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CORRELATION OF CLINICAL AND HISTOPATHOLOGICAL FINDINGS IN PATIENTS WITH CLINICALLY SUSPECTED PRIMARY SJÖGREN SYNDROME

POVEZANOST KLINIČKIH I PATOHISTOLOŠKIH NALAZA U BOLESNIKA SA SUSPEKTNIM PRIMARNIM SJÖGRENOVIM SINDROMOM

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Abstract

Introduction. Association of clinical and diagnostic findings is important in making correct diagnosis in patients with primary Sjogren's syndrome. The aim of this study was to evaluate the correlation of minor salivary gland biopsy, serological findings and sialometry, as components used to classify primary Sjögren's syndrome. *Materials and methods.* Thirty-six patients with subjective symptoms of possible primary Sjögren's syndrome underwent minor salivary gland biopsy and sialometry. Clinical and laboratory data were retrieved from the clinical files. We compared and correlated biopsy results, serological findings and salivary flow rate between Sjögren and non-Sjögren patients. Patients were classified into two groups, twenty-eight cases (77.8%) had a diagnosis of primary Sjögren's syndrome and eight cases (22.2%) did not fulfill the classification criteria for diagnosis. *Results.* Primary Sjögren's syndrome diagnosis strongly correlated with "positive" biopsy (rho= 0.93, p<0.001), serological findings (rho= 0.38, p=0.023) and negatively correlated with saliva flow rate (rho= –0.51, p=0.002). "Positive" biopsy results were in negative correlation with saliva flow rate (rho= –0.41, p=0.012), but in a stronger correlation with patients below the diagnostic flow rate cutoff (≤0.1 ml/minute, rho= 0.46, p=0.005). *Conclusion.* In conclusion, unstimulated whole salivary flow rate ≤0.1 ml/minute is highly predictive of "positive" biopsy and can be used as a supplemental method to biopsy in diagnosing the oral component of primary Sjögren's syndrome.

Key words: Sjögren's syndrome; Sialometry; Salivary; Gland; Biopsy; Classification

Sažetak

Uvod. Povezanost kliničkih i dijagnostičkih nalaza važna je u postavljanju točne dijagnoze primarnoga Sjögrenovog sindroma. Cilj ovog istraživanja bio je procijeniti korelaciju nalaza biopsije malih žlijezda slinovnica, seroloških nalaza i sijalometrije kao klasifikacijskih kriterija za dijagnozu primarnoga Sjögrenovog sindroma. *Materijali i metode.* U ovu studiju bilo je uključeno 36 pacijenata sa subjektivnim simptomima koji odgovaraju primarnom Sjögrenovom sindromu. Svim bolesnicima učinjena je biopsija malih žlijezda slinovnica i sijalometrija. Iz medicinske dokumentacije prikupljeni su klinički i laboratorijski podatci. Uspoređeni su rezultati biopsije, seroloških nalaza i brzine izlučivanja

sline u pacijenata sa Sjögrenovim sindromom i pacijenata koji ne ispunjavaju klasifikacijske kriterije za Sjögrenov sindrom. Bolesnici su razvrstani u dvije skupine; njih 28 (77,8 %) je ispunjavalo klasifikacijske kriterije za dijagnozu primarnoga Sjögrenovog sindroma, a osam (22,2 %) ih nije ispunjavalo klasifikacijske kriterije za dijagnozu. *Rezultati.* Rezultati analize pokazali su pozitivnu korelaciju dijagnoze primarnoga Sjögrenovog sindroma i „pozitivne" biopsije (rho = 0,93, p < 0,001) i seroloških nalaza (rho = 0,38, p = 0,023) te negativnu korelaciju s brzinom izlučivanja sline (rho = –0,41, p = 0,012). Rezultati „pozitivne" biopsije bili su u negativnoj korelaciji s brzinom izlučivanja sline (rho = –0,41, p = 0,012) dok je jača korelacija utvrđena kod pacijenata s nalazom ispod dijagnostičke razine brzine izlučivanja (≤ 0,1 ml/min, rho = 0,46, p = 0,005). *Zaključak.* Zaključno, nestimulirana brzina izlučivanja sline ≤ 0,1 ml/min objektivizirana sijalometrijom snažan je prediktor „pozitivne" biopsije te se može upotrebljavati kao dodatna metoda uz biopsiju u dijagnostici oralne komponente primarnoga Sjögrenovog sindroma.

Ključne riječi: Sjögrenov sindrom; Sijalometrija; Biopsija; Žlijezda; Slinovnica; Klasifikacija

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a chronic, lymphoproliferative, autoimmune disease affecting exocrine glands. The main features of the disease are dry mouth (xerostomia) and dry eyes (xerophthalmia) resulting from immune-mediated damage and dysfunction of the salivary and lacrimal glands (1, 2). The disease can be of varying intensity, from milder forms involving oral and ocular dryness, fatigue and pain, to more severe systemic conditions affecting multiple organs (3). Patients are at increased risk of developing lymphoma, particularly non-Hodgkin's lymphoma, compared with the general population (4). Thus, the appropiate and timely diagnosis is important due to clinical follow-up of the possible complications.

The disease occures in all age groups; however it mainly affects women, at ratio of 9:1 (5). The diagnosis of pSS commonly requires a multidisciplinary approach, including rheumatologists, oral medicine specialists, ophthalmologists and it relies on interpreting and integrating all aspects of the patient's medical history, clinical and laboratory testing and clinical experience of the physician (6, 7). The optimal management of pSS is not yet clear and the current treatments are mainly symptomatic.

The development of classification criteria for use in clinical practice as well as studies have occupied the scientific literature for many decades. Misdiagnosis of pSS is common and numerous studies have shown great variation in the frequency of pSS. Approximately half of all patients are thought to be undiagnosed (8). That makes it one of the least understood inflammatory diseases in current clinical practice. Although various classification criteria for pSS have been proposed, a revised version of the European criteria proposed by the American-European Consensus Group (AECG) has been the most widely used classification set (9). The revised version includes six criteria, four of the six criteria are necessary to confirm the diagnosis of pSS, among which should be either a „positive" histopathological finding or the presence of autoantibodies (anti-SSA and/or anti-SSB) (10). In daily clinical practice,

UVOD

Primarni Sjögrenov sindrom (pSS) kronična je, limfoproliferativna, autoimuna bolest koja zahvaća egzokrine žlijezde. Glavne su značajke ove bolesti suhoća usne šupljine (kserostomija) i očiju (kserofralmija) koje su posljedica imunološki posredovanog oštećenja i disfunkcije žlijezda slinovnica i suznih žlijezda (1, 2). Bolest može biti različitog intenziteta, od blažih oblika koji uključuju suhoću usne šupljine i očiju, umor i bol do težih oblika sistemskih bolesti koje zahvaćaju više organa (3). U usporedbi s općom populacijom, osobe koje boluju od primarnoga Sjögrenovog sindroma imaju povećan rizik od razvoja limfoma, osobito ne-Hodgkinovog limfoma (4). Stoga je odgovarajuća i pravovremena dijagnoza bitna zbog kliničkog praćenja bolesnika u slučaju razvoja mogućih komplikacija.

Ova bolest zahvaća sve dobne skupine, no češće se javlja u žena i to u omjeru 9:1 (5). Dijagnoza primarnoga Sjögrenovog sindroma (pSS) obično zahtijeva multidisciplinarni pristup koji uključuje više različitih stručnjaka, poput reumatologa, specijalista oralne medicine, oftalmologa i oslanja se na tumačenje i integraciju svih aspekata bolesnikove povijesti bolesti, kliničkih i laboratorijskih ispitivanja te kliničkog iskustva liječnika (6, 7). Optimalna metoda liječenja primarnoga Sjögrenovog sindroma (pSS) još uvijek nije jasno definirana, a terapije koje se danas upotrebljavaju uglavnom su simptomatske.

Razvoj klasifikacijskih kriterija te istraživanja za primjenu u kliničkoj praksi teme su koje se u znanstvenoj literaturi obrađuju već desetljećima. Pogrešna dijagnoza primarnoga Sjögrenovog sindroma (pSS) uobičajena je, a brojna istraživanja pokazala su i izrazita odstupanja u učestalosti pojave pSS-a. Smatra se da u otprilike 50% slučajeva bolest ostaje nedijagnosticirana (8). To je čini jednom od upalnih bolesti koje najmanje razumijemo u današnjoj kliničkoj praksi. Iako su predloženi različiti klasifikacijski kriteriji za pSS, najviše se upotrebljava klasifikacija utvrđena u revidiranoj verziji europskih kriterija koje je predložila organizacija *American-European Consensus Group* (AECG) (9). U revidiranoj verziji navodi se šest kriterija. Četiri od tih šest

the only reliable „gold" standard for the diagnosis of pSS is the clinical judgement of an experienced physician confirmed with objective complementary tests.

