Distinct clinical characteristics of anti-Ro/SSA-negative primary Sjögren's syndrome: data from a nationwide cohort for Sjögren's syndrome in Korea

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ABSTRACT

Objective. *To investigate clinical characteristics of patients with primary Sjögren's syndrome (SS) who were negative for anti-Ro/SSA antibody but positive for minor salivary gland biopsy (MSGB) compared to patients who presented positivity for anti-Ro/SSA antibody.*

Methods. *The data of 355 patients from the Korean Initiative of primary Sjögren's Syndrome (KISS), a nationwide prospective cohort for primary SS in Korea, were analysed. All patients fulfilled the 2016 American College of Rheumatology/European League Against Rheumatism (EULAR) classification criteria. Of these patients, 326 were positive for anti-Ro/SSA antibody and 29 were antibody-negative, although they had positive findings in MSGB. Various clinical features including all kinds of tests for evaluating secretory function, disease-related clinical indices and serological values available in the cohort were compared between the two groups.*

Results. *The anti-Ro/SSA-negative group showed less rheumatoid factor positivity ($p<0.001$), leucopenia ($p=0.003$), hypergammaglobulinaemia ($p<0.001$), lower serum β_2-microglobulin level ($p=0.034$), more anti-centromere antibody positivity ($p<0.001$), higher score in dryness domain of EULAR SS patient-reported index ($p=0.048$) and more positivity for peripheral nervous system domain in EULAR SS disease activity index and loss of teeth in SS disease damage index ($p=0.021$ and 0.041, respectively) than patients who were positive for anti-Ro/SSA antibody.*

Conclusion. *Primary SS patients who are negative for anti-Ro/SSA antibody have different clinical characteristics compared to patients who are positive for such antibody in Korea. Therefore, clinicians should consider MSGB in patients with suspicious symptoms who are anti-Ro/SSA-negative.*

Introduction

Primary Sjögren's syndrome (SS) is a systemic autoimmune disease that mainly affects exocrine glands by lymphocytic infiltration, resulting in dry eyes and dry mouth symptoms (1). This disease can induce various systemic manifestations ranging from chronic fatigue, arthralgia, and cutaneous lesions to life-threatening haematologic disorders such as lymphoma (2). Until recently there has not been a gold standard for diagnosing SS that endorsed by the majority of rheumatologic communities. Therefore, many clinical studies have been conducted to aim for establishing well defined criteria for SS and discovering major factors that lead to heterogeneity of its disease phenotypes. According to the most recently published 2016 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for primary SS, we should classify suspected patients as primary SS upon at least one positivity for either anti-Ro/SSA antibody or minor salivary gland biopsy (MSGB) (3). Anti-Ro/SSA antibody is presented in about 60-70% of primary SS patients. In actual clinical field, its positivity goes up to even 90%. So far, several studies have described the clinical importance of positivity for anti-Ro/SSA antibody in primary SS, reporting that the positivity for anti-Ro/SSA antibody is associated with an early disease onset, more severe exocrine glandular dysfunction and extraglandular manifestations, persistent B cell activation and higher risk of lymphoproliferative disease (4-7). Anti-Ro/SSA antibody is regarded a key marker which could de-

termine the disease and induce its typical features.

On the other hand, we can consider a patient to have primary SS without anti-Ro/SSA positivity if the patient shows positive findings in histopathology and dry signs fulfilling the 2016 classification criteria. However, anti-Ro/SSA-negative primary SS could have been underdiagnosed in actual clinical practice. MSGB cannot be easily performed in primary care settings due to its inconvenience and possible complications. Since the observed prevalence of anti-Ro/SSA-negative SS is relatively lower than anti-Ro/SSA-positive SS, only a few studies have been conducted to explore characteristics of anti-Ro/SSA-negative primary SS patients to date. Although these studies showed lower levels of B cell expansion and risk for lymphoma in anti-Ro/SSA-negative SS patients (8) reversely, they were insufficient to explain overall features of these patients. It has not been investigated how SS-specific features could be induced in these SS subgroup patients without the 'disease-determining' antibody, either.

Thus, the objective of this study was to determine clinical characteristics of patients with primary SS who were negative for anti-Ro/SSA antibody but positive for MSGB compared to patients who presented positivity for anti-Ro/SSA antibody. We focused on patients who fulfilled the 2016 ACR/EULAR classification criteria in order to follow the most recently accepted consensus for primary SS and compared all possible clinical information from our nationwide prospective cohort data between the two study groups. Our ultimate goal is to provide guidance for investigation into the underlying pathogenic mechanism and management for anti-Ro/SSA-negative primary SS subset.

