Positive histopathologic assessment in salivary glands shows little impact on clinical features of established primary Sjögren’s syndrome in a Korean population

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ABSTRACT
Objective. The presence and severity of focal lymphocytic sialadenitis in minor salivary glands is a pathognomonic feature in primary Sjögren’s syndrome (pSS). However, it has not been determined whether performing minor salivary gland biopsy (MSGB) in a setting of serologically and clinically established pSS provides additional clinical value. Therefore, we aimed to investigate the necessity of MSGB in established pSS patients with anti-Ro/SSA antibodies.

Methods. We extracted 185 patients with anti-Ro/SSA antibody-positive pSS from the Korean Initiative of pSS study, a prospective cohort study. We assigned them into two groups, 161 patients with focus scores ≥1 and another 24 with focus scores <1. The two groups were compared in various clinical aspects, including the severity of glandular dysfunction, systemic disease activity, extraglandular manifestations, and other clinical indices and laboratory values. We also evaluated the relationship between focus scores and clinically important variables in pSS.

Results. Between the two groups, there were no significant differences in the severity of secretory dysfunction, the frequency of extra-glandular manifestations, systemic disease activity represented by various clinical indices, and laboratory findings possibly predicting the risk for lymphoma. Rather, the Sjögren’s syndrome disease damage index was higher in the group with focus scores ≥1. Among all variables, only serum immunoglobulin G levels were correlated with focus scores.

Conclusion. Given the little influence on clinical phenotypes, routine MSGB could be omitted for serologically and clinically established pSS patients, especially in low-risk areas for lymphoproliferative diseases.

Introduction
Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease mainly presenting with dry eyes and dry mouth caused by the infiltration of inflammatory cells into the exocrine glands (1). Although the aetiologies and pathophysiologic mechanisms of pSS are still unclear, several criteria have been suggested to define the disease entity. According to the latest classification criteria from the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR), positivity in one of two items, either the presence of anti-Ro/SSA antibody or the histologic confirmation of lymphocytic infiltration in minor salivary glands, is needed in order to diagnose pSS (2).

Minor salivary gland biopsies (MSGB) are widely performed in many rheumatologic centres for pSS around the world to evaluate the histopathologic state of the exocrine glands (3). Focal lymphocytic sialadenitis (FLS) is the most specific pathologic findings for pSS (4). The focus score is a semi-quantitative value indicating the severity of FLS in lower lip biopsy specimens (5). Several studies reported a correlation between higher focus scores and specific clinical features, such as more severe secretory dysfunction and disease activity and elevated risk for lymphoproliferative disease in pSS (6-8). Focus scores ≥1 are regarded as a reasonable cut-off level for determining whether histopathologic results are sufficient for diagnosing pSS (6). However, pSS can be diagnosed based on decreased secretory functions, tested by the Schirmer I test or unstimulated salivary flow rate, and...
serologic positivity in the absence of histopathologic results according to the 2016 ACR/EULAR criteria (2). While measuring focus scores by MSGB could be used as a tool for diagnosing pSS and predicting significant consequences, such as lymphoproliferative diseases, the value of data provided by MSGB for disease outcomes is unknown if a diagnosis of pSS is already established, especially in relatively low lymphoma-risk areas. Although MSGBs can be done quickly in outpatient-settings, they can also result in several complications, such as permanent neural damage causing lower lip numbness (9). Therefore, the question arises as to whether MSGB is routinely needed in established pSS patients with positive anti-Ro/SSA antibodies.

In the present study, we aimed to assess the necessity of MSGB in established pSS patients. We chose anti-Ro/SSA antibody-positive pSS patients from our nationwide prospective cohort for pSS and divided them into two groups, one with focus scores <1 and the other with focus scores ≥1. Then, the two groups were compared in various aspects, including the severity of secretory dysfunctions, disease activity, and clinical parameters related to critical disease-related consequences. We also evaluated whether elevated focus scores were correlated with certain clinical variables of established pSS.

