

Ultrasonography predicts the results of labial salivary gland biopsy in patients with suspected Sjögren's syndrome: a matrix risk model

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Abstract

Objective: Although a positive result of labial salivary gland biopsy (LSGB) is critical for the diagnosis of Sjögren's syndrome, rheumatologists prefer assessing the non-invasive objective items and hope to learn the predicted probability of positive LSGB before referring patients with suspected Sjögren's syndrome to receive biopsy. This study aimed to explore the predictive value of combined B-mode ultrasonography (US) and shear-wave elastography (SWE) examination on LSGB results.

Methods: A derivation cohort and later a validation cohort of patients with suspected Sjögren's syndrome were recruited. All participants received clinical assessments, B-mode US and SWE examination on bilateral parotid and submandibular glands before LSGB. Positive LSGB was defined by a focus score ≥ 1 per 4 mm² of glandular tissue.

Results: In the derivation cohort of 91 participants, either the total US scores or the total SWE values of four glands significantly distinguished patients with positive LSGB from those with negative results (area under the curve [AUC]=0.956, 0.825, both $p < 0.001$). The positive predictive value (PPV) was 100% in patients with total US scores ≥ 9 or with total SWE values ≥ 33 kPa. The negative predictive value (NPV) was 100% in patients with total US scores < 5 , but 68% in patients with total SWE values < 27 kPa. A matrix risk model was derived based on the combination of total US scores and total SWE values. Patients can be stratified into high, moderate, and low risk of positive LSGB. In the validation cohort of 52 participants, the PPV was 94% in the high-risk subpopulation and the NPV was 93% in the low-risk subpopulation.

Conclusion: A novel matrix risk model based on the combined B-mode US and SWE examination can help rheumatologists to make a shared decision with suspected Sjögren's syndrome patients on whether the invasive procedure of LSGB should be performed.

Keywords: labial salivary gland biopsy, shear-wave elastography, Sjögren's syndrome, ultrasonography

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Introduction

Sjögren's syndrome (SS) is one of the most common connective tissue diseases (CTDs) for rheumatologists, with a high incidence rate per 100,000 person-years ranging from 6.0 to 11.8 in Asia, 3.9 to 5.3 in Europe, and 3.9 in North America.¹ It is characterized by lymphocytic infiltration into the

exocrine glands such as salivary and lacrimal glands, significant loss of secretory function, plus immune-mediated extra-glandular manifestations.² SS can be primary (pSS) as a stand-alone syndrome or secondary (sSS) as a complication of other CTDs. The latter is common in the clinic but sometimes the causal relationship between

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other CTDs and SS is uncertain when it is named Sjögren-overlap syndrome.^{3–5}

The disease progression, treatment, and prognosis of SS are distinct from other CTDs, so accurate disease classification or diagnosis of SS is essential for the correct intervention or management, preventing poor outcomes and reducing the overall mortality associated with the disease. However, it is always difficult to make a definite diagnosis of SS for the heterogeneity of the presenting symptoms. Autoantibodies such as anti-SSA/Ro, anti-SSB/La, antinuclear antibodies (ANAs) or rheumatoid factor are serological proof of autoimmunity that can help to distinguish pSS from other causes of sicca symptoms or salivary gland swelling.² However, they are not the specific autoantibodies to distinguish pSS from other CTDs. If sicca patients suffer from another suspected or diagnosed CTD such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis or primary biliary cirrhosis/cholangitis,^{6–8} anti-SSA/Ro together with anti-SSB/La can indicate a possibility of Sjögren-overlap syndrome or sSS, but do not confirm it.⁹ To date, there is still lack of a single clinical, laboratory, pathological, or radiological feature that could serve as a ‘gold standard’ of SS,¹⁰ and the closest in identifying such a feature is labial salivary gland biopsy (LSGB) with a subsequent histopathological evaluation.¹¹ All mainstream classification criteria utilize the same definition of positive LSGB as focal lymphocytic sialadenitis with a focus score ≥ 1 per 4 mm² tissue.^{12–14} The LSGB procedure is relatively simple, with a small incision in the lower lip which can be performed with local anesthesia on an outpatient basis.¹¹ However, this procedure for patients is invasive.

Considering various classification criteria for pSS or sSS,^{9,12–14} LSGB should be performed in patients with suspected SS but negative anti-SSA/Ro, or with positive anti-SSA/Ro but without abnormal sialometry or dry eye tests, or in association with another known CTD. However, both rheumatologists and the patients themselves care about the possible results before the invasive procedure of LSGB. In 1726 registry participants from the database of the Sjögren’s International Collaborative Clinical Alliance (SICCA), anti-SSA/Ro, unstimulated whole saliva flow rate (UWS), ocular signs, or sicca symptoms cannot recognize patients who tend to get positive LSGB or who tend to get negative

results.¹⁵ Ultrasonography (US) is now considered as the imaging modality of choice for the major salivary glands, and has been extensively studied as a diagnostic and monitoring tool for SS patients, as it is non-invasive, low cost, and has the potential for easily and readily accessible use.^{2,16,17} Mossel *et al.*¹⁸ showed an all-sided but subjective B-mode 0–48 US scoring system may predict the LSGB results, with a positive predictive value (PPV) of 84% and a negative predictive value (NPV) of 74% using a cut-off score of 14. Recently, shear-wave elastography (SWE), a new ultrasound technique, has generally been used to distinguish mass properties in solid tumors such as breast masses.¹⁹ SWE can quantitatively detect the stiffness of major salivary glands owing to a system’s quantification tool, so it is used not only for early diagnosis, biopsy guidance and treatment monitoring of the parotid non-Hodgkin lymphoma, but also increasing the diagnostic rate of pSS from 88.6% to 94.2% in patients with normal or non-specific B-mode US.^{20,21}