Methods

Study population

We selected targeted patients from participants of the Korean Initiative of primary Sjögren's Syndrome (KISS). KISS is a nationwide prospective cohort for primary SS in Korea. The purpose of this project was to establish a prospective cohort database and provide overall clinical data and samples of patients with primary SS to develop novel diagnostic and treatment tools. Informed consent was obtained from all participants according to the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital of the Catholic University of Korea (approval number: KC13ONMI0646). Recruitment began in Seoul St. Mary's Hospital, a tertiary care university hospital and referral centre in Seoul, Korea, in October 2013. By July 2017, the database included 502 patients with primary SS from 10 other university hospitals across Korea as well as Seoul St. Mary's Hospital. At enrolment for this cohort, all patients fulfilled the 2002 American-European Consensus group (AECG) classification criteria (9) and/or the 2012 ACR criteria (10). Exclusion criteria were radiation history of 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 and associated systemic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, mixed connective tissue disease, primary biliary cirrhosis, vasculitis, autoimmune hepatitis, and systemic sclerosis. Among all cohort enrolled participants, we finally chose 355 patients who fulfilled 2016 ACR/EULAR classification criteria (3) for primary SS. Of these patients, 29 showed negative findings for anti-Ro/SSA antibody but positive for minor salivary gland biopsy (MSGB). Thus, they were assigned into the anti-Ro/SSA-negative [anti-Ro (-)] group. The remaining 326 patients who presented positivity for anti-Ro/SSA antibody regardless of MSGB were assigned into the anti-Ro/SSA-positive [anti-Ro (+)] group.

Minor salivary gland biopsy and histopathologic assessment

All MSGB procedures and histopathologic assessments were performed according to the protocol announced by the Sjögren's International Collaborative Clinical Alliance (SICCA) at diagnosis or enrolment for primary SS. Harvesting procedures for minor salivary glands in the lower lip of patients were undergone by specialists in the department of otorhinolaryngology. Acquired specimens were sent to the department of pathology and assessed by experienced pathologists who specialised in oral pathology. The presence of focal lymphocytic sialadenitis which was defined as one or more dense aggregates of 50 or more lymphocytes around normal mucous acini was determined. Focus score was also calculated by giving the number of foci per 4 mm^2 of a specimen. The presence of ectopic germinal centre was also evaluated, but none of the patients in this study had this feature in their biopsy findings.

Clinical assessment

All clinical values were obtained from KISS cohort database. We used only baseline data at cohort enrolment. Symptoms about xerostomia and xerophthalmia were evaluated according to the first and second items of 2002 AECG classification criteria (9). In xerostomia tests, unstimulated salivary flow rate and xerostomia inventory (range, 11–55) (11) were measured. Schirmer I test, tear film break-up time, meibomian gland dysfunction, ocular stain score by both the SICCA method (12) and van Bijsterveld's system (13), and ocular surface disease index (range, 0-100) (14) were evaluated by ophthalmologists to assess ocular secretory function and related symptoms. All these ocular tests were performed for both eyes and worse results were selected for analysis. Disease severity and systemic involvement were evaluated according to the EULAR Sjögren's syndrome disease activity index (ESSDAI) (15) on top of other extraglandular manifestations such as Raynaud's phenomenon (RP) and autoimmune thyroid disease. ESSDAI scores of all enrolled patients were measured by rheumatologists. The Sjögren's syndrome disease damage index (SSDDI) was used to assess long-term disease-related damage (16). In addition to visual analogue scale (VAS) for patient's and physician's global assessment (range, 0-100 mm) about disease activity, the EULAR

Table I. Clinical features of 355 enrolled patients with primary Sjögren's syndrome.