Materials and methods
Study population
All the subjects in the present study were selected from participants in the Korean Initiative of primary Sjögren’s Syndrome (KISS) study. KISS is a nation-wide prospective cohort database established to provide overall clinical data and samples of patients with pSS in Korea. Recruitment for the cohort was conducted from October 2013 to July 2017. Finally, the database included 501 patients with pSS from 12 university hospitals across the entire nation, including Seoul St. Mary’s Hospital, a tertiary care university-affiliated hospital and referral centre in Seoul, Korea. Informed consent was obtained from all participants in the cohort according to the principles of the Declaration of Helsinki. All the studies related to this cohort including the present study were approved by the Institutional Review Board of Seoul St. Mary’s Hospital of the Catholic University of Korea (approval number: KC13ONMI0646). The inclusion criteria for the cohort enrolment was fulfilling the 2002 American-European Consensus Group (AECG) classification criteria and/or the 2012 ACR criteria (3, 10). The exclusion criteria were radiation history to the head and neck area, chronic hepatitis C or human immunodeficiency virus infections, previous lymphoproliferative disease, sarcoidosis, graft-versus-host disease, amyloidosis, and IgG4-related disease. We chose 185 patients who fulfilled the 2016 ACR/EULAR classification criteria for pSS with positivity for anti-Ro/SSA antibody and histopathologic results of MSGB. Two groups were assigned according to the focus scores based on an item of the 2016 ACR/EULAR criteria (2). Of the subjects, 161 showed focus scores ≥1 (FS ≥1 group), whereas the focus scores of the other 24 patients were <1 (FS <1 group).

Secretory function-related outcomes
We extracted data from the KISS database and used only baseline data in this study. Schirmer I test and the measurement of ocular staining scores were performed by ophthalmologists to evaluate the severity of keratoconjunctivitis sicca. The ocular staining scores were calculated by both the Sjögren’s International Collaborative Clinical Alliance (SICCA) method and the van Bijsterveld’s method (11, 12). Ocular tests were done for both eyes and the worse results were included for analysis. The unstimulated salivary flow rate (USFR) was measured to assess the degree of xerostomia (13). Positivity for each ocular and oral test was defined according to the 2016 ACR/EULAR classification criteria (2). The ocular surface disease index and the xerostomia inventory were evaluated as patient-reported indices for ocular and oral dryness, respectively (14, 15). All MSGBs were performed at the time of diagnosis for pSS or enrolment for the cohort, if not performed yet. MSGB and evaluation of the severity of lymphocytic infiltration into the salivary glands were done according to the SICCA protocol by experienced specialists in otorhinolaryngology and oral pathology at each centre involved in the cohort (16). In order to allow a robust and reliable analysis, biopsy specimens were acquired to include at least four labial salivary glands with 8 mm² of surface area of gland sections. Histopathologic assessment should determine whether FLS is present in specimens. If the initial cutting level of specimens was inconclusive, two additional cutting levels were included. FLS was defined as one or more dense aggregates of 50 or more lymphocytes around normal mucous acini (7). The focus score was calculated as the number of foci per 4 mm² of a specimen (5). The presence of an ectopic germinal centre (GC) was also evaluated (17).

Systemic activity-related outcomes
The EULAR Sjögren’s syndrome disease activity index (ESSDAI) and the Sjögren’s Syndrome Disease Damage Index (SSDDI) were generally used to assess systemic disease activity and long-term disease-related damage, respectively (18, 19). These values were assessed by rheumatologists in each cohort-participating centre. The EULAR Sjögren’s Syndrome Patient-Reported Index (ESSPRI) was assessed by the targeted subjects themselves (20). EuroQol (EQ)-5 dimensions (5D) time trade-off (TTO) values were derived from the South Korean reference data and EQ visual analog scale (VAS) scores were used to measure the disease-related quality of life (21). Information on extra-glandular manifestations, defined according to the case report form of the KISS study (Supplementary Table S1), was also collected. VASs assessing overall disease activity by both physicians and patients were also collected.

