Diagnostic and predictive evaluation using salivary gland ultrasonography in primary Sjögren's syndrome

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ABSTRACT

Objective. *We aimed to assess the diagnostic accuracy of salivary gland ultrasonography (SGUS) as a single test for the detection of primary Sjögren's syndrome (pSS) and examine the prognostic factors for severe structural damage of the salivary glands based on SGUS score.*

Methods. *Patients with pSS (n=94) and idiopathic sicca syndrome (n=44) were evaluated using the SGUS 0-48 scoring system, which comprises five parameters: parenchymal echogenicity, homogeneity, hypoechoic areas, hyperechogenic reflections, and clearness of posterior borders. The salivary gland volume and intraglandular power Doppler signal (PDS) were also assessed. A multivariate linear regression analysis was performed to determine the factors associated with SGUS score.*

Results. *Patients with pSS showed a significantly higher SGUS score than controls [median (IQR): 24.5 (13.0) vs. 6 (3.75), p<0.001]. An SGUS cut-off of ≥14 had a sensitivity of 80.9% and a specificity of 95.5% for the diagnosis of pSS. There were no significant differences in the measured volumes and PDS between pSS patients and controls. The SGUS score correlated with unstimulated salivary flow rate (USFR), serum rheumatoid factor and IgG. Double seropositivity with anti-Ro/SS-A and anti-La/SS-B (β=6.060, p=0.001) and USFR (β=-1.913, p<0.001) were independently associated with the SGUS score.*

Conclusion. *The SGUS scoring system is a valuable diagnostic method for pSS. Double seropositivity of anti-Ro/SS-A and La/SS-B along with USFR were independent predictive factors for structural damage of the salivary glands.*

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Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease characterised by lymphocytic infiltration and destruction of the salivary and lachrymal glands, leading to the symptoms of dry mouth and eyes (1). In 2016, new American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for pSS were developed to make the classification criteria uniform and improve recruitment for clinical trials (2). With the exclusion of sialography and salivary gland scintigraphy, the available methods for evaluation of pSS orally include minor salivary gland biopsy (MSGB) and unstimulated salivary flow rate (USFR) (3). To classify pSS, MSGB is required in patients with a negative anti-SSA/Ro. Although MSGB is a specific and confirmative method, it is an invasive procedure. A key issue with MSGB is disparity in the assessment methods and reporting of local pathologists (4, 5). Therefore, there is an increasing need for alternative, non-invasive and reliable diagnostic tools with the potential to improve and simplify the diagnostic process for pSS. The main advantage of salivary gland ultrasonography (SGUS) is the direct visualisation of structural abnormalities of the salivary glands. A number of publications have described and proven the value of SGUS for assessing major salivary gland involvement in pSS (6-10).

Despite these advantages of SGUS, a number of obstacles remain. Different SGUS scoring systems in B-mode were used in previous studies. All studies used parenchymal inhomogeneity with hypoechogenic areas to evaluate each salivary gland. However, in these studies, various other sonographic findings were noted in pSS patients, such as hyperechogenic bands, non-visible glandular border, and decreased echogenicity. The diagnostic usefulness of Doppler analysis and glandular size measurement has not been established and the feasibil-

ity of SGUS for detecting pSS in early stages of the disease is unclear. Specifically, SGUS could replace MSGB even in patients with early pSS.

Outcome measurement and prognostic prediction are challenging in patients with pSS because the disease course varies including glandular and extraglandular manifestations and lymphoma development. The assessment of the severity of glandular involvement in pSS is now feasible using SGUS (11). There is no proven prognostic factor for glandular damage in pSS, although numerous studies have revealed the risk factors for lymphoma. The question remains whether B-cell hyper-reactivity and increased disease activity play a role in glandular structural abnormalities in pSS.

We aimed to assess the diagnostic value of SGUS as a single test for pSS detection in an integrated manner. We assessed the diagnostic accuracy of three SGUS parameters: the US grey-scale scoring system, glandular volume measurement, and intraglandular power Doppler US (PDUS). The secondary aim was to examine the prognostic factors for severe structural changes in major salivary glands based on the SGUS scoring system.

Materials and methods

Study population

This was a single-centre prospective cohort study performed at Konkuk Medical Centre, Korea, from March 2016 to October 2017. We enrolled 138 patients with established pSS and suspected SS. The definitive diagnosis of pSS was made in accordance with American-European Consensus (AECG) criteria (12). Patients who did not fulfil the AECG criteria for pSS and received a diagnosis of idiopathic sicca syndrome were the controls. More specifically, idiopathic sicca syndrome was defined as a condition that manifests as persistent, dry eyes and dry mouth that is not immune-mediated of caused by a systemic disorder. Patients with secondary SS or who presented with dry mouth and dry eyes in the setting of other rheumatic systemic diseases, a history of hepatitis C infection, IgG4-related disease, acquired immunodeficiency syndrome, sarcoidosis, previous head and neck radiation treatment, graft versus host disease, and current use of anticholinergic or other drugs that might affect salivary gland function were excluded from the study. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for Human Research, Konkuk University Medical Center (KUH 1010776). The written consent was obtained from all participants.

