Hyperechoic bands detected by salivary gland ultrasonography are related to salivary impairment in established Sjögren's syndrome

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ABSTRACT

Objective. *In primary Sjögren's syndrome (pSS) dryness of eye and mouth is the cardinal referred symptom. Assessing the rate of activity and damage in the salivary glands of pSS patients is essential to improve disease management. Up to now, a differentiation of activity and damage ultrasonographic (US) lesions is an open issue. The aim of this preliminary study was to identify US lesions which better correlate with loss of function of salivary glands in pSS.*

Methods. *Salivary gland ultrasonography of consecutive patients with established pSS, fulfilling AECG and ACR/EULAR criteria was performed. The association between sialometry and Visual Analogue Scale (VAS) oral dryness and SGUS lesions was assessed through univariate and multivariate analysis.*

Results. *In 75 established pSS patients, mean disease duration 12.4±7.2 years, the hyperechoic bands of parotid gland (PG) and submandibular gland (SMG) were significantly associated with sialometry ($p<0.001$) and VAS oral dryness (PG $p=0.002$, SMG $p<0.001$). The global glandular involvement (scored according to De Vita et al., 1992) was associated with sialometry (PG $p=0.025$, SMG $p<0.001$) and with VAS oral sicca (PG $p=0.015$, SMG $p<0.001$). The multivariate analysis selected the hyperechoic bands of PG and SMG as the variables independently associated with sialometry and the hyperechoic bands and the homogeneity in the SMG as associated with VAS oral dryness.*

Conclusion. *These results indicate that salivary impairment in pSS, as objectively evaluated by sialometry, could be mainly associated with damage (i.e. hyperechoic bands) in established pSS. Additional follow-up studies and improved scoring tools are needed.*

Introduction

Salivary gland ultrasonography (SGUS) has proved useful for the diagnosis of primary Sjögren's syndrome (pSS), and abnormal homogeneity of glandular parenchymal is the most important sonographic feature discriminating pSS patients from controls by SGUS (1-4). In any inflammatory disease it is crucial to differentiate between active inflammatory lesions (reversible with therapy) and damage-related lesions (irreversible), and that is the case also of pSS. Of note, inhomogeneity of the glandular parenchymal detected by SGUS in pSS includes two distinct sonographic abnormalities, *i.e.* hypoechoic areas and hyperechoic bands (5). These have been mainly related to either activity/inflammation or to damage, respectively, although additional studies are definitely required to well correlate SGUS abnormalities with the corresponding histopathologic features (6, 7).

Dryness of eyes and dryness of mouth are two cardinal symptoms in pSS, and objective salivary and lacrimal glandular hypofunction could be explained by three principal mechanisms, which may contribute differently in different patient subsets. They include: i) active inflammation within the gland, due to the infiltration of immune cells; ii) chronic damage, with fibrotic or fatty lesions, and with loss of functional parenchyma, as the consequence of the aforementioned inflammation as well as of other possible pathologic events; and iii) functional impairment of the gland due to various mechanisms, *e.g.* autonomic dysfunction or downregulation of receptor-mediated secretion of saliva (8). To develop novel effective treatments for sicca symptoms in pSS, patients' stratification and the differentiation of the pathogenetic events in the

individual cases are crucial. The aim of this study was to identify the SGUS lesions more strictly associated with decreased salivary function in pSS, by means of a careful patient selection (*i.e.* established and anti-SSA/SSB-positive pSS) and of the accurate recording of SGUS abnormalities.

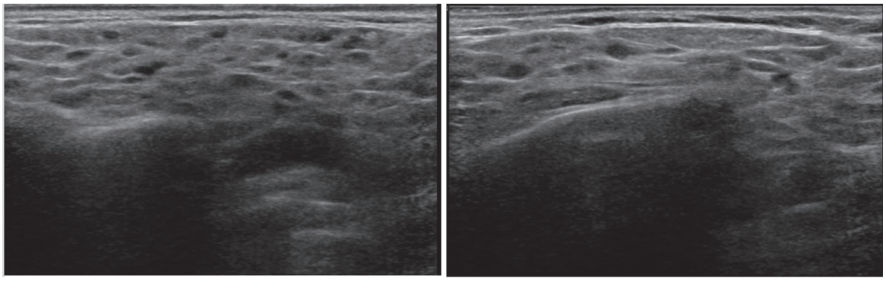

Fig. 1. Salivary gland ultrasonographic images characterised by abnormal parenchymal homogeneity with hypoechoic areas and hyperechoic bands.