However, whether B-mode US in addition to the SWE examination on major salivary glands can predict LSGB results with high PPV and high NPV remains elusive. In this study, we enrolled a derivation cohort of patients with suspected SS and analyzed the predictive value on LSGB results among the known non-invasive objective items and B-mode US and SWE examination of bilateral parotid glands (PGs) and submandibular glands (SMGs). According to the matrix risk model for predicting the probability of positive LSGB based on combined total US scores and total SWE values, all patients were stratified into three subpopulations of high, moderate, and low risk. A further newly enrolled validation cohort confirmed the PPV of 94% for predicting positive LSGB in the high-risk subpopulation, and the NPV of 93% for predicting negative LSGB in the low-risk subpopulation, which indicate that this matrix risk model is informative for both rheumatologists and patients to make decisions on whether the invasive procedure of LSGB should be performed.

Patients and methods

Patients

The patients who presented with at least one symptom of ocular or oral dryness based on American-European Consensus Group (AECG)

questions¹³ or systemic features derived from the European League Against Rheumatism (EULAR) Sjögren's syndrome disease activity index (ESSDAI) measure²² were recruited at the Department of Rheumatology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China. Exclusion criteria included any of the following situations: history of head and neck radiation treatment, active hepatitis C infection, acquired immune deficiency syndrome, sarcoidosis, amyloidosis, graft-versus-host disease, or IgG4-related disease. The patients enrolled from December 2018 to December 2019 were included in the derivation cohort to derive a matrix risk model for predicting the probability of positive LSGB. Another cohort of patients using the same inclusion and exclusion criteria were enrolled from April 2020 to September 2020 as a validation cohort. All subjects provided written informed consent forms. The protocol was approved by the Medical Ethics Committee of Sun Yat-sen Memorial Hospital (SYSEC-KY-KS-2018-012). This study was conducted in compliance with the Declaration of Helsinki.

Clinical assessments

Demographic and clinical data were collected before US examination. UWS was measured using standard methods and UWS ≤ 0.1 mL/min was considered as positive.^{23,24} Schirmer's test and Van Bijsterveld score were assessed by an ophthalmologist (Lan YQ) according to the standard procedures.²⁵ Positive ocular signs were defined as Van Bijsterveld score ≥ 4 and/or Schirmer's test ≤ 5 mm/5 min in at least one eye. Serological ANA was detected using indirect immunofluorescence on Hep2000 cells and commercial enzyme-linked immunosorbent assay (Aesku, Wendelsheim, Germany). Anti-SSA/Ro and anti-SSB/La were detected using line immunoassay (Euroimmun AG, Luebeck, Germany). Patients who fulfilled the 2002 AECG criteria¹³ were classified as pSS. Patients who fulfilled three items: (a) other definite CTDs; (b) positive LSGB; and (c) positive UWS and/or ocular signs, were classified as Sjögren-overlap syndrome.⁹ Patients who did not fulfil pSS or Sjögren-overlap syndrome were considered as non-SS in this study.

B-mode US and SWE examination

US examination was conducted on bilateral PGs and SMGs by two ultrasonologists (Hao SY and

Luo Y) who have related experience of more than 2 years and were blinded to clinical data. US images were obtained using Aixplorer (Supersonic Imagine, Aix en Provence, France) with a 15–4 MHz linear probe. The hypoechoic areas of each gland under B-mode US were scored using a well-accepted 0–4 scoring system.^{26,27} The intra-class correlation (ICC) was 0.976 ($p < 0.001$) for inter-observer agreement of US scores and 0.980 ($p < 0.001$) for intra-observer agreement.

For the lack of standard operating procedures of SWE examination on salivary glands, we set up a set of standard procedures in this study. All patients were asked to keep a relaxed position to avoid the skin tightening around the head and neck. The probe was kept vertical to the skin and the gland was positioned in the center of the elasticity box at a plane to demonstrate the representative glandular echo structure. SWE images were obtained without compression and saved when stabilized. In the center of the elasticity box, the system's quantification tool 'Q-Box' was used to measure the stiffness, avoiding blood vessels and lymph nodes. The 'Q-Box' designates a round region of interest with a diameter of 5 mm, measuring the quantitative stiffness as Young's modulus in kPa. At least five single measurements were taken for each gland, and the average Young's modulus was calculated.²⁸ The ICC was 0.825 ($p < 0.001$) for inter-observer agreement of SWE values and 0.905 ($p < 0.001$) for intra-observer agreement.

Labial salivary gland biopsy

LSGB was conducted by a stomatologist (Zhong JL) after US examination according to the standard procedures, which involved a small incision in the lower lip to harvest at least 4–6 minor salivary glands with a minimum surface area of 8 mm².^{11,15} Slides were stained with haematoxylin and eosin and then were scanned using ScanScope CS2 (Aperio CS2, Leica, German). The finding of dense mononuclear cell (mostly lymphocytic) aggregates of ≥ 50 cells in a periductal or peri-acinar distribution was referred to as focal lymphocytic sialadenitis. The total surface area of glandular tissue was measured using QuPath software (version 0.1.2). A focus score was calculated by dividing the number of foci by the total surface area, then multiplying by 4. A focus score ≥ 1 per 4 mm² of glandular tissue is considered as positive LSGB.^{12–14}

Table 1. Demographic and clinical characteristics of the derivation cohort.