Clinical and laboratory evaluation

A standardised clinical evaluation was performed and the following data were obtained and recorded: patient demographics, history of dry eyes/mouth and/or recurrent parotid enlargement, duration of subjective sicca symptoms, symptoms and signs suggestive of disease-related extraglandular involvement, comorbidities, medication use, Schirmer's test result (≤5 mm/5 minutes on at least one side is abnormal), USFR (abnormal if <0.1 ml/min), and sialo-scintigraphy result. MSGB was graded according to the focusing system (13, 14) To assess the disease activity and disease-related damage, we measured the EULAR Sjögren's syndrome disease activity index (ESSDAI) (15) and Sjögren's syndrome disease damage index (SSDDI) (16).

Routine laboratory tests included test assessing white blood cell (WBC) count; lymphocyte, haemoglobin, and platelet concentrations; erythrocyte sedimentation rate (ESR); C-reactive protein (CRP) level; immunoglobulin G (IgG), A (IgA), and I (IgM), cryoglobulin, and complement levels (C3, C4, CH50); and hepatitis B and C virus serum studies were conducted at the time SGUS was performed. Immunological data included antinuclear antibody (ANA) (assessed on HEp-2 cells, a titre ≥1.160 was considered positive), anti-SS-A/Ro, anti-SS-B/La, anti-centromere (by enzyme-linked immunosorbent assay [ELISA]), and rheumatoid factor ([RF] by nephelometry) levels.

Salivary gland ultrasonography (SGUS) examination

SGUS was performed on all patients by the same examiner (L.K.A.) who was blinded to the clinical data. We used a HD15 US (Philips Ultrasound, Bothell, WA, USA) device equipped with a multi-frequency linear probe at a frequency of 5-12 MHz. The settings were adjusted for increasing Doppler sensitivity by decreasing the pulse repetition frequency (800 Hz) and adjusting the Doppler gain to a level just below random noise. We assessed the 4 major salivary glands (bilateral parotid and submandibular glands). The US of the parotid glands was performed with the patient's head slightly tilted to the side opposite the side being scanned for better exposure of the examined area. Then, the patient was placed in a supine position with the head maximally tilted back to access the submandibular area. The major salivary glands were examined in the longitudinal and transverse planes. On each side, the parotid gland was scanned in the retromandibular fossa, anterior to the ear and sternocleiodomastoid muscle, and the submandibular gland was scanned in the posterior part of the submandibular triangle. The thyroid was also scanned to compare to the salivary glands for evaluation of parenchymal echogenicity. The following US parameters were analysed and recorded in a predefined form as: (1) semi-quantitative SGUS scores consisting of parenchymal echogenicity, parenchymal inhomogeneity, presence of hypoechoic areas, presence of hyperechoic foci, and clearance of SG posterior borders (2) volumes of the submandibular and parotid glands, and (3) intraglandular PDUS.

(1) Parenchymal echogenicity was evaluated in comparison with the thyroid parenchyma and surrounding soft tissue (muscle, subcutaneous fat). If the echogenicity of salivary gland was comparable to that of the thyroid the grade was 0 and if decreased, the grade was 1. Homogeneity of the parenchyma was graded from 0 to 3 (grade 0 for a homogeneous gland, grade 1 for mild inhomogeneity, grade 2 for evident inhomogeneity, and grade 3 for a grossly inhomogeneous gland. The presence of hypoechoic areas in the parenchyma was graded from 0 to 3 (grade 0=absent, grade 1=few scattered, grade 2=several, and grade 3=numerous hypoechoic ar-

