Ultrasound assessment of lacrimal glands: a cross-sectional study in healthy subjects and a preliminary study in primary Sjögren's syndrome patients

O. De Lucia[1], S. Zandonella Callegher[2], M.V. De Souza[1,3], N. Battafarano[4], N. Del Papa[4], M. Gerosa[1,3], I. Giovannini[2], A. Tullio[5], F. Valent[5], A. Zabotti[2], R. Caporali[1,3], S. De Vita[2]

[1]*Clinical Rheumatology Unit, Department of Rheumatology and Medical Sciences, ASST Centro Traumatologico Ortopedico G. Pini-CTO, Milan;*
[2]*Rheumatology Clinic, Department of Medical Area, University of Udine, Academic Hospital Santa Maria della Misericordia, Udine;*
[3]*Department of Clinical Sciences and Community Health, University of Milan;*
[4]*Rheumatology DH Unit, Department of Rheumatology and Medical Sciences, ASST Centro Traumatologico Ortopedico G. Pini-CTO, Milan;*
[5]*Institute of Epidemiology, Academic Hospital Santa Maria della Misericordia, Udine, Italy.*

Orazio De Lucia, MD, PhD
Sara Zandonella Callegher, MD
Mirian Veronica De Souza, MD
Norma Battafarano, MD
Nicoletta Del Papa, MD
Maria Gerosa, MD
Ivan Giovannini, MD
Annarita Tullio, MD
Francesca Valent, MD
Alen Zabotti, MD
Roberto Caporali, MD, PhD
Salvatore De Vita, MD, PhD

Please address correspondence to:
Orazio De Lucia,
Clinical Rheumatology Unit, Dept. of Rheumatology and Medical Sciences, ASST Centro Specialistico Ortopedico Traumatologico Gaetano Pini-CTO, Piazza Cardinal Ferrari 1, 20122 Milano, Italy.
E-mail: orazio.delucia@asst-pini-cto.it

Received on July 1, 2020; accepted in revised form on July 29, 2020.

Clin Exp Rheumatol 2020; 38 (Suppl. 126): S203-S209.

Key words: Sjögren's syndrome, ultrasound, lacrimal glands, healthy subjects, reliability

Competing interests: none declared.

ABSTRACT

Objective. *This study aimed to: i) perform an ultrasonographic (US) evaluation of the lacrimal glands (LGs) in healthy subjects in order to define the sonographic elementary lesions which could be identified in the LGs and describe their frequencies in healthy subjects; ii) test the intra and inter-rater agreement between four rheumatologists; iii) preliminary assess whether the elementary lesions of the LGs let us differentiate healthy subjects from primary Sjögren's syndrome (pSS) patients.*

Methods. *A consensus meeting was held to define the sonographic lesions to be evaluated. Healthy subjects and pSS patients underwent lacrimal glands ultrasound (LGUS) examinations in two Italian Rheumatology Clinics. A web-based reliability exercise was performed on healthy subjects' images by four rheumatologists. Afterward, images of pSS patients were evaluated for the presence of the sonographic lesions previously defined and compared to the US findings in healthy subjects.*

Results. *Fifty-seven healthy subjects and 17 pSS patients were evaluated. The intra and inter-rater reliability score was good-excellent for almost all the agreed US features assessed (glandular parenchyma visibility, size, homogeneity, hypoechoic areas, hyperechoic spots, fibrous gland appearance, fatty deposition). Among the LGUS elementary lesions in pSS patients compared with healthy subjects, we detected a significantly difference in glandular inhomogeneity [13/33 (39.4%) vs. 9/63 (14.3%), p=0.01], and in fibrous gland appearance [3/33 (9.1%) vs. 0/63 (0%), p=0.04].*

Conclusion. *In this preliminary study, LGUS proved to have a good-excellent intra and inter-rater reliability. The glandular parenchyma inhomogeneity and the fibrous gland appearance could help differentiate pSS patients from healthy subjects.*

Introduction

Primary Sjögren's syndrome (pSS) is a chronic, systemic, autoimmune connective tissue disease characterised by lymphocytic infiltrate of the exocrine glands, mainly the salivary and the lacrimal glands (LGs), resulting in oral and ocular dryness, as the principal symptoms, along with fatigue and musculoskeletal pain, and several possible extraglandular manifestations (1).

In recent years the salivary gland ultrasound (SGUS) has proven to be a valid and useful tool to evaluate the presence of salivary glands (SGs) involvement in patients with suspected or established pSS (2-6). Many sonographic lesions can be identified by SGUS (7), but the most reliable feature to distinguish pSS patients from controls and other pathological mimicker conditions has proved to be the inhomogeneity of the glandular parenchyma, described as the presence of hypoechoic/anechoic areas of various degrees within the glands. The majority of the SGUS scoring system in pSS are then focused on this sonographic feature (8-10). Based on the present knowledge, hypoechoic/anechoic areas are considered as a marker of glandular active inflammation (theoretically reversible with therapy), while hyperechoic bands as a marker of glandular damage, since they are related to objective salivary impairment (10, 11).

