Disease activity and damage in patients with primary Sjogren’s syndrome: Prognostic value of salivary gland ultrasonography

Vera Milic1,2*, Jelena Colic1☯, Andja Cirkovic2,3☯, Svetlana Stanojlovic2,4‡, Nemanja Damjanov1,2‡

1 Institute of Rheumatology, Belgrade, Serbia, 2 Faculty of Medicine, University of Belgrade, Belgrade, Serbia, 3 Department for Medical Statistics and Informatics Faculty of Medicine, Belgrade, Serbia, 4 Clinic for Eye Diseases, Clinical Centre of Serbia, Belgrade, Serbia

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* veramilic1409@gmail.com

Abstract

Objectives
To assess the association between salivary ultrasonography (sUS) findings and disease activity and damage in patients with primary Sjogren’s syndrome (pSS). We investigated the potential prognostic role of sUS as a tool in the assessment of disease activity.

Methods
In 303 pSS patients, disease activity was assessed by the European League Against Rheumatism (EULAR) Sjogren’s Syndrome Disease Activity Index (ESSDAI), the EULAR Sjogren’s Syndrome Patient Reported Index (ESSPRI), the Sjogren’s Syndrome Disease Activity Index (SSDAI) and the Sjogren’s Syndrome Disease Damage Index (SSDDI). The sUS parenchymal inhomogeneity (de Vita scoring system) was assessed in 303 pSS patients and 111 healthy controls. A receiver operating characteristic (ROC) curve was used to determine the cut-off value of the pathological sUS score. Logistic regression analysis was performed to assess risk factors for moderate and high disease activity.

Results
A pathological sUS score ≥ 2 was recorded in 271 (89.7%) patients and 8 (8.6%) healthy controls. Patients with moderate and high ESSDAI and SSDAI scores had significantly higher US activity in comparison to that of pSS patients with low disease activity (p = 0.006; p = 0.01, respectively). Additionally, pSS patients with moderate and high SSDAI scores had higher US activity (p = 0.031). Pathological sUS correlated with the glandular domain within the ESSDAI and SSDDI (p<0.001). The patients with a severe US score (5–6) had a 3.5 times greater chance of having moderate or high disease activity. The specificity of the severe de Vita sUS score for ESSDAI and SSDAI was 85.1% and 85.2%, respectively. In contrast, the sensitivity of a severe de Vita sUS score for ESSDAI was low, at 29.2%, while
the sensitivity for the SSDAI was higher, 42.3%. In the analysis of disease activity, a de Vita score ≥ 5 could be used as a risk factor for moderate and high ESSDAI (p = 0.042) and SSDAI (p = 0.006).

Conclusions
Pathological salivary gland ultrasonography is associated with high disease activity and damage in pSS. Consequently, sUS abnormalities might be surrogate items for glandular domains in the assessment of disease activity and damage. Thus, ultrasonography of the salivary gland combined with clinical and serological markers might be part of the next prognostic and therapeutic algorithm in the near future.

Introduction
Primary Sjogren’s syndrome (pSS) is a chronic systemic autoimmune disease characterized mainly by symptoms of ocular and oral dryness. However, up to 20% of patients have disease-related extra-glandular manifestations [1]. Autoantibodies to the autoantigens Ro/SS-A and La/SS-B are the most specific biomarkers for pSS, whereas cryoglobulins and hypocomplementaemia are the major prognostic markers of disease activity [2]. These patients are also at increased risk of having associated malignancies, particularly non-Hodgkin’s lymphoma [relative risk (RR)], (RR = 13.76) [3,4].

Treatment of patients with pSS is usually symptomatic (artificial tears and saliva replacement). None of the conventional immunosuppressant therapies are of proven effectiveness for systemic features of the disease. Thus, there is a growing interest in using current biological therapies in the treatment of SS [5–7]. In order to define key inclusion and response criteria in clinical trials with biologics, it is important to have objective measures of both disease activity and disease damage. Recently, standardized outcome tools for measuring disease-specific activity and patients’ reported symptoms have been developed by the European League Against Rheumatism (EULAR) SS study group: the EULAR SS Disease Activity Index (ESSDAI) for systemic features of pSS and the EULAR SS Patient-Reported Index (ESSPRI) for patient symptoms [8,9]. The distinction between disease activity (reversible) and damage (irreversible) has always been a matter of debate. For this purpose, two clinical indexes were derived from Italian authors in 2007: Sjogren’s Syndrome Disease Damage Index (SSDDI) for assessment of disease damage and Sjogren’s Syndrome Disease Activity Index (SSDAI) for disease activity [10]. The alterations in salivary glands are important parameters included in both disease activity indexes. The glandular domain in the ESSDAI and the new appearance or increased swelling of major salivary glands in the SSDAI contribute a significant number of points to the total score of disease activity. Apart from the size, the morphological changes in the salivary glands in pSS (either related to disease activity or damage) may be the important components of the clinical indexes. Among the modern imaging techniques, salivary ultrasonography (sUS) has an established role in the diagnosis and follow-up of pSS patients [11–16]. Recently, studies have shown that sUS is able to reveal improved salivary gland echostructure in patients with SS receiving rituximab [17,18]. These results indicate the reversibility of some of the salivary gland changes, most likely reflecting disease activity as opposed to disease-induced damage. Therefore, the presence of salivary gland fibrosis or atrophy detected by sUS could contribute to selecting the subset of pSS patients who are likely not to benefit from immunomodulatory treatment.
The purpose of our study was to assess the association between sUS findings and disease activity and damage in pSS patients. Here, we demonstrated the potential prognostic role of sUS as a tool in the assessment of disease activity.

