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Usefulness of Unstimulated and Stimulated Whole Saliva, Accuracy of Minor Labial Salivary Gland Biopsy in the Diagnosis of Primary Sjögren's Disease: A Croatian Single-Center, Cross-Sectional Study

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Abstract

The aim of this cross-sectional study was to determine the accuracy of minor labial salivary gland (MLSG) biopsy in the diagnosis of primary Sjögren's disease (pSD); to study the correlation between the focus score (FS) and anti-SSA/Ro, anti-SSB/La, anti-SSA and -SSB antibodies, unstimulated whole saliva (UWS) and stimulated whole saliva (SWS); to determine the role of UWS and SWS in the clinical evaluation of pSD patients and patients with *sicca* symptoms.

Methods. A total of 37 subjects were enrolled in the study and divided into two groups: the test group consisted of 15 patients diagnosed with pSD; the control group consisted of 22 patients who had *sicca* symptoms but did not meet the 2016 American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) diagnostic criteria. Clinical and laboratory tests, including saliva collection, MLSG biopsy, autoantibody titers, were performed in all patients.

Results. The median of the FS was 1.00 [IQR=1.00-1.50] in the test group, whereas in the control group, it was 0.00 [IQR=0.00-0.00] ($p < 0.001$). The sensitivity, specificity, and accuracy of MLSG biopsy were 86.7%, 100.0%, and 94.6%, respectively. The results showed a correlation between the FS and antinuclear antibodies (ANA) (p=0.002). In addition, Pearson's correlation showed a weak negative correlation between UWS (r=-0.058, p=0.73) and SWS (r=-0.022, p=0.90) and the FS. In the test group, 73.3% of patients had abnormal UWS values, while 86.7% had abnormal SWS values; among them, values of 0.00 ml/min for UWS and SWS were found in 60.0% and 26.7% of patients, respectively.

Conclusions. Although MLSG biopsy has great diagnostic value and accuracy in diagnosing pSD, it is not always definitive. Our study found a statistically significant association between the FS and ANA, and the greater utility of SWS in diagnosing pSD.

Keywords

Sjögren's Disease; Unstimulated Whole Saliva; Stimulated Whole Saliva; Minor Labial Salivary Gland Biopsy; Focus Score

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Introduction

Sjögren's disease (SD) is an autoimmune condition characterized by lymphocytic infiltration of the exocrine glands, resulting in dryness of the mouth (xerostomia) and eyes (xerophthalmia). *Sicca* symptoms are usually an organ-specific manifestation of the disease, although the disease may have a different spectrum of extraglandular manifestations. The diagnosis of primary Sjögren's disease (pSD) is based on a combination of *sicca* symptoms and objective autoimmune characteristics of the disease: the presence of autoantibodies (i.e., activation of B cells) and/or a positive biopsy finding of the minor labial salivary glands (MLSGs) (i.e., activation of T cells) [1]. SD can manifest as pSD, which occurs as an isolated condition, or as

secondary Sjögren's disease (sSD), when it occurs in association with other autoimmune diseases [2]. Secondary SD occurs most commonly in association with rheumatoid arthritis (RA) [3]. The term 'secondary' here does not imply a chronological course of disease manifestation. Diagnosis is often challenging because of overlapping disease entities, but it is very important to distinguish pSD from sSD as the disease course, including prognosis, is different [2]. The overall prevalence of SD is at least 0.4%, with the secondary form of the disease being more common [4]. SD shows a strong predilection for the female sex, with the difference between the sexes varying from 9:1 to 19:1. The average age at diagnosis is 56 years, with a peak between 20 and 40 years [4].

Genetic, environmental, epigenetic, and stochastic factors play a role in the development of SD. The precise etiopathogenetic mechanism is not yet clear, but it is known that the target cells of the immune system are the ribonuclear proteins (anti-SSA/Ro and anti-SSB/La) [5]. The disease has a genetic predisposition involving the major histocompatibility locus (MHC). An increased risk of developing SD has been demonstrated in individuals who have haplotypes in the human leukocyte antigen (HLA) -DQA and -DQB regions. Laboratory studies and some indirect epidemiological evidence point to the involvement of the Epstein-Barr virus (EBV) in the pathogenesis [6, 7]. In the damaged tissue, epithelial-mesenchymal transition is initiated, i.e., conversion of epithelial cells to mesenchymal-like cells occurs. This leads to pathological fibrosis, which is the main pathological feature of many chronic autoimmune diseases such as RA, systemic lupus erythematosus (SLE), and pSD [8]. Focal lymphocytic infiltrates may occur in any other affected organ as well. However, the salivary glands have been most thoroughly studied to date [9].