	Total n=355	Anti-Ro positive group n=326	Anti-Ro negative group n=29	*p*-value
Age (years)	53 (43-60)	53 (43-59)	56 (47-67)	0.111
Gender (female)	351 (98.9)	322 (99.1)	28 (96.6)	0.290
Dry eyes	340 (95.8)	311 (95.4)	29 (100)	0.622
Dry mouth	342 (96.3)	313 (96.0)	29 (100)	0.611
Xerostomia related items				
Unstimulated salivary flow rate <1.5 ml in 15 min	181/199 (91.0)	159/171 (87.8)	22/28 (78.6)	0.025
Unstimulated salivary flow rate (ml/15 min)	0.3 (0.0-1.2)	0.3 (0.0-1.2)	0.3 (0.1-3.2)	0.505
Xerostomia inventory	38 (30-43)	38 (30-43)	40 (28-44)	0.650
Xerophthalmia related items				
Schirmer I test <5 ml/5 min	264/333 (79.3)	242/307 (78.8)	22/26 (84.6)	0.619
Schirmer I test (ml/5 min)	3 (2-5)	3 (2-5)	3 (2-5)	0.885
Tear break up time (sec)	3 (2-4)	3 (2-4)	3.5 (2-5)	0.353
Meibomian gland dysfunction	171/243 (70.4)	156/221 (70.6)	15/22 (68.2)	>0.999
OSS by SICCA method	4 (1-7)	4 (1-7)	4 (1.8-6.3)	0.964
OSS by van Bijsterveld's method	3 (1-6)	3 (1-6)	4 (1-5)	0.916
OSS by SICCA ≥5 (or Bijsterveld ≥4)	112/256 (43.8)	100/234 (42.7)	12/22 (54.5)	0.286
Ocular surface disease index	36 (20-54)	38 (20-55)	28 (17-53)	0.234
Minor salivary gland biopsy positivity*	190/213 (89.2)	161/184 (87.5)	29 (100)	0.050
Focus score	3 (2-4)	3 (2-4)	2.5 (1-4)	0.333
VAS for physician's global assessment	30 (18-45)	30 (19-50)	20 (6-41)	0.008
VAS for patient's global assessment	62 (47-76)	61 (45-75)	75 (63-84)	0.001
ESSPRI	5 (4-6)	5.3 (4-6.7)	5.3 (4.3-6.7)	0.586
ESSPRI pain	3 (0-5)	3 (0-5)	3 (0-5)	0.764
ESSPRI fatigue	5 (5-7)	5 (5-7)	5 (5-7)	0.378
ESSPRI Dryness	7 (5-8)	7 (5-8)	8 (5-10)	0.048
EuroQol-5 dimensions time tradeoff value	0.85 (0.78-0.91)	0.85 (0.78-0.91)	0.88 (0.75-0.91)	0.985
EuroQol VAS	67 (50-80)	70 (50-80)	65 (50-75)	0.710

All data are n (%) or median (interquartile). *Positive minor salivary gland biopsy is defined as focal lymphocytic sialadenitis with a focus score of ≥1 focus/4 mm^2. OSS: ocular staining score; SICCA: Sjögren's international collaborative clinical alliance; VAS: visual analogue scale; ESSPRI: EULAR Sjögren's syndrome patient-reported index

Sjögren's syndrome patient-reported index (ESSPRI) was used to evaluate patient-reported dryness, fatigue, and pain (17). To investigate patients' health-related quality-of-life, EuroQol (EQ)-5 dimensions (5D) time tradeoff (TTO) values derived from South Korean reference data (18) and EQ VAS were used.

Haematological and serological assessment

Besides clinical information, all laboratory data were also collected from each participant at study enrolment. Cytopenia possibly resulting from vitamin or iron deficiency, drugs, or anaemia of chronic disease was excluded. Leucopenia was defined as white blood cell count <4.00 x $10^3/mm^3$. Neutropenia (neutrophil <1.5 x $10^3/mm^3$), anaemia (haemoglobin concentration <12 g/dl), thrombocytopenia (platelet count <150 x $10^3/mm^3$), and hypergammaglobulinaemia (immunoglobulin G >16 g/L) were defined according to the haematological and biological domain of the ESSDAI (15). Antinuclear antibody (ANA) titre was determined using an indirect immunofluorescence assay on HEp2 cells, and a titre of 1:320 was considered positive. Rheumatoid factor (RF) was determined by immunoturbidimetric assay, and a value over 20 IU/ml was defined as a positive finding. Anti-Ro/SSA antibodies and all other autoantibodies were tested using commercial enzyme-linked immunosorbent assay.

Statistical analysis

All data analyses were carried out using IBM-SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA). After confirming that data were not normally distributed by Kolmogorov-Smirnov test, continuous variables are expressed as median and interquartile range (IQR) and analysed with Mann-Whitney test. Chi-square test and Fisher's exact test were used for categorical variables. Statistical significance was considered at $p<0.05$.