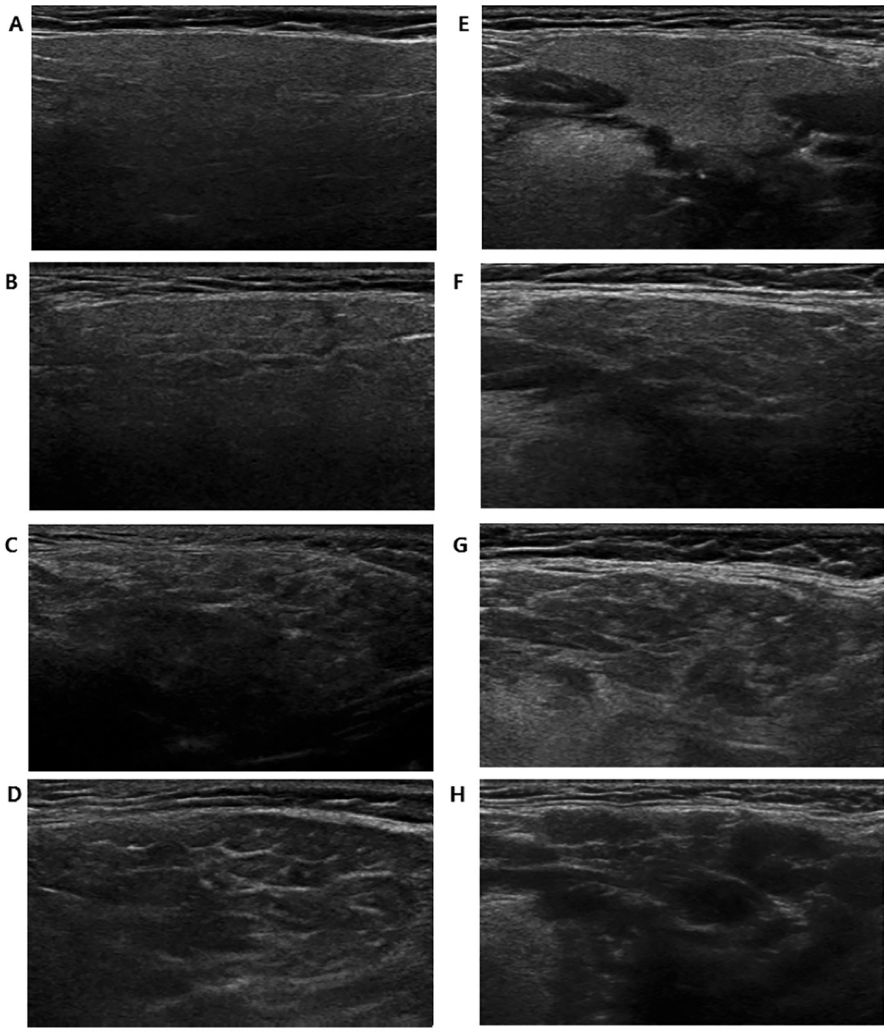

Fig. 1. Representative images showing salivary gland ultrasonography in primary Sjögren's syndrome. (A-D) Parotid ultrasonographic grade of homogeneity, (**A**) Normal homogenous parotid gland (Grade 0), (**B**) Mild inhomogeneous parotid gland (Grade 1), (**C**) Evident inhomogeneous parotid gland (Grade 2), (**D**) grossly inhomogeneous parotid gland (Grade 3), (E-H) Submandibular ultrasonographic grade of homogeneity, (**E**) Grade 0, (**F**) Grade 1, (**G**) Grade 2, (**H**) Grade 3.

eas). The presence of hyperechoic foci was graded from 0 to 3 in the parotid glands (grade 0=absent, grade 1=few scattered, grade 2=several, and grade 3=numerous hyperechoic foci) and from 0 (absent) to 1 (present) in the submandibular glands. Delineation of the SGs from surrounding tissues (visibility of glandular borders) was graded from 0 to 3 (grade 0=well defined borders, grade 1=slightly less defined borders, grade 2=ill-defined borders, and grade 3=borders not visible, blurred). Finally, the SGUS score was calculated by summation of the grades of the 5 parameters described above for all 4 glands according to the scoring system of Hocevar *et al*. (17). The SGUS score ranged from 0 to 48 (Fig. 1).

(2) The volumes of the submandibular and parotid glands were calculated as longitudinal diameter (cm)×transverse diameter (cm)×sagittal diameter (cm)×0.5 and expressed in ml (18).

(3) Intraglandular PDUS was interpreted through a 4-grade semi-quantitative scoring system as follows: grade 0=no parenchymal flow, grade 1=up to three single spots signals or up to two confluent spots or one confluent spot plus up to two single spots, grade 2=flow signals in less than half of the cross section of a gland (≤50%), and grade 3=flow signals in more than half of the cross section of a gland (>50%). The normal large vessels visible within the salivary glands (external carotid artery and retromandibular vein in the parotid gland and facial artery and vein in the submandibular gland) were excluded from the PDUS score.