The spectrum of clinical symptoms that occur is broad and can range from dry mouth and/or eyes to joint (atralgia, non-erosive polyarthritis), kidney (tubulointerstitial changes), and lung damage (interstitial lung disease, follicular bronchitis) [10]. *Sicca* symptoms are present in 98.0% of cases. Patients with keratoconjunctivitis sicca complain of feeling a foreign body in the eyes, burning and pain in the eyes, and sensitivity to light. Xerostomia affects speech and swallowing, especially of solid and dry foods. Caries in physiologically clean areas (class V) and early tooth loss are twice as common in patients with SD. Other oral SD manifestations include recurrent oral infections with *Candida albicans*, angular cheilitis, atrophic glossitis, and recurrent oral ulceration [11]. Acute or chronic swelling of the parotid gland occurs in 34.0% of patients, and here it is important to rule out malignant non-Hodgkin B-cell lymphoma (NHL) [12].

Numerous studies have attempted to define the classification criteria for SD, as it is impossible to confirm or exclude the diagnosis of the disease with a single diagnostic test [13]. Unstimulated whole saliva (UWS) is an objective diagnostic classification criterion adopted by the American-European Consensus Group (AECG) in 2002, and by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) in 2016 [14, 15]. Secretion of UWS is significantly affected by various factors (e.g., age, circadian rhythm, room temperature, medications, diseases, collection technique), hence, the use of stimulated whole saliva (SWS) is suggested as a much more reliable method [13, 16].

Anti-Sjögren's-syndrome-related antigen A (anti-SSA/Ro) and anti-Sjögren's-syndrome-related antigen B (anti-SSB/La) autoantibodies are found in 33.0-74.0% and 23.0-52.0% of patients, respectively, and are considered the most important immunological markers of SD. A positive finding of these antibodies correlates with an early onset of the disease, a longer duration of the disease, and a greater involvement of the salivary glands. Their presence is also associated with extraglandular systemic manifestations and risk of neonatal lupus [17]. The new ACR-EULAR classification excluded an isolated positive finding of anti-SSB/La as a criterion, since anti-SSA/Ro antibodies are usually detected alone or together with anti-SSB/La, whereas findings of positive anti-SSB/La and negative anti-SSA/Ro are extremely rare [18, 19]. These antibodies are present in other connective tissue diseases and even in healthy individuals as well [20]. Other serologic disorders include the presence of rheumatoid factor (RF) (60.0-75.0%) and nonspecific antinuclear antibodies (ANA) [21].

MLSG biopsy is the gold standard in the diagnosis of SD. However, some data from the literature question its diagnostic value, citing nonstandard histopathologic criteria as evidence [22–24]. Although it is very important for the diagnosis of SD, the histopathological findings of MLSG biopsy may be negative in some patients. Then, if SD is suspected, anti-SSA/Ro antibodies should be present [25]. The focus score (FS) is the main histopathologic measure for SD. The FS is calculated by dividing the total number of foci in the specimen by the gland surface area and multiplying by four to obtain the number of foci per 4 mm^2. A value of 12 represents the maximum, while for a FS > 10, foci are usually confluent [26].

Thus, **the objectives** of our study were to determine the accuracy of MLSG biopsy in diagnosing pSD; to study the correlation between the FS and specific autoantibodies such as anti-SSA/Ro60, anti-SSA/Ro52, anti-SSB/La, anti-SSA/Ro and -SSB/La, as well as ANA, UWS and SWS; to determine and compare the values of UWS and SWS in both groups of subjects (pSD patients and patients with *sicca* symptoms not meeting the ACR-EULAR classification criteria for diagnosis); to compare the usefulness of UWS and SWS in pSD patients.

Materials and Methods

Study Design and Subjects

This single-center, cross-sectional study was conducted between June 2019 and June 2022 at the Dental Poyclinic Split, teaching base of the School of Medicine, Study of Dental Medicine, University of Split, Split, Croatia.