Laboratory values
The presence of cytopenia and hypergammaglobulinaemia were defined according to the haematological and biological domains of the ESSDAI, respectively (18). We excluded cytopenia...
possibly caused by other medical conditions, such as nutrient deficiencies, drugs, or anaemia of chronic disease. Anti-nuclear antibody (ANA) positivity was defined as a titre ≥1:320. Positivity for anti-Ro/SSA and anti-La/SSB antibodies were tested using commercial enzyme-linked immunosorbent assay.

Relationships between focus scores and clinical variables
The association between clinical variables possibly influenced by the severity of FLS, such as secretory function, serological variables, and indices related to systemic disease activities, and focus scores were evaluated (6). Relationships with laboratory values which showed statistically significant differences between the two study groups were also assessed.

Statistical analysis
Continuous variables were assessed for departures from normal distribution with the Kolmogorov-Smirnov test. As they were not normally distributed, all the continuous variables were expressed as medians and interquartile ranges (IQR) in the tables and figures and were analysed with the Mann-Whitney U-test. Categorical variables and were analysed with the Mann-Whitney U-test. Categorical variables were assessed by the Chi-squared test and Fisher’s exact test. Spearman’s rank correlation coefficient was performed to assess the relationship between focus scores and other clinical values. IBM-SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Statistical significance was considered at a level of p<0.05.

Results
Basal demographics and clinical indices
At the time of enrolment for the cohort, the FS <1 group was marginally older than the other group (median 56 vs. 50, p=0.052). Nearly all subjects in both study groups were female (98.8% and 100%, respectively). The median focus score in the FS ≥1 group was 3 (IQR 2–5) as shown in Table I. Among all the specimens acquired from the MSGBs, 2–5) as shown in Table I. Among all the specimens acquired from the MSGBs.
there was no single ectopic germinal centre-like lesion. Median disease duration was about 4 months with similarity between the two study groups and medication status made no inter-group differences according to the data shown in Table I. VAS scoring of disease activity by physicians (median 30 vs. 43) and patients (median 57 vs. 53) was similar between the two groups. Disease-related quality of life estimated by the EQ-5D index and VAS also showed no inter-group differences. The ESSPRI assessed by the patients for pain, fatigue, and dryness showed similar tendency regardless of focus score (median overall scores 5.3 vs. 5.2).

Glandular functions
Most patients in both groups suffered from dry eyes and dry mouth symptoms according to questionnaires from the first and second items of the 2002 AECG criteria (3). The severity of xerostomia presented by the USFR was not different between the two groups, as shown in Table II and Figure 1A. Neither the absolute values nor positive USFR ratios was affected by focus scores, whether they were ≥1 or not. Among clinical variables related to xerophthalmia, ocular staining scores by both SICCA (median 5 vs. 4) and the van Bijsterveld methods (median 4 vs. 2) were not different between the groups. The proportion of patients with Schirmer I tests ≤5 mm/5 minutes was higher in the FS <1 group (Table II), whereas the absolute values of the Schirmer I test were not different between the two groups (Fig. 1B). The xerostomia inventory and the ocular surface disease index, questionnaires assessing the severity of dry eyes and dry mouth respectively, showed similar median values between the two groups (Table II).

Indices for systemic disease activity and disease-related long-term damage
The systemic activity assessed by the ESSDAI was similar between the two groups (Fig. 1C). There were no inter-group differences in the positive ratio of each domain in the ESSDAI (Supplementary Table S2). The median value of the SSDDI in the FS <1 group was higher than that in the FS ≥1 (Fig. 1D). Among all domains of the SSDDI, only ‘structural abnormalities’ showed significant difference, presenting with a higher prevalence in the FS <1 group (Supplementary Table S3).