Characteristics	N=91
Age, years	43 ± 15
Female, n (%)	85 (93)
Smoking history	1 (1)
UWS ≤ 0.1 mL/min, n (%)	28 (31)
Van Bijsterveld score ≥ 4, n (%)	7 (8)
Schirmer's test ≤ 5 mm/5 min, n (%)	23 (25)
Positive ANA	83 (91)
Positive anti-SSA/Ro, n (%)	77 (85)
Positive anti-SSB/La, n (%)	25 (27)
Positive LSGB, n (%)	57 (63)
Total US scores	8.9 ± 4.1
Total SWE values, kPa	29.8 ± 5.0
Disease classification	
pSS, n (%)	59 (65)
Sjögren-overlap syndrome, n (%)	9 (10)
Non-SS, n (%)	23 (25)
ANA, anti-nuclear antibodies; LSGB, labial salivary gland biopsy; SS, Sjögren's syndrome; SWE, shear-wave elastography; US, ultrasonography; UWS, unstimulated whole saliva flow rate.	

Statistical analysis

Statistical analyses were performed with SPSS for Windows 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Data were presented as the frequencies and percentages for categorical variables, and the mean with standard deviation (SD) or median with interquartile range (IQR) for continuous variables according to distributions. Independent-samples *t*-test was used for comparison between two independence groups. The chi-square test or Fisher's exact test were used for categorical variables among groups. Pearson correlation analysis was used to identify the correlation between two independence groups with continuous variables. An independent *t*-test was used for comparison between two independence groups. Receiver operating characteristic (ROC) curve analysis with area under the curve (AUC) was used to determine the cut-off values of total

US scores in distinguishing patients with positive LSGB from those with negative LSGB. Logistic regression models were built to analyze the probability of classifying positive and negative LSGB depending on non-invasive objective items. The matrix risk model for predicting positive LSGB was developed based on the combination of two independent variables such as total US scores and total SWE values. The probabilities of positive LSGB with 95% confidence intervals (CIs) were tested using RStudio (version 1.2.5019). To get a high PPV (>90%) in the high-risk subpopulation and a high NPV (>90%) in the low-risk subpopulation, the patients whose probability of positive LSGB was greater than 90% were classified as the high-risk subpopulation, and the patients whose probability of positive LSGB was less than 5% were classified as the low-risk subpopulation. The patients whose probability of positive LSGB was between 5% and 90% were classified as the moderate-risk subpopulation. All significance tests were two-tailed and were conducted at the 5% significance level, unless otherwise specified.

Results

Baseline characteristics of the derivation cohort

Ninety-one patients were enrolled in the derivation cohort (Table 1). The mean age was 43 ± 15 years and 93% of them were women. Only one patient (1%) had a smoking history. Twenty-eight patients (31%) showed UWS ≤ 0.1 mL/min, 26 patients (29%) had positive ocular signs, 77 patients (85%) had positive anti-SSA/Ro, and 57 patients (63%) had positive LSGB. Finally, 59 patients (65%) were classified as pSS, nine patients (10%) were diagnosed as Sjögren-overlap syndrome due to SS complicating with RA (*n*=6) or SLE (*n*=3), and 23 patients (25%) were non-SS.

Non-invasive objective criteria items cannot predict positive LSGB results

We explored the predictive value on LSGB results among non-invasive objective criteria items including UWS, ocular signs, and anti-SSA/Ro in the derivation cohort of 91 participants. Neither ROC curve analysis nor logistic regression showed these items could distinguish patients with positive LSGB from those with negative results (all *p* > 0.05, Figure 1A). The incidence of positive LSGB was 80% in 20 patients with positive UWS, which was not

significantly different from 58% in 71 patients with negative UWS ($p > 0.05$, Figure 1B). Likewise, the incidence of positive LSGB was not significantly different between patients with positive or negative ocular signs (62% *versus* 63%, $p > 0.05$), or between patients with positive or negative anti-SSA/Ro (66% *versus* 43%, $p > 0.05$). Taken together, there was no significant relationship between these non-invasive objective criteria items and the focus score of salivary gland biopsy.

The characteristics of B-mode US and SWE finding on major salivary glands

B-mode US and SWE findings on bilateral PGs and SMGs were explored in the derivation cohort of 91 participants. A heatmap revealed equal US scores in each pair of PGs or SMGs (Figure 2A). There were 65% of individual patients showing equal scores among four glands, while the differences between PGs and SMGs were one score in 30 patients (33%) or two scores in two patients (2%). Likewise, the SWE values were highly correlated in each pair of PGs or SMGs ($r = 0.964$, 0.942 , respectively, both $p < 0.001$, Figure 2B), and the SWE values in SMGs were significantly correlated with those in PGs ($r = 0.664$, $p < 0.001$, Figure 2C). These results indicated the symmetric morphological change and stiffness among four major salivary glands.

The relationship between SWE values and US scores in the same gland was explored among 182 PGs or 182 SMGs. The SWE values in the glands with US scores of 1–3 were significantly higher than the glands with scores of 0 (all $p < 0.001$), but the SWE values in the glands with US scores of 4 declined to a level similar to the glands with scores of 0 (Figure 2D). Among 12 patients who had at least one gland with US scores of 4, the total SWE values in four patients (33%) with multiple hyperechoic areas were 22.0 ± 0.8 kPa, significantly lower than those in patients without multiple hyperechoic areas (27.5 ± 2.1 kPa, $p = 0.001$). To make clear the potential pathological changes in multiple hyperechoic areas, we conducted ultrasound-guided biopsy on one parotid gland with a US score of 4 (Supplemental Figure 1a, b). Lymphocytic infiltration was similar between the parotid gland and labial glands, but the former had abundant adipose tissue but loss of parenchymal glandular tissue (Supplemental Figure 1c, d), implying multiple hyperechoic areas

