS might represent a sensitive screening tool to identify negative patients on subsequent lip biopsy, thus avoiding in selected cases invasive procedures.

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