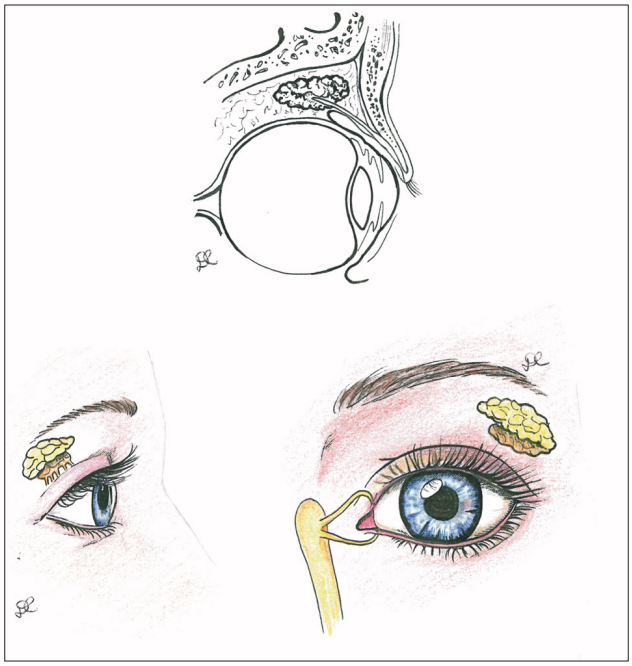
The LGs are paired, almond-shaped glands, located in the upper lateral region of the orbit within the lacrimal fossa. They normally measure approxi-

mately 20 × 12 × 5 mm, although the size decreases with age, and they secrete the aqueous layer of the tear film (Fig. 1) (12-14). The LGs consist of an orbital and a palpebral lobe, which are separated anatomically by the lateral horn of the aponeurosis of the superior levator palpebrae muscle (12, 13). Infiltrative and inflammatory processes, as well as LGs lymphoma, tend to have a diffuse pattern involving both lobes, while the epithelial neoplasms are more frequent located in the orbital lobe (12, 13, 15). Except for the Schirmer's test and the ocular surface staining, the other tools to evaluate the LGs involvement in pSS patients, such LGs computed tomography, LGs biopsy, etc., are much more difficult to be done in routine clinical practice (16, 17).

The ultrasonography of the LGs (LGUS), if useful, would represent a major advance for the management of pSS. The literature on LGUS is however scant and mostly focused on LGs masses and on the Doppler evaluation of the lacrimal artery (15, 18, 19). The LGUS evaluation in pSS was described only in few case reports (20-22), while Giovagnorio *et al*. were the first to perform LGUS in 15 pSS patients and 15 healthy controls in 2000. In their experience, atrophic and normal-sized glands were difficult or impossible to detect (9/15 pSS patients, 60%), while detectable glands were correlated with lacrimal impairment (6/6, 100%). Changes of echotexture, namely fatty infiltration (3/6, 50%) or the presence of multiple small cyst-like lesions in two patients with LGs lymphoma, were identified (23).

The LGUS, if adequate for the assessment of pSS, would represent an objective, easy, non-invasive, and repeatable test. Since the LGs are located superficially, with the new US machines and high frequencies transducers, the LGs could be better and easier evaluated than in the past years (24). Unlike the Schirmer's test, the LGUS could hypothetically provide information about the real attribution of the lacrimal impairment to pSS rather than to other conditions (age or drug-related dry eye syndrome, etc.) (13, 17). It could investigate anatomic alterations, such as LGs masses, but above all, it could differentiate inflammatory from damage-related lesions, then facilitating therapeutic choices (11, 15, 25). Moreover, in some pSS patients ocular and oral manifestations are not synchronous, and then it could be relevant to fully characterise both SGs and LGs involvement in the same patient (26).

Fig. 1. Lacrimal gland anatomy.



This study has two major aims. First, to perform an US evaluation of the LGs in healthy subjects in order to: i) define the sonographic elementary lesions which could be identified in the LGs and describe their frequencies in healthy subjects, ii) test the intra and inter-rater agreement between four rheumatologists. Secondly, to preliminary assess whether the elementary lesions of the LGs, previously defined, let us differentiate healthy subjects from pSS patients, highlighting a possible relevance of further LGUS studies in pSS.

Materials and methods

A consensus meeting was held in December 2019 to define the sonographic lesions to be evaluated in the LGs. Two rheumatologists with more than 10 years of experience in US imaging and two rheumatology residents participated.