Materials and methods

Participants

The cross-sectional study enrolled 303 pSS patients who fulfilled the American-European Consensus Group (AECG) classification criteria [19]. For sUS evaluation, the control group included 111 healthy subjects without any symptoms of dryness and concomitant autoimmune or thyroid disease. The study was approved by the local Ethics Committee Institute of Rheumatology (number 20/1-51). All patients gave informed written consent.

The questionnaire-based evaluation included the following: ocular symptoms; oral symptoms; ocular signs (Rose Bengal test); salivary gland involvement (sialo-scintigraphy and/or biopsy of minor salivary glands (MSGs) evaluated by the Chisholm and Mason scale); serological tests, including rheumatoid factor (RF), antinuclear antibody (ANA), and antibodies to the extractable nuclear antigens SS-A and SS-B; symptoms/signs suggestive of disease-related extra-glandular manifestations; and related current treatments. The sUS examination was performed simultaneously with the other diagnostic procedures.

Assessment of SS disease activity and damage by clinical indexes

At enrolment, physicians completed the ESSDAI for each pSS patient. The ESSDAI (0–123) proposes the evaluation of 12 domains or organ systems (constitutional, lymphadenopathy, glandular, articular, cutaneous, pulmonary, renal, peripheral nervous system, central nervous system, muscular, haematological and biology). Low activity (ESSDAI < 5), moderate activity (5 ≤ ESSDAI ≤ 13) and high activity (ESSDAI ≥ 14) levels were defined [8]. Physicians completed the Sjogren’s Syndrome Disease Activity Index (SSDAI) and the Sjogren’s Syndrome Disease Damage Index (SSDDI) [10]. The SSDAI is a global score (0–21) including the following items (constitutional, change in salivary gland swelling, articular symptoms, haematologic features, pleuropulmonary symptoms, change in vasculitis, renal involvement and peripheral neuropathy). An SSDAI score ≥ 5 was defined as a high level of activity [10]. The SSDDI comprises a three-domain assessment (ocular, oral and systemic damage). The systemic domain was further classified into neurological, renal, pulmonary, cardiovascular, gastrointestinal, musculoskeletal, endocrine and malignancy sub-domains. The maximum score for each item was 1, and it has 27 items in total [10].

All patients completed the ESSPRI, a VAS scale (0–10) for dryness, fatigue and pain [9].

Salivary gland ultrasonography

The parotid and submandibular glands were examined by US using a GE LogiqE9 with a linear high-frequency transducer (6–15 MHz). The parotid glands were evaluated in a longitudinal and cross-sectional plane and the submandibular glands in a longitudinal plane. All ultrasound scans were performed by the same examiner (VM), blinded to the clinical diagnosis. The de Vita scoring system [20] was used for graded changes in the parenchymal homogeneity of salivary glands: 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 hyperechoic areas and diffuse hyperechoic bands) (Fig 1). The sUS score (0–6) represents the sum of the single scores of each pair of parotid and
submandibular glands. Disease activity by US was graded into three groups: normal (US score 0–1), moderate (US score 2–4) and severe (US score 5–6).

**Statistical analysis**

All statistical analyses were performed using Statistical Package for Social Science (SPSS) software version 21.0. For statistical comparison, Student’s t test, chi-square test, and Fisher’s exact test were used as appropriate. Spearmen’s test was used for correlation analysis. A receiver operating characteristic (ROC) curve was used to determine the cut-off value of the pathological sUS score with the highest level of accuracy. We calculated the diagnostic accuracy of a de Vita score ≥ 5 in comparison with ESSDAI, ESSPRI, SSDDI and SSDAI. All possible variables were first analysed through univariate logistic regression, and only significant variables were then summarized in a multivariate logistic regression model. The de Vita score was analysed as a potential predictor with a normal score as the reference. For all statistical analyses, p values less than 0.05 were considered statistically significant.
Results

Characteristics of patients with pSS

A total of 303 pSS patients (96.7% females) were enrolled in the study. The control group consisted of 111 healthy controls (97.3% females). There was no statistically significant difference in the mean age between pSS patients and healthy controls (54 ±12 vs. 52 ±15, p = 0.131). The general characteristics of pSS patients are presented in Table 1. The frequencies of domains in different ESSDAI and ESSPRI scores are presented in S1 and S2 Tables.