Methods

Patients

Consecutive patients with pSS, referred to the Clinic of Rheumatology, University Hospital of Udine, Italy, from January until April 2019 were recruited. The inclusion criteria were: a) fulfilment of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2016 classification criteria for pSS (9) and the American European Consensus Group 2002 classification criteria (10); b) presence of anti-Ro/SSA serum antibodies; and c) pSS duration ≥5 years. The study was conducted according to a protocol approved by the Regional Ethical Committee (CEUR-2017-Os-027-ASUIUD) (11). All patients gave oral and written informed consents for all procedures, which were carried out in accordance with the Declaration of Helsinki and with the guidelines for good clinical practice.

Healthy controls

Since fibrosis in salivary glands could be attributable to aging, sex and age-matched healthy individuals (HCs) were also evaluated by SGUS. Exclusion criteria were: concomitant autoimmune or thyroid disease, active smoking, concomitant antidepressant or diuretic therapy.

Clinical and laboratory data

Data collected included: gender, date of birth, pSS duration, previous minor salivary gland biopsy, presence of serum anti-Ro/SSA and anti-La/SSB antibodies and rheumatoid factor (RF).

Oral dryness was evaluated by the presence of both subjective symptoms, *i.e.* the Visual Analogue Scale (VAS) oral sicca, and by validated objective evaluation, *i.e.* unstimulated salivary flow rate. Unstimulated saliva was collected in pre-weighed containers and the volume of secreted saliva determined by weighing, with 1 gram of saliva corresponding to 1 ml. Levels ≤1.5 ml/15 minutes was considered pathologically. Sialometry was performed the same day of the SGUS evaluation, according to the recommended procedure (9, 10, 12).

Ultrasonographic assessment of major salivary glands

Parotid glands (PG) and submandibular glands (SMG) were examined using a SAMSUNG RS85 machine with a linear high-frequency transducer (LM4-15B). Both PG and SMG were scanned with patients lying in supine position with the neck hyper-extended and the head slightly turned to the opposite site. PG were evaluated in longitudinal and cross-sectional plane and SMG in longitudinal plane. The US examination was performed by only one clinical investigator expert in SGUS (AZ), blinded to clinical data of the patients. The US definitions of lesions (echogenicity, homogeneity, hyperechoic bands, location of the hypoechoic/anechoic areas in the gland, number of abnormal lymph nodes in the glands, calcification, posterior border visible) were defined according to previous studies (13, 14) (Fig. 1). Changes in the homogeneity of the glands were also evaluated according to the original score proposed by *De Vita el al*. in 1992 (SGUS score) (1). This score has four levels: grade 0 (normal homogenous parenchyma), grade 1 (mild level of inhomogeneity, with isolated and small hypoechoic areas, without hyperechoic bands), grade 2 (moderate inhomogeneity with multiple hypoechoic areas and/or few hyperechoic bands), grade 3 (severe inhomogeneity with large and confluent hypoechoic areas and diffuse hyperechoic bands). We evaluated also fatty deposition in the gland, defined as irregular area with increased echogenicity of the normal parenchyma, scored as absent (0) or present (1).

Statistical analyses

Ultrasonographic (US) lesions in each gland (left and right PG and left and right SMG) were either dichotomised (echogenicity: normal/abnormal; homogeneity: normal/abnormal; abnormal lymph nodes: no/yes; parenchymal calcifications: no/yes; posterior glandular border visible: no/yes; fatty deposition: no/yes) or categorised into ordinal levels (hyperechoic bands: none/ <50% of the parenchyma/ ≥50%; distribution of the hypoechoic areas in the gland: none/ isolated (<25% area)/ localised (25-50%)/ scattered (>50%) /diffused; SGUS score: 0/1/2/3. For both the PG and the SMG, the worse finding of the two sides was used in the analyses. Results of sialometry were dichotomised assuming 1.5 mL/15 minutes as the cut-off value.