Minor salivary gland biopsy (MSGB) is widely accepted and currently remains the best method for diagnosing the salivary gland damage (11), but it is not a *sine qua non* criterion for diagnosis of pSS. Histopathological finding of lymphocytic sialoadenitis is highly informative, but represents an invasive diagnostic method. Focal lymphocytic infiltrates of minor labial salivary glands are considered target-organ specific for pSS, but it may also be present in other autoimune diseases as well as in aquired immunodeficiency syndrome (AIDS) (12, 13, 14). External factors such as smoking and medications have also been suggested to influence the focal lymphocytic infiltrates (15).

The anti-SSA and/or anti-SSB autoantibodies are considered typical among pSS patients. Depending on different laboratory methods, anti-SSA and/or anti-SSB autoantibodies are detected in about 50% to 70% of patients with pSS (16). It is well known that negative serology results occur in 10% to 50% of patients with pSS and correlate with a milder form of the disease (17). The presence of autoantibodies (anti-SSA and/or anti-SSB) have been included in all classifications and usually precede MSGB on routine pSS diagnosis.

The approach to the definition of glandular involvement in pSS is constantly evolving, but in daily practice, clinicians tend to use less invasive diagnostic methods. Thus having access to less invasive but sensitive confirmatory tests that complement existing immunological and/or histopathological findings would be a great advantage. The objective assessment of oral and ocular dryness could be one of such complementary tests. Current literature suggests good agreement between ocular component and pSS, but the previous results regarding xerostomia are inconclusive and possibly confusing. Evaluation of xerostomia in patients with clinically suspected pSS is a diagnostic challenge. Sialometry results can vary considerably because xerostomia can be a side effect of numerous medications (antidepressants, antihistamines, diuretics, etc.) or previous treatment (e.g., radiotherapy of the head and neck) (18).

Sialometry is widely applied in diagnosing xerostomia. Several methods for collecting saliva have been reported. Salivary flow measurement is simple and quick diagnostic method, needs no special equipment and was shown to have good reproducibility. Sialometry has a sensitivity (56.1%) and specificity (80.7%) that is lower than the sensitivity (84.4%) and specificity (86.2%) of MSGB (19); however, sialometry, unlike MSGB, is completely uninvasive diagnostic method. Unstimulated whole saliva (UWS) flow rate ≤0.1 ml/

potrebno je za potvrdu dijagnoze pSS-a, a moraju uključivati pozitivan patohistološki nalaz ili prisutnost autoantitijela (anti-SS-A i/ili anti-SS-B) (10). U svakodnevnoj kliničkoj praksi jedinim pouzdanim „zlatnim" standardom za dijagnozu pSS-a smatra se klinička prosudba iskusnog liječnika potvrđena objektivnim komplementarnim ispitivanjima.

Biopsija malih žlijezda slinovnica (engl. *minor salivary gland biopsy*, *MSGB*) široko je prihvaćena i trenutačno najbolja metoda za dijagnozu oštećenja žlijezda slinovnica, ali nije *condicio sine qua non* za dijagnozu pSS-a. Patohistološki nalaz limfocitnog sijaloadenitisa pruža mnogo informacija, ali predstavlja invazivnu dijagnostičku metodu. Fokalni infiltrati limfocita malih labijalnih žlijezda slinovnica smatraju se ciljnim organom specifičnim za pSS, ali mogu biti prisutni i kod drugih autoimunih bolesti, kao i kod sindroma stečene imunodeficijencije (AIDS) (12, 13, 14). Također se smatra da vanjski čimbenici poput pušenja i uzimanja lijekova utječu na fokalne infiltrate limfocita (15).

Anti-SS-A i/ili anti-SS-B autoantitijela tipična su za bolesnike koji boluju od pSS-a. Ovisno o različitim laboratorijskim metodama, anti-SS-A i/ili anti-SS-B autoantitijela otkrivaju se u otprilike 50 – 70% bolesnika koji boluju od pSS-a (16). Poznato je da se negativni serološki rezultati javljaju u 10 – 50% bolesnika koji boluju od pSS-a te da su u korelaciji s blažim oblikom bolesti (17). Pretrage za utvrđivanje prisutnosti autoantitijela (anti-SS-A i/ili anti-SS-B) uključene su u sve klasifikacije i obično se izvode prije biopsije malih žlijezda slinovnica u rutinskoj dijagnostici pSS-a.

Pristup definiciji zahvaćenosti žlijezda u pSS-u stalno se razvija, ali u svakodnevnoj praksi kliničari su više skloni upotrebljavati manje invazivne dijagnostičke metode. Stoga bi pristup manje invazivnim, ali osjetljivim potvrdnim ispitivanjima koja bi se obavljala kao komplementarna uz postojeće imunološke i/ili patohistološke nalaze bio jako koristan. Objektivna procjena suhoće usne šupljine i očiju mogla bi biti jedno od takvih komplementarnih ispitivanja. U novijoj literaturi ističe se povezanost između očne komponente i pojave pSS-a, ali raniji rezultati u vezi s kserostomijom nepotpuni su i zbunjujući. Procjena kserostomije u bolesnika sa suspektnim pSS-om postupak je koji predstavlja pravi dijagnostički izazov. Rezultati sijalometrije mogu pokazivati značajna odstupanja jer kserostomija može biti nuspojava uzimanja brojnih lijekova (antidepresivi, antihistaminici, diuretici itd.) ili prethodnih terapija (npr. radioterapija glave i vrata) (18).

Sijalometrija se u velikoj mjeri primjenjuje u dijagnostici kserostomije. U literaturi se navode razne metode za prikupljanje sline. Mjerenje izlučivanja sline jednostavna je i brza dijagnostička metoda koja ne zahtijeva uporabu posebne opreme i za koju je dokazano da ima dobru ponovljivost. Osjetljivost (56,1%) i

minute is one of the classification criteria for the diagnosis of pSS (10, 20, 21).

In the study by Liquidato *et al.*, authors concluded that both isolated MSGB and sialometry can be used in screening patients with clinically suspected pSS because those diagnostic methods have shown no difference in sensitivity. If there are limitations in the classification of patients, the combination of a „positive" histopathological finding and sialometry increases the chance of making a correct diagnosis (22).

The main objective of our study was to retrospectively evaluate correlation between MSGB, serological findings and sialometry in patients with clinically suspected pSS.

SUBJECTS AND METHODS

This is a retrospective study involving patients with subjective complaint of oral and ocular dryness who were examined at the Department of Internal Medicine, Clinical Hospital Centre „Sestre milosrdnice", Zagreb in a period of three years. Clinical oral examination with appropriate diagnostic tests was performed at the Department of Oral Medicine, School of Dental Medicine, University of Zagreb and classification was made based on revised AECG criteria (10). The symptoms of ocular and/or oral dryness or various extraglandular symptoms were the initial reason for specialist consultation and raised suspicion of pSS.

The study was conducted after approval by the Ethics Committee of the Clinical Hospital Centre „Sestre milosrednice" regarding the retrospective collection of data. After signing the informed consent, the patients were included in the study. The study was performed in accordance with the World Medical Association, Declaration of Helsinki (23).

The diagnosis of pSS was made by experienced rheumatologists in collaboration with oral medicine specialists and ophthalmologists. Patients were classified into two groups, pSS group and non-pSS group. Primary Sjögren's syndrome was diagnosed based on revised AECG classification criteria, considering the results of the 6-item screening questionnaire for oral and ocular dryness, ocular test (Schirmer's test), measurement of timed UWS flow rate, MSGB, presence of autoantibodies (anti-SSA and/or anti-SSB), and expert clinical judgement (10). Non-pSS group included patients who have not been diagnosed with pSS because they were not fulfilling the revised AECG diagnostic criteria. Only patients who underwent MSGB were included in the study after signing informed consent.

Exclusion criteria were: non signing informed consent; patients with radiation-induced xerostomia, sarcoidosis-associated xerostomia, current use of anticholinergic drugs; patients with history of secondary SS,

specifičnost (80,7%) sijalometrije niže su od osjetljivosti (84,4%) i specifičnosti (86,2%) biopsije malih žlijezda slinovnica (19), no, za razliku od biopsije malih žlijezda slinovnica, sijalometrija je u potpunosti neinvazivna dijagnostička metoda. Nestimulirana brzina izlučivanja sline ≤ 0,1 ml/min jedan je od klasifikacijskih kriterija za dijagnozu pSS-a (10, 20, 21).

U istraživanju koje su proveli Liquidato i sur. autori su zaključili da se i izolirana biopsija malih žlijezda slinovnica i sijalometrija mogu upotrebljavati u probiru bolesnika sa suspektnim pSS-om jer te dijagnostičke metode nisu pokazale razliku u osjetljivosti. Ako postoje ograničenja u klasifikaciji bolesnika, kombinacija „pozitivnoga" patohistološkog nalaza i sijalometrije povećava vjerojatnost za postavljanje točne dijagnoze (22).