Healthy subjects were then evaluated between January 2020 and February 2020 in two Rheumatology Clinics (Milan and Udine, Italy), while unselected pSS patients were evaluated between March 2020 and May 2020 in the Clinic of Rheumatology, University Hospital of Udine, Italy.

All participants gave informed consent for all procedures, which were carried out in accordance with the Declaration of Helsinki and with the guidelines for good clinical practice. The study was conducted according to a protocol approved by the local Ethical Committee (CEUR-2017-Os-027-ASUIUD).

Ultrasonographic assessment of lacrimal glands

All lacrimal glands ultrasound (LGUS) examinations were performed by two rheumatologists (ODL and AZ), using two commercially available real-time scanners (Esaote Mylab X8 with a linear high-frequency transducer L4-15, and Samsung RS85 with a linear high-frequency transducer LA4-18B). The subjects were examined in a supine position and with the eyelids closed. The lacrimal region was scanned obliquely, with a scanning plane almost parallel to the anterior outline of the orbit, using part of the ocular globe as an acoustic window (Fig. 2). Static images were acquired.

Healthy subjects

Only subjects older than 18 years were evaluated. Exclusion criteria were pres-

ence of sicca symptoms or autoimmune diseases, use of medications with the potential to influence saliva and tears production, a medical history of radiation exposure to head and neck region, hepatitis B, hepatitis C, human immunodeficiency virus infections, sarcoidosis, lymphoma, and graft versus host reaction. The data collected at the time of US evaluation were gender and age.

Web-based reliability exercise on LG images of healthy subjects

Afterward, a web-based reliability exercise was performed on healthy subjects' images. The images were reviewed by the four rheumatologists in two rounds, with an interval of one week between the first and the second round.

pSS patients

The inclusion criterion for the pSS patients was the fulfillment of the American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) classification criteria (27). Data collected from medical charts were gender, age, disease duration, presence of anti-Ro/SSA, anti-La/SSB antibodies and rheumatoid factor, Schirmer's I test, unstimulated salivary flow rate, lip biopsy focus score (28), Visual Analogue Scale (VAS) ocular and oral dryness, EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) (29), and EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) (30).

Analysis of sonographic lesions detected in pSS patients

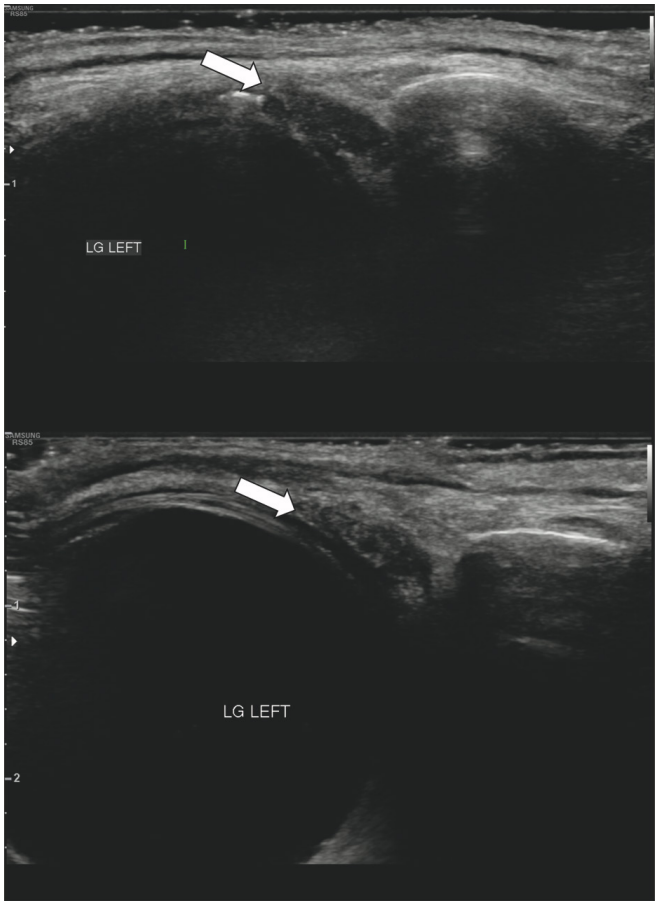
The images collected were evaluated for the presence of the sonographic lesions defined in the consensus meeting by one rheumatologist (AZ).

Statistical analysis

The study population features have been investigated performing descriptive statistics on categorical and numerical variables. Frequency distributions were used for categorical variables. For numerical variables, we considered mean, median, interquartile range, standard deviation, 25° and 75° percentile, minimum and maximum values. Kolmogorov-Smirnov test was per-

Fig. 2. Ultrasound scanning technique.

Fig. 3. Examples of sonographic pictures of lacrimal glands in healthy subjects.



formed for checking the normality of the distribution for numerical variables. Based on the results, parametric or non-parametric tests have been performed.