The proportion of patients with reduced salivary flow rate (≤1.5 mL/15 minutes) was calculated in each category of US lesions, and the statistical significance of differences across categories was assessed through the chi-square test. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) of each US lesion for abnormal sialometry, were calculated. To this end, hyperechoic bands were dichotomised into absent *vs* present, distribution of the hypoechoic areas in the gland into ≤1 *vs*. >1 and the SGUS score into ≤1 *vs*. >1. VAS oral sicca distribution in each category of the US lesions was described through the mean ± standard deviation and quartiles, and the statistical differ-

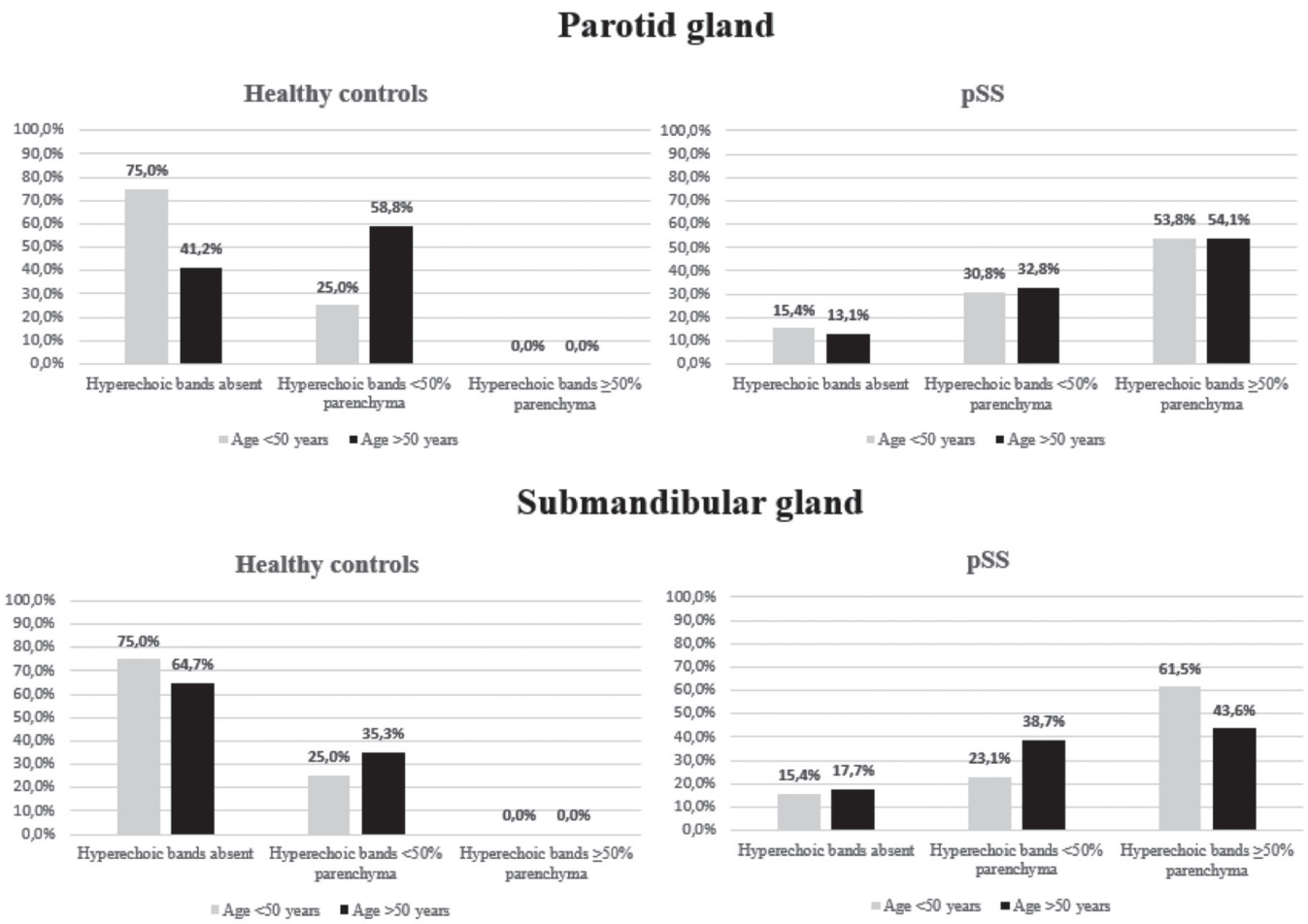

Fig. 2. Hyperechoic bands in healthy controls *vs*. primary Sjögren's syndrome (pSS).

ences across categories were assessed though Wilcoxon Rank Sums tests (for dichotomous variables) or Kruskal-Wallis tests (for variables with more than 2 levels). Results with *p*-value <0.05 were considered statistically significant.

To assess which US lesions were independently associated with abnormal sialometry, a stepwise logistic regression analysis was conducted, including all the US lesions as potential covariates. Significance levels of 0.15 and 0.10 were chosen to entry the model and to stay in the model, respectively. The association between each US finding with abnormal sialometry is expressed through the odds ratio (OR) with 95% confidence interval (95% CI). The area under the ROC curve (AUC) is presented as a measure of the final model discrimination capacity. To assess the US lesions which were independently associated with the VAS oral dryness value, a stepwise linear regression analysis was conducted, including