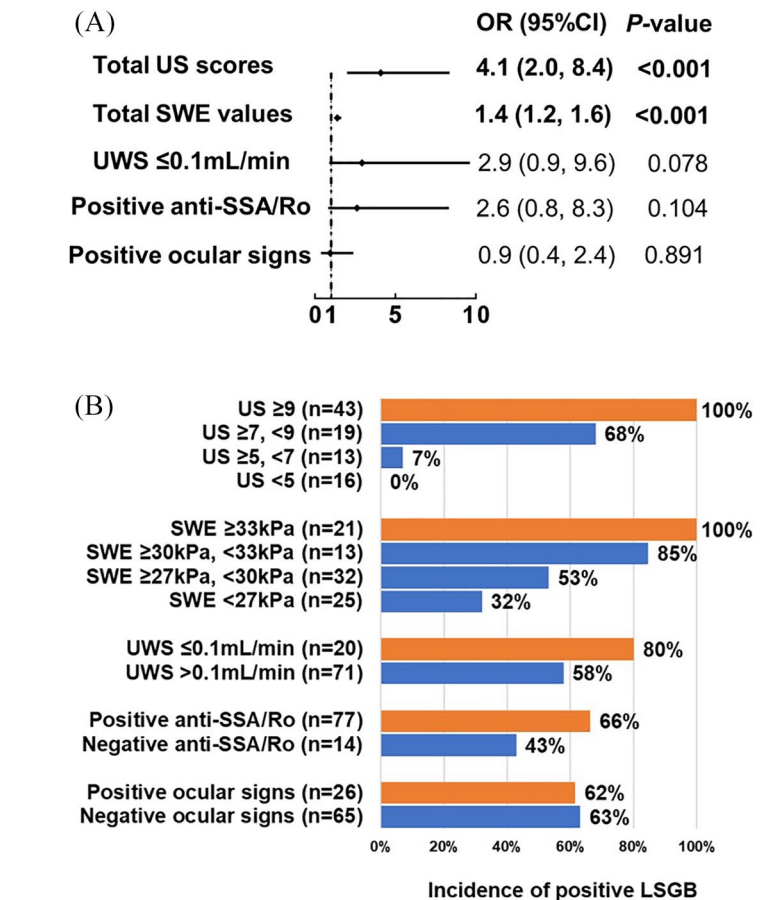


Figure 1. The predictive value on positive LSGB results among various non-invasive objective items. A. Logistic regression modeled the probability for classifying LSGB (+) and LSGB (-) using non-invasive objective items as independent variables. The odds ratio (OR) with 95% confidence interval (CI) and p values were shown. B. Bar chart demonstrated the incidence of positive LSGB in each subgroup of patients. LSGB, labial salivary gland biopsy; SWE, shear-wave elastography; US, ultrasonography; UWS, unstimulated whole saliva.

may indicate adipose tissue replacement which decrease the stiffness of salivary glands.

The total US score predicts LSGB results with both high PPV and NPV

To test the hypothesis that lymphocytic infiltration was consistent among major and minor salivary glands, we analyzed the correlation between total US scores of four major glands and histological focus scores in tissues from LSGB in the derivation cohort of 91 participants. The total US scores were positively correlated with histopathological focus scores ($r = 0.529$, $p < 0.001$). The patients with positive LSGB showed significantly higher total US scores than those with negative