Statistical analysis

Statistical analyses were performed using the SPSS software package for Windows v. 17.0 (SPSS Inc., Chicago, IL, USA). Data were compared using the unpaired Student *t*-test, Chi-square test, and Mann-Whitney U-test, as appropriate. On the receiver operating characteristic (ROC) curve, theoptimal cut-off value producing the best combination of sensitivity and specificity was located nearest the upper left corner of the curve. Correlations between the SGUS score and various parameters of pSS were evaluated by Spearman correlation coefficient. To identify items independently associated with SGUS scores, we performed multivariate linear regression analyses. All items that were associated with a diagnosis of pSS by univariate analysis with *p*-values less than 0.1 were entered into the multivariate model, applying backward elimination. Results were considered statistically significant when $p<0.05$.

Results

Characteristics of the study population

The study cohort included 138 patients. Ninety-four patients fulfilled the AECG criteria for pSS, and 44 patients were diagnosed with idiopathic sicca syndrome. Table I summarises the baseline characteristics of study population. MSGB was performed in 21 patients with pSS and 10 patients with idiopathic sicca syndrome. The focus score was significantly higher in pSS group than controls [median (IQR) 1 (2) vs. 0 (0), $p=0.001$]. No differences were observed between the two groups with respect to age, gender, and duration of sicca symptoms. Among the 94 patients with pSS, 32 (34.0%) had a history of parotidomegaly, 32 (34.0%) reported Raynaud's phenomenon, 38 (40.4%) had arthritis or arthralgia, 4 (4.3%) had biopsy proven vasculitis, 7 (7.4%) had pulmonary involvement, 9 (9.6%) had peripheral nervous system involvement, 6 (6.4%) were undergoing treatment for thyroiditis.

Table I. Baseline characteristics of the study population.

	pSS (n=94)	Idiopathic sicca syndrome (n=44)	*p*-value
Age, mean (SD), years	55.6 (12.2)	59.2 (11.8)	0.093
Female, n (%)	89 (94.7)	42 (95.5)	0.476
Duration of sicca symptoms, mean (SD), years	6.4 (4.1)	5.2 (4.7)	0.109
Xerostomia, n (%)	77 (81.9)	39 (88.6)	0.869
Xerophthalmia, n (%)	75 (79.8)	37 (84.1)	0.976
Abnormal Schirmer's test, n (%)	67 (71.3)	33 (75)	0.886
USFR, mean (SD), ml/15 minutes	2.2 (2.3)	3.4 (3.5)	**0.040**
Positive ANA, n (%)	64 (68.1)	3 (6.8)	**<0.001**
Positive anti-Ro/SSA, n (%)	84 (89.4)	3 (6.8)	**<0.001**
Positive anti-La/SSB, n (%)	47 (50.0)	2 (4.5)	**< 0.001**
Positive anti-centromere, n (%)	10 (10.6)	1 (2.3)	0.446
Positive RF, n (%)	53 (56.4)	11 (25)	**0.002**
RF, mean (SD), IU/dl	28.8 (38.1)	21.8 (40.9)	0.341
IgG, mean (SD), mg/dl	1643.0 (424.1)	1288.3 (431.3)	**<0.001**
C3, mean (SD), mg/dl	98.3 (19.2)	114.8 (36.8)	**0.015**
C4, mean (SD), mg/dl	24.7 (20.1)	25.9 (7.6)	0.723
Abnormal sialo-scintigraphy, n (%)	79 (84.0)	32 (72.7)	0.692
ESR, mean (SD), mm/h	19.5 (14.9)	14.5 (18.6)	0.100
CRP, mean (SD), mg/dl	0.1 (0.2)	0.3 (0.9)	0.208
Raynaud phenomenon	28 (29.8)	4 (9.1)	0.248

pSS: primary Sjögren's syndrome; USFR: unstimulated salivary flow rate; ANA: antinuclear antibody, RF: rheumatoid factor; Ig: immunoglobulin; C: complement; ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.

Diagnostic value of SGUS scoring system

The SGUS scores of bilateral parotid, submandibular glands and the sum of all four major salivary glands in the pSS group were significantly higher than those in the idiopathic sicca syndrome [median (IQR); 24.5 (13) *vs*. 6 (3.75), 11 (11) *vs*. 3 (2), and 14 (7) *vs*. 3 (2); all four glands, bilateral parotid glands, and submandibular glands, respectively] (Fig. 2).

By setting a SGUS score cut-off value of 14 [area under the curve (AUC) 0.941 (SD 0.019), 95% CI 0.904, 0.978], SGUS had 80.9% sensitivity and 95.5% specificity with a positive predictive value (PPV) of 97.4% and a negative predictive value (NPV) of 70.0%. Combined evaluation of bilateral parotid and submandibular glands showed better diagnostic accuracy than did single gland evaluation (Supplementary Figure 1). Table II presents the diagnostic accuracy of the SGUS in comparison with anti-Ro/SSA, Schirmer's test, USFR, and sialo-scintigraphy. Schirmer's test and USFR test were less sensitive and specific than SGUS. Sialo-scintigraphy had higher sensitivity, but much lower specificity in differentiating pSS from idiopathic sicca syndrome.