A total of 37 subjects participated in the study, 15 patients with diagnosis of pSD and 22 control subjects.

Inclusion criteria were subjective dryness in the oral cavity and/or eyes for at least three months.

Exclusion criteria were previous radiation therapy to the head and neck, hepatitis C (HCV), acquired immunodeficiency syndrome (AIDS), lymphoma, sarcoidosis, graft-versus-host disease (GvHD), medications causing salivary gland dysfunction (anticholinergics, tricyclic antidepressants, antihypertensives, antihistamines), smoking.

All subjects were sent to an oral medicine specialist for the initial examination with a suspected diagnosis of SD due to the presence of *sicca* symptoms. The control group consisted of patients who did not meet the ACR-EULAR diagnostic criteria but had *sicca* symptoms, as for ethical reasons it was not possible to include healthy subjects.

Outcome Measures

A comprehensive medical and dental history was obtained from all subjects. The subjective presence of dry eyes and/or dry mouth was noted, and patients underwent clinical and laboratory tests.

Saliva Collection

The amount of saliva was determined by sialometry. Saliva was collected in the morning between 9 and 11 a.m. using the spit method. In fertile women, whole saliva was collected during the follicular phase of the cycle. In the first measurement, UWS samples were collected by asking patiens to spit saliva into a measuring tube calibrated to 0.10 ml with an opening of 1.50 cm in diameter for five minutes. An abnormal value of UWS $\leq$ 0.10 ml/min was considered a positive finding. For the second measurement, SWS samples were collected in the same manner after asking patients to shake and drink or spit out a 1.0% vitamin C solution prepared by dissolving 1.00 g ascorbic acid in 1.00 dcl water. An abnormal SWS value $<$ 0.70 ml/min was considered a positive finding [27].

Minor Labial Salivary Gland Biopsy

Tissue samples for pathohistological analysis of MLSG were obtained from clinically healthy lower lip mucosa. The same oral medicine specialist performed biopsy, i.e., a horizontal incision of 1.50-2.00 cm was made parallel to the vermilion of the lower lip under local anesthesia. This method is used most often, since the incidence of complications is less than 1.0% [26, 28, 29]. Four to six small salivary glands were excised. The specimens were then placed in a container containing 10.0% formaldehyde and sent to the Clinical Institute of Forensic Medicine, Pathology and Cytology, Clinical Hospital Center Split, Split, Croatia, for further examination. The same experienced pathologist performed histopathological examination and the FS was assessed [26].

Autoantibody Titer

Antibody titers of anti-SSA/Ro60, anti-SSA/Ro52, anti-SSB/La, and ANA were determined by laboratory testing at the Department of Medical Laboratory Diagnostics, Clinical Hospital Center Split, Split, Croatia. Titers of antiSSA/Ro60, anti-SSA/Ro52, anti-SSB/La were determined by enzyme-linked immunosorbent assay (ELISA) (Cat. No. BI-5000; Biomedica, Vienna, Austria) according to the manufacturer instructions. A positive finding of anti-SSA and -SSB antibodies was considered evidence of concomitantly elevated levels of anti-SSA and anti-SSB antibodies (above reference values).

Statistical Analysis

The data obtained were entered into a previously created spreadsheet in the Microsoft Excel 2007 program (Microsoft Corporation, Redmond, Washington, USA) and statistically processed, and the sensitivity, specificity, and accuracy of MLSG biopsy were calculated. Analysis was performed using the statistical program SPSS Statistics (25, IBM, Armonk, New York). Data normality was determined using the Kolmogorov-Smirnov test. The statistical difference between categorical and continuous variables was analyzed with the Chi-square test and the Fisher's exact test, depending on the characteristics of the sample. The demographic characteristics of the respondents are presented in a table, and the difference in the representation of the respondents with respect to each demographic characteristic was examined with the Fisher's exact test and the Mann-Whitney U test. The conclusion about the association between the FS and UWS and between the FS and SWS was based on the Pearson correlation coefficient. In addition, the association between the FS and anti-SSA/Ro60, anti-SSA/Ro52, anti-SSB/La antibodies, ANA, and anti-SSA and -SSB antibodies was tested using the Fisher's exact test. The justification for using the Fisher's exact test comes from the qualitative feature of the data (nominal feature) with low frequencies. A significance level of $p < 0.05$ was used for all statistical tests conducted in this study.