Results

Baseline clinical characteristics and glandular functions

The median age of 355 patients enrolled for this study was 53 years (IQR, 43-63 years). Although the anti-Ro (-) group was slightly older than the anti-Ro (+) group, the difference was not statistically significant. The majority of them were females (98.9%) and had symptoms of xerostomia (96.3%) and xerophthalmia (95.8%). Almost all clinical variables about dry eyes and mouth including xerostomia inventory, Schirmer I test, tear break up time, meibomian gland dysfunction, ocular staining score (both SICCA method and van Bijsterveld's method), and ocular surface disease index were similar between the two groups except the proportion of patients who showed positive finding for unstimulated salivary flow rate (defined as <1.5 ml in 15 minutes, $p=0.025$) as shown in Table I. Focus score which indicates the severity of lymphocytic infiltration in

Table II. Clinical indices for disease-related systemic activity, extraglandular manifestations and long-term damage.

	Total n=354	Anti-Ro positive group n=325	Anti-Ro negative group n=29	*p*-value
ESSDAI total score	3 (1-6)	3 (1-6)	2 (0-5)	0.195
Positivity for each ESSDAI domain				
Constitutional	48 (13.6)	46 (14.2)	2 (6.9)	0.399
Lymphadenopathy	17 (4.8)	17 (5.2)	0 (0)	0.380
Glandular	38 (10.7)	38 (11.7)	0 (0)	0.057
Articular	81 (22.9)	74 (22.8)	7 (24.1)	0.866
Cutaneous	23 (6.5)	22 (6.8)	1 (3.4)	0.708
Pulmonary	34 (9.6)	29 (8.9)	5 (17.2)	0.178
Renal	4 (1.1)	3 (0.9)	1 (3.4)	0.291
Muscular	3 (0.8)	3 (0.9)	0 (0)	>0.999
Peripheral nervous system	14 (4.0)	10 (3.1)	4 (13.8)	0.021
Central nervous system	3 (0.8)	3 (0.9)	0 (0)	>0.999
Haematological	103 (29.1)	100 (30.8)	3 (10.3)	0.019
Biological	191 (54.0)	179 (55.1)	12 (41.4)	0.156
Other extraglandular manifestations				
Raynaud's phenomenon	61 (17.2)	52 (16.0)	9 (31.0)	0.067
Autoimmune thyroid disease	44 (12.4)	42 (12.9)	2 (6.9)	0.556
SSDDI total score	2 (1-3)	2 (1-3)	2 (1-3)	0.970
Positivity for each SSDDI item				
Oral/salivary damage				
Salivary flow impairment	210 (59.3)	194 (59.7)	16 (55.2)	0.635
Loss of teeth	33 (9.3)	27 (8.3)	6 (20.7)	0.041
Ocular damage				
Tear flow impairment	245 (69.2)	226 (69.5)	19 (65.5)	0.653
Structural abnormalities	209 (59.0)	192 (59.1)	17 (58.6)	0.962
Neurologic damage				
Central nervous system involvement	3 (0.8)	3 (0.9)	0 (0)	>0.999
Peripheral neuropathy	11 (3.1)	9 (2.8)	2 (6.9)	0.225
Pleuropulmonary damage	6 (1.7)	6 (1.8)	0 (0)	>0.999
Renal impairment	5 (1.4)	5 (1.5)	0 (0)	>0.999
Lymphoproliferative disease	0 (0)	0 (0)	0 (0)	

All values are n (%) or median (interquartile). ESSDAI: EULAR Sjögren's syndrome disease activity index; SSDDI: Sjögren's syndrome disease damage index.

salivary glands showed no inter-group difference. Interestingly, opinions about disease severity were conflicting among physicians and patients according to results of VAS. Patients of anti-Ro (-) group considered their states of SS more serious than those of anti-Ro (+) group [median: 75 (IQR, 63–84) *vs*. 61 (IQR, 45–75), p=0.001], although physicians assessed them reversely [20 (IQR, 6–41) *vs*. 30 (IQR, 19–50), p=0.008]. Similarly, in dryness domain of ESSPRI in which patients scored pain, fatigue, and dryness by themselves, the anti-Ro (-) group felt sicca symptoms worse than the anti-Ro (+) group [8 (IQR, 5–10) *vs*. 7 (IQR, 5-8), p=0.048] despite both groups had comparable secretory functions as shown in Table I. However, there was no disparity in the EQ-5D TTO values and EQ VAS between the two groups.