Diagnostic value of salivary gland volumes and PDUS findings

The volumes of the salivary glands were measured for bilateral parotid and submandibular glands in pSS and idiopathic sicca syndrome. The average volumes of parotid and submandibular glands in patients with pSS [mean (SD); 39.8 (11.4), and 6.6 (3.0), respectively] were smaller than those with idiopathic sicca syndrome [43.2 (13.1), and, 7.4 (2.6), respectively], but there was no significant difference between two groups (p=0.121, and p=0.124, respectively) (Supplementary Table I). No difference was found between the pSS and the idiopathic sicca group in terms of PDUS; total PDUS scores [median (IQR), 3.5 (5) *vs*. 4.5 (4), respectively, p=0.313] (Supplementary Table II).

Diagnostic accuracy of SGUS for early pSS

To test the accuracy of SGUS scores for detection of pSS in early stages, we categorised the patients into two groups, according to symptom duration (symptom duration ≤5 years *vs*. >5 years). The patients with a sicca symptom duration ≤5 years (n=72) showed a sensitivity of 77.1%, a specificity of 95.8%, a PPV of 97.4%, and a NPV of 67.6%. The pSS patients with a sicca symptom duration >5 years (n=66) showed a sensitivity of 84.8%, a specificity 95.0%, a PPV of 97.5%, and an NPV 73.1%. SGUS performance in pSS patients with longer symptom duration showed better diagnostic accuracy. However, no significant differences between early and late pSS were observed with respect to SGUS scores [median (IQR) 24 (12.5) *vs*. 27

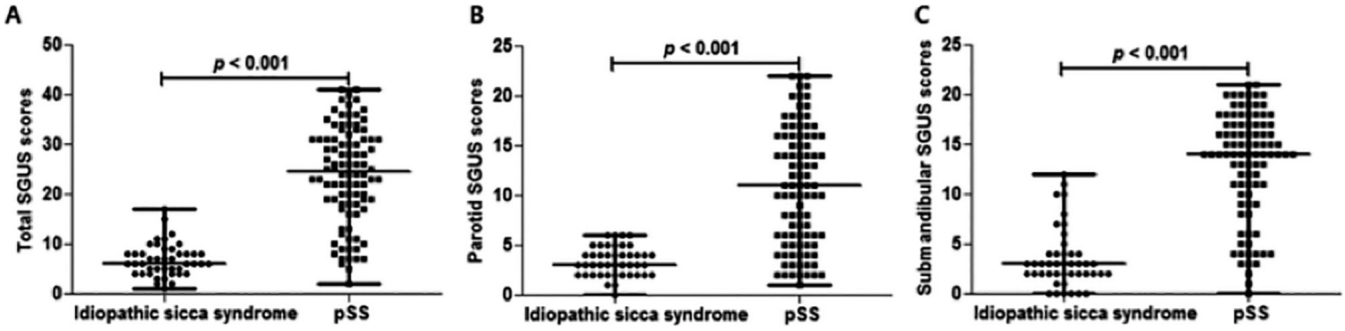

Fig. 2. Salivary gland ultrasound scores in patients with primary Sjogren's syndrome and idiopathic sicca syndrome.
(**A**) Distribution of scores for four glands (0-48). (**B**) Distribution of scores for both parotid glands (0-26) (**C**) Distribution of scores for both submandibular glands (0-22).

Table II. Diagnostic accuracy of SGUS for primary Sjögren's syndrome.

	Sensitivity	Specificity	PPV	NPV
Total SGUS scores	80.9	95.5	97.4	70.0
SGUS scores of parotid glands	74.5	90.9	94.6	62.5
SGUS scores of submandibular glands	81.9	88.6	93.9	69.6
Anti-Ro/SSA antibodies	89.4	93.2	96.6	80.4
Schirmer's test ≤5ml/15min	76.1	25.0	76.1	34.4
USFR ≤1.5ml/15min	61.5	68.2	80.0	46.2
Sialo-scintigraphy	94.0	8.6	71.2	37.5

SGUS: salivary gland ultrasound; PPV: positive predictive value; NPV: negative predictive value; USFR: unstimulated salivary flow rate.

Table III. Clinical, laboratory, and salivary gland ultrasound features of positive and negative salivary gland ultrasound scores.