Results

Study Subjects

Fifteen patients diagnosed with pSD and 22 control subjects were enrolled in the study. Of the 37 subjects, 32 (86.5%) were women and 5 (13.5%) were men, whose ages ranged from 27 to 84 years. In both observed groups, the number of female patients was higher (93.3% and 81.8%, respectively). No statistically significant difference was found between gender in relation to group (p=0.31) (Table 1).

Table 1. Demographic data.

	Test group	Control group	Total
Number of subjects	15 (40.5)	22 (59.5)	37 (100.0)
Age, median (IQR)	68.00 (45.0-76.0)	62.00 (50.0-70.0)	65.00 (49.0-72.0)
Gender, n (%)	M: 1 (6.7) F: 14 (93.3)	M: 4 (18.1) F: 18 (81.8)	M: 5 (13.5) F: 32 (86.5)

Notes: F - female; M – male; IQR - interquartile range.

Minor Labial Salivary Gland Biopsy

The median of the FS was 1.00 [IQR=1.00-1.50] in the test group, whereas in the control group, it was 0.00 [IQR=0.00-0.00]. The test revealed a statistically significant difference in the FS between the test and control groups ($p < 0.001$, Mann-Whitney U test). Out of the total number of patients,

MLSG biopsy was positive in 13 patients with pSD and negative in 24 patients (two patients were diagnosed with pSD and 22 were not). Sensitivity, specificity, and accuracy were 86.7%, 100.0%, and 94.6%, respectively.

Autoantibody Titer

Anti-SSA/Ro60 antibodies were present in four patients in the test group and two patients in the control group. The same results were obtained for anti-SSA/Ro52 antibodies. Anti-SSB/La antibodies were present in two pSD patients and one control patient. No statistically significant difference in the presence of anti-SSA/Ro60 (p=0.17), anti-SSA/Ro52 (p=0.17), and anti-SSB/La (p=0.36) antibodies was detected between the test and control groups. The distribution of positive anti-SSA/Ro60, anti-SSA/Ro52, anti-SSB/La, anti-SSA and -SSB, and ANA depending on the FS is shown in Table 2.

Table 2. Distribution of postive anti-SSA/Ro60, anti-SSA/Ro52, anti-SSB/La antibodies, ANA and anti-SSA and -SSB antibodies depending on the focus score.

	FS=0	FS <1	FS ≥1	*p
	N=21	N=3	N=13	
Anti-SSA/Ro60	3 (14.3)	1 (33.3)	2 (15.4)	0.65
Anti-SSA/Ro52	3 (14.3)	1 (33.3)	2 (15.4)	0.65
Anti-SSB/La	2 (9.5)	1 (33.3)	0 (0.0)	0.37
ANA	3 (14.3)	1 (33.3)	9 (69.2)	**0.002**
Anti-SSA and -SSB	2 (9.5)	1 (33.3)	0 (0.0)	0.37

Notes: FS - focus score. *Fisher's exact test; statistically significant results are in bold.

Sialometric Evaluation

Pearson's correlation revealed a weak negative correlation with no statistical significance between the FS and UWS (r= -0.058, p=0.73) and SWS (r= -0.022, p=0.90). The increase in the FS was accompanied by a decrease in UWS and SWS (Fig. 1 a, b). The median value of UWS was 0.00 ml/min [IQR=0.00-0.20] in the test group, whereas it was 0.10 ml/min [IQR=0.00-0.20] in the control group. The median value of SWS was 0.20 ml/min [IQR=0.00-0.40] in the test group, whereas it was 0.30 ml/min [IQR=0.20-0.50] in the control group. No statistically significant difference was found between UWS (p=0.35) and SWS (p=0.25) with respect to the group (Mann-Whitney U test). In the test group, 11 (73.3%) patients had abnormal UWS values, while 13 (86.7%) patients had abnormal SWS values (Table 3). Among them, in the test group, nine (60.0%) patients had a finding of UWS=0.00 ml/min, whereas a finding of SWS=0.00 ml/min was present in four (26.7%) patients. In the control group, eight (36.4%) patients had UWS=0.00 ml/min, whereas no patient had SWS=0.00 ml/min ($p < 0.05$).