Glavni cilj našeg istraživanja bio je retrospektivno procijeniti korelaciju između biopsije malih žlijezda slinovnica, seroloških nalaza i sijalometrije u bolesnika sa suspektnim pSS-om.

ISPITANICI I METODE

Ovo je retrospektivna studija koja uključuje bolesnike sa subjektivnim pritužbama na suhoću usne šupljine i očiju koji su tijekom razdoblja od tri godine bili pregledani u Klinici za unutarnje bolesti KBC-a Sestre milosrdnice u Zagrebu. Klinički pregled usne šupljine s odgovarajućim dijagnostičkim pretragama obavljen je na Zavodu za oralnu medicinu Stomatološkog fakulteta Sveučilišta u Zagrebu, a klasifikacija je izvršena na temelju revidiranih AECG kriterija (10). Simptomi suhoće usne šupljine i/ili očiju ili razni ekstraglandularni simptomi bili su primarni razlog za savjetovanje sa specijalistom te su upravo oni izazvali sumnju na pSS.

Studija je provedena nakon odobrenja Etičkog povjerenstva KBC-a Sestre milosrdnice u pogledu retrospektivnog prikupljanja podataka. Nakon potpisivanja informiranog pristanka bolesnici su uključeni u istraživanje. Studija je provedena u skladu s Helsinškom deklaracijom Svjetskoga liječničkog udruženja (23).

Dijagnozu pSS-a postavili su iskusni reumatolozi u suradnji sa specijalistima oralne medicine i oftalmolozima. Bolesnici su razvrstani u dvije skupine: pSS skupinu i ne-pSS skupinu. Primarni Sjögrenov sindrom dijagnosticiran je na temelju revidiranih AECG klasifikacijskih kriterija, uzimajući u obzir rezultate probirnog upitnika od 6 stavki koji je uključivao suhoću usne šupljine i očiju, test suhog oka (Schirmerov test), mjerenje brzine izlučivanja sline (UWS), biopsiju malih žlijezda slinovnica (MSGB), prisutnost autoantitijela (anti-SS-A i/ili anti-SS-B) te kliničku procjenu stručnjaka (10). U ne-pSS skupinu svrstani su bolesnici kojima nije dijagnosticiran pSS jer nisu ispunjavali revidirane AECG dijagnostičke kriterije. U studiju su bili uključeni samo bolesnici kojima je učinjena biopsija

hepatitis C virus (HCV) infection, human immunodeficiency virus (HIV) infection, pre-existing lymphoma, graft-versus-host disease (GvHD); patients without clinical, laboratory and histopathological findings.

The results of clinical, laboratory and histopathological findings were stored in medical files. The data collected included age, gender, family history, body mass index (BMI), subjective symptoms of xerostomia and xerophthalmia, ocular tests, cerebrovascular risk factors (hypertension, *diabetes mellitus* (DM), smoking, hyperlipidemia), as well as history of other comorbidities. The time (in months) from the initial onset of symptoms to the diagnosis of pSS was also recorded as well as information of previous or current use of immunosuppressants before the MSGB.

Clinical oral examination included measurement of UWS flow rate according to a sialometry protocol (20, 24). Patients were asked to refrain from eating, drinking, smoking and brushing their teeth for at least two hours prior to saliva sampling. Saliva was collected in graduated tubes for five minutes using the spitting method. The UWS flow rate ≤0.1 ml/minute indicated reduced secretion of salivary glands (10, 20, 21).

Minor salivary gland biopsy was performed from the inner surface of the lower lip mucosa under local anesthesia at the Department of Oral Medicine, School of Dental Medicine, University of Zagreb. Histopathological analysis of MSGB was performed at the Department of Pathology and Cytology, University Hospital Centre Zagreb. A "positive" biopsy finding is a classification criteria that highly indicates pSS and refers to the presence of focal lymphocytic sialoadenitis with a focus score ≥1 at 4 mm^2 of glandular tissue (1 focus is a cluster of 50 CD4 + cells) (25).

We also used EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) to measure perception of patients' symptoms (26). It is a very simple questionnaire to assess disease symptoms and activity on a 10-point scale for pain, fatigue and dryness. The scores from three questions are added together, resulting in a total score that varies from 3 (very mild symptoms) to 30 (maximum symptoms). With good construct validity, it is used as an outcome measure in clinical trials (27).

Statistical analysis was performed using MedCalc version 11.4 software. The normal distribution of continuous variables was assessed by the Kolomogrov-Smirnov test. The continuous variables are expressed as mean ± standard deviation (SD) or median (interquartile range) as appropriate. Categorical variables are expressed as a number (%). Student's t-test was used to compare mean values between groups where the distribution was normal. Mann-Whitney nonparametric test was used when the distribution of values was not normal. The significance level (P value) for all analyses was defined as P <.05. The correlation be-

malih žlijezda slinovnica i to nakon potpisivanja informiranog pristanka.

Kriteriji isključenja bili su: nepotpisivanje informiranog pristanka; bolesnici koji boluju od kserostomije uzrokovane zračenjem, kserostomije povezane sa sarkoidozom, koji trenutačno uzimaju antikolinergike, bolesnici koji u anamnezi imaju sekundarni Sjögrenov sindrom, infekciju virusom hepatitisa C (HCV), infekciju virusom humane imunodeficijencije (HIV), bolesnici s već postojećim limfomom, oni koji imaju bolest presatka protiv primatelja (GvHD) te bolesnici bez kliničkih, laboratorijskih i patohistoloških nalaza.

Rezultati kliničkih, laboratorijskih i patohistoloških nalaza pohranjeni su u medicinskoj dokumentaciji. Prikupljeni podatci uključivali su dob, spol, obiteljsku anamnezu, indeks tjelesne mase (BMI), subjektivne simptome kserostomije i kseroftalmije, očne pretrage, rizične čimbenike cerebrovaskularne bolesti (hipertenzija, *diabetes mellitus* (DM), pušenje, hiperlipidemija) te anamnezu drugih komorbiditeta. Također je zabilježeno vrijeme (u mjesecima) od početne pojave simptoma do dijagnoze pSS-a, kao i informacije o prethodnoj ili trenutačnoj primjeni imunosupresiva prije biopsije malih žlijezda slinovnica.

Klinički pregled usne šupljine uključivao je mjerenje brzine izlučivanja sline (UWS) u skladu s protokolom sijalometrije (20, 24). Bolesnici su zamoljeni da se suzdrže od jela, pića, pušenja i pranja zubi najmanje dva sata prije uzimanja uzorka sline. Slina je sakupljena metodom pljuvanja u graduirane epruvete tijekom razdoblja od pet minuta. Brzina izlučivanja sline ≤ 0,1 ml/min ukazuje na smanjeno lučenje žlijezda slinovnica (10, 20, 21).

Biopsija malih žlijezda slinovnica izvedena je s unutarnje strane sluznice donje usne, u lokalnoj anesteziji, na Zavodu za oralnu medicinu Stomatološkog fakulteta Sveučilišta u Zagrebu. Patohistološka analiza biopsije malih žlijezda slinovnica provedena je na Kliničkom zavodu za patologiju i citologiju KBC-a Zagreb. Rezultat „pozitivne" biopsije klasifikacijski je kriterij koji s velikom točnošću ukazuje na pSS i odnosi se na prisutnost fokalnoga limfocitnog sijaloadenitisa s brojem fokusa ≥ 1 na 4 mm^2 žljezdanog tkiva (nakupina od 50 CD4 + stanica čini jedan fokus) (25).

Također smo upotrebljavali indeks subjektivnih bolesnikovih tegoba Europske lige protiv reumatizma (engl. *European League Against Rheumatism Sjögren's syndrome patient report index*, dalje u tekstu: ESSPRI) za mjerenje percepcije simptoma bolesti u bolesnika (26). To je vrlo jednostavan upitnik za procjenu simptoma bolesti i aktivnosti na skali od 1 do 10 prema kojoj bolesnici procjenjuju subjektivni osjećaj boli, umora i suhoće. Bodovi iz tri pitanja zbrajaju se te se time dobiva ukupan rezultat u rasponu od 3 (vrlo blagi simptomi) do 30 bodova (najteži simptomi). Zbog

tween clinical, serological and histological findings was analysed using Spearman correlation coefficient.

RESULTS

A total of 56 MSGB were performed at the Department of Oral Medicine, School of Dental Medicine, University of Zagreb. Of the initial number of patients, 36 patients (34 female and 2 male) were included in the study because they met the inclusion criteria. Twenty patients were excluded due to lack of clinical or/and laboratory findings or presence of other exclusion criteria.

The mean age of the patients was 53.4 years (ranging from 23 to 77 years) and 34 patients were female (94.4%). According to the revised AECG criteria, 36 patients were divided into two groups, a pSS group (n=28; male/female: 2/26) and a non-pSS group (n=8; male/female: 0/8). Patients in the non-pSS group had subjective oral and ocular dryness but did not meet the diagnostic criteria for pSS diagnosis because they had negative serology and/or histopathological findings that were not consistent with the revised AECG diagnostic criteria. Eighteen patients (64.3%) did not meet serological criteria for the diagnosis of pSS but met the histopathologic criteria. The demographic, clinical and laboratory characteristics of pSS and non-pSS patients are showed in Table 1.