all the US findings as potential covariates. Significance levels of 0.15 and 0.10 were chosen to entry the model and to stay in the model, respectively. The increase or decrease in VAS oral sicca associated with each US lesions is expressed through the β-coefficient. The R^2 is presented as the percentage of the variation in VAS oral dryness that is explained by the final model.

Sample size

Since the aim of our study was the assessment of glandular damage and considering the presence of hyperechoic bands as a possible marker of glandular damage, assuming that 75%±10% of patients had hyperechoic bands and admitting an alpha-error of 0.05 using a two-tailed test, a sample size of 73 patients was finally estimated.

Results

Clinical characteristics of patients

Seventy-five patients with pSS were enrolled: 69/75 (92%) were females, the mean age (±SD) at evaluation was 62.1±11.8 years and mean disease duration was 12.4±7.2 years. Patients positive for anti-Ro/SSA were 75/75 (100%), as required by inclusion criteria, while for both anti-Ro/SSA and anti-La/SSB were 43/75 (57.3%). Mean VAS oral dryness was 6.9±2.8 (median 7, range 0–10), while an abnormal unstimulated salivary flow rate was found in 54/75 (72%) patients. Detailed clinical data are summarised in Supplementary Table S1.

Healthy controls

Twenty-three HCs were evaluated, 20/23 (86.9%) were female, mean age (± SD) at evaluation was 61.3±14.9 years, none had subjective or objective sicca symptoms. At SGUS evaluation HCs had none or few hyperechoic bands (*i.e*. in <50% parenchyma), but no one had hyperechoic bands in ≥50% parenchyma. Distribution of hyperecho-

Table I. Univariate association between unstimulated salivary flow rate and ultrasound lesions.