Discussion

The diagnosis of pSD was made according to the 2016 ACR-EULAR classification criteria, which include the 2012 AECG and ACR criteria. The classification refers to any individual who meets the inclusion criteria and has a total of ≥ 4 points. Inclusion criteria are symptoms of dry eyes and/or mouth for at least three months [15]. This classification is adapted for clinical research, whereas the AECG classification is used both in research and clinical settings. In contrast to the AECG classification, the new ACR-EULAR classification is based solely on objective tests and is appropriate for classifying pSD, although further research is needed to confirm its applicability to sSD [18].

Although MLSG biopsy is the most accurate diagnostic procedure in SD classification according to the ACR-EULAR classification criteria, it should be remembered that it is not always completely accurate [30], as it was confirmed by our study results. According to Vitali *et al.*, MLSG biopsy is a diagnostic test with high sensitivity (82.4%) and specificity (86.2%) [31]. A study by

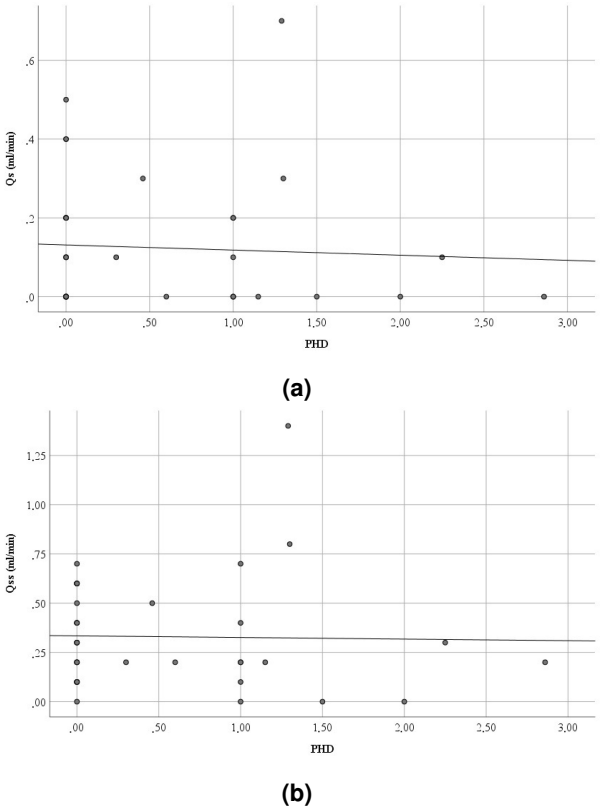

Figure 1. An increase in the FS (FS test group 1.00 [IQR=1.00-1.50]; FS control group 0.00 [IQR=0.00-0.00]) leads to a decrease in the values of UWS (a) and SWS (b). *Abbreviations:* PHD - pathohistologic diagnosis, i.e., the FS; Qs (UWS) - unstimulated whole saliva; Qss (SWS) - stimulated whole saliva; IQR - interquartile range.

Table 3. Abnormal unstimulated and stimulated whole saliva values by groups.

	Test group (N=15)	Control group (N=22)	p
	n (%)	n (%)	
UWS (≤0.10 ml/min)	11 (73.3)	13 (59.1)	0.37*
SWS (<0.70 ml/min)	13 (86.7)	22 (100.0)	0.16**

Notes: * - χ^2 test; ** - Fisher's exact test.

de Azevedo *et al.* showed a sensitivity of 72.0% and a specificity of 83.8%, while a study by Giovelli *et al.* showed a sensitivity of 86.6% and a specificity of 97.4% [14, 32]. Edelstein *et al.* reported a sensitivity range of 63.5% to 93.7% and a specificity range of 61.2% to 100.0% [33]. The results of the above studies are consistent with our results. In our study, the sensitivity of MLSG biopsy was 86.7%. Not a single patient in the control group had a positive MLSG biopsy result, i.e., the specificity was 100.0%. The results of the above studies probably differ due to the different number of subjects included in the study, but all studies showed high sensitivity and specificity of this diagnostic test. The high sensitivity and specificity of this diagnostic method in a small number of subjects underlines its value as an irreplaceable diagnostic criterion. This confirms that MLSG biopsy is the "gold standard" for diagnosing SD when findings are inconclusive. However, further studies on the composition of the mononuclear lymphocytic infiltrate are needed to improve the diagnostic and therapeutic approach to SD.