Table 1. General characteristics of pSS patients.

| Characteristic | pSS patients (N = 303) |
|---------------|------------------------|
| Age (years), mean ±SD | 54.03±11.95 |
| Female sex, number (%) | 293 (96.7) |
| Disease duration (years), med (min-max) | 5 (1–22) |
| < 5 years, n (%) | 128 (43) |
| 5–10 years, n (%) | 113 (38) |
| >10 years, n (%) | 59 (20) |
| Clinical signs | |
| Ocular symptoms, n (%) | 275 (91.4) |
| Oral symptoms, n (%) | 278 (92.4) |
| Lymphoma, n (%) | 8 (2.6) |
| Diagnostic tests | |
| Positive keratoconjunctivitis sicca, n (%) | 197 (96.1) |
| Positive scialo-scintigraphy†, n (%) | 150/152 (98.7) |
| Positive biopsy of MSG, n (%) | 190/219 (86.8) |
| Positive RF, n (%) | 212 (70) |
| Positive ANA, n (%) | 223 (73.6) |
| Positive Anti-SSA Ab, n (%) | 254 (83.8) |
| Positive Anti-SSB Ab, n (%) | 153 (50.5) |
| Disease activity indexes | |
| ESSDAI, med (min-max), IQR | 6 (0–75) 8 |
| ESSDAI ≥5, n (%) | 201 (66.3) |
| ESSPRI, med (min-max), IQR | 6 (0–10) 2.67 |
| ESSPRI ≥5, n (%) | 219 (72.3) |
| SSDAI, med (min-max), IQR | 5 (0–18), 3 |
| SSDAI ≥5, n (%) | 160 (53.2), 3 |
| SSDDI med (min-max), IQR | 2 (0–12) |
| Current treatments | |
| Glucocorticoids, n (%) | 161 (53.1) |
| Hydroxychloroquine (n%) | 225 (74.3) |
| Azatioprine, n (%) | 17 (4.1) |
| Methotrexate, n (%) | 18 (4.3) |
| Cyclophosphamide, n(%) | 3 (1) |

Except where indicated otherwise, values are the number n, (%); ESSDAI, EULAR Sjogren’s Syndrome Disease Activity Index; ESSPRI, EULAR Sjogren’s Syndrome Patient Reported Index; EULAR European League Against Rheumatism; SSDAI, Sjogren’s Syndrome Disease Activity Index (SSDAI); SSDDI, Sjogren’s Syndrome Disease Damage Index; MSG: minor salivary glands; RF: rheumatoid factor; ANA: antinuclear antibody; Anti-SSA Ab, anti-SSA antibody; Anti-SSB Ab, anti-SSB antibody.

† Values of objective tests given as rates of positive results (positive/total)

https://doi.org/10.1371/journal.pone.0226498.t001
Ultrasonography findings and correlations

In comparison to healthy controls, the calculated sUS AUC-ROC was 0.96 (0.009) (95% CI 0.94–0.97), which reached a range of very high accuracy. The optimal cut-off for sUS score was set at ≥ 2, with the best ratio of sensitivity (89.6%) and specificity (86.7%). Two hundred seventy-one (89.7%) patients with pSS and only 8 (8.6%) healthy controls had sUS scores ≥ 2. Out of 271 pSS patients with pathological sUS, 197 (65.2%) had moderate sUS activity, and 74 (24.5%) had severe sUS activity.

The overall sUS score correlated directly with the age of patients (r = 0.09, p = 0.05), biopsy of MSG (r = 0.17, p = 0.01), SSDDI (r = 0.16, p = 0.003), SSDAI (r = 0.22, p<0.0001) and ESSDAI (r = 0.428, p<0.0001). Furthermore, pathological sUS correlated with the constitutional domain (r = 0.16, p = 0.004), lymphadenopathy (r = 0.25, p<0.001), glandular domain (r = 0.25, p<0.001) and muscular domain (r = 0.16, p = 0.008) within the ESSDAI. Additionally, pathological sUS correlated with the glandular domain within the SSDDI (r = 0.16, p = 0.004). Disease duration and ESSPRI did not correlate with sUS score (p>0.005).

Characteristics of pSS patients and ESSDAI and ESPRI disease activity

Table 2 presents the characteristics of pSS patients with different grade ESSDAI and ESSPRI indexes. Patients with moderate and high ESSDAI scores exhibited significantly higher US activity (p = 0.006), were more frequently positive for anti-SSA (p = 0.014) and had more common lymphoma (p = 0.043) in comparison to those of SS patients with low ESSDAI scores. These patients were taking corticosteroids more frequently (p = 0.008), azathioprine (p = 0.003) and methotrexate (p = 0.038). Patients with moderate ESSPRI scores were older (p = 0.024) and more often had both xerophthalmia (p = 0.003) and xerostomia (p = 0.022) in comparison to those of pSS patients with low disease activity.