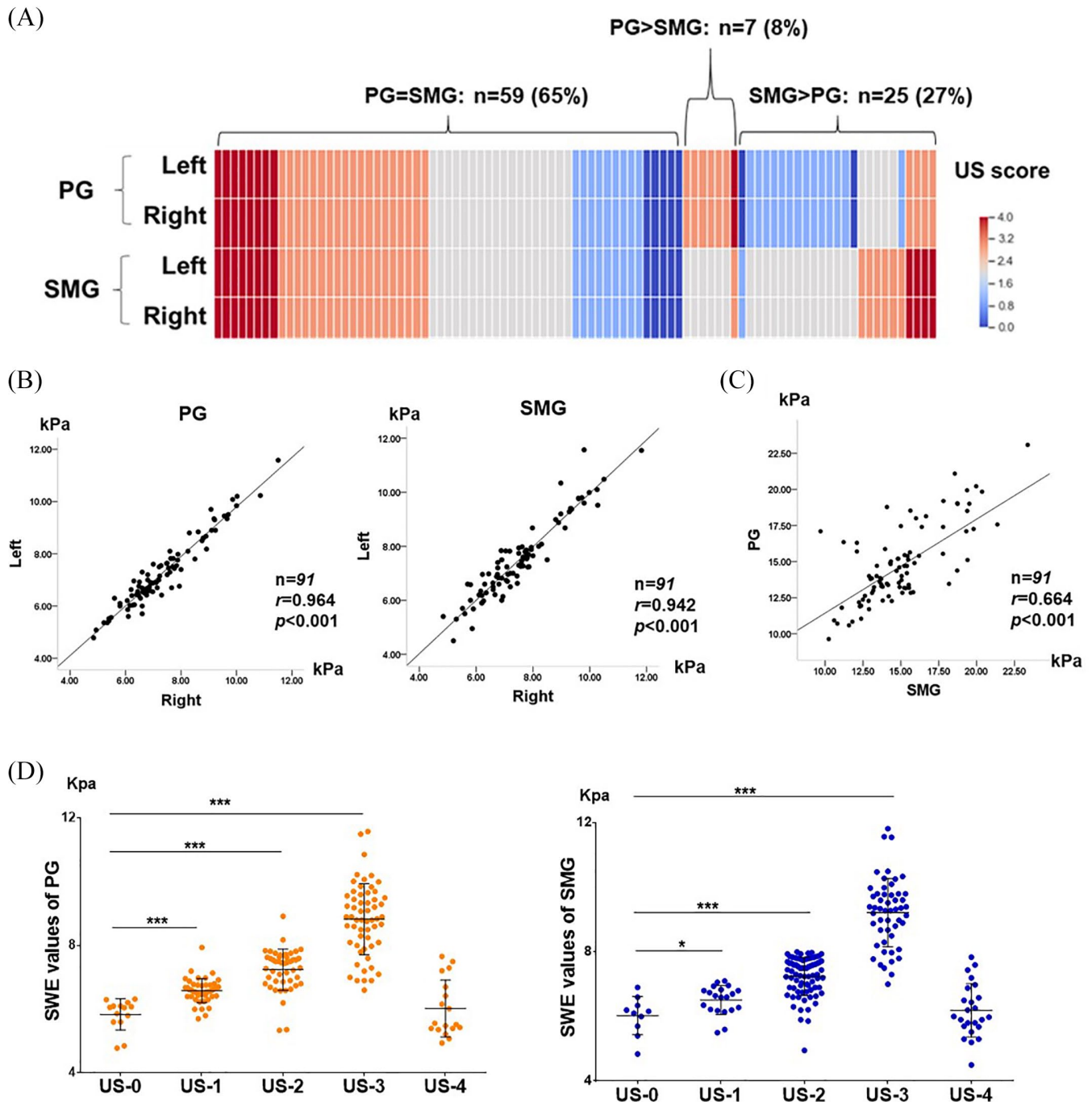


Figure 2. The findings of major salivary glands detected by B-mode US or SWE in the derivation cohort of 91 participants. A. A heatmap illustrated US scores in bilateral parotid glands (PGs) and submandibular glands (ss). B. Scatter plots with Pearson correlation analysis showed the positive correlation of SWE values in the left glands with the right glands. C. Scatter plots with Pearson correlation analysis showed the positive correlation of SWE values in the PGs with the SMGs. D. SWE values among 182 PGs or SMGs were compared based on different degrees of US scores. SWE, shear-wave elastography; US, ultrasonography. *** $p<0.001$, * $p<0.05$.

LSGB (11.3 ± 2.8 versus 4.8 ± 2.5 , $p<0.001$, Figure 3A). The ultrasonographic and histopathological images, respectively, in one patient

with positive LSGB or another patient with negative LSGB are shown in Figure 3B. Logistic regression showed the increased total US score

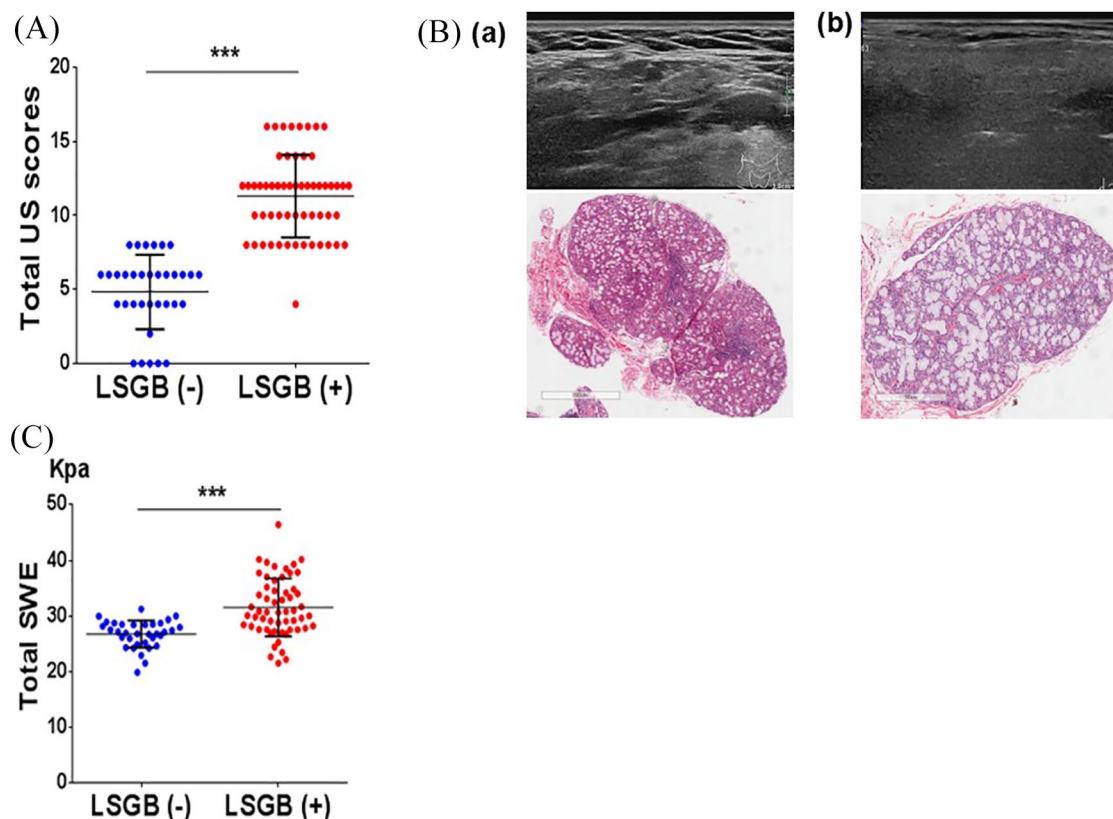


Figure 3. The relationship between findings of major salivary glands detected by B-mode US or SWE and the labial salivary gland biopsy (LSGB) results. A. The total US scores of four glands were compared between LSGB (+) and LSGB (-) patients. B. Ultrasonographic and histopathological images were shown in two representative patients. (a) One patient had the total US score 12 and the focus score 2.2. (b) The other patient had the total US score 6 and the focus score 0. (c) The total SWE values of four glands were compared between LSGB (+) and LSGB (-) patients.

SWE, shear-wave elastography; US, ultrasonography.