The results showed that the two groups shared most of the features similarly. There are statistically significant differences between these two groups in BMI and obesity. Proportion of obese patients in the pSS group was higher than in the non-pSS group (71.4% *vs* 12.5%, p=0.005). There was a higher proportion of smokers in the pSS group (17.9% *vs* 0%, p=0.566), although it was not statistically signifficant. The time from onset of symptoms to diagnosis of pSS ranged from 1 to 84 months, with a mean of 36 months. Two (5.6%) of a total of 36 patients underwent immunosuppressive therapy prior to MSGB.

The perception of symptoms was similar among the groups according to the ESSPRI assessment (Table 2). It is very interesting to notice that the perception of pain measured by the ESSPRI questionnaire was statistically significant lower (p=0.041) in the pSS group than in the non-pSS group, but we must take into account the small number of patients included in interpreting the results. Studies with a larger number of patients are needed to make relevant conclusions.

Serological testing was performed in all 36 patients. At least one autoantibody (anti-SSA or anti-SSB) was positive in 14 patients (38.9%) (Table 1). The frequency of anti-SSA autoantibodies in the pSS group was statistically significant higher than in the non-pSS group (42.9% *vs* 0%, p=0.033), while the frequency of anti-

dobre konstruktivne valjanosti ovaj se upitnik upotrebljava za mjerenje rezultata u kliničkim ispitivanjima (27).

Statistička analiza provedena je pomoću softvera *MedCalc* (verzija 11.4). Normalna raspodjela kontinuiranih varijabli procijenjena je pomoću Kolomogrov-Smirnovljeva testa. Kontinuirane varijable izražene su kao srednja vrijednost ± standardna devijacija (SD) ili medijan (interkvartilni raspon, IQR) prema potrebi. Kategoričke varijable izražavaju se u brojčanom obliku (%). Studentov t-test upotrijebljen je za usporedbu srednjih vrijednosti između skupina u kojima je raspodjela bila normalna. Mann-Whitneyjev neparametrijski test upotrijebljen je u slučajevima u kojima raspodjela vrijednosti nije bila normalna. Razina značajnosti (p-vrijednost) definirana je kao p <,05 za sve analize. Povezanost kliničkih, serоloških i histoloških nalaza analizirana je s pomoću Spearmanovog koeficijenta korelacije.

REZULTATI

Na Zavodu za oralnu medicinu Stomatološkog fakulteta Sveučilišta u Zagrebu provedeno je ukupno 56 biopsija malih žlijezda slinovnica. Od početnog broja bolesnika u istraživanje je uključeno njih 36 (34 žene i 2 muškarca) koji su zadovoljili kriterije uključenja. Dvadeset bolesnika isključeno je zbog nedostatka kliničkih i/ili laboratorijskih nalaza ili prisutnosti drugih kriterija isključenja.

Prosječna dob bolesnika bila je 53,4 godine (u rasponu od 23 do 77 godina), a od ukupnog broja bolesnika njih 34 bilo je ženskog spola (94,4 %). Prema revidiranim AECG kriterijima, 36 bolesnika podijeljeno je u dvije skupine, pSS skupinu (n = 28; broj muškaraca / broj žena: 2/26) i ne-pSS skupinu (n = 8, broj muškaraca / broj žena: 0/8). Bolesnici u ne-pSS skupini imali su simptom subjektivne suhoće usne šupljine i očiju, ali nisu ispunjavali dijagnostičke kriterije za dijagnozu pSS-a jer su imali negativne serološke i/ili patohistološke nalaze koji nisu bili u skladu s revidiranim AECG dijagnostičkim kriterijima. Osamnaest bolesnika (64,3%) nije zadovoljilo serološke kriterije za dijagnozu pSS-a, ali je zadovoljilo patohistološke kriterije. Demografske, kliničke i laboratorijske karakteristike bolesnika koji boluju od pSS-a i bolesnika koji ne boluju od pSS-a prikazane su u tablici 1.

Rezultati su pokazali da obje skupine imaju većinu sličnih karakteristika. Između ove dvije skupine postoje statistički značajne razlike u indeksu tjelesne mase i pretilosti. Udio pretilih bolesnika u pSS skupini bio je veći nego u ne-pSS skupini (71,4% prema 12,5%, p = 0,005). Skupina pSS imala je veći udio pušača (17,9% prema 0%, p = 0,066), iako taj broj nije bio statistički značajan. Vremensko razdoblje od pojave simptoma

Table 1. Demographic, clinical and laboratory characteristics of pSS group according to the revised AECG criteria
Tablica 1. Demografske, kliničke i laboratorijske karakteristike pSS skupine prema revidiranim AECG kriterijima

Variable / Varijabla	Patients with pSS (n=28) / Bolesnici koji boluju od pSS-a (n = 28)	Non-pSS patients (n=8) / Bolesnici koji ne boluju od pSS-a (n =28)	p value* / p-vrijednost*
Age, mean (SD) / Dob, srednja vrijednost (SD)	63.9 (13.0)	67.5 (10.0)	0.472
Age at diagnosis, mean (SD) / Dob pri postavljanju dijagnoze, srednja vrijednost (SD)	52.3 (12.6)	57.6 (11.4)	0.285
Gender – Female / Spol – ženski	26 (92.9%)	8 (100%)	1
Immunotherapy / Imunoterapija	7 (25%)	1 (12.5%)	0.651
History of cardiovascular diseases / Bolesnici koji u anamnezi imaju kardiovaskularne bolesti	17 (60.7%)	3 (37.5%)	0.422
Smoking / Pušenje	5 (17.9%)	0 (0.0%)	0.566
Exercise / Fizička aktivnost	10 (35.7%)	2 (25.0%)	0.691
BMI, mean (SD) / Indeks tjelesne mase (BMI), srednja vrijednost (SD)	26.5 (4.1)	23.4 (2.0)	**0.006**
Overweight / Pretilost	20 (71.4%)	1 (12.5%)	**0.005**
Hyperlipidemia / Hiperlipidemija	9 (32.1%)	3 (37.5%)	1
Diabetes mellitus	11 (39.3%)	2 (25.0%)	0.682
Hypertension / Hipertenzija	13 (46.4%)	6 (75.0%)	0.236
"positive" MSGB / „Pozitivna" biopsija malih žlijezda slinovnica	27 (96.4%)	0 (0.0%)	< **0.001**
anti-SSA / anti-SS-A	12 (42.9%)	0 (0.0%)	**0.033**
anti-SSB / anti-SS-B	10 (35.7%)	2 (25.0%)	0.691
Either anti-SSA or SSB / Anti SS-A ili anti-SS-B	12 (42.9%)	2 (25.0%)	0.441
Saliva flow, median (IQR) / Brzina izlučivanja sline, medijan (IQR)	0.1 (0.04 – 0.2)	0.48 (0.3 – 0.6)	**0.003**
Low saliva flow / Niska brzina izlučivanja sline	21 (75.0%)	1 (12.5%)	**0.003**

Legend / Legenda: pSS – primary Sjögren's syndrome / primarni Sjögrenov sindrom; AECG: American-European Consensus Group
** Fischer's exact test and t-test were used, significant differences indicated in bold / Upotrebljavani su Fisherov test i t-test, značajne razlike označene su podebljanim slovima*

SSB autoantibodies was similar in both groups (35.7% *vs* 25.0%, p=0.69).

Mann–Whitney test showed that UWS flow rate was statistically significant lower in pSS group (mean UWS flow rate 0.148 +/– 0.147 *vs* 0.463 +/– 0.259 ml/minute, p=0.003). Unstimulated whole salivary flow rate is an objective diagnostic test to assess the involvement of the salivary glands. It was performed in all patients (n=36). The results of 22 (61.1%) patients showed hyposalivation according to the revised AECG diagnostic criteria for diagnosing pSS (abnormal UWS flow rate ≤ 0.1 ml/ minute) (Table 1). In the pSS group, 75% of patients had low UWS flow rate (*vs* 12.5% in non-pSS group). The results of UWS flow rates are presented in Figure 1. The difference is statistically significant (Mann–Whitney test p=0.003).

The correlation between histopathological and serological findings, salivary gland involvement and clinical features were assessed by Spearman rank correlation. As expected, pSS diagnosis strongly correlated

do dijagnoze pSS-a bilo je u rasponu od 1 do 84 mjeseca, a u prosjeku je iznosilo 36 mjeseci. Dva (5,6%) od ukupno 36 bolesnika uzimala su imunosupresivnu terapiju prije biopsije malih žlijezda slinovnica.