	Patient with positive unstimulated salivary flow rate, n, (%)	*p*-value
PG echogenicity		0.053
0= normal	13/53, (24.5%)	
1= abnormal	40/53, (75.5%)	
SMG echogenicity		<0.001
0= normal	6/54, (11.1%)	
1= abnormal	48/54, (88.9%)	
PG homogeneity		0.123
0= normal	9/53, (17.0%)	
1= abnormal	44/53, (83.0%)	
SMG homogeneity		0.006
0= normal	3/54, (5.6%)	
1= abnormal	51/54, (94.4%)	
PG hyperechoic bands		<0.001
0= none	2/53, (3.8%)	
1= <50% parenchyma	17/53, (32.1%)	
2= ≥50% parenchyma	34/53, (64.1%)	
SMG hyperechoic bands		<0.001
0= none	4/54, (7.4%)	
1= <50% parenchyma	19/54, (35.2%)	
2= ≥50% parenchyma	31/54, (57.4%)	
PG location of the hypoechoic areas in the gland		0.012
0= none	9/53, (17.0%)	
1= isolated (<25% area)	6/53, (11.3%)	
2= localised (25-50% area)	4/53, (7.5%)	
3= scattered (>50% area)	8/53, (15.1%)	
4= diffused	26/53, (49.1%)	
SMG location of the hypoechoic areas in the gland		0.014
0= none	6/54, (11.1%)	
1= isolated (<25% area)	7/54, (13.0%)	
2= localised (25-50% area)	9/54, (16.7%)	
3= scattered (>50% area)	10/54, (18.5%)	
4= diffused	22/54, (40.7%)	
PG abnormal lymph nodes in the glands		0.655
0= no	35/53, (66.0%)	
1= yes	18/53, (34.0%)	
SMG abnormal lymph nodes in the glands		0.371
0= no	52/54, (96.3%)	
1= yes	2/54, (3.7%)	
PG calcification		0.080
0= no	46/53, (86.8%)	
1= yes	7/53, (13.2%)	
SMG calcification		0.681
0= no	47/54, (87.0%)	
1= yes	7/54, (13.0%)	
PG posterior border visible		0.324
0= no	2/53, (3.8%)	
1= yes	51/53, (96.2%)	
SMG posterior border visible		0.482
0= no	1/54, (1.8%)	
1= yes	53/54, (98.2%)	
PG SGUS score		0.025
0	7/53, (13.2%)	
1	5/53, (9.4%)	
2	20/53, (37.8%)	
3	21/53, (39.6%)	
SMG SGUS score		<0.001
0	4/54, (7.4%)	
1	6/54, (11.1%)	
2	24/54, (44.5%)	
3	20/54, (37.0%)	
PG fatty deposition		0.990
0= no	48/53, (90.6%)	
1= yes	5/53, (9.4%)	
SMG fatty deposition		0.807
0= no	45/54, (83.3%)	
1= yes	9/54, (16.7%)	

PG: parotid glands; SMG: submandibular glands.

ic bands according to age is shown in Figure 2.

Univariate analyses

• *Association between unstimulated salivary flow rate and SGUS lesions*

By univariate analyses, hyperechoic bands detected by SGUS in PG and in SMG were significantly associated with the unstimulated salivary flow rate ($p<0.001$ for both). The SGUS score for the worst PG and SMG was associated with the unstimulated salivary flow rate ($p=0.025$ for PG and $p<0.001$ for SMG). Other lesions associated with unstimulated salivary flow rate were PG and SMG abnormal echogenicity ($p=0.053$ for PG and $p<0.001$ for SMG), and PG and SMG distribution of the hypoechoic areas in the gland (respectively $p=0.012$ for PG and $p=0.014$ for SMG). With regard to abnormal homogeneity, the association with the unstimulated salivary flow rate was significant only for the SMG ($p=0.006$). Full results are shown in Table I.

• *Association between the VAS of oral dryness and SGUS lesions*

The hyperechoic bands were significantly associated with VAS oral dryness for both PG and SMG ($p=0.002$ and $p<0.001$, respectively). Also, in case of the SGUS score for the worst PG and SMG was associated with the VAS of oral dryness ($p=0.015$ for PG and $p<0.001$ for SMG). The distribution of the hypoechoic areas in the gland was associated with the VAS of oral dryness in the SMG ($p=0.009$), while in the PG the association was weaker ($p=0.076$). The abnormal echogenicity and homogeneity were associated with VAS oral dryness ($p=0.003$ and $p=0.001$, respectively) only in the SMG, while there was no association in the PG. Full results are shown in Supplementary Table S2.