In our study, the test group consisted of 15 (40.5%) patients diagnosed with SD according to the ACR-EULAR classification criteria. Out of the 15 pSD patients, 13 had a positive MLSG biopsy result, while two had a negative result. These results show a high biopsy accuracy (94.6%), which is consistent with the data reported in the literature (89.0%) [30]. However, the biopsy accuracy was not 100.0%, which supports the hypothesis of this study that MLSG biopsy is not always accurate. The studies performed so far on MLSG biopsy accuracy have mostly been retrospective and used a small sample size, with evaluating biopsy and its accuracy not being their main objective. A study by de Azevedo *et al.* showed a biopsy accuracy of 79.0%, whereas a study by Giovelli *et al.* showed a biopsy accuracy of 93.3% [14, 32]. Their results are consistent with the results of our study.

The accuracy of MLSG biopsy is compromised by the impossibility of repeating the same results. The reason for this is the scattering of the lymphocytic infiltrate, due to which the sampling and interpretation of an inadequate area can lead to incorrect test results. The result depends on the pathologist who examines the patient's sample as well, so that in 12.6% of cases, a different diagnosis is made on the second evaluation by another pathologist [34]. Fisher *et al.* emphasized the need for standardization of histopathologic interpretation, from specimen collection to processing and interpretation of lymphocyte focus [26]. Interpretation can be complicated or even impossible by a very dense lymphocytic infiltrate, because it is then difficult to distinguish individual foci and determine their number [35, 36]. Some authors question the accuracy of MLSG biopsy and point out that lymphocytic infiltration is present in other diseases such as non-specific chronic sialoadenitis (NSCS), chronic sclerosing sialadenitis (Küttner's tumor), granulomatous inflammation, acinar atrophy, interstitial fibrosis, and ductal dilatation. All of these conditions are relatively common and their incidence increases with age [37, 38].

Daniels *et al.* analyzed 1,726 histopathologic samples of MLSG biopsies (i.e., the FS) from patients enrolled in the Sjögren's International Collaborative Clinical Alliance (SICCA) registry and demonstrated the correlation between the FS and the detection of anti-SSA (Ro60 and Ro52 together) or anti-SSB, in contrast to our study which examined the correlation between the FS and individual antibodies [39]. The results of Daniels *et al.* showed a statistically significant correlation between the FS and anti-SSA/-SSB ($p < 0.001$). Of positive anti-SSA/-SSB patients, 76.0% had the FS ≥ 1, meaning that patients with positive antibodies were nine times more likely to have the FS > 1 [39]. A study by Sharma *et al.* on 229 subjects diagnosed with pSD found that patients with the FS=0 had a lower chance of being positive for anti-SSB antibodies than those with the FS ≥ 1 [25]. Their results are not consistent with the results of our study. In this study, no statistically significant difference was found between anti-SSA/Ro60, anti-SSA/Ro52 and anti-SSB/La antibodies depending on the FS (p=0.65, p=0.65 and p=0.37, respectively). The difference in the obtained results can be explained by the different number of subjects in the studies, i.e., the small sample of our subjects, the fact that we analyzed single autoantibodies (anti-SSA/Ro60 and anti-SSA/Ro52), and different reference limits for the determination of autoantibodies of each laboratory. In addition, in this study, no statistically significant difference was found between anti-SSA and -SSB antibodies depending on the FS (p=0.37). Daniels *et al.* reported that 72.0% of subjects with a positive ANA result had the FS ≥ 1, i.e., a statistically significant difference was demonstrated between the FS and ANA ($p < 0.001$) [39]. Their results were consistent with the results of our study, i.e., 69.2% of subjects with a positive ANA result had the FS ≥ 1, and a statistically significant difference was also found between the FS and ANA (p=0.002).