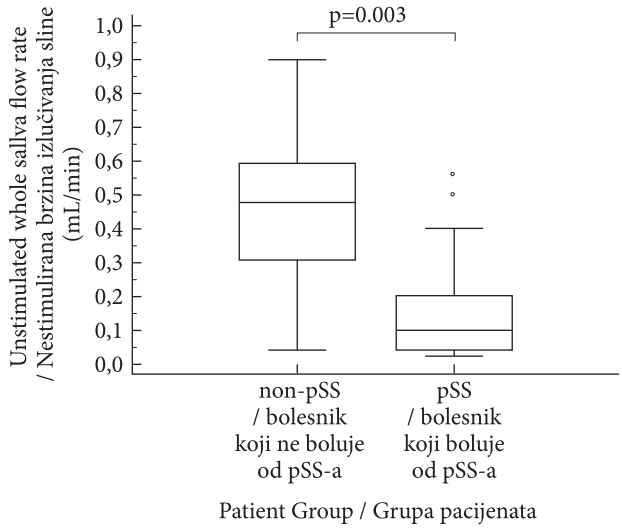
Prema ESSPRI upitniku za procjenu, percepcija simptoma bolesti u obje skupine bila je slična (tablica 2). Zanimljivo je da je percepcija boli mjerena ESSPRI upitnikom statistički značajno niža (p = 0,041) u pSS skupini nego u ne-pSS skupini, ali moramo uzeti u obzir mali broj bolesnika uključenih u tumačenje rezultata. Za donošenje relevantnih zaključaka potrebno je provesti studije s većim brojem bolesnika.

Svih 36 bolesnika podvrgnuto je serološkim ispitivanjima. Najmanje jedno autoantitijelo (anti-SS-A ili anti-SS-B) bilo je pozitivno kod 14 bolesnika (38,9%) (tablica 1). Učestalost anti-SS-A autoantitijela u pSS skupini bila je statistički značajno veća nego u ne-pSS skupini (42,9% prema 0%, p = 0,033), dok je učestalost anti-SS-B autoantitijela bila slična u obje skupine (35,7% prema 25,0%, p = 0,69).

Table 2. Results of ESSPRI measurements in the pSS and non-pSS groups.
Tablica 2. Rezultati ESSPRI mjerenja u pSS i ne-pSS skupini

Self-reported measure / Mjera samoprocjene	Patients with pSS (n=28), Median (IQR) / Bolesnici koji boluju od pSS-a (n = 28), Median (IQR)	Non-pSS patients (n=8), Median (IQR) / Bolesnici koji ne boluju od pSS-a (n = 8), Median (IQR)	p value* / p-vrijednost*
Dryness score / Ocjena suhoće (prema bodovnoj skali)	8 (7.5 – 9)	8 (7 – 9)	0.533
Pain score / Ocjena boli (prema bodovnoj skali)	5 (5 – 7)	6.5 (6 – 8)	**0.041**
Fatigue score / Ocjena umora (prema bodovnoj skali)	5 (4 – 7)	6.5 (6 – 8)	0.070
Total score / Ukupan rezultat	19 (16 – 22)	21 (20 – 22.5)	0.147

Legend / Legenda: pSS – primary Sjögren's syndrome / primarni Sjögrenov sindrom
ESSPRI: European League Against Rheumatism Sjögren's syndrome patient report index
** Mann-Whitney test, significant p-values highlighted in bold / Mann-Whitneyjev test, značajne p-vrijednosti istaknute su podebljanim slovima*



Legend / Legenda: pSS – primary Sjögren's syndrome / primarni Sjögrenov sindrom

Figure 1. Unstimulated whole saliva flow rate in pSS and non-pSS patients
Slika 1. Nestimulirana brzina izlučivanja sline u bolesnika koji boluju od pSS-a i bolesnika koji ne boluju od pSS-a

with MSGB (rho=0.93, p<0.001), BMI (rho=0.41, p=0.013), the presence of anti-SSA autoantibody (rho=0.38, p=0.023) and negatively correlated with UWS flow rate (rho= –0.51, p=0.002) and ESSPRI pain score (rho= –0.35, p=0.037). Also, positive MSGB results were in negative correlation with UWS flow rate (rho= –0.41, p=0.012) but in a stronger correlation with patients below the diagnostic flow rate cutoff (≤0.1 ml/minute, rho=0.46, p=0.005). The detection of either autoantibody (anti-SSA or anti-SSB) was not in correlation with other parameters but both were correlated well with diagnosis (rho= –0.48, p=0.003 and rho= –0.42, p=0.011, for A and B, respectively).

It is interesting to note that diagnostic criteria did not correlate statistically significant with ESSPRI scores or most demographic or clinical parameters. A good

Mann-Whitneyjevim testom utvrđeno je da je brzina izlučivanja sline statistički značajno niža u pSS skupini (prosječna brzina izlučivanja sline 0,148 +/– 0,147 prema 0,463 +/– 0,259 ml/minuti, p = 0,003). Nestimulirana brzina izlučivanja sline objektivna je dijagnostička pretraga za procjenu zahvaćenosti žlijezda slinovnica. Ova je pretraga obavljena na svim bolesnicima (n = 36). Kod 22 (61,1%) bolesnika rezultati pretraga ukazali su na hiposalivaciju prema revidiranim AECG dijagnostičkim kriterijima za dijagnozu pSS-a (abnormalna brzina izlučivanja sline ≤ 0,1 ml/min) (tablica 1). U pSS skupini kod 75% bolesnika utvrđena je niska brzina izlučivanja sline (u odnosu na 12,5% u ne-pSS skupini). Rezultati brzine izlučivanja sline prikazani su na slici 1. Razlika je statistički značajna (Mann-Whitney test, p = 0,003).

Povezanost patohistoloških i seroloških nalaza, zahvaćenosti žlijezda slinovnica i kliničkih slika procijenjena je Spearmanovim koeficijentom rang korelacije. Očekivano, dijagnoza pSS-a bila je u snažnoj korelaciji s biopsijom malih žlijezda slinovnica (rho = 0,93, p < 0,001), indeksom tjelesne mase (rho = 0,41, p = 0,013), prisutnošću anti-SS-A autoantitijela (rho = 0,38, p = 0,023) i u negativnoj korelaciji s brzinom izlučivanja sline (rho = –0,51, p = 0,002) i ocjenom boli prema bodovnoj skali upitnika ESSPRI (rho = –0,35, p = 0,037). Također, rezultati „pozitivne" biopsije malih žlijezda slinovnica bili su u negativnoj korelaciji s brzinom izlučivanja sline (rho = –0,41, p = 0,012), dok je jača korelacija utvrđena kod pacijenata s nalazom ispod dijagnostičke razine brzine izlučivanja (≤ 0,1 ml/minuti, rho = 0,46, p = 0,005). Otkrivanje bilo kojeg autoantitijela (anti-SS-A ili anti-SS-B) nije bilo u korelaciji s drugim parametrima, ali su oba autoantitijela bila u dobroj korelaciji s dijagnozom (rho = –0,48, p = 0,003 i rho = –0,42, p = 0,011, za A i B).

Zanimljivo je da dijagnostički kriteriji nisu bili u statistički značajnoj korelaciji s ESSPRI ocjenama ili većinom demografskih ili kliničkih parametara. Dobra ne-

negative correlation was observed only for anti-SSA and anti-SSB autoantibodies and the age of the patient at the time of diagnosis (rho= –0.48, p=0.003 and rho= –0.42, p=0.011 for A and B, respectively). ESSPRI pain and fatigue scores correlated between themselves (rho= 0.73, p<0.0001) but neither correlated with ESSPRI dryness score. Interestingly the dryness score correlated well with patient BMI (rho= 0.57, p=0.0003). Additionally, exercising was negatively correlated with both ESSPRI fatigue (rho –0.37, p=0.026) and ESSPRI total scores (rho= –0.41, p=0.014) indicating that it can alleviate some of the subjective symptoms and improve overall self-perception.

DISCUSSION

The objective of our study was to retrospectively evaluate correlation between MSGB, serological findings and sialometry in patients with clinically suspected pSS. From our experience, in early stage pSS can be misdiagnosed as burning mouth symptoms. Several reports frequently show a delay in the diagnosis of pSS that can range from 3 to 11 years (18.). Already in early stage of disease patients have reduced UWS flow rate, sometimes even without salivary gland ultrasonography abnormalities (28). Using UWS flow rate may improve the timely diagnosis of the disease and should be part of the classification criteria for diagnosing pSS.

The fact that multiple classification criteria for pSS are available could introduce some confusion in clinical trials and research, resulting in different cohorts of patients and in non-comparable results (29, 30). It is interesting to mention that in the classification criteria of the American College of Rheumatology (ACR) (31) the oral component is completely left out. Many medical experts in this field did not agree with such classification criteria (29). In order to develop international consensus on classification criteria, the ACR-EULAR (European League Against Rheumatism) criteria were introduced, endorsed by both ACR and EULAR (21, 32).