Characteristics of pSS patients and SSDDI and SSDAI disease activity

Table 3 presents the characteristics of pSS patients with different grades of the SSDDI and SSDAI. Patients with moderate and high SSDDI scores showed higher US activity (p = 0.031) and more common lymphoma (p<0.001) compared to SS patients with low disease activity. Patients with moderate SSDAI scores more frequently had positive biopsies of MSG (p = 0.015), positive anti-SSB (p = 0.033), concurrent therapy of corticosteroids (p = 0.019) and higher US activity (p = 0.01) compared to those of pSS patients with low disease activity.

Predictive value of clinical, serological and sUS variables for moderate and high pSS activity and damage

The results of logistic regression analyses (Table 4) showed that severe US activity is an independent predictor of moderate and high pSS activity according to the ESSDAI score. The patients with a severe de Vita score (5–6) had a 3.5 times greater chance of having moderate or high disease activity. The presence of lymphoma increased extremely, with a 263.41-fold higher risk for moderate disease activity according to SSDAI. SSDAI greater than or equal to 5 highly correlated with positive biopsy of MSG (OR 3.061).

The diagnostic accuracy of a de Vita score ≥ 5 in comparison with ESSDAI, ESSPRI, SSDDI and SSDAI ≥ 5 is presented in Table 5. The specificity of a severe de Vita sUS score for ESSDAI and SSDAI was 85.1% and 85.2%, respectively. In contrast, the sensitivity of a severe de Vita sUS score for ESSDAI was low, 29.2%, while the sensitivity for SSDAI was higher, 42.3%. In the analysis of disease activity, a de Vita score ≥ 5 could be a risk factor for moderate
and high ESSDAI (p = 0.042) and SSDAI (p = 0.006). However, this could not be applied for ESSPRI and SSDDI (p > 0.05).

**Discussion**

In the clinical setting, the assessment of disease activity indexes is essential in patients with pSS. The relationship between different sUS scores and disease severity has been recently demonstrated [21–23]. However, these studies have focused only on EULAR indexes or associations between some of the clinical aspects of pSS with sUS damage. In our study, we tested the correlation of sUS using sets of indexes for disease activity developed by both Vitali et al. and the EULAR group. We found that a higher sUS score was associated with higher ESSDAI and SSDAI indexes of disease activity, with an estimated US diagnostic specificity for ESSDAI and SSDAI of 85.1% and 85.2%, respectively. To our knowledge, this was not previously reported.

The predictive value of different factors for disease activity, including parenchymal inhomogeneity as the sUS hallmark for pSS, has been demonstrated [24,25]. Inhomogeneity of the salivary parenchyma detected by sUS includes the presence of hypoechoic areas and/or hyperechoic bands [26]. These sUS changes indicate active glandular inflammation due to the infiltration of immune cells and/or chronic damage with fibrotic lesions and loss of functional...
Several SUS scoring systems have been proposed for the evaluation of typical SUS changes in pSS; however, no consensus has been reached yet [25]. In our study, grade 2 of the de Vita scoring system was denoted as abnormal SUS findings, and this cut-off value had the best diagnostic sensitivity (89.6%) and specificity (86.7%) for pSS, according to ROC curve analyses. Likewise, Jouse-Joulin et al. [28] reported that the diagnostic sensitivity of SUS for pSS ranged from 45.8% to 91.6% and that the specificity ranged from 73% to 98.1%.

Interestingly, in our cohort, 15 out of 202 (7.5%) pSS patients with ESSDAI \( \geq 5 \) had normal SUS. The preserved SUS structure of the salivary glands with functional impairment in these cases indicates that other mechanisms may be involved in the pathogenesis of pSS, including abnormalities in parasympathetic neurotransmission [29]. In our cohort, 43% of the pSS patients had a duration of disease \( < 5 \) years, while 89.7% of these patients had pathological SUS. We found no correlation between disease duration and SUS change, similar to Theander E et al. [16]. This finding implies that changes in the salivary gland parenchymal echostructure are likely to be present in the early course of disease. On the other hand, we found positive correlations between SUS findings and indexes of disease activity and damage as well as serological tests, lymphoma and immunosuppressive therapy. Consistent with our findings, Fidelix et al. [15] reported an association of more severe SUS scores with an ESSDAI \( \geq 5 \) and serological tests. Additionally, Kimura-Hayama E et al. [30] have also reported that elastography