Extraglandular manifestations and disease related damage

Overall scores of both ESSDAI and SSDDI presented no considerable difference between the two study groups as shown in Table II. However, in separate assessment for each domain of ESSDAI, the positivity for domain of peripheral nervous system (PNS) was more frequent in the anti-Ro (-) group (13.8% *vs*. 3.1%, p=0.021). The anti-Ro (+) group showed more haematological abnormalities (30.8% *vs*. 10.3%, p=0.019). These will be described in detail in the next part. Among items of SSDDI, loss of teeth occurred more in the anti-Ro (-) group (20.7% *vs*. 8.3%, p=0.041). Less positivity for glandular domain of ESSDAI and more frequent RP were also observed in the anti-Ro (-) group, although differences between the two groups were not statistically significant (p=0.057 and p=0.067, respectively).

Haematological and serological features

The anti-Ro (-) group showed higher median values of total white blood cell count, absolute neutrophil count, and haemoglobin concentration (p=0.002, p=0.003 and p=0.005, respectively). Results are shown in Table III. Because of these differences in blood cell count results, proportion of patients who presented leukopenia was lower in the anti-Ro (-) group (6.9% *vs*. 34.1%, p=0.003). Rheumatoid factor (RF) positivity (p<0.001), level of anti-cyclic citrullinated peptides (CCP) antibody and β_2-microglobulin (p=0.012 and p=0.034, respectively) were also lower in the anti-Ro (-) group. Regarding parameters about B-cell expansion, the anti-Ro (-) group showed less hypergammaglobulinaemia and lower serum immunoglobulin G and A levels (all p<0.001). Actual

Table III. Haematologic and serologic features.

	Total n=355	Anti-Ro positive group n=326	Anti-Ro negative group n=29	*p*-value
White blood cell count (x 10^3/mm^3)	4.520 (3.700-5.592)	4.440 (3.600-5.500)	5.155 (4.632-6.147)	0.002
Leukopenia (<4.00 x 10^3/mm^3)	112/352 (31.8)	110/323 (34.1)	2 (6.9)	0.003
Absolute neutrophil count (x 10^3/mm^3)	2.355 (1.817-3.250)	2.300 (1.760-3.210)	3.120 (2.342-3.475)	0.003
Neutropenia (<1.50 x 10^3/mm^3)	47/350 (13.4)	46/321 (14.3)	1 (3.4)	0.100
Haemoglobin concentration (g/dl)	12.8 (12.0-13.6)	12.8 (12.0-13.6)	13.4 (12.7-14.0)	0.005
Anaemia (<12 g/dl)	80/352 (22.7)	77 (23.8)	3 (10.3)	0.097
Platelet count (x 10^3/mm^3)	221 (188-257)	222 (188-258)	228 (181-273)	0.591
Thrombocytopenia (<150 x 10^3/mm^3)	24/352 (6.8)	23/323 (7.1)	1 (3.4)	0.707
Anti-nuclear antibody positivity (titre ≥320)	204/325 (62.8)	188/297 (63.3)	16/28 (57.1)	0.519
Rheumatoid factor positivity (>20 IU/ml)	211/320 (65.9)	205/292 (70.2)	6/28 (21.4)	<0.001
Anti-cyclic citrullinated peptides antibody (U/ml)	1.5 (0.8-4.0)	1.6 (0.9-4.5)	0.8 (0.5-1.3)	0.012
β_2-microglobulin (μg/ml)	1.952 (1.609-2.437)	1.988 (1.656-2.473)	1.538 (1.250-2.205)	0.034
Hypergammaglobulinaemia	160/322 (49.7)	158/293 (53.9)	2 (6.9)	<0.001
Immunoglobulin G (mg/dl)	1593 (1355-1983)	1651 (1385-2016)	1288 (1109-1379)	<0.001
Immunoglobulin A (mg/dl)	268 (202-366)	271 (209-377)	202 (154-262)	<0.001
Immunoglobulin M (mg/dl)	115 (85-151)	113 (82-149)	120 (96-173)	0.094
Cryoglobulin	9/299 (3.0)	9/273 (3.3)	0/26 (0)	>0.999
Low C3 (<76 mg/dl)	55/336 (16.4)	47/308 (15.3)	8/28 (28.6)	0.104
Low C4 (<12 mg/dl)	17/336 (5.1)	17/308 (5.5)	0/28 (0)	0.379
C3 (mg/dl)	92 (81-102)	91 (81-102)	84 (74-99)	0.079
C4 (mg/dl)	22 (18-26)	22 (18-26)	22 (18-26)	0.956
Anti-La/SSB antibody positivity	186/354 (52.5)	186/325 (57.2)	0 (0)	<0.001
Anti-centromere antibody positivity	38/302 (12.6)	23/274 (8.4)	15/28 (53.6)	<0.001
Anti-topoisomerase antibody positivity	5/282 (1.8)	3/255 (1.2)	2/27 (7.4)	0.074
Anti-ribonucleoprotein antibody positivity	6/207 (2.9)	6/189 (3.2)	0 (0)	>0.999
Anti-Jo-1 antibody positivity	3/295 (1.0)	2/267 (0.7)	1/28 (3.6)	0.259
Anti-DNA antibody positivity	10/315 (3.2)	10/287 (3.5)	0 (0)	0.609