	SGUG score <14 (n=18)	SGUG score ≥14 (n=76)	*p*-value
Age, median (IQR), years	57.5 (20)	57.0 (16)	0.874
Female, n (%)	3 (16.7)	2 (2.6)	0.243
Duration of sicca symptoms, median (IQR), years	5.0 (6.5)	6.0 (7.0)	0.172
Xerostomia, n (%)	14 (77.8)	63 (82.9)	0.482
Xerophthalmia, n (%)	13 (72.2)	62 (81.6)	0.329
Abnormal Schirmer's test, n (%)	10 (55.6)	57 (75)	**0.031**
Abnormal USFR, n (%)	4 (22.2)	52 (68.4)	**<0.001**
USFR, mean (SD), ml/15 minutes	3.3 (3.8)	1.3 (1.5)	**0.001**
Positive ANA, n (%)	7 (38.9)	57 (75)	**0.003**
Positive anti-Ro/SSA, n (%)	16 (22.9)	68 (89.5)	1.000
Positive anti-La/SSB, n (%)	5 (27.8)	42 (55.3)	**0.036**
Double positive Ro/La	3 (16.7)	40 (52.6)	**0.001**
Positive anti-centromere, n (%)	1 (5.6)	9 (11.8)	0.676
RF, median (IQR)	9.5 (20.3)	27.0 (31.0)	**0.006**
Positive RF, n (%)	6 (33.3)	47 (61.8)	**0.028**
IgG, median (IQR), mg/dl	1460.0 (407.8)	1746.0 (295.5)	**0.016**
C3, mean (SD), mg/dl	94.3 (24.9)	95.2 (20.3)	0.909
C4, mean (SD), mg/dl	23.7 (7.8)	22.4 (7.8)	0.222
Abnormal sialo-scintigraphy, n (%)	12 (66.7)	67 (88.2)	**0.004**
ESR, median (IQR), mm/h	12.0 (13.8)	18.0 (20.5)	0.160
CRP, median (IQR), mg/dl	0.03 (0.05)	0.05 (0.06)	0.231
ESSDAI, median (IQR)	3 (11.25)	3 (3.75)	0.067
SSDDI, median (IQR)	2 (2)	2 (2)	0.552
Total SGUS score	9 (4)	26 (9)	**<0.001**
Average volume of parotid glands, median (IQR), ml	43.1 (16.9)	39.3 (14.9)	**0.048**
Average volume of submandibular glands, median (IQR), ml	7.5 (4.7)	5.7 (2.9)	**0.031**
PDS sum scores of four salivary glands, median (IQR)	6 (5)	3 (6)	**0.008**

SGUG: salivary gland ultrasonography; USFR: unstimulated salivary flow rate; ANA: antinuclear antibodies; RF: rheumatoid factor; C: complement; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ESSDAI: European league against rheumatism Sjögrens' syndrome disease activity; SSDDI: Sjögren's syndrome disease damage index; PDS: power Doppler signal.

(13.25), p=0.226], total PDUS scores [4 (5) *vs*. 3 (5), p=0.094], and the average volumes of bilateral parotid [40.5 (18.3) *vs*. 39.7 (10.7), p=0.432] and submandibular glands [6.1 (3.6) *vs*. 5.85 (3.3), p=0.311].

Associations between SGUS scores with pSS disease features

The patients with pSS were classified into two groups according to cut-off value of SGUS scores: positive SGUS group (SGUG score≥14) and negative SGUS group (SGUG score<14). According to the SGUS scores, 80.9% of patients had positive SGUS findings and 19.1% patients had negative SGUS findings. Positive SGUS group had lower USFR, more often abnormal sialo-scintigraphy, positive ANA, double seropositive anti-Ro/SSA and anti-La/SSB, and significantly higher levels of RF and IgG than negative SGUS group. Among the pSS patients who underwent MSGB (n=21), focus score was significantly higher in positive SGUS group than negative SGUS group [median (IQR); 2 (1.5) *vs*. 0 (0.75), p<0.001]. The average volumes of bilateral submandibular and parotid glands were significantly smaller (p=0.048, and p=0.031, respectively), and PDUS sum scores of major four salivary glands were lower in positive SGUS group than negative SGUS group (p=0.008). However, those in positive SGUS group did not differ significantly from those in negative SGUS group with regard to disease duration, age, gender, dry symptoms, anti-Ro/SSA, anti-centromere, levels of complement, ESR, and CRP, ESSDAI, and SSDDI (Table III). The total SGUS scores correlated with levels of RF and IgG (r=0.370, p<0.001 and r=0.242, p=0.022, respectively), and inversely correlated with USFR (r=-0.578, p<0.001) and total PDUS scores (r=-0.303, p=0.003) (Supplementary Table III).