All images evaluation produced dichotomous data (0-1). Intra-rater and inter-rater reliability was assessed by using unweighted Fleiss', Cohen's and Light's kappa coefficients for considered scores. Intra-rater and inter-rater agreement have also been calculated. Agreement values of 0–20% were considered slight, 21-–0% fair, 41–60% moderate, 61–80% good, and 81–100% excellent.

We calculated the proportion of agreement observed and the proportion of agreement expected by chance alone. Of these results then we computed mean, median, 1st and 3rd quartile, minimum (min) and maximum (max) values, and the 95% confidence interval (CI) with the bootstrap percentile method.

We compared the data from healthy subjects with the data obtained from pSS patients. We considered both demographic data, compared with the t-test and Fisher's exact test, and US data, compared with Fisher's exact test.

All statistical analyses were performed using R software, v. 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria). The significance level was set at 0.05.

Results

Consensus on ultrasonographic elementary lesions in LGs

The agreed US features for the LGUS evaluation were glandular parenchyma visibility (visible or not-visible), glandular size (normal or increased/decreased), glandular parenchyma homogeneity (homogenous or inhomogeneous), hypoechoic areas (absent or present), hyperechoic spots (absent or present), fibrous gland appearance (absent or present – defined as hyperechoic appearance covering the whole surface of the gland, similarly to OMERACT definition in SGUS (9)), and fatty deposition (absent or present). Examples in Figure 3.

Healthy subjects

Fifty-seven healthy subjects were evaluated, 42/57 (73.7%) were female. The mean age (±SD) was 51 years (±14.9) and the median age was 51.7 years (range 22–81 years) (Table I). One hundred and thirteen images of the LGs were acquired (56 right LGs and 57 left LGs). The frequency of the US elementary lesions for each observer for the first and the second round was reported in Table II.

Table I. Clinical characteristics of heathy subjects and pSS patients.

Demographic data	Healthy subjects	pSS patients	*p*-value
Number of patients	57	17	
Gender, female, n (%)	42/57, (73.7%)	16/17, (94.1%)	0.10
Age at disease onset, years, mean ± SD	-	38.1 ± 9	
Age at evaluation, years, mean ± SD	51.6 ± 14.9	52.2 ± 6.9	0.78
Disease duration, years, mean ± SD	-	14.1 ± 8.7	
Serological features			
Anti-Ro/SSA positive, n (%)	-	16/17, (94.1%)	
Anti-La/SSB positive, n (%)	-	11/17, (64.7)	
Rheumatoid factor positive, n (%)	-	11/17, (64.7)	
Clinical features			
Abnormal Schirmer's I test*, n (%)	-	14/17, (82.3%)	
Abnormal unstimulated salivary flow rate§, n (%)	-	14/17, (82.3%)	
Lip biopsy focus score ≥1, n (%)	-	8/11, (72.7%)	
VAS ocular dryness, mean ± SD; median	-	5.8 ± 2.6; 6	
VAS oral dryness, mean ± SD; median	-	7.9 ± 1.5; 8	
ESSPRI, mean ± SD; median	-	6.4 ± 1.7; 7	
ESSDAI, median (range)	-	8 (1-19)	

*Schirmer's I test values <5 mm/5 minutes were considered pathological.
§Unstimulated salivary flow rate ≤1.5 mL/15 minutes was considered pathological.

Web-based reliability exercise in LG images of healthy subjects

After calculating the kappa coefficients and the proportions of agreements observed and the proportion of agreement expected by chance alone, a very high agreement but low kappa values was found. As known (31), kappa values are affected by prevalence, and paradoxical results can be obtained in particular situations with asymmetrical unbalanced observed marginal values, like in ours.

In our study, we observed this problem in inter-rater data. We had very low or missing values in contingency tables relatively to visible alterations by all the readers or visible by one of the readers. Unfortunately, there is no resolutive correction for this paradox (32) and we decided to keep and illustrate only the agreement results for both intra and inter-rater reliability, even if intra-rater kappa coefficients were not paradox affected, to give more uniform results.

The mean value of the data obtained from the four readers about healthy subjects was considered. Regarding the size, glandular parenchyma homogeneity, hypoechoic areas, hyperechoic spots, fibrous gland appearance, and fatty deposition, the mean value of the data obtained from the four readers (only for the 63 images in which the gland was visible by all the readers) was considered. Glandular parenchyma visibility has been evaluated on 113 images. The intra-rater reliability was excellent with an agreement of 95% (min 86%, max 100%; 95% CI 91–98%) (Supplementary Table S1). The inter-rater agreement for the first round was 79% (min 73%, max 91%; 95% CI 75–83%) (Supplementary Table S2), and similar results were reported in the second round (77%, min 66%, max 91%, 95% CI 72–82%). The intra-rater reliability of glandular size presented an agreement of 94% (min 87%, max 100%; 95% CI 91–97%). No difference for inter-rater agreement was found in the first (80%; min 68%, max 92%; 95% CI 75–86%) and in the second round (79%; min 67%, max 92%; 95% CI 73–85%). Regarding glandular parenchyma homogeneity, the intra-rater reliability presented an agreement of 92% (min 87%, max 97%; 95% CI 90–94%). The inter-rater agreement was good in the first (71%; min 65%, max 84%; 95% CI 68–75%) and in the second round (72%, min 65%, max 80%, 95% CI 69–75%). The intra-rater reliability of hypoechoic areas presented an agreement of 94% (min 87%, max

Table II. Frequencies of the sonographic elementary lesions in healthy subjects for each observer in the first and second round.