Multivariate analysis

• *Association between unstimulated salivary flow rate and SGUS lesions*

The stepwise logistic regression analysis showed the presence of hyperechoic bands of salivary glands, both PG and SMG, as the sole SGUS variable independently associated with abnormal

unstimulated sialometry. The likelihood increased by 2.5 times for each increase in the scoring of hyperechoic bands (*i.e.* from no bands to <50% of parenchyma, and from 50% to ≥50) (Table II). The area under the ROC curve indicates a good accuracy of this final model (Fig. 3).

• *Association between the VAS of oral dryness and SGUS lesions*

The linear regression analysis highlighted the hyperechoic bands in the SMG and the abnormal homogeneity in the SMG as SGUS variables independently associated with the VAS oral dryness (β-coefficient respectively 1.26 and 2.25), while the presence of abnormal lymph nodes in the PG was inversely associated with VAS oral sicca (β-coefficient -1.8) (Suppl. Table S3).

Sensitivity and specificity of the different SGUS lesions for unstimulated salivary flow rate

Among the SGUS lesions, the hyperechoic bands in the PG had the highest sensitivity (96.2%) for an abnormal unstimulated salivary flow rate, but the specificity was low (38.1%). In the SMG the sensitivity and the specificity of the hyperechoic bands were similar (92.6% and 42.9%, respectively). The SGUS score in the SMG had the best performance in terms of balance between sensitivity and specificity (81.5% and 66.7%), with a PPV of 86.3% and a NPV of 58.3%. Full results are shown in Table III.

Association of SGUS hyperechoic bands with clinical features of pSS

Although this was not the purpose of the study, the SGUS abnormalities significantly associated with unstimulated salivary flow rate in pSS, *i.e.* hyperechoic bands, were also investigated in all patients for a possible association with some other clinical features, including patient age at the time of SGUS, disease duration, anti-SSB and rheumatoid factor seropositivity, as well as the focus score in lip biopsy (the latter data was available only in 25/75 patients). Among these different variables, the multivariate regression analysis found a significant positive association between hyperechoic bands and anti-SSB positivity (p=0.01).

Table II. Logistic regression model after stepwise selection for unstimulated salivary flow rate.

Variable	Odds ratio	95% Confidence interval
PG* hyperechoic bands§	2.51	1.01-6.23
SMG** hyperechoic bands§	2.57	1.05-6.27

*PG: parotid glands; **SMG: submandibular glands; §Likelihood increase for increase in hyperechoic bands from none to <50% parenchyma and from <50% to ≥50% parenchyma.

Fig. 3. ROC curve for linear regression model for unstimulated salivary flow rate.

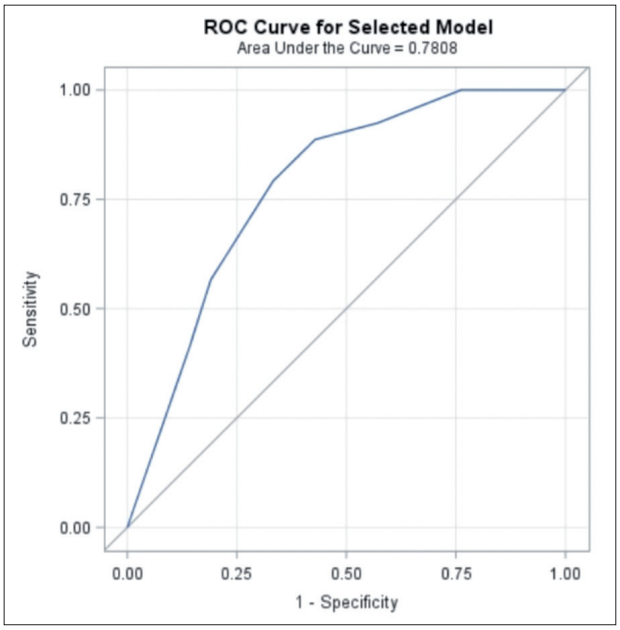

Table III. Sensitivity, specificity, positive predictive value and negative predictive value of ultrasound lesions associated with unstimulated salivary flow rate.

