Implications of thyroid-related autoantibodies in patients with Sjögren's syndrome without overt thyroid diseases

Y.-J. Ha[1,2], J.-I. Lee[1], S.R. Choi[1,2], E.H. Kang[1,2], K.-S. Kim[3], W.-J. Jeong[4], J.Y. Hyon[5], M.J. Kim[6], S. Cha[7], Y.J. Lee[1,2,8]

[1]Division of Rheumatology, Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul; [3]Department of Periodontology, Section of Dentistry, Seoul National University Bundang Hospital, Seongnam; [4]Department of Otorhinolaryngology, Head & Neck Surgery, Seoul National University Bundang Hospital, Seongnam; [5]Department of Ophthalmology, Seoul National University Bundang Hospital, Seongnam; [6]Division or Endocrinology, Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea; [7]Division of Oral Medicine, Department of Oral and Maxillofacial Diagnostic Sciences, University of Florida College of Dentistry, Gainesville, FL, USA; [8]Department of Medical Device Development, Seoul National University College of Medicine, Seoul, Republic of Korea.

Abstract

Objective

We investigated the prevalence of anti-thyroid autoantibodies and thyroid dysfunction, and their association with clinical and laboratory features in Korean patients with primary Sjögren's syndrome (pSS) without overt thyroid illnesses.

Methods

We consequently included 196 pSS patients (190 women) and cross-sectionally collected clinical and laboratory data including the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and Patient Reported Index (ESSPRI). The fatigue-dominant group was defined as those in the highest quartile of the fraction of fatigue, calculated as the ESSPRI fatigue score divided by the total ESSPRI score. Thyroid stimulating hormone (TSH), free T4 (fT4), anti-thyroglobulin (TG), anti-thyroid peroxidase (TPO), and anti-TSH receptor antibody (TRAb) were measured.

Results

Of 196 patients, 31 (15.8%) were positive for anti-TG, 28 (14.3%) for anti-TPO, and 28 (14.3%) for TRAb. Subclinical hypothyroidism (S-Hypo) was identified in 23 (11.7%) patients. Anti-TG had no correlations with thyroidal function or pSS-related features. Meanwhile, anti-TPO was significantly associated with TSH levels or anti-centromere antibody. TRAb-positive patients exhibited significantly higher ESSDAI and clinical ESSDAI scores. Moreover, the most influential independent predictor for TRAb was lymphopenia (odds ratio [OR] = 5.541), which has been known as a prognostic factor. Additionally, significant bidirectional associations were found between the fatigue-dominant group (OR = 3.482) and anti-TPO-positive S-Hypo (OR = 7.586) in multivariate regression analyses.

Conclusion

Latent thyroid autoimmunity, particularly TRAb and anti-TPO, may be associated with clinical and laboratory manifestations of pSS, such as disease activity or fatigue, even without overt thyroid dysfunction.

Key words

Sjögren's syndrome, anti-thyroid autoantibodies, thyroid function tests, fatigue

You-Jung Ha, MD, PhD
Jee-In Lee, MD
Se Rim Choi, MD
Eun Ha Kang, MD, PhD, MPH
Keun-Suh Kim, DDS
Woo-Jin Jeong, MD, PhD
Joon Young Hyon, MD, PhD
Min Joo Kim, MD, PhD
Seunghee Cha, DDS, PhD
Yun Jong Lee, MD, PhD

Please address correspondence to:
Yun Jong Lee
Department of Internal Medicine,
Seoul National University
Bundang Hospital,
82 Gumi-ro, 173 Beongil,
Bundang-gu, Seongnam-si,
Gyeonggi-do 13620, Republic of Korea.
E-mail: yn35@snu.ac.kr

ORCID iD: 0000-0001-7615-8611

Received on March 17, 2024; accepted in revised form on May 6, 2024.

Funding: this work was supported by the Ministry of Health & Welfare, Republic of Korea (grant no. HI21C150101 to LYJ), Seoul National University Hospital (grant no. 21-2023-0079 to LYJ), and the National Institutes of Health (NIDCR DE032707 to S.C.).

Competing interests: none declared.