The MSGB has been standardized as one of major parameter used in a composite of criteria to provide reliable sensitivity and specificity for the classification of pSS (10, 21, 31). It is an invasive diagnostic method and there is no algorithm or any recommendation when to indicate MSGB. Unfortunately, there are no clear guidelines when MSGB is indicated in the treatment of patients with pSS. There is no data in the available literature on how rheumatologists use MSGB results in daily clinical work. Patients with clinically suspected pSS are preselected for having difficulty with their oral mucosa, thus, any operative intervention in the oral area should be undertaken only with good reason. Given the diversity of classification criteria used throughout the years, the lack of experience with MSGB, inconsistent interpretation of results and pa-

gativna korelacija uočena je samo za anti-SS-A i anti-SS-B autoantitijela i dob bolesnika pri postavljanju dijagnoze (rho = –0,48, p = 0,003 i rho = –0,42, p = 0,011 za A i B). Ocjene boli i umora prema bodovnoj skali upitnika ESSPRI bile su u međusobnoj korelaciji (rho = 0,73, p < 0,0001), ali niti jedna od njih nije bila u korelaciji s ocjenom suhoće prema bodovnoj skali upitnika ESSPRI. Zanimljivo je da je ocjena suhoće bila u dobroj korelaciji s indeksom tjelesne mase bolesnika (rho = 0,57, p = 0,0003). Nadalje, fizička aktivnost bila je u negativnoj korelaciji s ocjenom umora prema bodovnoj skali upitnika ESSPRI (rho = –0,37, p = 0,026) i ukupnim rezultatom upitnika ESSPRI (rho = –0,41, p = 0,014), što ukazuje na to da fizička aktivnost može ublažiti neke od subjektivnih simptoma i poboljšati opću samopercepciju.

RASPRAVA

Cilj našeg istraživanja bio je retrospektivno procijeniti korelaciju između biopsije malih žlijezda slinovnica, seroloških nalaza i sijalometrije u bolesnika sa suspektnim pSS-om. Prema našem iskustvu, pSS se u ranoj fazi može pogrešno dijagnosticirati kao simptom sindroma pekućih usta. U raznim izvješćima često se ukazuje na kašnjenje u dijagnozi pSS-a koje može varirati od 3 do 11 godina (18). Već u ranoj fazi bolesti bolesnici imaju smanjenu brzinu izlučivanja sline, ponekad čak i bez abnormalnosti ultrazvuka žlijezda slinovnica (28). Čimbenik brzine izlučivanja sline može poboljšati pravovremenu dijagnozu bolesti i trebao bi biti uključen u klasifikacijske kriterije za dijagnosticiranje pSS-a.

Činjenica da su dostupni razni kriteriji klasifikacije za pSS mogla bi unijeti određenu dozu pomutnje u klinička ispitivanja i istraživanja, što bi rezultiralo različitim skupinama bolesnika i neusporedivim rezultatima (29, 30). Zanimljivo je da je u klasifikacijskim kriterijima organizacije *American College of Rheumatology* (ACR) (31) oralna komponenta potpuno izostavljena. Mnogi medicinski stručnjaci u ovom području nisu se složili s takvim kriterijima klasifikacije (29). S ciljem postizanja međunarodnog konsenzusa o kriterijima klasifikacije uvedeni su kriteriji ACR-EULAR (Europska liga protiv reumatizma) koje su odobrile obje prethodno navedene organizacije – ACR i EULAR (21, 32).

Biopsija malih žlijezda slinovnica standardizirana je kao jedan od glavnih parametara koji se upotrebljava u skupu kriterija kako bi se osigurala pouzdana osjetljivost i specifičnost za klasifikaciju pSS-a (10, 21, 31). To je invazivna dijagnostička metoda i ne postoji algoritam niti ikakva preporuka kada postaviti indikaciju za biopsiju malih žlijezda slinovnica. Nažalost, ne postoje jasne smjernice koje bi određivale kada bi trebalo postaviti indikaciju za biopsiju malih žlijezda slinovnica u liječenju bolesnika s pSS-om. U dostupnoj literaturi nema podataka o tome kako reumatolozi upotrebljavaju rezultate

tient benefit, it is hard to assess in which cases it would be convenient to have MSGB in defining diagnosis of pSS. From our experience, in select clinical situations, the diagnosis of pSS can be made based on noninvasive tests i.e., it can be confirmed by positive serology findings (anti-SSA and/or anti-SSB). Clearly, dryness of the exocrine glands and serologic markers are not a complete replacement for tissue diagnosis, which does provide additional data that may be helpful in certain clinical situations.

Over the past years, salivary gland ultrasound has gained more attention. It was proven to be effective for the detection of typical structural abnormalities in pSS (33, 34). Parotid gland ultrasound can be directly compared to parotid and submandibular glands histopathology (35). Salivary gland ultrasound has a good sensitivity and high specificity, it appeared to mirror dysfunction of the salivary glands, even at the early stages of the disease (36). It is non-invasive, relatively inexpensive, easily accessible and quick (37). Despite the potential of salivary gland ultrasound in diagnosis and classification of pSS, the value of salivary gland ultrasound to assess disease activity and disease progression needs to be established (38).

There are many methods of measuring salivary gland function, although not all of them are practical and adaptable for clinical trials (28, 39). As a measure of salivary gland function, the UWS flow rate has been adopted as one validated measure for the revised AECG and ACR-EULAR criteria for pSS (10, 21). It provides great sensitivity and specificity in differentiating patients with pSS from non-pSS patients. It is simple to administer and non-invasive, needs no special equipment, has a good reproducibility and reflects the physiologic state of the gland. The results of our study showed statistically significant correlation between "positive" biopsy score and clinically reduced (≤0.1 ml/minute) saliva secretion (rho= 0.461, p=0.0047). Our results illustrate the correlation between MSGB, which shows morphological changes, and UWS flow rate, representing the functional aspect. Therefore, UWS flow rate may serve as a noninvasive surrogate biomarker of inflammation and fibrosis as well as an indicator of treatment success in patients with pSS. Unstimulated whole salivary flow rate reflects the basal saliva flow. It should be included in all pSS trials to document glandular parenchymal damage and its possible deterioration over time in pSS. Considering the statistical correlation between a "positive" biopsy score and the serological findings, it was noted that there was not statistically significant correlation between these two parameters (p=0.10). Our results reaffirm that "positive" histopathology finding is a requirement for the diagnosis of pSS in the absence of anti-SSA autoantibodies (22, 40, 41). We highly recommend that physi-

biopsije malih žlijezda slinovnica u svakodnevnoj kliničkoj praksi. Bolesnici sa suspektnim pSS-om odabrani su postupkom predselekcije na temelju postojanja poteškoća s oralnom sluznicom, stoga se svaki operativni zahvat u oralnom području treba obavljati samo ako za to postoji dobar razlog. S obzirom na raznolikost kriterija klasifikacije koji su se upotrebljavali tijekom godina, nedostatak iskustva s postupkom biopsije malih žlijezda slinovnica, nedosljedno tumačenje rezultata i koristi za bolesnike, teško je procijeniti u kojim bi slučajevima bilo korisno upotrebljavati biopsiju malih žlijezda slinovnica u postavljanju dijagnoze pSS-a. Prema našem iskustvu, u odabranim kliničkim slučajevima dijagnoza pSS-a može se postaviti na temelju neinvazivnih testova, odnosno može se potvrditi pozitivnim serološkim nalazima (anti-SS-A i/ili anti-SS-B). Jasno je da suhoća egzokrinih žlijezda i serološki markeri ne mogu u potpunosti zamijeniti tkivnu dijagnozu koja pruža dodatne podatke koji mogu biti korisni u određenim kliničkim slučajevima.

Proteklih godina pažnja znanstvene zajednice sve je više usmjerena na ultrazvuk žlijezda slinovnica. Ova metoda pokazala se učinkovitom za otkrivanje tipičnih strukturnih abnormalnosti u pSS-u (33, 34). Ultrazvuk parotidnih žlijezda može se izravno usporediti s patohistologijom parotidnih i submandibularnih žlijezda (35). Ultrazvuk žlijezda slinovnica ima dobru osjetljivost i visoku specifičnost te se čini da odražava disfunkciju žlijezda slinovnica, čak i u ranim stadijima bolesti (36). Ova je metoda neinvazivna, relativno jeftina, lako dostupna i brza (37). Unatoč potencijalu koji ultrazvuk žlijezda slinovnica ima u dijagnostici i klasifikaciji pSS-a, potrebno je utvrditi njegovu vrijednost za procjenu aktivnosti bolesti i progresije bolesti (38).