| Characteristic | Low (n = 277) | Moderate and high (n = 26) | p value | Low (n = 142) | Moderate and high (n = 161) | p value |
|---------------|---------------|-----------------------------|---------|---------------|-----------------------------|---------|
| Age, mean±SD  | 54.13±12.17   | 54.73±9.24                  | 0.808   | 54.71±11.99   | 53.71±11.90                | 0.467   |
| Female, n (%) | 270 (97.1)    | 24 (92.3)                   | 0.207f  | 140 (97.9)    | 154 (95.7)                 | 0.272   |
| Duration of disease, med (min-max) | 5 (0–22) | 7 (1–17) | 0.059 | 5 (1–20) | 5 (0–22) | 0.404 |
| Xerophthalmia, n (%) | 253 (91.0) | 24 (92.3) | 0.824 | 129 (90.2) | 148 (91.9) | 0.600 |
| Xerostomia, n (%) | 255 (91.9) | 25 (96.2) | 0.451 | 131 (92.2) | 149 (92.5) | 0.923 |
| Positive keratoconjunctivitis sicca, n (%) | 182 (96.3) | 16 (94.1) | 0.504f | 90 (96.8) | 108 (95.6) | 0.658 |
| Positive sialo-scintigraphy, n (%) | 139 (98.6) | 11 (100.0) | 1.000f | 68 (98.6) | 82 (98.8) | 1.000f |
| Positive biopsy of MSG, n (%) | 173 (88.7) | 18 (78.3) | 0.150 | 80 (81.6) | 111 (92.5) | 0.015 |
| De Vita score (US activity), n (%) | 0.031 | 0.001 | | | | |

Except where indicated otherwise, values are the number (%); SUS, Sjogren’s Syndrome Disease Activity Index; SSDDI, Sjogren’s Syndrome Disease Damage Index; MSG, minor salivary gland; RF, rheumatoid factor; ANA, anti-nuclear antibody; Anti-SSA Ab, anti-SSA antibody; Anti-SSB Ab, anti-SSB antibody; according to the chi-square test or Fisher’s exact test where appropriate (f)

https://doi.org/10.1371/journal.pone.0226498.t003

Table 3. Characteristics of pSS patients and comparison according to the level of its activity using SSDDI and SSDAI scores.
ultrasound of the major salivary glands correlates with the ESSDAI. However, this study did not find a correlation with the ESSPRI, similar to our results.

In our study group, 66.3% of pSS patients exhibited disease activity with an ESSDAI score ≥5, while only 3.1% of pSS patients had no systemic disease activity (ESSDAI = 0). According to Brito-Zeron et al. [31], pSS patients with high systemic disease activity are at high risk of death, and close follow-up (3–6 months) is strongly advised. In our study, analyses of domains of the ESSDAI revealed articular involvement as the most frequent finding (85%), followed by haematological (67.2%) and glandular (44%) involvement. This is in line with previously

| Predictor                  | ESSDAI Moderate and high | ESSPRI Moderate and high | SSDDI Moderate | SSDAI Moderate |
|----------------------------|--------------------------|--------------------------|----------------|----------------|
| De Vita score Normal (0–1) | ref                      | ref                      | ref            | ref            |
| De Vita score Moderate (2–4) | 1.598                    | 0.232                    | NA             | 0.998          |
| De Vita score Severe (5–6)  | 3.556                    | 0.007                    | NA             | 0.998          |
| Age                        | 1.013                    | 0.299                    | NA             | 1.230          |
| Xerophthalmia              | 2.075                    | 0.113                    |                |                |
| Xerostomia                 | 2.147                    | 0.116                    |                |                |
| Lymphoma                   | 263.428                  | <0.001                   |                |                |
| Positive of biopsy of MSG  | 3.061                    | 0.020                    |                |                |
| Positive anti-SSB          | 1.585                    | 0.133                    |                |                |
| Glucocorticoids            | 1.533                    | 0.153                    |                |                |

ESSDAI, EULAR Sjogren’s Syndrome Disease Activity Index; ESSPRI, EULAR Sjogren’s Syndrome Patient Reported Index; EULAR European League Against Rheumatism; SSDAI, Sjogren’s Syndrome Disease Activity Index (SSDAI); SSDDI, Sjogren’s Syndrome Disease Damage Index; MSG minor salivary gland; Anti-SSB Ab, anti-SSB antibody; https://doi.org/10.1371/journal.pone.0226498.t004

Table 5. Diagnostic accuracy of a de Vita score ≥5 in comparison with ESSDAI, ESSPRI, SSDDI and SSDAI ≥5.