Introduction

Sjögren's syndrome (SS) frequently coexists with other systemic or organ-specific autoimmune diseases. Autoimmune thyroid disease (AITD) is considered the most commonly associated autoimmune disease in patients with SS (1), and a recent meta-analysis demonstrated a greater risk of thyroid disease (odds ratio [OR] = 3.29) and AITD (OR = 3.48) in patients with primary SS (pSS) than in controls (2). Additionally, anti-thyroid peroxidase (TPO) or anti-thyroglobulin (TG), well-known markers of Hashimoto's thyroiditis (HT), can be detected in up to 40% of patients with pSS (3). Anti-TPO and anti-TG positivity rates in patients with SS were estimated to be approximately three times higher than that in controls (4). Therefore, it is widely accepted that pSS and HT are immunologically and pathogenetically correlated.

HT manifests as arthralgia/myalgia, fatigue, and sicca symptoms and leads to the development of non-Hodgkin's lymphoma (NHL), similar to pSS (3-5). However, whether concomitant AITD or thyroid dysfunction has a clinical impact on pSS remains controversial. One study reported that AITD was more common in SS patients who were positive for rheumatoid factor (RF) and anti-SSA/Ro antibodies (6), whereas another study reported a lower prevalence of anti-SSA/Ro antibodies in SS with AITD (7). An Italian study revealed that HT was related to the mild phenotype of SS (8). In contrast, a Chinese study showed a higher prevalence of extra-glandular manifestations and active immunological profiles in pSS patients with thyroid disorders (9). A single retrospective study reported comparable EULAR SS Disease Activity Index (ESSDAI) scores between pSS with and without AITD (10).

Anti-thyroid stimulating hormone (TSH) receptor autoantibodies (TRAbs), the gold standard markers for Graves' disease (GD), have not been commonly investigated in pSS. Previous studies have described hyperthyroidism in 0–7.5% of patients with pSS (11, 12) and reported TRAb detection rates of 0–12.6% (12, 13). Nevertheless, a recent systematic review showed a higher prevalence of hyperthyroidism in pSS (OR=2.61) than in controls, similar to hypothyroidism (OR=3.81) (2). Until now, there have been no studies on clinical implications of thyroid dysfunction or thyroid-related autoantibodies in Korean patients with pSS.

Therefore, we aimed to investigate the prevalence of subclinical thyroid dysfunction and anti-thyroid autoantibodies, including TRAb, as well as their association with pSS-related features in Korean pSS patients without overt thyroid diseases.

Methods

Patients and clinical data

We prospectively and consequently recruited 228 patients with pSS from September 2021 to February 2023. Of these, 196 patients were ultimately included in our analysis after excluding those on levothyroxine or anti-thyroid drugs due to overt thyroid disease (21 overt hypothyroidism and 1 GD), patients with thyroid cancer (n = 4), and those who withdrew from the study (n = 6). All patients were younger than 80 years of age and met the 2016 ACR/EULAR criteria for pSS (14).

We collected EULAR SS Patient Reported Index (ESSPRI), ESSDAI, clinical ESSDAI (ClinESSDAI), and EuroQol 5-dimensional (EQ-5D) data at enrolment (15-18). The ESSPRI score is a composite measure of the severity of the key three symptoms of pSS (dryness, limb pain, and fatigue) using 0-10 numerical scale (16). The ESSDAI is a systemic disease activity index of pSS and includes 12 organ-specific domains (constitutional, glandular, cutaneous, respiratory, renal, articular, muscular, peripheral and central nervous system, haematological, lymphoid, and biological) (15). The ClinESSDAI is a modified ESSDAI without the biological domain (17).

To estimate the relative contribution of fatigue to the ESSPRI, the fraction of fatigue ($F_{fatigue}$) was calculated as the ESSPRI fatigue visual analog scale (VAS) score divided by the total ESSPRI score. Patients in the highest quartile of $F_{fatigue}$ were defined as the fatigue-dominant group. Clinical manifestations were classified according to

the ESSDAI domain. Laboratory data, including the complete blood count, erythrocyte sedimentation rate (ESR), and levels of IgG, β2-microglobulin, cryoglobulin, and complement C3 and C4, were assessed. Lymphopenia was defined as absolute lymphocyte counts <1,000/mm^3. Data on RF, antinuclear antibody, anti-SSA/Ro, and anti-centromere antibody (ACA) were retrieved from medical records.