Postoje razne metode mjerenja funkcije žlijezda slinovnica, iako nisu sve praktične i pogodne za klinička ispitivanja (28, 39). Budući da se upotrebljava kao metoda za mjerenje funkcije žlijezda slinovnica, brzina izlučivanja sline usvojena je kao valjana mjera za revidirane AECG i ACR-EULAR kriterije za dijagnozu pSS-a (10, 21). Ta metoda omogućuje veliku osjetljivost i specifičnost u razlikovanju bolesnika koji boluju od pSS-a i onih koji ne boluju od pSS-a. Jednostavna je za primjenu i neinvazivna, ne zahtijeva uporabu posebne opreme, ima dobru ponovljivost i odražava fiziologiju žlijezde. Rezultati našeg istraživanja pokazali su statistički značajnu korelaciju između „pozitivnog" rezultata biopsije i smanjenog (≤ 0,1 ml/min) lučenja sline (rho = 0,461, p = 0,0047). Naši rezultati ukazuju na korelaciju između biopsije malih žlijezda slinovnica, koja pokazuje morfološke promjene, i brzine izlučivanja sline, koja predstavlja funkcionalni aspekt. Stoga, brzina izlučivanja sline može poslužiti kao neinvazivni zamjenski biomarker u slučajevima upale i fibroze, kao i pokazatelj uspješnosti u liječenju bolesnika s pSS-om. Nestimulirana brzina izlučivanja sline odražava brzinu izlučivanja bazalne sline. Taj čimbenik

cians should consider performing MSGB in patients with glandular dysfunctions and negative serology. Evidence-based algorithms need to be developed in screening patients who have dryness of the exocrine glands to prevent delaying the diagnosis of pSS, and to avoid unnecessary MSGB in patients who already have oral complications. In patients diagnosed with pSS, objective diagnostic tests for ocular and oral dysfunction should be performed regularly to monitor salivary gland function over time. Future studies aimed at developing effective screening and treatment algorithms are needed to improve the quality of life of these patients. In that respect, continuing research in understanding the underlying etiopathogenesis and possibly incorporating genetic and genomic alterations, might prove beneficial in constructing improved criteria for this complex disorder.

We acknowledge some limitations of our study. The study was conducted in a single center with a retrospective nature. The study was performed at the Clinical Hospital Center „Sestre milosrdnice", which belongs to tertiary health care. Patients with various diseases were included in the study and we performed MSGB and UWS flow rate in all patients. Being a single-centre study, the number of patients included was limited and most importantly, the control group was represented by patients clinically suspected for pSS but diagnosed with non-pSS, which is a heterogeneous entity. However, patients with xerostomia (non-pSS patients) had all undergone MSGB, which to date, remains a cornerstone for pSS diagnosis. It is not possible to exactly determine how many patients with clinically suspected pSS were not biopsied and therefore not included, a factor which could therefore have had an influence on the results. The limited number of cases and the long time interval for the indication of the MSGB and UWS flow rate in some cases under pSS investigation may have modulated the results. Eight of our patients were receiving immunosuppressive therapy, which can downregulate the production of autoantibodies and also altered some histological parameters. Because of the retrospective nature of the study we were not able to evaluate the temporality of the autoantibody presentation. Thus, prospective carrying out of other serological tests (rheumatoid factor, C3, C4, cryoglobulins) would have improved the validity of the clinical judgment and possibly modified the sensitivity of the classification criteria. The methods adopted for ocular staining varied among the participating institutions, forcing us to modify certain items in some criteria.

In conclusion, in our study according to the revised AECG classification criteria only „positive" histopathological findings, reduced saliva secretion and existence of anti-SSA autoantibody confirmed to have a significant diagnostic value in detecting pSS patients. Also,

treba uključiti u sva ispitivanja pSS-a u svrhu dokumentiranja oštećenja parenhima žlijezda i njegova mogućeg pogoršanja do kojeg s vremenom može doći u slučaju bolesnika koji boluju od pSS-a. Uzimajući u obzir statističku korelaciju između „pozitivnog" rezultata biopsije i seroloških nalaza, uočeno je da ne postoji statistički značajna korelacija između ova dva parametra ($p = 0,10$). Naši rezultati potvrđuju da je „pozitivan" patohistološki nalaz uvjet za postavljanje dijagnoze pSS-a u odsutnosti anti-SS-A autoantitijela (22, 40, 41). Naša je preporuka da liječnici razmotre provođenje postupka biopsije malih žlijezda slinovnica u bolesnika s disfunkcijama žlijezda i negativnom serološkom analizom. Algoritme utemeljene na dokazima potrebno je razviti u probiru bolesnika koji imaju simptom suhoće egzokrinih žlijezda kako bi se spriječilo odgađanje dijagnoze pSS-a i kako bi se izbjegla nepotrebna biopsija malih žlijezda slinovnica u bolesnika koji već imaju oralne komplikacije. U bolesnika s dijagnozom pSS-a potrebno je redovito provoditi objektivne dijagnostičke pretrage za disfunkciju očiju i usne šupljine u svrhu praćenja funkcije žlijezda slinovnica tijekom određenog razdoblja. U budućnosti je potrebno provesti studije usmjerene na razvoj učinkovitih algoritama za probir i liječenje ove bolesti kako bi se poboljšala kvaliteta života ovih bolesnika. U tom pogledu, daljnje provođenje istraživanja vezanih za razumijevanje temeljne etiopatogeneze te mogućnost uključivanja čimbenika poput genetskih i genomskih promjena mogli bi se pokazati korisnim u postavljanju poboljšanih kriterija za ovaj složeni poremećaj.

Priznajemo da u sklopu naše studije postoje i određena ograničenja. Studija je provedena u jednom bolničkom centru te je po svojoj prirodi retrospektivna. Provedena je u Kliničkom bolničkom centru Sestre milosrdnice koji je zdravstvena ustanova na tercijarnoj razini. U studiju su uključeni bolesnici s raznim bolestima te su na svim bolesnicima provedeni postupci biopsije malih žlijezda slinovnica i brzine izlučivanja sline. Budući da je ova studija provedena u jednom bolničkom centru, broj bolesnika koji su u nju uključeni bio je ograničen i, što je najbitnije, predstavnici kontrolne skupine bili su bolesnici sa suspektnim pSS-om, ali kojima nije dijagnosticiran pSS, što je heterogeni entitet. No, svim bolesnicima koji boluju od kserostomije (bolesnici koji ne boluju od pSS-a) bila je učinjena biopsija malih žlijezda slinovnica, koja je i dandanas temeljni postupak za dijagnozu pSS-a. Nije moguće točno utvrditi broj bolesnika sa suspektnim pSS-om kojima nije učinjena biopsija i koji stoga nisu uključeni u studiju, što je čimbenik koji bi sigurno utjecao na rezultate. Čimbenici poput ograničenog broja slučajeva i dugoga vremenskog intervala za postavljanje indikacije za postupke biopsije malih žlijezda slinovnica i brzine izlučivanja sline možda su utjecali na konačne rezultate u nekim slučajevima u kojima se tek trebalo utvrditi postojanje dijagnoze pSS-a. Osam bole-

UWS flow rate appears to be a useful aid in diagnosis of pSS. Unstimulated whole salivary flow rate ≤0.1 ml/minute is highly predictive of "positive" MSGB and can be used as a supplemental method to MSGB in diagnosing the oral component of pSS. As a non-invasive, easy to perform, inexpensive method it might be good monitoring tool to follow the progress of the disease.

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snika iz naše studije uzimalo je imunosupresivnu terapiju koja može smanjiti proizvodnju autoantitijela i također promijeniti neke histološke parametre. Budući da je ova studija bila retrospektivna, nismo bili u mogućnosti procijeniti vremensko razdoblje prikaza autoantitijela. Stoga bi prospektivno provođenje drugih seroloških pretraga (reumatoidni faktor, C3, C4, krioglobulini) poboljšalo valjanost kliničke procjene i možda modificiralo osjetljivost klasifikacijskih kriterija. Ustanove koje su sudjelovale u provođenju studije upotrebljavale su različite metode za bojenje okularne površine, zbog čega smo morali modificirati određene stavke u nekim kriterijima.

U konačnici možemo zaključiti da je u našoj studiji prema revidiranim AECG klasifikacijskim kriterijima potvrđeno da samo „pozitivni" patohistološki nalazi, smanjeno lučenje sline i postojanje anti-SS-A autoantitijela imaju značajnu dijagnostičku vrijednost u otkrivanju bolesnika koji boluju od pSS-a. Nadalje, možemo zaključiti da je brzina izlučivanja sline korisna metoda koja može pomoći u dijagnozi pSS-a. Nestimulirana brzina izlučivanja sline ≤ 0,1 ml/min snažan je prediktor „pozitivne" biopsije te se može upotrebljavati kao dodatna metoda uz biopsiju u dijagnostici oralne komponente primarnoga Sjögrenovog sindroma. Budući da se radi o neinvazivnoj i jeftinoj metodi koja je jednostavna za izvođenje, ona može biti korisna za praćenje progresije bolesti.