Extra-glandular manifestations
Beyond the ESSDAI, the prevalence of other systemic manifestations in pSS was also evaluated. As presented in Table III, though lymphadenopathy was marginally more frequent in the focus <1 group, the difference was not statistically significant. The frequencies of other manifestations from articular symptoms to renal involvement were similar between the two groups. Focus scores were not significantly associated with the prevalence of any the extra-glandular features in this study.

Laboratory data
The absolute values of all blood cell measurements, including hemoglobin levels, were not different between the two groups (Table IV). Positivity for cytopenia and serological markers, such as rheumatoid factor and antinuclear antibody, showed the same tendency. Whereas the prevalence of hypogammaglobulinemia, low C3 and low C4 was similar, the absolute immunoglobulin G levels were higher in the FS ≥1 group (median 1670 mg/dl vs. 1360 mg/dl) and the C4 levels were higher in the FS <1 group (median 28 mg/dl vs. 21 mg/dl).

Relationship between focus scores and other clinical variables
Because higher focus scores could reflect more severe secretory dysfunctions, we tried to evaluate the relationships between the degree of inflammation in exocrine glands and other clinical values related to glandular functions. The severity of FLS presented by the focus scores was not correlated with USFR or OSS in established pSS patients with positive anti-Ro(SSA) antibodies in the present study (Fig. 2A and 2B). The ESSDAI and SSDDI showed slightly negative relationships with focus scores but the relationships were not statistically significant (Fig. 2C and 2D). However, immunoglobulin G levels were positively correlated with focus scores, as shown in Figure 2E (r=0.299, p<0.001). C4 levels, an-
other variable showing a significant difference in the previous analysis, was not (Fig. 2F).

### Discussion

In the present study, we aimed to determine the necessity of MSGB for predicting disease severity or clinical phenotypes, beyond a mere diagnostic tool, by comparing the clinical features of established pSS patients with positive anti-Ro/SSA antibodies and focus scores of ≥1 or <1. A focus score of 1, which is regarded as a diagnostic threshold in pSS, was not correlated with clinical outcomes in a setting of established pSS patients with anti-Ro/SSA antibodies. Focus scores also did not correlate with glandular dysfunction or systemic disease severities according to data from the KISS study. MSGBs have been performed in order to diagnose pSS and assess the extent of inflammation in the glands since the Chisholm and Mason criteria were announced (22). The infiltration of inflammatory cells mainly comprised of lymphocytes, called FLS, was found to be a principal pathologic finding in salivary glands of patients with pSS (4). Focus score, defined as the number of aggregated lymphocytic foci per 4 mm² of a specimen, has been calculated for the purpose of diagnosing pSS since it was introduced in 1974 (23). After two decades, a focus threshold score of ≥1 was suggested (24). Several reports validated the appropriateness of the focus threshold score in discriminating pSS from other medical conditions (4, 25). A study performed with the SICCA registry reported that FLS was a principal pathologic finding in salivary gland biopsies of patients with anti-Ro/SSA antibodies, whether or not the respective anti-Ro/SSA antibodies were present (26). Such unpredicted findings in the present study could result from the older age of the patients and the longer disease duration in the FS <1 group. Aging itself can reduce acinar cell secretion and increase fat and fibrovascular tissue, resulting in dry mouth (27).  

Though the age difference between the two groups was modest in the present study, these results suggest that higher focus scores do not always mean more severe secretory dysfunction in a setting of established pSS.

Several papers reported that the severity of FLS, represented by focus scores, was correlated with specific EGMs and laboratory values. The prevalence of EGMs, such as Raynaud’s phenomenon, cutaneous vasculitis, and enlarged lymph node or spleen, was evaluated in pSS patients with higher focus scores (7). Focus scores ≥1, where 1 was defined as a threshold for diagnosing pSS according to the classification criteria, also had statistically significant rela-
tionships with several serologic findings, such as positivity for rheumatoid factor, high ANA titres, and higher immunoglobulin G concentrations in previous studies (6, 28). However, in the setting of anti-Ro/SSA antibody positivity, none of the EGMs and systemic disease activities, including the ESSDAI scores and patient-reported indices, were significantly different according to the severity of the FLS (Fig. 1C, Table II, and Table III). Such tendency was also maintained in an analysis of binary comparisons between focus scores and other clinical variables. Serum immunoglobulin G levels presented the only significant inter-group differences and positive correlation with the focus scores (Fig. 2E).