The current study protocol was reviewed and approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB No. B-0506/021-004), and informed written consent was obtained from all participants.

Measurement of thyroid hormones and thyroid-related autoantibodies

Thyroid function tests including serum TSH (reference range, 0.3–4.0 μIU/mL) and free T4 (fT4; 0.89–1.79 ng/dL) were measured by an immune radio-metric assay (IMRA; BRAHMS GmbH, Hennigsdorf, Germany) and a radioimmunoassay (RIA; Beckman Coulter/Immunotech, Prague, Czech Republic), respectively. Anti-TG (BRAHMS GmbH) and anti-TPO (BRAHMS GmbH) antibodies were measured using commercial RIA kits. TRAbs were determined using an electrochemiluminescence immunoassay (Elecsys® anti-TSH-R, Roche Diagnostics Ltd, Basel, Switzerland). Positivity for anti-thyroid antibodies was defined as a value of ≥60 IU/mL for anti-TG and anti-TPO, and >1.22 IU/L for TRAb, based on manufacturer's instructions. Subclinical hypothyroidism (S-Hypo) or hyperthyroidism (S-Hyper) was defined as TSH levels above (>4.0 μIU/mL) or below (TSH<0.3 μIU/mL) the reference range, respectively, with normal fT4 levels (19). Anti-TPO-positive patients with S-Hypo (anti-TPO-positive S-Hypo) were classified as those at a high risk for overt hypothyroidism (20).

Statistical analysis

Data are presented as median [interquartile range, IQR] for continuous variables and number (percentages) for categorical variables. Comparisons between groups were performed using the Mann-Whitney U-test for continuous variables because most variables did not follow a normal distribution. Chi-square or Fisher's exact tests were used to compare categorical variables. Univariate regression analysis was performed to identify clinical variables associated with thyroid dysfunctional status or thyroid-related autoantibodies. All variables with a *p*-value of <0.1 in the univariable analyses were included in the multivariable logistic forward regression analyses. All analyses were performed with SPSS Statistics for Windows, v. 26 (IBM Corp., Armonk, NY, USA). A *p*-value <0.05 was considered statistically significant.

Table I. Baseline characteristics of study population.

Characteristics	
Women	190 (96.9%)
Age (year)	
At diagnosis	50.4 [40.0-59.0]
At enrolment	58.6 [46.5-67.3]
Disease duration (year)	6.2 [2.7-12.4]
Clinical features at diagnosis	
Oral dryness	148 (75.5%)
Ocular dryness	147 (75.0%)
Salivary gland dysfunction	167/191 (87.4%)
USWSF ≤ 0.1 mL/min	152/185 (82.2%)
Lacrimal gland dysfunction	157/194 (80.9%)
Schirmer test ≤ 5/5 mm	129/181 (71.3%)
Ocular stain score ≥ 5	101/189 (53.4%)
Focal lymphocytic sialadenitis with focus score ≥1	124/155 (80.0%)
Anti-SSA/Ro positivity	175 (89.3%)
Anti-SSB/La positivity	104 (53.1%)
ANA positivity ≥1:160	177 (90.3%)
RF positivity	108/192 (56.3%)
ACA positivity	22/194 (11.3%)
no. of ESSDAI domains ever affected*	1.0 [1.0-2.0]
Disease activity	
ESSDAI	2.0 [1.0-4.0]
ESSDAI ≥ 5	34 (17.3%)
ClinESSDAI	0.0 [0.0-0.0]
ClinESSDAI ≥ 5	33 (16.8%)
ESSPRI	5.0 [3.3-6.0]
Dryness VAS	5.0 [3.0-7.0]
Fatigue VAS	5.0 [3.3-7.0]
Pain VAS	3.0 [1.0-6.0]
ESSPRI ≥ 5	99 (50.5%)
$F_{fatigue}$	1.13 [0.93-1.36]
EQ-5D index score	0.93 [0.89-0.97]
EQ VAS	75.0 [70.0-80.0]
Current medications	
Pilocarpine	117 (59.7%)
Hydroxychloroquine	59 (30.1%)
Methotrexate	12 (6.1%)
Leflunomide	6 (3.1%)
Azathioprine or mycophenolate	6 (3.1%)
Tacrolimus or cyclosporine	3 (1.5%)
Glucocorticoids	20 (10.2%)
Prednisolone equivalent dose (mg/day)	2.5 [1.3-4.4]