Round 1	RATER 1	RATER 2	RATER 3	RATER 4	Mean	Range
Non-visible glandular parenchyma	29/113 (25.7%)	20/113 (17.7%)	3/113 (2.6%)	7/113 (6.2%)	13.05%	2.6-25.7%
Abnormal glandular size	4/63 (6.3%)	1/63 (1.6%)	6/63 (9.5%)	18/63 (28.6%)	11.5%	1.6-28.6%
Inhomogeneity	12/63 (19.1%)	16/63 (25.4%)	7/63 (11.1%)	7/63 (11.1%)	16.675%	11.1-25.4%
Hypoechoic areas	0	6/63 (9.5%)	7/63 (11.1%)	16/63 (25.4%)	11.5%	0-25.4%
Hyperechoic spots	10/63 (15.9%)	2/63 (3.2%)	0	8/63 (12.7%)	7.95%	0-15.9%
Fibrous gland appearance	1/63 (1.6%)	0	0	0	0.4%	0-1.6%
Fatty deposition	2/63 (3.2%)	9/63 (14.3%)	0	2/63 (3.2%)	1.6%	0-14.3%
Round 2	**RATER 1**	**RATER 2**	**RATER 3**	**RATER 4**	**Mean**	**Range**
Non-visible glandular parenchyma	29/113 (25.7%)	19/113 (16.8%)	3/113 (2.6%)	6/113 (5.3%)	12.6%	2.6-25.7%
Abnormal glandular size	5/63 (7.9%)	1/63 (1.6%)	3/63 (4.8%)	20/63 (31.8%)	11.525%	1.6-31.8%
Inhomogeneity	7/63 (11.1%)	12/63 (19.1%)	5/63 (7.9%)	6/63 (9.5%)	15.875%	7.9-19.1%
Hypoechoic areas	0	1/63 (1.6%)	5/63 (7.9%)	16/63 (25.4%)	8.725%	0-25.4%
Hyperechoic spots	8/63 (12.7%)	2/63 (3.2%)	0	6/63 (9.5%)	6.35%	0-12.7%
Fibrous gland appearance	1/63 (1.6%)	0	0	0	0.4%	0-1.6%
Fatty deposition	0	9/63 (14.3%)	0	0	3.575%	0-14.3%

100%; 95% CI 91–97%) and the inter-rater agreement presented an excellent proportion of agreement in the first round (84%; min 75%, max 97%; 95% CI 79–89%) and good in the second round (80%, min 69%, max 97; 95% CI 74–86%). Considering hyperechoic spots, the intra-rater reliability presented an agreement of 95% (min 87%, max 100%; 95% CI 92–99%). The proportion of inter-rater agreement was excellent in the first (86%, min 78%, max 97%; 95% CI 82–90%) and in the second round (85%, min 75%, max 97%; 95% CI 81–89%). The intra-rater reliability of fibrous gland appearance presented an agreement of 99% (min 97%, max 100%; 95% CI 98–100%). The inter-rater agreement was the same in the first and in the second round (51%, min 2%, max 100%, 95% CI 21–80%). Concerning fatty deposition, the intra-rater reliability presented an agreement of 98% (min 97%, max 100%; 95% CI 97–98%). The inter-rater reliability was excellent both in the first and in the second round (90%, min 83%, max 97%, 95% CI 86–94%, similar result for both rounds) (Intra and inter-rater agreement results are reported respectively in Supplementary Tables S1 and S2).

pSS patients

Seventeen pSS patients were evaluated, 16/17 (94.1%) were female, the mean age (± SD) at LGUS evaluation was 52.2 years (±6.9), the mean (± SD) disease duration was 14.1 years (± 8.7). An objective lachrymal functional impairment, evaluated by Schirmer's I test, was present in 14/17 (82.3%) patients (Table I).

Thirty-four LGs images of these pSS patients were evaluated, LGs were visualised in 33/34 (97%). The main detected alterations were glandular parenchyma inhomogeneity (13/33, 39.4%), presence of hypoechoic areas (8/33, 24.2%), and abnormal glandular size (6/33, 18.2%). More details in Table III.