All values are n (%) or median (interquartile).

values of serum immunoglobulin G and A in the anti-Ro (-) group were mostly distributed within normal reference range. Due to well-known close relation with anti-Ro/SSA antibody, anti-La/SSB antibody was more frequently observed in the anti-Ro (+) group (57.2% *vs*. 0%, $p<0.001$). Among other autoantibodies, only anti-centromere antibody (ACA) showed difference in positivity frequency between the two groups. Patients in the anti-Ro (-) group presented significantly more positivity for this antibody (53.6% *vs*. 8.4%, $p<0.001$). Three patients of the anti-Ro (+) group and two patients of the anti-Ro (-) group showed positivity for anti-topoisomerase antibody, whereas 2 of the anti-Ro (+) group patients and 1 of the anti-Ro (-) group patients were positive for anti-Jo-1 antibody. None of them had clinical features such as myositis or interstitial lung disease which could exist in other autoimmune diseases with positivity for those autoantibodies. Those two patients of the anti-Ro (-) group with positivity for anti-topoisomerase antibody presented RP.

Discussion

In primary SS, anti-Ro/SSA antibody has been reported to be related to disease-specific symptoms and disease severity (2). As anti-La/SSA antibody has little influence on clinical manifestations in primary SS without anti-Ro/SSA antibody (19, 20), most recently published 2016 ACR/EULAR classification criteria include anti-Ro/SSA antibody as the only serologic marker (3). However, 20–30% of SS patients still show lymphocytic infiltration of exocrine gland in despite of the absence of such disease-determinant antibody (2). Because several previous studies have described that anti-Ro/SSA-negative patients have less concerns about critical consequences of SS-like lymphoma (8), researchers have not focused on these population sufficiently. Therefore, whether another possible key marker that can exist in anti-Ro/SSA-negative patients and induce SS specific symptoms has not been clarified yet. In our study, we could find some important information about such uncertain pathogenesis and clinical characteristics of anti-Ro/SSA-negative SS. First, secretory dysfunctions of both lacrimal and salivary glands in anti-Ro/SSA-negative patients were comparable to those in patients with anti-Ro/SSA antibody. Focus score indicating the severity of lymphocytic infiltration in exocrine glands showed little difference between the two groups. These findings suggest that the mechanism and the degree of glandular pathology might be similar regardless whether there is anti-Ro/SSA antibody in primary SS. Subjective dryness could be even more severe in anti-Ro/SSA-negative patients according to results of ESSPRI shown in Table II. Although such 'dry feeling' did not affect the overall health-related quality of life, this could not just be patients' subjective emotions because complete or partial loss of teeth occurred more in anti-Ro/SSA-negative patients as possible consequences of xerostomia. Other disease-related systemic activity and extraglandular manifestations measured with ESSDAI were similar between the two groups except the PNS

domain in ESSDAI. These documented peripheral neuropathies that were dominant in the anti-Ro/SSA-negative group might be pure sensory neuropathies according to the study performed by Jamilloux *et al*. (21), although there is no certainty of this due to the lack of detailed information about these in the present study. B-cell activating factor also could affect this result (22). However, we did not measure it in the present study.