Data are presented as number (%) or median [interquartile ranges].
*Among 11 domains excluding the biological domain.
USWSF: unstimulated whole salivary flow; ANA: antinuclear antibody; RF: rheumatoid factor; ACA: anticentromere antibody; ESSDAI: EULAR SS disease activity index; ClinESSDAI: clinical ESSDAI; ESSPRI: EULAR SS Patient Reported Index; VAS: visual analogue scale; $F_{fatigue}$: fraction of fatigue; EQ-5D: EuroQoL-5 dimensional.

Results

Baseline characteristics of study participants

The baseline characteristics of the 196 participants with pSS (190 women and 6 men) are summarised in Table I. Their median age at enrolment was 58.6 (IQR, 46.5–67.3) years, and median disease duration was 6.2 (2.7–12.4)

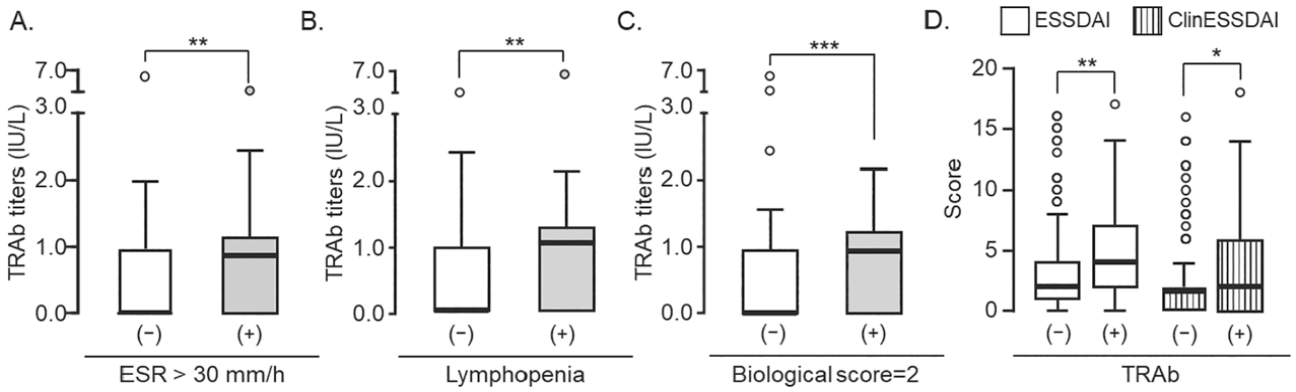

Fig. 1. Associations between TRAb and primary SS-related clinical features. Box plots denote the median (bold center lines) and the 25th and 75th percentiles. *P* values were calculated by the Mann-Whitney U test.
**p*<0.05; ***p*<0.01; ****p*<0.001.
TRAb: anti-TSH receptor antibodies; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR SS disease activity index; ClinESSDAI: clinical ESSDAI.

years. The median ESSDAI, ClinESSDAI, and ESSPRI scores were 2.0, 0.0, and 5.0, respectively. The top five of involved ESSDAI domains were the haematological (58.2%), articular (25.5%), glandular (17.3%), cutaneous (16.3%), and respiratory (7.7%). Thirty-four (17.3%) patients had an ESSDAI ≥5 (moderate-to-high activity) and 99 (50.5%) had an ESSPRI ≥5 (unsatisfactory symptom state).