| De Vita score vs. | ESSDAI      | ESSPRI      | SSDDI      | SSDAI       |
|------------------|-------------|-------------|-------------|-------------|
| Sensitivity      | 29.2%       | 26.4%       | 42.3%       | 32.9%       |
| (95 CI 23.0–36.0)| (95 CI 20.7–32.7)| (95 CI 23.4–63.1)| (95 CI 25.7–40.8)|
| Specificity      | 85.1%       | 80.7%       | 77.3%       | 85.2%       |
| (95 CI 76.7–91.4)| (95 CI 70.6–88.6)| (95 CI 71.9–82.1)| (95 CI 78.3–90.6)|
| Overall accuracy | 47.9%       | 41.3%       | 74.3%       | 57.4%       |
| (95 CI 42.1–53.6)| (95 CI 35.7–47.0)| (95 CI 68.9–79.1)| (95 CI 51.6–63.1)|
| Positive predictive value | 79.7%   | 78.4%       | 14.9%       | 71.6%       |
| (95 CI 68.8–88.2)| (95 CI 67.3–87.1)| (95 CI 77.7–25.0)| (95 CI 59.9–81.5)|
| Negative predictive value | 37.6%  | 29.3%       | 93.4%       | 52.8%       |
| (95 CI 31.3–44.2)| (95 CI 23.5–35.6)| (95 CI 89.4–96.3)| (95 CI 46.2–59.4)|
| Likelihood ratio + | 1.97       | 1.37        | 1.86        | 2.23        |
| (95 CI 1.18–3.29)| (95 CI 0.84–2.24)| (95 CI 1.13–3.06)| (95 CI 1.42–3.50)|
| Likelihood ratio - | 0.83       | 0.91        | 0.75        | 0.79        |
| (95 CI 0.73–0.94)| (95 CI 0.80–1.04)| (95 CI 0.53–1.04)| (95 CI 0.69–0.89)|
| Area under the ROC | 57.2%      | 53.5        | 59.8        | 59.1        |
| P                | 0.042       | 0.341       | 0.099       | 0.006       |

ESSDAI, EULAR Sjogren’s Syndrome Disease Activity Index; ESSPRI, EULAR Sjogren’s Syndrome Patient Reported Index; EULAR European League Against Rheumatism; SSDAI, Sjogren’s Syndrome Disease Activity Index (SSDAI); SSDDI, Sjogren’s Syndrome Disease Damage Index. https://doi.org/10.1371/journal.pone.0226498.t005
reported studies [32, 33]. Here, we found that pathological sUS correlated with the glandular
domain within the ESSDAI and SSDDI. This is one possible reason for the higher disease
activity, so sUS might be a surrogate item for the glandular domain in an objective measure of
disease-related damage to salivary glands. Interestingly, disease longevity > 5 years, de Vita
scores ≥2, and current therapy with glucocorticoids and methotrexate were identified as vari-
ables independently associated with a higher ESSDAI score. In addition, we noted that abnor-
mal sUS was an independent prognostic factor, whereas a severe de Vita score had high
predictive value for high and moderate levels of ESSDAI and SSDAI.

However, some limitations of our study are worth noting. A progression of sUS changes in
pSS patients could not be observed due to the cross-sectional design of this study. The main
limitation of our research is related to the assessment of sUS score by a single ultrasonogra-
pher. Although the experienced ultrasonographer was blinded to the diagnosis, it is well
known that sUS is a subjective method of imaging.

In conclusion, pathological salivary gland ultrasonography is associated with high disease
activity and damage in pSS. Consequently, sUS abnormalities might represent a surrogate item
for the glandular domain in the assessment of disease activity and damage. Thus, ultrasonogra-
phy of the salivary glands combined with clinical and serological markers might be part of the
next prognostic and therapeutic algorithm in the near future.

Supporting information

S1 Table. The frequencies of domains in different ESSDAI scores. Low activity: ESSDAI<5; Moderately activity: 5≤ESSDAI≤13; High activity: ESSDAI≥14.
(DOCX)

S2 Table. The frequencies of domains in different ESSPRI scores. Absence activity: ESSPRI 0; Low activity ESSPRI 5; Moderate and high activity: ESSPRI high ≥5.
(DOCX)

Author Contributions

Conceptualization: Vera Milic, Andja Cirkovic, Nemanja Damjanov.

Data curation: Vera Milic, Andja Cirkovic, Svetlana Stanojlovic, Nemanja Damjanov.

Formal analysis: Vera Milic, Jelena Colic, Andja Cirkovic, Svetlana Stanojlovic, Nemanja Damjanov.

Funding acquisition: Vera Milic, Jelena Colic, Svetlana Stanojlovic.

Investigation: Vera Milic, Jelena Colic, Andja Cirkovic, Svetlana Stanojlovic, Nemanja Damjanov.

Methodology: Vera Milic, Jelena Colic, Andja Cirkovic, Svetlana Stanojlovic, Nemanja Damjanov.

Project administration: Vera Milic, Jelena Colic, Andja Cirkovic, Nemanja Damjanov.

Resources: Vera Milic, Jelena Colic.

Software: Vera Milic, Jelena Colic, Andja Cirkovic.

Supervision: Vera Milic, Andja Cirkovic, Svetlana Stanojlovic, Nemanja Damjanov.

Validation: Vera Milic, Jelena Colic, Andja Cirkovic, Svetlana Stanojlovic, Nemanja Damjanov.
References

1. Jonsson R, Bowman SJ, Gordon TP. Sjogren’s syndrome. Ch.78 in: Koopman Wj, Moreland LW, eds. Arthritis and allied conditions 15th edition. Lippincott Williams and Wilkins; 2005:1681–1705.