*** $p < 0.001$.

was an independent risk factor for positive LSGB (OR: 4.1, 95% CI: 2.0–8.4, $p < 0.001$, Figure 1A), indicating a high total US score may predict positive LSGB while a low total US score may predict negative LSGB.

ROC curve analysis confirmed the total US scores did significantly distinguish patients with positive LSGB from those with negative LSGB (AUC = 0.956, $p < 0.001$). As one cut-off point cannot get high enough specificity for predicting positive or negative LSGB, we divided the total US scores into four categories in which the incidences of positive LSGB were, respectively, 100% (the total US scores ≥ 9 , $n = 43$), 68% ($< 9, \geq 7$, $n = 19$), 7% ($< 7, \geq 5$, $n = 13$) and 0% (< 5 , $n = 16$, Figure 1B). The PPV in patients with the total US scores ≥ 9 was 100% and the NPV in patients with the total US scores < 5 was 100%.

The total SWE value predicts LSGB results with high PPV but low NPV

Likewise, the patients with positive LSGB had significantly higher total SWE values of four major glands than those with negative LSGB (32 ± 5 kPa versus 27 ± 2 kPa, $p < 0.001$, Figure 3C). Logistic regression showed the increased total SWE value was an independent risk factor for positive LSGB (OR: 1.4, 95% CI: 1.2–1.6, $p < 0.001$, Figure 1A). ROC curve analysis showed the total SWE values could significantly distinguish patients with positive LSGB from those with negative LSGB (AUC = 0.825, $p < 0.001$). The total SWE values were divided into four categories in which the incidence of positive LSGB were, respectively, 100% (total SWE values ≥ 33 kPa, $n = 21$), 85% (< 33 kPa, ≥ 30 kPa, $n = 13$), 53% (< 30 kPa, ≥ 27 kPa, $n = 32$) and 32% (< 27 kPa, $n = 25$, Figure 1B). The PPV in patients with total SWE

Total US scores	≥ 9	95.2% (69.2%~99.4%)	97.7% (88.0%~99.6%)	98.9% (92.3%~99.9%)	99.5% (92.2%~100%)
	$< 9, \geq 7$	50.0% (18.4%~81.5%)	68.1% (47.8%~83.3%)	82.0% (53.2%~94.8%)	90.7% (46.5%~99.1%)
	$< 7, \geq 5$	4.8% (0.7%~25.7%)	9.8% (2.3%~33.6%)	18.8% (3.2%~61.7%)	33.1% (2.9%~89.1%)
	< 5	0.3% (0~5.0%)	0.5% (0~8.5%)	1.2% (0.1%~20.6%)	2.4% (0.1%~50.9%)
		< 27	$\geq 27, < 30$	$\geq 30, < 33$	≥ 33
		Total SWE values (kPa)			

Figure 4. A matrix risk model for predicting positive labial salivary gland biopsy (LSGB) based on the combination of the total ultrasonography (US) scores and the total shear-wave elastography (SWE) values. Four categories of the total US scores were combined with four categories of the total SWE values to generate a matrix and then the relative probability of positive LSGB in each grid was calculated. The 95% confidence intervals of the probabilities are shown in parentheses. According to the probability of positive LSGB, patients with suspected SS could be stratified into three subpopulations: high risk for positive LSGB (in red, the probability $> 90\%$), moderate risk for positive LSGB (in yellow, the probability between 5% and 90%), and low risk for positive LSGB (in green, the probability $< 5\%$).

values ≥ 33 kPa was 100%, while the NPV in patients with total SWE values < 27 kPa was only 68%, indicating a high total SWE value predicts positive LSGB but a low total SWE value could not predict negative LSGB.

A matrix risk model for predicting positive LSGB based on the combination of US and SWE findings

The above data indicated low predictive value in patients with total US scores ≥ 5 and < 9 and low NPV if only based on total SWE values. Therefore, we combined four categories of the total US scores with four categories of the total SWE values to generate a matrix and then calculated the predicted probability of positive LSGB in each grid (Figure 4). According to the probability of positive LSGB in this matrix risk model, patients with suspected SS could be stratified into three subpopulations: (a) high risk for positive LSGB: the patients with total US scores ≥ 9 had the probability 95.2–99.5% ($n = 43$), and the patients with total US scores ≥ 7 and < 9 as well as total SWE values ≥ 33 kPa had the probability 90.7% ($n = 0$); (b) moderate risk for positive LSGB: the patients with total US scores ≥ 7 and < 9 as well as total SWE values < 33 kPa had the probability 50–82% ($n = 19$), and the patients with total US scores ≥ 5 and < 7 as well as total SWE values

≥ 27 kPa had the probability 9.8–33.1% ($n = 11$); (c) low risk for positive LSGB: the patients with total US scores < 5 had the probability 0.3–2.4% ($n = 16$), and the patients with total US scores ≥ 5 and < 7 as well as total SWE values < 27 kPa had the probability 4.8% ($n = 2$).

Validation of the matrix risk model

To validate the accuracy of the matrix risk model on the prediction of positive LSGB, an additional 52 patients were enrolled in the validation cohort. Their demographic and clinical characteristics are shown in Supplemental Table 1. Similar to the derivation cohort, the US scores and SWE values were symmetric among four major salivary glands (Supplemental Figure 2a–b). The total US scores were positively correlated with histopathological focus scores ($r = 0.548$, $p < 0.001$). The patients with positive LSGB had significantly higher total US scores or total SWE values of four major glands than those with negative LSGB (Supplemental Figure 2c–d). According to the matrix risk model of positive LSGB, 16 patients were in the high-risk subpopulation, nine patients in the moderate-risk subpopulation, and 27 patients in the low-risk subpopulation. Age, sex, UWS, autoantibodies, LSGB, and disease classification were comparable between the derivation and validation cohort in each subpopulation (all

Table 2. Comparison of demographic and clinical characteristics in each risk subpopulation for positive LSGB between the derivation and validation cohort.