Prevalence of thyroid-related auto-antibodies and thyroid dysfunction

A total of 71 (36.2%) patients were positive for any thyroid-related autoantibodies. The prevalence of anti-TG, anti-TPO, and TRAb antibodies was 31 (15.8%), 28 (14.3%), and 28 (14.3%), respectively. The anti-TPO titres were observed to exceed the threshold for positivity by more than 15 times, while the levels of other autoantibodies remained below 2-3 times their positivity criteria (Supplementary Table S1).
Of 196 patients, 24 (12.2%) had TSH > 4.0 μIU/mL, but none had TSH > 10.0 μIU/mL. Among those with TSH>4.0 μIU/mL, 23 (11.7%) patients were S-Hypo, and 6 (3.1%) were TPO-positive S-Hypo. Three patients (1.5%) had TSH <0.3 μIU/mL but did not show thyroid-related antibodies including TRAb. Among them, two patients had S-Hyper.

Clinical features related to thyroid-related autoantibodies

When stratified by anti-TPO, anti-TPO positivity was associated with old age, S-Hypo, ACA positivity, and anti-TG positivity (Suppl. Table S2). Anti-TPO positivity or anti-TG positivity were not associated with ESSDAI, ESSPRI, or other pSS-related clinical features. In multivariate regression analysis, anti-TPO positivity was independently associated with TSH levels (OR=1.461 [95% CI 1.123–1.901], *p*=0.005), anti-TG (OR=7.163 [95% CI 2.737–18.749], *p*<0.001), and ACA (OR=3.541 [95% 1.179–10.634], *p*=0.024) (Suppl. Table S3).
Anti-TG positivity was not associated with clinical and laboratory variables with the exception for RF (OR=2.594 [95% CI 1.022–6.588], *p*=0.045) and anti-TPO positivity (OR=5.490 [95% CI 2.208–13.654], *p*<0.001) in multivariate regression analysis. But, among 12 patients with both anti-TPO and anti-TG positivity, only 3 (25%) belonged to the S-Hypo group.
When stratified by the presence of TRAb, the TRAb-positive subgroup had a shorter disease duration and a lower likelihood of lacrimal gland dysfunction at diagnosis (Suppl. Table S4). The TRAb-positive subgroup showed a significantly increased prevalence of pulmonary involvement (17.9% *vs*. 4.8%, *p*=0.010) and a significantly higher IgG level (median 2.00 g/dL *vs*. 1.74 g/dL, *p*=0.027). Furthermore,

Table II. Age- and sex-adjusted logistic regression analysis to determine factors related to TRAb positivity.

Variables	Univariate		Multivariate	
	OR [95% CI]	*p* value	OR [95% CI]	*p*-value
Disease duration (year)	0.914 [0.843-0.991]	**0.029**	-	-
Lacrimal dysfunction at diagnosis	0.350 [0.146-0.840]	**0.019**	0.307 [0.117-0.804]	**0.016**
ESSDAI ≥ 5	3.333 [1.375-8.082]	**0.008**	-	-
ClinESSDAI ≥ 5	2.288 [0.909-5.761]	0.079	-	-
Respiratory domain*	4.348 [1.310-14.432]	**0.016**	-	-
Biological domain*	2.362 [0.853-6.541]	0.098	-	-
Domains ever affected ≥3**	2.400 [0.950-6.604]	0.064	-	-
ANC	1.000 [1.000-1.001]	**0.048**	-	-
ESR	1.028 [1.008-1.048]	**0.005**	1.031 [1.009-1.054]	**0.005**
Serum protein	1.923 [0.967-3.822]	0.062	-	-
IgG	1.001 [1.000-1.002]	**0.026**	-	-
Lymphopenia[c]	4.500 [1.748-11.586]	**0.002**	5.541 [1.966-15.618]	**0.001**
RF positivity	2.159 [0.900-5.182]	0.085	-	-

*Based on the definition of ESSDAI domain; **among 11 domains excluding biological domain; [c]absolute lymphocyte counts <1,000/mm^3.
TRAb: anti-TSH receptor antibodies; ESSDAI: EULAR SS disease activity index; ClinESSDAI: clinical ESSDAI; ESSPRI: EULAR SS Patient Reported Index; ANC: absolute neutrophil count; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor.