Table III. Ultrasound characteristics of pSS patients and healthy subjects' images.

	pSS images	healthy subjects' images	*p*-value
Non-visible glandular parenchyma	1/34, 3%	13/113, 11.5%	0.19
Abnormal glandular size	6/33, 18.2%	7/63, 11.1%	0.36
Inhomogeneity	13/33, 39.4%	9/63, 14.3%	**0.01**
Hypoechoic areas	8/33, 24.2%	6/63, 9.5%	0.07
Hyperechoic spots	1/33, 3%	5/63, 8%	0.66
Fibrous gland appearance	3/33, 9.1%	0/63, 0%	**0.04**
Fatty deposition	0/33, 0%	3/63, 4,77%	0.55

Table IV. Clinical characteristics of pSS patients with positive and negative lacrimal gland ultrasound (LGUS).

Demographic data	Positive LGUS	Negative LGUS	*p*-value
Number of patients	10	7	
Gender, female, n (%)	9/10, (90%)	7/7, (100%)	
Age at disease onset, years, mean ± SD	39.5 ± 8.4	36 ± 10	0.463
Age at evaluation, years, mean ± SD	54.5 ± 5.7	48.9 ± 7.6	0.203
Disease duration, years, mean ± SD	15 ± 9.8	12.9 ± 7.2	0.845
Serological features			
Anti-Ro/SSA positive, n (%)	9/10, (90%)	7/7, (100%)	1
Anti-La/SSB positive, n (%)	7/10, (70%)	4/7, (57.1%)	0.644
Rheumatoid factor positive, n (%)	7/10, (70%)	4/7, (57.1%)	0.644
Clinical features			
Abnormal Schirmer's I test*, n (%)	8/10, (80%)	6/7, (85.7%)	1
Abnormal unstimulated salivary flow rate§, n (%)	8/10, (80%)	6/7, (85.7%)	1
Lip biopsy focus score ≥1, n (%)	7/8, (87.5%)	1/3, (33.3%)	0.152
VAS ocular dryness, mean ±SD; median	6.5 ± 1.7; 6	4.9 ± 3.4; 5	0.372
VAS oral dryness, mean ±SD; median	8 ± 1.2; 8	7.7 ± 1.9; 8	0.759
ESSPRI, mean ±SD; median	7 ± 1.6; 7.5	5.5 ± 1.6; 5.7	0.096
ESSDAI, median (range)	7 (1-19)	8 (2-18)	0.961

Positive LGUS: at least one ultrasound lesion in at least one lacrimal gland at LGUS; Negative LGUS: no lesion in both the lacrimal glands at LGUS.
*Schirmer's I test values <5 mm/5 minutes were considered pathological.
§Unstimulated salivary flow rate ≤1.5 mL/15 minutes was considered pathological.

Comparison between healthy subjects and pSS patients

The two cohorts of subjects considered were matched for age and sex (Table I). Among US elementary lesions in pSS patients compared with healthy subjects, we detected a significantly difference in glandular parenchyma inhomogeneity [13/33 (39.4%) *vs*. 9/63 (14.3%), p=0.01], and in fibrous gland appearance [3/33 (9.1%) *vs*. 0/63 (0%), p=0.04]. More details in Table III.

Comparison between pSS patients with and without ultrasound lesions

No significant differences between demographic, serological and clinical data of pSS patients with positive (*i.e*. at least one US lesion in at least one LG) and negative (no lesion in both the LGs) LGUS were found (Table IV).

Discussion

The present study provides pilot data on the reliability of the LGUS in healthy subjects. Furthermore, it represents a preliminary attempt to detect differences in LGUS appearance between pSS patients and healthy subjects, defining the most relevant sonographic elementary lesions which could help identify pSS patients.

In this study, after the preliminary agreement on the LGUS lesions to be assessed, four rheumatologists performed a reliability exercise to evaluate the US features of LGs in healthy subjects, finding a good-excellent intra and inter-rater reliability. The intra-rater reliability was excellent regarding all the features assessed (glandular parenchyma visibility, glandular size, glandular parenchyma homogeneity, hypoechoic areas, hyperechoic spots, fibrous gland appearance, and fatty deposition). The inter-rater reliability was excellent regarding the hypoechoic areas in the first round (while it was good in the second round), the hyperechoic spots and fatty deposition in both rounds. Finally, in both rounds the inter-rater agreement for the glandular parenchyma visibility, homogeneity, and size was good, and moderate for fibrous gland appearance. Few previous studies evaluated the reliability of the SGUS in pSS, usually with variable results (33-35), and recently the authors of the OMERACT score for the SGUS in pSS highlighted a good level of reliability for their score (9), however, no studies up to now evaluated the reliability of the LGUS in either healthy subjects or pSS patients.