Factors associated with SGUS scores in patients with pSS

To assess the independent determinants of SGUS scores in patients with pSS, a linear regression analysis model was built. In multivariate analysis, double positivity of anti-Ro/SSA and anti-La/SSB (β=6.060, p=0.001) and USFR (β=-1.913, p<0.001) were independently associated with SGUS scores (Table IV).

Discussion

The present study aimed to evaluate the diagnostic accuracy of SGUG in patients with pSS using various methods, including a scoring system, volume measurement, and PDUS. The study confirmed the high sensitivity and specificity of the SGUS 0–48 scoring system for distinguishing patients with pSS from those with idiopathic sicca syndrome. However, there was no significant difference in the volumes and PDUS of the major salivary glands between patients with pSS and controls.

Table IV. Factors associated with salivary gland ultrasound scores by univariate and multivariate linear regression analysis.

Variable	Univariate			Multivariate		
	β	95% CI	*p*-value	β	95% CI	*p*-value
Disease duration	0.388	-0.102, 0.874	0.120			
Age	0.077	-0.086, 0.240	0.349			
Double positivity of anti Ro/SSA and La/SSB*	7.490	3.791, 11.189	**< 0.001**	6.060	2.672, 9.448	**0.001**
Positive anti Ro/SSA	2.690	-3.753, 9.134	0.409			
Positive anti La/SSB	6.319	2.552, 10.087	**0.001**			
Positive anti centromere	3.818	-2.792, 10.428	0.253			
IgG	0.006	0.001, 0.011	**0.011**			
RF	0.068	0.017, 0.119	**0.009**			
Positive ANA	6.518	2.458, 10.577	**0.002**			
ESR	0.146	0.013, 0.278	**0.031**			
CRP	3.894	-7.101, 14.888	0.483			
Positive Schirmer's test**	6.031	1.273, 10.789	**0.014**			
USFR	-2.185	-2.966, -1.404	**< 0.001**	-1.913	-2.664, -1.163	**< 0.001**
SSDDI	0.168	-1.420, 1.756	0.834			
ESSDAI	-0.408	-0.916, 0.100	0.114			

Total R^2: Coefficient of determination (% of variability explained by the model). Total R^2: 0.351, Adjusted R^2: 0.336, $p<0.001$, β: regression coefficient.
*Compared with patients without double positivity of anti Ro/SSA and La/SSB antibodies (reference category). **Compared with patients with negative Schirmer's test (reference category).

To date, various SGUS scoring system have been published (8). In contrast with other scoring system, 0-48 scoring system uses five components including not only homogeneity and hypoechogenic areas, but also parenchymal echogenicity, clearness of boarder margin, and hyperechogenic reflections. These distinct variables could ameliorate the diagnostic accuracy of SGUS in patients with pSS (19). Among the studies using SGUS 0-48 scoring system, there were the discrepancies in sensitivity and specificity. Previous studies showed that a cut-off of 15, 17 and 19 yielded a good sensitivity/specificity (88.6/84.2, 58.8/98.7%, and 87.1%/90.8%, respectively) (17, 19, 20). In this study, lowering optimal cut-off SGUS value at 14 resulted in the maximal sensitivity (82.7%) and specificity (92.9%). The different set of cut-off values and control groups could affect inconsistent results. A large scale multicentre and international study is needed to validate the accuracy of SGUS and set the cut-off value. Concordance with previous study (19), combined evaluation of submandibular and parotid glands showed better diagnostic accuracy than the evaluation of single gland. Therefore, SGUG examination of all four salivary glands is necessary to make a correct diagnosis of pSS.

The MSGB remains the gold standard for confirming the diagnosis. In recent years, SGUS has been introduced as a convenient and non-invasive imaging tool for pSS. The good agreement between SGUS and MSGB was demonstrated in several studies (21, 22). But the question remains: can SGUS replace the salivary gland biopsy? The common points between our study and previous reported SGUS studies are quite lower sensitivity and NPV compared to specificity and PPV (6, 8, 22, 23). SGUS is more useful in establishing pSS than in making an exclusive diagnosis of pSS, because its PPV is greater than NPV. And in our study, there were two patients with abnormal MSGB who showed normal SGUS findings. Thus, our opinion is that the SGUS could not replace the MSGB, but SGUS could be the first-line diagnostic tool along with anti-SSA/Ro, when patients are suspected to pSS. Then MSGB could be the next step for exclusion of pSS in patients who are negative for anti-Ro antibody and SGUS.