As mentioned above, previous studies suggested that focus scores correlated with clinical values related to increased risk for life-threatening consequences and could predict the development of lymphoproliferative diseases (8). However, none of the possibly involved variables, such as low C4 or hypergammaglobulinaemia, were different in the present study (Table IV). Serum cryoglobulins were lower in the patients in this study compared with other region of the world and there were no inter-group differences in our cohort (29). Moreover, there was no single formation of germinal centre (GC)-like lesions among all MSGB specimens of patients from the KISS study. Together with high focus scores, the persistent presence of GC-like lesions was sug-
gested for predicting the development of lymphoma (30). A previously published paper reported that Korean patients with pSS had relatively low risk for and prevalence of lymphoma compared to Caucasians with pSS (31). Whereas pSS patients from European countries had about a 15 times increased risk of lymphoma compared to the general population, Korean patients showed a less than 7-fold risk (32). Such tendencies were also seen in patients from other nations in eastern Asia (32, 33). Therefore, although genetic backgrounds resulting in relatively lower risk for lymphoproliferative diseases are scarcely revealed to date, if a population in a certain area presented with a relatively low risk for lymphoma, the additional performance of an MSGB provided limited clinical information to the already-established diagnosis of pSS.

MSGBs are done by a minimally invasive technique and clinicians insist that the procedure is easy and harmless. However, MSGBs are associated with some discomfort and the possibility of complications. Many patients suffered pain at the incision site for at least one week and up to 6% of them may experience permanent sensory loss in the lower lip due to damages to branches of the mental nerve (9). Therefore, routinely performing MSGBs in established pSS patients should be reconsidered to reduce avoidable complications.

In the present study, the authors conducted a cross-sectional study to elucidate the clinical influences of the severity of FLS on the clinical features of pSS by comparing data gathered on the study cohort at the time of enrolment. However, to clarify a prognostic role of MSGB in pSS, a longitudinal and observational study on the incidence of critical EGMs or changes in secretory function over a period of time should be undertaken. The present study showed short periods of disease duration which was represented as 4 months of median values. Although the time between symptoms onset and the diagnosis established is relatively long in pSS, the median values of disease duration presented in this study are insufficient to reveal the prevalence of critical consequences. As the KISS cohort study was designed to follow participants at least for five years, it would be worthwhile to plan a further retrospective study by analysing the long-term influences of inflammatory changes in salivary glands after the follow-up period. Another pitfall of the present study was that acquisition of a biopsy specimen and the evaluation of FLS were done by each rheumatologic centre included in the cohort. Focus scores themselves did not show good reproducibility even when assessed by the same experienced pathologist repeatedly. Inter-observer variability of calculating focus score showed poor agreement according to a recent paper (34). In order to minimise such variabilities, all evaluators in the present study were made to abide by the SICCA protocol (16). Nevertheless, there could be some confounded data among the histopathologic findings.

In conclusion, the severity of FLS, represented by focus score, did not have significant influences on clinical features in established pSS patients with anti-Ro/SSA antibodies. Higher focus scores were not correlated with clinically important variables, such as predictors of lymphoproliferative diseases, except for serum immunoglobulin G levels. Moreover, the prognostic role of focus scores could be more limited in low-risk areas for lymphoma, such as Korea. Given these findings, routine MSGBs in a setting of an established diagnosis for pSS could be omitted in Korean population, unless specific signs suggestive of significant conditions are present.

References