Over the past years, the SGUS has received a growing interest as a non-invasive and easily performed technique in the management of pSS, while very few data on the LGUS are available. To our knowledge, only Giovagnorio *et al*. performed, twenty years ago, LGUS in a cohort of healthy subjects and pSS patients, using a very different US equipment and reporting a high rate of non-visible LGs in pSS patients (9/15 patients, 60%) (23). Furthermore, that study did not evaluate all the US features we herein considered. Nowadays, modern high-performance US machines seem to allow to differentiate LGs of healthy subjects from pSS patients, indeed in the present study, the glandular parenchyma inhomogeneity (p=0.01) and the fibrous gland appearance (p=0.04) are significantly more frequent in pSS patients than in healthy subjects. No differences were found in the characteristics of pSS patients with and without US lesions. Considering the preliminary nature of our study, more data are needed to confirm and better understand these results.

As reported by Milic *et al*., some abnormalities may also be detected by SGUS in asymptomatic subjects or non-pSS sicca patients. Indeed they found that glandular structural changes, including abnormal glandular size, abnormal echogenicity, inhomogeneity, focal changes, and non-visible posterior glandular borders, were detected in 11.1% asymptomatic controls and 50% subjects with sicca symptoms, whereas these US alterations were found in 93% patients with pSS and 27.3% patients with secondary SS (36). Similarly, in our study, some US lesions were detected by LGUS both in healthy subjects and pSS patients, namely abnormal glandular size, hypoechoic areas, and hyperechoic spots. Therefore, further investigations are required.

In our opinion, LGUS is feasible, easy to perform, non-invasive, and with a good inter e intra-rater agreement, then it could be a valuable objective tool to assess LGs lesions in pSS patients.

Acknowledgements

We thank Dr Donatella Colatutto, Rheumatology Clinic, Department of Medical Area, University of Udine, for her contributions to this work through her drawings.

References

1. GOULES AV,TZIOUFAS AG: Primary Sjögren's syndrome: clinical phenotypes, outcome and the development of biomarkers. *Immunol Res* 2017; 65: 331-44.
2. DEVAUCHELLE-PENSEC V, ZABOTTI A, CARVAJAL-ALEGRIA G, FILIPOVIC N, JOUSSE-JOULIN S, DE VITA S: Salivary gland ultrasonography in primary Sjögren's syndrome: opportunities and challenges. *Rheumatology* (Oxford) 2019 Mar 19.
3. DELLI K, DIJKSTRA PU, STEL AJ, BOOTSMA H, VISSINK A,SPIJKERVET FK: Diagnostic properties of ultrasound of major salivary glands in Sjögren's syndrome: a meta-analysis. *Oral Dis* 2015; 21: 792-800.
4. CORNEC D, JOUSSE-JOULIN S, MARHADOUR T *et al*.: Salivary gland ultrasonography improves the diagnostic performance of the 2012 American College of Rheumatology classification criteria for Sjögren's syndrome. *Rheumatology* (Oxford) 2014; 53: 1604-7.
5. BALDINI C, ZABOTTI A, FILIPOVIC N *et al*.: Imaging in primary Sjögren's syndrome: the 'obsolete and the new'. *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S215-21.
6. ZABOTTI A, ZANDONELLA CALLEGHER S, LORENZON M *et al*.: Ultrasound-guided core needle biopsy compared to open biopsy: a new diagnostic approach to salivary gland enlargement in Sjögren's syndrome? *Rheumatology* (Oxford) 2020.
7. JOUSSE-JOULIN S, NOWAK E, CORNEC D *et al*.: Salivary gland ultrasound abnormalities in primary Sjögren's syndrome: consensual US-SG core items definition and reliability. *RMD Open* 2017; 3: e000364.
8. JOUSSE-JOULIN S, MILIC V, JONSSON MV *et al*.: Is salivary gland ultrasonography a useful tool in Sjögren's syndrome? A systematic review. *Rheumatology* (Oxford) 2016; 55: 789-800.
9. JOUSSE-JOULIN S, D'AGOSTINO MA, NICOLAS C *et al*.: Video clip assessment of a salivary gland ultrasound scoring system in Sjögren's syndrome using consensual definitions: an OMERACT ultrasound working group reliability exercise. *Ann Rheum Dis* 2019; 78: 967-73.
10. DE VITA S, LORENZON G, ROSSI G, SABELLA M, FOSSALUZZA V: Salivary gland echography in primary and secondary Sjögren's syndrome. *Clin Exp Rheumatol* 1992; 10: 351-6.
11. ZABOTTI A, ZANDONELLA CALLEGHER S, GANDOLFO S *et al*.: Hyperechoic bands detected by salivary gland ultrasonography are related to salivary impairment in established Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S146-52.