Another are of interest is whether the SGUS could correctly detect patients with early stages of pSS. Two previous studies evaluated the good diagnostic accuracy of SGUS in pSS patients with disease duration ≤5 years (sensitivity/specificity, 65.8%/95.3%, and 66%/98%) (6, 22). Similar to these data, we showed a sensitivity of 77.1% and a specificity of 95.8% in early stage of pSS (≤5 years). But the SGUS performance in pSS patients with disease duration >5 years showed better diagnostic accuracy than in pSS patients with disease duration ≤5 years. Our study suggested that the role of SGUS in patients with recent onset sicca symptoms could be limited. Carful interpretation of negative SGUS results is needed to avoid misdiagnosis of pSS, especially in patients with recent onset sicca symptoms. Further studies are needed to reveal the diagnostic validity of SGUS in the patients with early pSS.

In terms of PDUS and volume measurement, our results disagreed with previously published data. Besides our study, the PDUS was used in one study to score only parotid glands based on the number of spots in their regions of interest. In that study, patients with pSS showed significantly higher vascularity than controls (24). Werniche *et al*. demonstrated the detection of reduced volumes of submandibular glands in patients with pSS had high specificity at the cut-off point of 3.0 ml (18). On the other hand, in our study, glandular volume measurement and PDUS failed to contribute to distinguish pSS from idiopathic sicca syndrome. However, our study suggested the time course of SGUS changes in patients with pSS. We demonstrated smaller volumes of both parotid and submandibular glands, and more decreased power Doppler signal (PDS) in patients with advanced stages of pSS (SGUG score ≥14) than those without definite SGUS structural abnormality (SGUG score <14). Hypervascularity and glandular enlargement in the early inflammatory phase of pSS could lead to glandular hypo-vascularity and volume reduction in the late phase of pSS. Therefore, increased PDS without definite structural US changes could be the early pSS findings. So far, clear definitions of glandular enlargement and shrinkage are not established. In the future, a longitudinal study is required to identify whether it would be reasonable to repeat SGUS in a patient with an initially increased PDS and

normal SGUS score. Volumetric data of SGUS in patients with pSS should be collected as well.

We found a good correlation of SGUS scores with clinical and laboratory findings. The SGUS scores were related to the USFR and levels of RF and IgG. The assessment of disease activity and treatment outcome in patients with pSS is challenging because of the lack of simple and validated tools. Despite some authors suggesting that SGUS may have promising potential to evaluate the disease activity (19, 23), we did not find any correlation between SGUS scores and both ESSDAI and SSDDI. The use of SGUS as marker for disease activity and damage could be insufficient, as pSS is a systemic autoimmune disorder that affects not only exocrine glands but also extra-glandular organs.

To evaluate the predictive factor for glandular destruction, we used linear regression model. Though the multivariate linear regression model, we found that double seropositivity of anti-SSA/Ro and anti-SSB/La and USFR were independently associated with SGUS scores. There was a correlation between focus score and Ro 52 kD and La 48 kD in saliva, suggesting a strong relationship between local inflammation and autoantibody production (25). Gerli et al showed the degree of infiltration in the salivary gland tissue was significantly greater in patients with anti-SSA/Ro plus anti-SSB/La antibodies in their sera than in those with anti-SSA/Ro alone (26). Similarly, we showed double seropositivity of anti-SSA/Ro and anti-SSB/La was a predictive factor for severe glandular destruction. In ACR/EULAR classification criteria for pSS, positive serology for anti-SSB/La was excluded, because anti-SSB/La positivity did not affect classification performance in absence with anti-SSA/Ro positivity (3). Although anti-SSB/La was excluded in the new classification criteria, we recommend performing the test for both anti-SSA/Ro and anti-SSB/La to predict the severity of exocrine damage.

Our study did have some limitations. First, this study was a single-centre study, and the number of patients with idiopathic sicca syndrome was relatively small. Another limitation of our study was insufficient in MSGB data. MGSB was performed only in patients who were negative for anti-SSA/Ro and SSB/La but were suspected of pSS according to the AECG classification criteria. Because the number of patients who underwent the MSGB was limited, we could not compare the SGUS score with the focus score. Previously reported studies showed the overall concordance between the SGUS score and focus scores. However, to data, there are no studies that have compared major salivary gland histology to the SGUS findings. In the future, further studies of comparing the histological examination of major salivary glands to the SGUS findings are needed.

In conclusion, the present study evaluated the use of SGUS for the diagnosis of pSS in various ways including SGUS scoring system, PDUS, and glandular volume measurement. Our results showed that only SGUS scoring system represented a diagnostic usefulness for detection of pSS. Double seropositivity of anti-Ro/SSA and anti-La/SSB, and USFR could independently predict the severity of the structural damage of the major salivary glands in patients with pSS.

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