TRAb titres were higher in subgroups with ESR >30 mm/h (p=0.007), lymphopenia (p=0.008), or biological domain level = 2 (p<0.001) (Fig. 1A-C). Interestingly, both the ESSDAI (4.0 [2.0–7.0] *vs*. 2.0 [1.0–4.0], p=0.004) and ClinESSDAI (2.0 [0.0–5.9] *vs*. 2.0 [0.0–2.0], p=0.014) scores were significantly higher in the TRAb-positive subgroup than in the TRAb-negative group (Fig. 1D). Accordingly, TRAb-positive patients showed a higher prevalence of ESSDAI ≥5.0 (35.7% *vs*. 14.3%, p=0.006) (Suppl. Table S4). However, TRAb positivity was not associated with thyroid functional status or focus scores of labial gland biopsies. Age-and sex-adjusted multivariate regression showed a significant association between TRAb positivity and lymphopenia (OR=5.541 [95% CI 1.966–15.618], p=0.001), ESR (OR=1.031 [95% CI 1.009–1.054], p=0.005), or lacrimal gland dysfunction at diagnosis (OR=0.307 [95% CI 0.117–0.804], p=0.016) (Table II).

Clinical features related to thyroid functional states

The S-Hypo subgroup (n=23) had a significantly longer disease duration (11.2 years) compared to the euthyroid subgroup (n=171, 5.3 years, p=0.003) (Suppl. Table S5), with no age differences between the groups. This subgroup exhibited significantly higher frequencies of ACA and anti-TPO positivity (both p<0.05). The prevalence of fatigue-dominant group was significantly higher in patients with S-Hypo (43.5% *vs*. 23.1%, p<0.05) or anti-TPO-positive S-Hypo (66.7% *vs*. 24.2%, p<0.05) than in those without (Fig. 2). However, this trend was not observed in patients with the highest quartile of ESSPRI fatigue VAS score or total ESSPRI score.

When compared to the fatigue non-dominant patients, the fatigue-dominant group was younger and less likely to have dry eye at diagnosis, unsatisfactory symptom state, or ≥3 ever affected ESSDAI domains (all p<0.05) (Suppl. Table S6). They also had a higher prevalence of S-Hypo (20.0% *vs*. 8.9%, p=0.035) or anti-TPO-positive S-Hypo (8.0% *vs*. 1.4%, p=0.038) and lower lymphocyte counts (p=0.015). Logistic regression analysis revealed age (OR = 0.967 [95% CI 0.943–0.991], p=0.008) and anti-TPO-positive S-Hypo (OR=6.770 [95% CI 1.180–38.851], p=0.032) as significant predictors for the fatigue dominant group (Table III). The fatigue-dominant group itself was independently associated with S-Hypo (OR=3.482 [95% CI 1.298–9.342], p=0.013) (Suppl. Table S7) or anti-TPO-positive S-Hypo (OR=7.586 [95% CI 1.211–47.522], p=0.03) (Table IV) in further multivariate regression analyses.

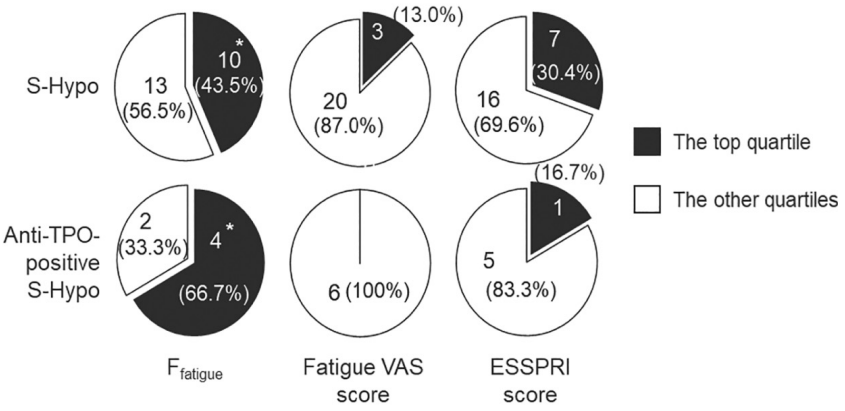

Fig. 2. Association between thyroid functional status and the fractions of fatigue ($F_{fatigue}$). The prevalence of patients in the top quartile was significantly higher in pSS patients with subclinical hypothyroidism (S-Hypo, p=0.035 by Chi-square test) or anti-TPO-positive S-Hypo (p=0.038 by the Fisher's exact test) than those without, when those were categorised by $F_{fatigue}$. But no significant difference was observed when categorised by fatigue VAS score or ESSPRI score. Black sectors indicate the percentage of patients in the top quartile and white sectors represent that of patients in the other quartiles. ESSPRI: EULAR SS Patient Reported Index.