Characteristics	High risk			Moderate risk			Low risk		
	Derivation (n=43)	Validation (n=16)	p Value	Derivation (n=30)	Validation (n=9)	p Value	Derivation (n=18)	Validation (n=27)	p Value
Age, years	44.7 ± 14.1	48.4 ± 13.5	0.361	39.9 ± 15.2	45.4 ± 17.2	0.354	44.0 ± 15.2	42.0 ± 13.2	0.653
Women, n (%)	42 (98)	16 (100)	1.000	28 (93)	9 (100)	1.000	15 (83)	26 (96)	0.286
UWS ≤ 0.1 mL/min, n (%)	14 (33)	5 (31)	0.924	11 (37)	4 (44)	0.711	3 (17)	4 (15)	1.000
Van Bijsterveld score ≥ 4, n (%)	4 (9)	4 (25)	0.194	3 (10)	0	1.000	0	3 (11)	0.264
Schirmer's test ≤ 5 mm/5 min, n (%)	11 (26)	7 (44)	0.212	12 (40)	3 (33)	1.000	0	10 (37)	0.003
Positive ANA	43 (100)	16 (100)	NA	29 (97)	9 (100)	1.000	11 (61)	17 (63)	0.900
Positive anti-SSA/Ro, n (%)	42 (98)	15 (94)	0.472	25 (83)	8 (89)	1.000	10 (56)	16 (59)	0.805
Positive anti-SSB/La, n (%)	16 (37)	10 (63)	0.082	7 (23)	3 (33)	0.669	2 (11)	7 (26)	1.000
Positive LSGB, n (%)	43 (100)	15 (94)	0.271	13 (43)	4 (44)	1.000	1 (6)	2 (7)	1.000
Total US scores	12.4 ± 2.1	12.3 ± 2.0	0.843	7.3 ± 1.0	7.3 ± 1.0	0.860	3.0 ± 2.1	3.7 ± 1.8	0.237
Total SWE values, kPa	32.4 ± 5.7	29.6 ± 5.1	0.097	28.6 ± 2.0	28.1 ± 0.8	0.416	25.4 ± 1.9	26.4 ± 2.5	0.139
Disease classification									
pSS, n (%)	39 (91)	11 (69)	0.052	18 (60)	3 (33)	0.277	2 (11)	7 (26)	0.470
Sjögren-overlap syndrome, n (%)	4 (9)	5 (31)		4 (13)	3 (33)		1 (6)	1 (4)	
Non-SS, n (%)	0	0		8 (27)	3 (33)		15 (83)	19 (70)	
ANA, anti-nuclear antibodies; LSGB, labial salivary gland biopsy; SS, Sjögren's syndrome; SWE, shear-wave elastography; US, ultrasonography; UWS, unstimulated whole saliva flow rate.									

$p > 0.05$, Table 2). The PPV was 94% (15/16) in the high-risk subpopulation for predicting positive LSGB, and the NPV was 93% (25/27) in the low-risk subpopulation for predicting negative LSGB, confirming the predictive value of this matrix risk model.

Discussion

With regard to the suspected patients with SS, rheumatologists prefer assessing the non-invasive objective items and hope to learn the predicted probability of positive LSGB before referring

patients to receive the biopsy, because positive LSGB has a high weight for the classification of SS.^{12,13} We found that the classic non-invasive objective items included in the classification criteria of SS such as UWS, ocular signs and anti-SSA/Ro, did not recognize patients who tend to get positive LSGB or who tend to get negative results, consistent with the publications from the SICCA database.¹⁵ However, total US scores ≥ 9 have a PPV of 100% for predicting positive LSGB, while total US scores < 5 have a high NPV of 100% for predicting negative LSGB. In addition, for the first time we introduced the SWE

examination for predicting LSGB results, and found the total SWE values ≥ 33 kPa can recognize the high-risk subpopulation with positive LSGB among patients with total US scores ≥ 7 and < 9 , or total SWE values < 27 kPa can recognize the low-risk subpopulation with positive LSGB among patients with total US scores ≥ 5 and < 7 . A novel matrix risk model based on the combination of total US scores and total SWE values showed the PPV in the high-risk subpopulation was 94% for predicting positive LSGB and the NPV was 93% for predicting negative LSGB in the low-risk subpopulation. This study provides a protocol for predicting the probability of positive or negative LSGB before invasive procedures that helps rheumatologists make a shared decision on the biopsy with suspected patients with SS.

So far, none of the imaging techniques can directly examine labial minor salivary glands, while evaluation of the major salivary glands could be the second best to estimate the pathological changes in LSGB tissues. The US examination of major salivary glands has been considered as a useful tool in disease classification, patient stratification and the monitoring of therapeutic response in SS patients.^{2,17,29–33} Due to the superficial localization, PGs and SMGs are always the salivary glands for US examination. B-mode US can display parenchymal echogenicity, homogeneity, the presence of hypoechoic areas, hyperechoic reflections, and clearness of salivary gland borders. These five parameters made up an early 0–48 scoring system (0–12 for one gland; a total of bilateral PGs and SMGs), in which each parameter relies on subjective assessment.³⁴ Given the fact that multiple hypoechoic areas correlate with lymphocytic infiltration within salivary glands,³⁵ an update 0–16 scoring system (0–4 for one gland; a total of four glands) was solely based on the diameter of multiple hypoechoic areas,²⁶ which is simpler and more objective than the 0–48 scoring system.³⁴ In this study, we adopted the 0–16 scoring system for predicting LSGB results, and expectedly found the positive correlation of the total US scores with focus scores of LSGB tissues. Both the PPV in patients with total US scores ≥ 9 and the NPV in patients with total US scores < 5 are 100%. These results suggest that routine B-mode US examination on major salivary glands with a simple 0–16 scoring system can help rheumatologists to predict the LSGB results before the invasive procedure of biopsy.