12. MACHIELE R, LOPEZ MJ, CZYZ CN: Anatomy, head and neck, eye lacrimal gland. *In*: StatPearls, Treasure Island (FL), 2020.
13. CONRADY CD, JOOS ZP, PATEL BC: Review: The lacrimal gland and its role in dry eye. *J Ophthalmol* 2016; 2016: 7542929.
14. IZUMI M, EGUCHI K, UETANI M *et al*.: MR features of the lacrimal gland in Sjögren's syndrome. *AJR Am J Roentgenol* 1998; 170: 1661-6.
15. GAO Y, MOONIS G, CUNNANE ME, EISENBERG RL: Lacrimal gland masses. *AJR Am J Roentgenol* 2013; 201: W371-81.
16. BJORDAL O, NORHEIM KB, RODAHL E, JONSSON R, OMDAL R: Primary Sjögren's syndrome and the eye. *Surv Ophthalmol* 2020; 65: 119-32.
17. WHITCHER JP, SHIBOSKI CH, SHIBOSKI SC *et al*.: A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjögren's Syndrome International Registry. *Am J Ophthalmol* 2010; 149: 405-15.
18. BILGILI Y, TANER P, UNAL B *et al*.: Doppler sonography of the normal lacrimal gland. *J Clin Ultrasound* 2005; 33: 123-6.
19. BADARINZA M, SERBAN O, MAGHEAR L *et al*.: Multimodal ultrasound investigation (grey scale, Doppler and 2D-SWE) of salivary and lacrimal glands in healthy people and patients with diabetes mellitus and/or obesity, with or without sialosis. *Med Ultrason* 2019; 21: 257-64.
20. SECELEANU A, POP S, PREDA D, SZABO I, ROGOJAN L, SECELEANU R: Ultrasound features of lacrimal gland in Sjögren's syndrome: case report. *Acta Clin Croat* 2012; 51 (Suppl. 1): 135-40.
21. SECELEANU A, POP S, PREDA D, SZABO I, ROGOJAN L, SECELEANU R: Imaging aspects of the lacrimal gland in Sjögren syndrome--case report. *Oftalmologia* 2008; 52: 35-9.
22. AHUJA AT, METREWELI C: Ultrasound features of Sjögren's syndrome. *Australas Radiol* 1996; 40: 10-4.
23. GIOVAGNORIO F, PACE F, GIORGI A: Sonography of lacrimal glands in Sjögren syndrome. *J Ultrasound Med* 2000; 19: 505-9.
24. KANG T, HORTON L, EMERY P, WAKEFIELD RJ: Value of ultrasound in rheumatologic diseases. *J Korean Med Sci* 2013; 28: 497-507.
25. CAFARO G, CROIA C, ARGYROPOULOU OD *et al*.: One year in review 2019: Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S3-15.
26. PSIANOU K, PANAGOULIAS I, PAPANASTASIOU AD *et al*.: Clinical and immunological parameters of Sjögren's syndrome. *Autoimmun Rev* 2018; 17: 1053-64.
27. 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. *Ann Rheum Dis* 2017; 76: 9-16.
28. GREENSPAN JS, DANIELS TE, TALAL N, SYLVESTER RA: The histopathology of Sjögren's syndrome in labial salivary gland biopsies. *Oral Surg Oral Med Oral Pathol* 1974; 37: 217-29.
29. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69: 1103-9.
30. SEROR R, RAVAUD P, MARIETTE X *et al*.: EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI): development of a consensus patient index for primary Sjögren's syndrome. *Ann Rheum Dis* 2011; 70: 968-72.
31. FEINSTEIN AR, CICCHETTI DV: High agreement but low Kappa: I. the problems of two paradoxes. *J Clin Epidemiol* 1990; 43: 543-49.
32. CICCHETTI DV, FEINSTEIN AR: High agreement but low kappa: II. Resolving the paradoxes. *J Clin Epidemiol* 1990; 43: 551-58.
33. HOCEVAR A, AMBROZIC A, ROZMAN B, KVEDER T, TOMSIC M: Ultrasonographic changes of major salivary glands in primary Sjögren's syndrome. Diagnostic value of a novel scoring system. *Rheumatology* (Oxford) 2005; 44: 768-72.
34. DELLI K, ARENDS S, VAN NIMWEGEN JF *et al*.: Ultrasound of the major salivary glands is a reliable imaging technique in patients with clinically suspected primary Sjögren's syndrome. *Ultraschall Med* 2018; 39: 328-33.
35. SALAFFI F, CAROTTI M, IAGNOCCO A *et al*.: Ultrasonography of salivary glands in primary Sjögren's syndrome: a comparison with contrast sialography and scintigraphy. *Rheumatology* (Oxford) 2008; 47: 1244-9.
36. MILIC VD, PETROVIC RR, BORICIC IV *et al*.: Major salivary gland sonography in Sjögren's syndrome: diagnostic value of a novel ultrasonography score (0-12) for parenchymal inhomogeneity. *Scand J Rheumatol* 2010; 39: 160-6.