2. Brito-Zerón P, Ramos-Casals M, Bove A, Sentis J, Font J. Predicting adverse outcomes in primary Sjogren’s syndrome: identification of prognostic factors. Rheumatology 2007; 46:1359–1362. https://doi.org/10.1093/rheumatology/kem079 PMID: 17569749

3. Liang Y, Yang Z, Qin B, Zhong R. Primary Sjogren’s syndrome and malignancy risk: a systematic review and meta-analysis. Ann Rheum Dis 2014; 73:1151–1156. https://doi.org/10.1136/annrheumdis-2013-203305 PMID: 23687261

4. Theander E, Vasallis L, Baecklund E, Nordmark G, Warff G, Liedholm R, et al. Lymphoid organisation in labial salivary gland biopsies is a possible predictor for the development of malignant lymphoma in primary Sjogren’s syndrome. Ann Rheum Dis 2011; 70:1363–1368. https://doi.org/10.1136/ard.2010.144782 PMID: 21715359

5. Bowman SJ, Everett CC, O’Dwyer JL, Emery P, Pitzalis C, Ng WF, et al. Randomized controlled trial of rituximab and cost-effectiveness analysis in treating fatigue and oral dryness in primary Sjogren’s syndrome. Arthritis Rheum 2017; 69:1440–1450.

6. Mariette X, Seror R, Quaruccio L, Barou G, Salvis S, Fabris M, et al. Efficacy and safety belimumab in primary Sjogren’s syndrome. Results of the BELISS open-label phase II study. Ann Rheum Dis 2015; 74:526–531. https://doi.org/10.1136/annrheumdis-2013-203991 PMID: 24345769

7. Mariette X, Ravaud P, Steinfeld S, Barou G, Goetz J, Hachulla E, et al. Inefficacy of infliximab in primary Sjogren’s syndrome: results of the randomized, controlled trial of remicade in primary Sjogrens syndrome (TRIPSS). Arthritis Rheum 2004; 50:1270–1276. https://doi.org/10.1002/art.20146 PMID: 15077311

8. Seror R, Ravaud P, Bowman SJ, Barou G, Tzioufas A, Theander E, et al. EULAR Sjogren’s syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogrens syndrome. Ann Rheum Dis 2010; 69:1103–1109. https://doi.org/10.1136/ard.2009.110619 PMID: 19561361

9. Seror R, Ravaud P, Mariette X, Bootsma H, Theander E, Hansen A, et al. EULAR Sjogren’s syndrome patient reported index (ESSPRI): development of a consensus patient index for primary Sjogren’s syndrome. Ann Rheum Dis 2011; 70:968–972. https://doi.org/10.1136/ard.2010.143743 PMID: 21345815

10. Vitali C, Palombi G, Baldini C, Benucci M, Bombardieri S, Covelli M, et al. Sjogren’s syndrome disease damage index and disease activity index: scoring system for the assessment of disease damage and disease activity in Sjogren’s syndrome, derived from an analysis of a cohort of Italian patients. Arthritis Rheum 2007; 56:2223–2231. https://doi.org/10.1002/art.22658 PMID: 17599741

11. Milic V, Petrovic R, Boricic I, Radunovic G, Marinovic-Eric J, Jeremic P, et al. Ultrasonography of major salivary glands could be an alternative tool to sialografin in the American-European classification criteria for primary Sjogren’s syndrome. Rheumatology 2012; 51:1081–1085. https://doi.org/10.1093/rheumatology/ker431 PMID: 22302061

12. van Nijnwegen JF, Mossel E, Delli K, van Ginkel MS, Stel AJ, Kroese FMG, et al. Incorporation of salivary gland ultrasonography into the ACR-EULAR criteria for primary Sjogren’s syndrome. Arthritis Care Res (Hoboken) 2019; 29. https://doi.org/10.1002/acr.24017 PMID: 31254494

13. Takagi Y, Nakamura H, Sumi M, Shimizu T, Hirai Y, Horai Y et al. Combined classification system based on ACR/EULAR and ultrasonographic scores for improving the diagnosis of Sjogrens syndrome. PLoS One 2018; 13 (4):e0195113. https://doi.org/10.1371/journal.pone.0195113 PMID: 29614092

14. Lee KA, Lee SH, Kim HR. Diagnostic and predictive evaluation using salivary gland ultrasonography in primary Sjogren’s syndrome. Clin Exp Rheumatol 2018; Suppl 112(3):165–172.

15. Fidelix T, Czapkowski A, Azjen S, Andriolo A, Treviáni VFM. Salivary gland ultrasonography as a predictor of clinical activity in Sjogren’s syndrome. PLoS One 2017; 12(8):e0182287. https://doi.org/10.1371/journal.pone.0182287 PMID: 28783737
16. Theander E, Mand T. Primary Sjogren’s syndrome: diagnostic and prognostic value of salivary gland ultrasonography using a simplified scoring system. Arthritis Res Ther. 2014; 66:1102–1107.