Recently, the stiffness of salivary glands can be measured using ultrasound elastography such as SWE or strain-based elastography.³⁶ SWE is a dynamic analysis of the propagation speed of a shear wave, characterized by a system's quantification tool and low dependence on the operators. SWE is superior to the strain-based elastography which achieves only semi-quantitative measurement and is more affected by the operators.³⁷ In addition to determining the mass properties in salivary glands and early diagnosis of parotid non-Hodgkin lymphoma, SWE was also applied to assessing the chronic inflammation of major salivary glands.^{20,21,35,38,39} Bădărăinză *et al.*²⁰ showed the SWE values of PGs have high specificity of 99.9% in distinguishing pSS patients without lymphoma from those with parotid lymphoma development, but the specificity in distinguishing pSS patients from healthy subjects was only 80%. Arslan *et al.*³⁹ also reported the specificity of the SWE values of PGs in distinguishing pSS patients from healthy volunteers was 88.3%, the specificity of the SWE values of SMGs was 80%. In this study, the SWE values increased as US scores increased from 0 to 3, suggesting lymphocytic infiltration contributes to an early increase of stiffness in salivary glands of suspected SS patients, consistent with the previous small sample study.³⁹ However, for the first time we reported that the SWE values in the glands with severe sialadenitis (US scores of 4) became lower especially when accompanied by multiple hyperechoic areas. This interesting finding may explain the low distinguishing value of SWE examination between SS patients and healthy controls.^{20,39} We conducted a biopsy in one PG with a US score of 4 and found the histological change of multiple hyperechoic areas was adipose tissue, suggesting that replacement of adipose tissue may lead to the low stiffness of salivary glands, although it needs to be further validated in large sample studies. Furthermore, the PPV in patients with the total SWE values ≥ 33 kPa was 100% for predicting positive LSGB, which extends the application of the SWE examination in major salivary glands, although the NPV is low if only based on the total SWE values.

Considering the uncertainty in those patients with total US scores ≥ 5 and < 9 , we combined the B-mode US and SWE values to derive a matrix risk model. The patients with total US scores ≥ 7 and < 9 as well as total SWE values ≥ 33 kPa had the probability of positive LSGB of 90.7%, who belong to the high-risk subpopulation for positive

LSGB, likewise the patients with total US scores ≥ 9 . On the other hand, those patients with total US score ≥ 5 and < 7 as well as total SWE values < 27 kPa had the probability of positive LSGB of 4.8%, who belong to the low-risk subpopulation for positive LSGB, likewise the patients with total US scores < 5 . Further validation cohort results confirmed the PPV of predicting positive LSGB is high (94%) in the high-risk subpopulation, and the NPV of predicting negative LSGB is high (93%) in the low-risk subpopulation. The suspected SS patients in the high-risk subpopulation are suggested to receive the biopsy procedure for final disease classification. Whether the combination of B-mode US and SWE values may be a surrogate item of positive LSGB for the classification of SS in the high-risk subpopulation, or invasive procedure of LSGB can be default in the high-risk subpopulation especially in some special cases who are strongly against the biopsy or have contraindications for LSGB (e.g. severe thrombocytopenia) are worth further studies. Those patients in the low-risk subpopulation are suggested to suspend the invasive biopsy procedure and this subpopulation may be considered as non-SS if they cannot fulfil any criteria of SS based on UWS, ocular signs, or anti-SSA/Ro. Dynamic monitoring of the progression of salivary gland lesions through the combined B-mode US and SWE examination is recommended to the low-risk subpopulation for the risk assessment. In case of the risk rising from low to moderate, the patients are suggested to receive the biopsy procedure. It is worth noting that US or SWE findings of salivary glands in the early stage of IgG4-related sialadenitis, infection-related chronic parotitis, Kimura's disease, and radiation-induced salivary gland injuries may be similar to SS.^{40,41} We should distinguish these diseases, based on the clinical features, serological indicators, ultrasonographic findings, and histological characteristics.

There were several limitations in this study. First, we used the findings of B-mode US and SWE on major salivary glands to predict the pathological changes in LSGB tissues. We cannot directly examine the labial minor salivary glands because it is out of the scope of present imaging techniques. However, there are significant correlations between the total US scores of four major glands and histological focus scores in tissues from LSGB in both the derivation cohort and validation cohort. Second, the predicted probability of positive LSGB in the moderate risk

subpopulation is 9.8–82.0%, so neither PPV nor NPV is high in this subpopulation. These patients are suggested to receive the biopsy procedure for final disease classification. Third, there are no standard operating procedures of ultrasound elastography examination on salivary glands so far. In this study, we adopted SWE which is more reproductive than strain-based elastography. We also set up a set of standard procedures for the SWE examination in our own center that needs further validation. Finally, this is a single-center study with a small sample size which would cause bias or imprecision to a certain extent. Although we can calculate the predicted probability of positive LSGB in the given grid of the matrix risk model using statistical methods, there was only one patient with the total US scores ≥ 7 and < 9 but the total SWE values ≥ 33 kPa in the validation cohort, and no patients in the derivation cohort. Further validation in a large cohort from multiple centers is needed in future.

To sum up, this study raises a novel application of conventional B-mode US and extends the application of SWE examination to the prediction of LSGB results, and raises a novel matrix risk model that can recognize patients who tend to get positive LSGB or who tend to get negative results. This model can help rheumatologists to make a shared decision with the patients with suspected SS on whether the invasive procedure of LSGB should be performed.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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