	Sensitivity	Specificity	PPV	NPV
PG* echogenicity	75.5%	47.6%	78.4%	43.5%
SMG** echogenicity	88.9%	47.6%	81.4%	62.5%
SMG homogeneity	94.4%	28.6%	77.3%	66.7%
PG hyperechoic bands^	96.2%	38.1%	79.7%	80.0%
SMG hyperechoic bands^	92.6%	42.9%	80.6%	69.2%
PG location of the hypoechoic areas in the gland§	71.7%	61.9%	82.6%	46.4%
SMG location of the hypoechoic areas in the gland§	75.9%	66.7%	85.4%	51.8%
PG SGUS score #	77.4%	52.4%	80.4%	47.8%
SMG score #	81.5%	66.7%	86.3%	58.3%

*PG: parotid glands; **SMG: submandibular glands; ^hyperechoic bands were dichotomised into: 0 (absent) *vs*. 1 (<50% parenchyma) and 2 (≥50% parenchyma) considered together; §location of the hypoechoic areas in the gland was dichotomised into: 0 (absent) and 1(isolated) considered together *vs*. 2 (localised) 3 (scattered) and 4 (diffused) considered together; #score by *De Vita et al*. was dichotomised into: grade 0 and 1 considered together *vs*. grade 2 and 3 considered together.

Discussion

Dryness of the eyes and of the mouth are the most prevalent symptoms in pSS (15). Then, they should be adequately assessed and possibly treated. Oral dryness may however result from different pathologic mechanisms, likely mixed, *i.e.* salivary gland inflammation, damage and functional abnormalities (8). To decide the proper treatment choices and to investigate novel therapies in pSS-related dryness, a clear distinction between the aforementioned mechanisms is therefore required (16). Currently, salivary gland scintigraphy, sialography and salivary flow rate are tools included in pSS classification criteria (9,

10), while for follow-up studies data are limited (5). Salivary gland biopsy may be also useful to this end, although the low number of glandular lobules studied may not well reflect the entire pathologic process (17). SGUS may represent an important future step for the diagnosis and follow-up of pSS patients, potentially able to detect changes in glandular inflammation and damage (1, 6, 7, 18-21). Artificial intelligence and image segmentation studies are in course to improve the reliability of this tool, by means of automatic scoring.

Based on the present knowledge, SGUS hypoechoic areas are mainly the expression of parenchymal inflammation, while hyperechoic bands mainly of chronic damage (5, 7, 22). This study suggests for the first time, to our knowledge, that the detection of hyperechoic bands in both PG and SMG, consistent with glandular damage, are significantly associated with objective salivary impairment and, to a lesser extent, with subjective oral dryness in pSS. Abnormal glandular homogeneity, which is the key, comprehensive abnormality in the SGUS scores (1, 23-27), was herein associated only with subjective salivary dryness, which may be much less reliable. In addition, abnormal homogeneity may result also from hypoechoic areas, and this generates confusion to clearly establish the role of activity *versus* damage in salivary gland involvement. As a second point, only the subset of established pSS patients, all anti-Ro/SSA positive and with a disease duration of at least 5 years, was chosen for the present, initial study. This is of major importance in our opinion, since the contribution of inflammation, damage and dysfunction may carry a different weight in different patient subsets, as well as in pSS of different duration (28-31).

Globally, the present results suggest that salivary impairment is mainly associated with glandular damage, as visualised by SGUS in established and seropositive pSS patients. Besides anti-SSA positivity, a relationship between hyperechoic bands and anti-SSB positivity was also noticed in this study. Furthermore, this study indirectly supports the notion that salivary glands fibrosis is mainly a consequence of pSS itself, rather than mainly a consequence of the increased age (32, 33). In fact, hyperechoic bands were much rarer and much less prominent in matched HCs. Although the present results are clearly limited, further studies by SGUS are needed in pSS. Other accurate and easy-to-perform tools to evaluate the salivary glands are actually lacking, while many potential novel therapies are ready to be tested. Novel SGUS approaches in pSS, able to better differentiate inflammation and damage in different patient subsets, and to better score them with computer-assisted algorithms, are under investigation and might better allow to detect subtler, but clinically relevant changes over time (34).

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