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REFERENCES / LITERATURA

1. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM i sur. Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum. 1993;36(3):340–7.
2. Fox R. Sjögren's syndrome. The Lancet. 2005;366:321–31.
3. Ng W, Bowman S. Primary Sjogren's syndrome: too dry and too tired. Rheumatology (Oxford). 2010;49(5):844–53.
4. Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjögren syndrome. Nat Rev Rheumatol. 2018;14(3):133–45.
5. Psianou K, Panagoulias I, Papanastasiou AD, de Lastic AL, Rodi M, Spantidea PI i sur. Clinical and immunological parameters of Sjögren's syndrome. Autoimmun Rev. 2018;17(10):1053–64.
6. Cartee DL, Maker S, Dalonges D, Manski MC. Sjögren's syndrome: oral manifestations and treatment, a dental perspective. J Dent Hyg. 2015;89(6):365–71.
7. Holdgate N, St. Clair E. Recent advances in primary Sjogren's syndrome. F1000Res. 2016;5:1412.
8. Kassan S, Moutsopoulos H. Clinical Manifestations and Early Diagnosis of Sjögren Syndrome. Arch Intern Med. 2004;164(12):1275.
9. Rasmussen A, Ice JA, Li H, Grundahl K, Kelly JA, Radfar L i sur. Comparison of the American-European Consensus Group Sjögren's syndrome classification criteria to newly proposed American College of Rheumatology criteria in a large, carefully characterised sicca cohort. Ann Rheum Dis. 2013;73(1):31–8.
10. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE i sur. 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(6):554–8.
11. Vitali C, Bombardieri S, Moutsopoulos HM, Coll J, Gerli R, Hatron PY i sur. Assessment of the European classification criteria for Sjögren's syndrome in a series of clinically defined cases: results of a prospective multicentre study. The European

Study Group on Diagnostic Criteria for Sjögren's Syndrome. Ann Rheum Dis. 1996;55(2):116–21.

12. Greenspan J, Daniels T, Talal N, Sylvester R. The histopathology of Sjögren's syndrome in labial salivary gland biopsies. Oral Surg Oral Med Oral Pathol. 1974;37(2):217–29.
13. Lindhal G, Hedfors E. Lymphocytic infiltrates and epithelial HLA-DR expression in lip salivary glands in connective tissue disease patients lacking sicca: a prospective study. Br J Rheumatol. 1989;28(4):293–8.
14. Guellec D, Cornec D, Jousse-Joulin S, Marhadour T, Marcorelles P, Pers JO i sur. Diagnostic value of labial minor salivary gland biopsy for Sjögren's syndrome: a systematic review. Autoimmun Rev. 2013;12(3):416–20.
15. Stone DU, Fife D, Brown M, Earley KE, Radfar L, Kaufman CE i sur. Effect of tobacco smoking on the clinical, histopathological, and serological manifestations of Sjögren's syndrome. PLOS ONE. 2017;12(2):e0170249.
16. Oni C, Mitchell S, James K, Ng WF, Griffiths B, Hindmarsh V i sur. Eligibility for clinical trials in primary Sjögren's syndrome: lessons from the UK Primary Sjögren's Syndrome Registry. Rheumatology (Oxford). 2016;55(3):544–52.
17. Luo J, Xu S, Lv Y, Huang X, Zhang H, Zhu X i sur. Clinical features and potential relevant factors of renal involvement in primary Sjögren's syndrome. Int J Rheum Dis. 2019;22(2):182–90.
18. Lee Y, Song R, Yang Y, Eun Y. Prevalence and Predictors of Sjögren's Syndrome in Patients with Burning Mouth Symptoms. J Oral Facial Pain Headache. 2018;32(1):91–6.
19. Vitali C, Moutsopoulos H, Bombardieri S. The European Community Study Group on diagnostic criteria for Sjögren's syndrome. Sensitivity and specificity of tests for ocular and oral involvement in Sjögren's syndrome. Ann Rheum Dis. 1994;53 (10):637–47.
20. Navazesh M. Methods for Collecting Saliva. Ann N Y Acad Sci. 1993;694:72–7.
21. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM i sur. 2016 American College of Rheumatology/ European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. Ann Rheum Dis. 2017;76(1):9–16.
22. Liquidato B, Filho I. Evaluation of sialometry and minor salivary gland biopsy in classification of Sjögren's Syndrome patients. Braz J Otorhinolaryngol. 2005;71(3):346–54.
23. World Medical Association Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191.
24. Navazesh M, Kumar S. Measuring salivary flow: challenges and opportunities. J Am Dent Assoc. 2008;139:35S–40S.
25. Daniels TE, Cox D, Shiboski CH, Schiødt M, Wu A, Lanfranchi H i sur. Associations between salivary gland histopathologic diagnoses and phenotypic features of Sjögren's syndrome among 1,726 registry participants. Arthritis Rheum. 2011;63 (7):2021–30.
26. Cho HJ, Yoo JJ, Yun CY, Kang EH, Lee HJ, Hyon JY i sur. The EULAR Sjogren's syndrome patient reported index as an independent determinant of health-related quality of life in primary Sjogren's syndrome patients: in comparison with non-Sjogren's sicca patients. Rheumatology (Oxford). 2013;52(12): 2208–17.
27. Seror R, Theander E, Brun JG, Ramos-Casals M, Valim V, Dörner T i sur. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). Ann Rheum Dis. 2015;74(5):859–66.
28. Jousse-Joulin S, Milic V, Jonsson MV, Plagou A, Theander E, Luciano N i sur. Is salivary gland ultrasonography a useful tool in Sjögren's syndrome? A systematic review. Rheumatology (Oxford). 2016;55(5):789–800.
29. Cornec D, Saraux A, Cochener B, Pers JO, Jousse-Joulin S, Renaudineau Y i sur. Level of agreement between 2002 American–European Consensus Group and 2012 American College of Rheumatology classification criteria for Sjögren's syndrome and reasons for discrepancies. Arthritis Res Ther. 2014;16 (2):R74.
30. Goules AV, Exarchos TP, Pezoulas VC, Kourou KD, Venetsanopoulou AI, De Vita S i sur. Sjögren's syndrome towards precision medicine: the challenge of harmonisation and integration of cohorts. Clin Exp Rheumatol. 2019;118(3):175–84.
31. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H i sur. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance Cohort. Arthritis Care Res (Hoboken). 2012;64(4):475–87.
32. Billings M, Amin Hadavand M, Alevizos I. Comparative analysis of the 2016 ACR-EULAR and the 2002 AECG classification criteria for Sjögren's syndrome: Findings from the NIH cohort. Oral Dis. 2018;24(1–2):184–90.
33. Carotti M, Salaffi F, Di Carlo M, Barile A, Giovagnoni A. Diagnostic value of major salivary gland ultrasonography in primary Sjögren's syndrome: the role of grey-scale and colour/ power Doppler sonography. Gland Surg. 2019;8(S3):S159–67.
34. Devauchelle-Pensec V, Zabotti A, Carvajal-Alegria G, Filipovic N, Jousse-Joulin S, De Vita S. Salivary gland ultrasonography in primary Sjögren's syndrome: opportunities and challenges. Rheumatology (Oxford). 2019;kez079.
35. Mossel E, Delli K, van Nimwegen JF, Stel AJ, Kroese FGM, Spijkervet FKL i sur. Ultrasonography of major salivary glands compared with parotid and labial gland biopsy and classification criteria in patients with clinically suspected primary Sjögren's syndrome. Ann Rheum Dis. 2017;76(11):1883–9.
36. Baldini C, Luciano N, Tarantini G, Pascale R, Sernissi F, Mosca M i sur. Salivary gland ultrasonography: a highly specific tool for the early diagnosis of primary Sjögren's syndrome. Arthritis Res Ther. 2015;17(1):146.
37. Ramsubeik K, Motilal S, Sanchez-Ramos L, Ramrattan L, Kaeley G, Singh J. Diagnostic accuracy of salivary gland ultrasound in Sjögren's syndrome: A systematic review and meta-analysis. Ther Adv Musculoskelet Dis. 2020;12:1759720X20973560.
38. van Ginkel MS, Glaudemans AWJM, van der Vegt B, Mossel E, Kroese FGM, Bootsma H i sur. Imaging in primary Sjögren's syndrome. J Clin Med. 2020;9(8):2492.
39. Liu S, Chen W, Wang M, Wu T, Dong L, Pan C i sur. Quantitative analysis of parotid gland secretion function in Sjögren's syndrome patients with dynamic magnetic resonance sialography. Korean J Radiol. 2019;20(3):498.
40. Pereira D, Vilela V, dos Santos T, Pires F. Clinical and laboratorial profile and histological features on minor salivary glands from patients under investigation for Sjögren's syndrome. Med Oral Patol Oral Cir Bucal. 2014;19(3):e237–41.
41. Liapi A, Horisberger A, François S, Ribi C. Sjögren's syndrome: when to suspect and how to confirm? Rev Med Suisse. 2016; 12(513):698–702.