Table III. Age- and sex-adjusted logistic regression analysis to determine factors related to the fatigue-dominant group.

Variables	Univariate		Multivariate	
	OR [95% CI]	p value	OR [95% CI]	p-value
Age (year)	0.968 [0.945-0.992]	**0.010**	0.967 [0.943-0.991]	**0.008**
Dry eye at the diagnosis	0.475 [0.232-0.971]	**0.041**	-	-
Domains ever affected ≥ 3*	0.258 [0.075-0.886]	**0.031**	-	-
TSH (uIU/mL)	1.236 [1.003-1.523]	**0.047**	-	-
S-Hypo	2.558 [1.043-6.272]	**0.040**	-	-
Anti-TPO-positive S-Hypo	6.261 [1.110-35.299]	**0.038**	6.770 [1.180-38.851]	**0.032**
ALC (/mm^3)	0.999 [0.999-1.00]	**0.028**	-	-

*Among 11 domains excluding biological domain.
S-Hypo: subclinical hypothyroidism; TPO-positive S-Hypo: Anti-TPO-positive subclinical hypothyroidism; ALC: absolute lymphocyte count.

Table IV. Age- and sex-adjusted logistic regression analysis to determine factors related to anti-TPO-positive subclinical hypothyroidism.

Variables	Univariate		Multivariate	
	OR [95% CI]	p value	OR [95% CI]	p-value
Articular domain ever affected*	6.261 [1.110-35.299]	**0.038**	7.586 [1.211-47.522]	**0.030**
Anti-TG positivity	5.786 [1.111-30.120]	**0.037**	-	-
Fatigue-dominant group**	6.261 [1.110-35.299]	**0.025**	7.586 [1.211-47.522]	**0.030**
ANC (/mm^3)	0.999 [0.995-1.000]	0.058	-	-

*Based on the definition of ESSDAI domain; **the highest quartile of $F_{fatigue}$.
Anti-TPO: anti-thyroid peroxidase; anti-TG: anti-thyroglobulin; ANC: absolute neutrophil count.

Discussion

This study demonstrated that, of 196 Korean patients with pSS without overt thyroid diseases, 36% had at least one

thyroid-related autoantibody, including anti-TPO (14.3%) and TRAb (14.3%). However, the prevalence of S-Hypo and S-Hyper were only 11.7% and 1.0%, respectively. The TRAb-positive subgroup showed significantly higher ESSDAI and ClinESSDAI than the TRAb-negative subgroup. Lymphopenia and ESR levels were independent factors associated with TRAb positivity, while S-Hypo was significantly associated with anti-TPO positivity but not anti-TG. S-Hypo was significantly associated with a high prevalence of the fatigue-dominant group, characterised by higher levels of fatigue than other pSS-related main symptoms such as dryness and arthralgia. Additionally, in multivariate analyses, the fatigue-dominant group was a significant predictor for anti-TPO-positive S-Hypo and *vice-versa*.

The association between pSS and thyroid disease has been discussed since the 1960s (21). Although many studies have reported a greater prevalence of AITD in patients with pSS than in controls (1, 3), its prevalence was heterogeneous across studies (10.5% to 45%) (3, 22, 23). This could be attributed to racially disparate populations, varying classification criteria for pSS or definitions of AITD, and different methods for detecting thyroid-related autoantibodies. S-Hypo is the most common form of thyroid dysfunction in pSS (7). Our results also demonstrated that S-Hypo (11.7%) was more common than S-Hyper (1.0%). Unlike the results of Caramaschi *et al*., we did not observe any differences in disease severity between patients with and without S-Hypo (8).