17. Jousse-Joulin S, Devauchelle-Pensec V, Cornec D, Marhadour T, Bressollette L, Gestin S, et al. Brief report: ultrasonographic assessment of salivary gland response to rituximab in primary Sjogren’s syndrome. Arthritis Rheumatol 2015; 67:1623–1628. https://doi.org/10.1002/art.39088 PMID: 25708147

18. Fisher BA, Everett CC, Rout J, ODwyer JL, Emery P, Pitzalis C et al. Effect of rituximab on a salivary gland ultrasound score in primary Sjogren’s syndrome: results of the TRACTISS randomized double-blind multicenter substudy. Ann Rheum Dis 2018; 77:412–416. https://doi.org/10.1136/annrheumdis-2017-212268 PMID: 29275334

19. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE et al. Classification criteria for Sjogren’s syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002; 61:554–558. https://doi.org/10.1136/ard.61.6.554 PMID: 12006334

20. De Vita S, Lorenzon G, Rossi G, Sabella M, Fossaluzz a V. Salivary gland echography in primary and secondary Sjogren’s syndrome. Clin Exp Rheumatol 1992; 10:351–156. PMID: 13952220

21. Coiffier G, Martel A, Albert JD, Lescoat A, Blunzen A, Perdriger A, de et al. Ultrasonographic dam-

22. Zambotti A, Zandonella Calleghe r S, Gandolfo S, Valent F, Giovannini I, Cavallaro E et al. Hyperec choic bands detected by salivary gland ultrasonography are related to salivary impairment in established Sjogren s syndrome. Clin Exp Rheumatol 2019; 37 Suppl 118 (3):146–152. PMID: 31365337

23. Inanc N, Sahinkaya Y, Mumcu G, Ture Ozdemir F, Paksoy A, Erturk Z et al. Evaluation of salivary gland ultrasonography in primary Sjogren’s syndrome: does it reflect clinical activity and outcome of the disease. Clin Exp Rheumatol 2019; 37 Suppl 118 (3):140–145. PMID: 31287407

24. Milic VD, Petrovic RR, Boricic I, Radunovic GL, Pejnovic NN, Soldatovic I, et al. Major salivary gland sonography in Sjogren’s syndrome: diagnostic value of a novel ultrasonography score (0–12) for parenchymal inhomogeneity. Scan J Rheumatol 2010; 39:1891–1898.

25. Martel A, Coiffier G, BLEuyen A, Goa sguen J, de Bandt M, Delignz C, et al. What is the best salivary gland ultrasonography scoring system methods for the diagnosis of primary or secondary Sjogren s syndrome? Joint Bone Spine 2019; 86:211–217. https://doi.org/10.1016/j.jbspin.2018.06.014 PMID: 30053612

26. Baldini C, Luciano N, Tarantini G, Pascale R, Sernissi F, Mosca M, et al. Salivary gland ultrasonography: a highly specific tool for the early diagnosis of primary Sjogren’s syndrome. Arthritis Res Ther 2015; 17:146. https://doi.org/10.1186/s13075-015-0657-7 PMID: 26022333

27. Ramos-Casals M, Font J. Primary Sjogren’s syndrome: current and emergent aetiopathogenic con-

28. Borchers AT, Naguwa SM, Keen CL, Gershwin ME. Immunopathogenesis of Sjogren’s syndrome. Clin Rev Allergy Immunol. 2003; 25:89–104. https://doi.org/10.1385/CRIAI:25:1:89 PMID: 12794264

29. Kimura-Hayama E, Criales-Vera S, Azpeitia-Espinoza L, Pacheco-Molina C, Reyes E, Lima G, et al. Elastographic ultrasound: an additional image tool in Sjogrens syndrome. International Journal of Rheumatic Diseases 2018; 21:1293–1300. https://doi.org/10.1111/1756-185X.13292 PMID: 29624878

30. Brito-Zeron P, Kostov B, Solans R, Fraile G, Suarez-Cuervo C, Casanovas, et al. Systemic activity and mortality in primary Sjogren’s syndrome: predicting survival using the EULAR-SS Disease Activity Index in 1045 patients. Ann Rheum Dis 2016; 75:348–355. https://doi.org/10.1136/annrheumdis-2014-206418 PMID: 25433020

31. Ramos-Casals M, Brito-Zeron P, Solans R, Camps MT, Casanovas A, Sopena B, et al. Systemic- involvement in primary Sjogren’s syndrome evaluated by the EULAR-SS disease activity index: analysis of 921 Spanish patients (GEAS-SS Registry). Rheumatology 2014; 53:321–331. https://doi.org/10.1093/rheumatology/ket349 PMID: 24162151

32. Ramos-Casals M, Solans R, Rosas J, Camps MT, Gil A, Del Pino-Montes J, et al. Primary Sjogren’s syndrome in Spain: clinical and immunologic expression in 1010 patients. Medicine (Baltimore) 2008; 87:210–219.