Data from the literature on the association between UWS and SWS in relation to the FS are contradictory. Bookman *et al.* showed a significant correlation between the FS and SWS ($p < 0.001$), whereas there was a less significant correlation between the FS and UWS (p=0.031) [40]. According to Daniels *et al.* patients with UWS < 0.10 ml/min had twice the chance of the FS ≥ 1, i.e., there was a significant correlation between the FS and UWS. However, they did not compare SWS values with the FS [39]. On the other hand, Sharma *et al.* reported no correlation between the FS and UWS in patients with pSD [25]. De Azevedo *et al.* found no correlation between the FS and UWS as well [14]. Their results are consistent with those of our study, which found a weak, negative, and statistically non-significant correlation between UWS and SWS with respect to the FS (p=0.73 and p=0.90, respectively). The heterogeneity of the results is probably due to a different number of subjects included, i.e. the small number of our subjects, non-standardization of MLSG biopsy, and non-standardized methods of saliva collection. In addition, some studies included only pSD patients, whereas others included both pSD and sSD patients.

A study by Alvariño *et al.* on 103 patients showed the mean values of the UWS and SWS test groups as

0.07 ml/min and 0.38 ml/min. Their results are consistent with those of our study. The mean values of the UWS and SWS control groups were 0.33 ml/min and 1.20 ml/min, respectively [41]. The difference in the results obtained (compared with our study) can be explained by the fact that their control group of 50 subjects included only healthy individuals without symptoms of dry mouth. Bookman *et al.* reported that 82.4% of test group patients and 57.9% of controls had abnormal UWS values. In this study, similar results were obtained (73.3% and 59.1%, respectively). Within the test and control groups, 61.8% and 15.2% of subjects had abnormal SWS values, respectively [40]. Our study yielded the following results: 86.7% of test group patients and 100.0% of controls had abnormal SWS values. The SWS results were significantly different from the results of our study. This difference can probably be explained by the different inclusion criteria for an abnormal SWS finding. They considered SWS value abnormal if it was < 0.60, whereas our value was < 0.70. Moreover, the studies differed in the method of saliva collection. In a study by Bookman *et al.*, saliva was collected for one minute, whereas in our study, it was collected for five minutes. Alvariño *et al.* reported that 35.9% of subjects had UWS of 0.00 ml/min, whereas 11.7% of control subjects had SWS of 0.00 ml/min [41]. In our study, 60.0% of pSD patients had UWS of 0.00 ml/min, whereas 26.7% of patients had SWS of 0.00 ml/min. The difference in the results obtained can be explained by the fact that our test group consisted only of pSD patients and the different number of subjects who participated in the studies. In our study, we did not compare patients with pSD and sSD since numerous studies have shown that it is not necessary to distinguish between UWS and SWS. However, the results of both studies suggest that SWS is of greater utility than UWS in diagnosing pSD [41].

Limiations

One of the limitations of our study was the small sample size. In addition, we included pSD patients as the test group and patients who had *sicca* symptoms but did not meet the ACR-EULAR classification criteria for diagnosis as the control group. If the control group had consisted of healthy individuals, we would have obtained different results. Histopathologic evaluation of MLSG biopsy was performed by the same experienced pathologist. In this way, we made a small contribution to reduce the bias of the histopathological MLSG biopsy findings and FS calculation. Therefore, the results obtained should be interpreted with caution in the context of study limitations.

Conclusions

Nevertheless, we can conclude that the results demonstrate the great diagnostic value (sensitivity - 86.7%, specificity - 100.0%) and accuracy (94.6%) of MLSG biopsy in distinguishing pSD patients from those with *sicca* symptoms. In addition, the study found a statistically significant association between the FS and ANA. However, the expected volumes of UWS and SWS and the frequency of abnormal values (≤ 0.10 ml/min and < 0.70 ml/min, respectively) did not differ significantly between pSD patients and those with *sicca* symptoms. At the same time, UWS values of 0.00 ml/min were twice as frequent as SWS values of 0.00 ml/min in pSD patients, indicating a greater diagnostic utility of SWS in diagnosing pSD.

Ethical Statement

The study was approved by the Ethics Committee of the School of Medicine, University of Split, Split, Croatia (Class: 003-08/22-03/0003; Reg. No: 2181-198-03-04-22-0057). This study was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its subsequent amendments. All patients participated voluntarily in the study and were informed in detail (verbally and in writing) about the purpose of the study before signing the informed consent form.

Informed Consent

Informed consent was obtained from all subjects participating in the study.

Data Availability

Data are available upon request from the corresponding author's email.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Financial Disclosure

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