Second, results about haematological abnormalities such as cytopenia (leukopenia, neutropenia and anaemia) and serological features including RF positivity, hypergammaglobulinaemia and β_2-microglobulin that showed significant difference between the two groups in our study were consistent with those of previous studies (4-6). Although all types of cytopenia are usually subclinical in primary SS (23), their relationship with anti-Ro/SSA antibody is supposed to be obvious in our study. Hypergammaglobulinaemia and higher level of β_2-microglobulin have been considered to be due to persistent B-cell stimulation and activation. The presence of RF could initiate this process (1, 2, 8). This could mean that B-cell targeted treatment could be less effective in anti-Ro/SSA-negative SS patients because of their relatively lower chronic B-cell proliferation. The positivity for anti-Ro/SSA antibody has shown to have close association with these pre-lymphomatous conditions in other studies (2, 4, 5, 8, 24). Because we ruled out cases with a history of lymphoproliferative disease at enrolment and only selected data from baseline, we did not observe actual occurrence of lymphoma in the present study. Therefore, we should take a close look during follow-up period of cohort to discover whether anti-Ro/SSA-negative patients will show lower lymphoma incidence.

Third, among other autoantibodies, only ACA showed a significant difference in prevalence between the two study groups. Over 50% of anti-Ro/SSA-negative patients were positive for ACA in our study. This subset of patients showed high frequency of RP, elevated ESSDAI scores and more compromised secretory functions without elevated biologic markers of B cell hyperactivity. This could be the reason why anti-Ro/SSA-negative patients showed such clinical characteristics described above. ACA-positive primary SS has been thought to be another subset of the disease (25, 26). According to several recent studies, ACA positivity can affect SS patients with older age, less hypergammaglobulinaemia, RF, anti-Ro/SSA positivity, and more RP (27). These findings are quite similar to previously mentioned characteristics of anti-Ro/SSA-negative group found in our study. However, ACA positivity also causes more severe dry symptoms and higher ESSDAI score in primary SS as well (28). These points could contribute to results of our study by increasing heterogeneity of the anti-Ro/SSA-negative group. We could have only seen characteristics of ACA-positive patient-mixed study population, not pure anti-Ro/SSA-negative patients. Therefore, ACA-positive SS patients should have been separated from anti-Ro/SSA-negative SS group as a different subset of SS in this respect. Otherwise, ACA could be the key marker that induces SS-specific manifestations in patients without anti-Ro/SSA antibody. Considering its influence on clinical feature of primary SS, ACA should be carefully examined for next criteria in the future.

Our study has several limitations. The study population of the anti-Ro/SSA-negative group was relatively small compared to that of the anti-Ro/SSA-group to maximise analysis power. Lower prevalence of anti-Ro/SSA-negative SS which was observed in our study could originate from conservative implementation of MSGB at enrolment for our cohort data. There has been no data that described the rate of anti-Ro/SSA positivity in Korea is different from that in other countries. The prevalence of anti-Ro/SSA-negative primary SS in overall KISS cohort was 12%, which is still low compared to that of other series. During early periods of cohort enrolment between 2013 and 2015 when 2016 criteria had not been introduced yet, definite primary SS patients diagnosed with positivity for anti-Ro/SSA antibody not requiring further MSGB could have been included in the database preferentially. Obviously, we underwent all the evaluations for primary SS in patients with sicca symptoms including MSGB during late periods of enrolment when 2016 criteria had been announced. There might have been a sort of selection bias at early stage of cohort enrolment that could result in such discrepancies of the rate of positivity for the antibody. Second, there might be anti-Ro52-positive patients in the anti-Ro/SSA-negative group (2). Peene *et al*. (29) have published that commercial anti-Ro/SSA assays mainly detect anti-Ro60 antibodies. Therefore, anti-Ro52-positive patients could have been missed by standard anti-Ro/SSA assays. Because these patients are supposed to have strong features as observed in anti-Ro/SSA-positive SS patients (30), the actual clinical manifestations about anti-Ro/SSA-negative group could have been confounded by them. A future study must have a clarified criterion about this. Nevertheless, the strong point of our study was that it included the most various clinical variables in the analysis to date.

In conclusion, primary SS patients who show an absence of anti-Ro/SSA antibody have discriminating clinical manifestations compared to anti-Ro/SSA-positive patients. Therefore, clinicians should consider minor salivary gland biopsy in patients with suspicious symptoms who are anti-Ro/SSA-negative in order to diagnose primary SS. Considering their features, treatment and follow-up plans that are adapted for anti-Ro/SSA-negative SS subset must be conducted in clinic. Further study is also needed to investigate related mechanisms.

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