Higher detection rates of anti-TPO (OR=2.73) and anti-TG (OR=3.09) in patients with pSS have also been frequently reported compared with those in the general population (4). The reported prevalence of anti-TPO or anti-TG positivity ranges from 10% to 42% in patients with pSS (3, 22, 24, 25). In our study, the seropositive rate of anti-TG and anti-TPO was 15.8% and 14.3%, respectively. The prevalence of anti-TPO positivity was higher than that of the general population in Korea, which was 7.3% in a nationwide representative Korean population (26). Our study demonstrated that anti-TPO positivity was associated with old age, S-Hypo, and ACA positivity (Suppl. Table S2), consistent with previous studies in pSS (27). The association between anti-TPO positivity and TSH levels or ACA positivity was further confirmed in multivariate analysis. Although anti-TG was most frequently observed thyroid-related autoantibodies in the present study, it did not demonstrate any relationship with the features of pSS.

Studies regarding TRAb positivity in patients with pSS are limited. In this study, TRAb was measured using a third-generation competition assay which is composed of a porcine TSH receptor and ruthenium-labelled human thyroid-stimulating monoclonal antibody M22. So far, only two studies have reported the prevalence of TRAb in patients with pSS: 0% in 29 Japanese patients using a third-generation ELISA (13) and 12.6% in 95 Hungarian patients using western blot (12). Interestingly, in the current study, TRAb positivity was significantly associated with high ESSDAI even though it was not related to thyroid dysfunction. The ESSDAI is reported to be correlate with the type I interferon signature or BAFF levels in SS (28, 29). TRAb titres have been reported to be associated with interferon and BAFF levels in patients with GD (30, 31). TRAb may stimulate TSH receptor (TR)-positive immune cells, including B cells (32). TSH can induce the proliferation of lymphocytes and the production of proinflammatory cytokines from dendritic cells via TR (33). However, TRAb positivity may simply result from polyclonal B cell hyperactivity.

Graves' ophthalmopathy (GO) is the most famous and common extra-thyroidal manifestation of GD. GO can occur in subjects without any signs of hyperthyroidism, but the majority have TRAb. Anti-α-fodrin antibody, considered related to pSS, was also reported to be observed in patients with GO (34). Additionally, a recent Korean cohort study showed that the risk for SS was higher (HR=1.89 [95% CI, 1.30–2.74]) in GD patients with GO than those without GO (35). But we did not observe any GO cases in our patients with pSS.

In multivariate analysis, lymphopenia was the most prominent independent predictor for TRAb positivity. Lymphopenia in pSS could be secondary to premature aging of naive $CD4^+$ T cells, and the low number of $CD4^+$ T cells is correlated with disease activity or severe systemic disease (36-38). Moreover, lymphopenia is a significant risk factor for overall mortality and lymphoma development (39, 40). Therefore, TRAb positivity may carry prognostic implications in pSS, and further studies are needed to confirm the effect of TRAb positivity on the long-term outcome in pSS.

Another unique feature of our study was the association between the fatigue-dominant group and S-Hypo. Fatigue is a common symptom of hypothyroidism (7) and is the complaint that severely affects a patient's quality of life in pSS (41, 42). Nevertheless, a pSS-specific tool for measuring fatigue has not yet been established. Furthermore, no study has shown significant associations between fatigue and thyroid function status or indices in patients with pSS. It can be a possible explanation that fatigue is strongly influenced by other pSS-related confounding factors, including pain, sicca symptoms, and depression (43). Therefore, it is unsurprising that S-Hypo was associated with the fatigue-focused groups defined by the highest quartile of the $F_{fatigue}$, but not based on the top quartile of the ESSPRI fatigue VAS or ESSPRI score, in our study.

The mutual correlations between the fatigue-dominant group and S-Hypo suggest the concept that neuroendocrine dysfunction contributes to fatigue in pSS. Johnson *et al*. observed hypoactivity of the hypothalamic-pituitary-adrenal axis in patients with pSS and lower adrenocorticotropic hormone and cortisol levels, with a blunted response to corticotrophin-releasing hormone stimulation and elevated basal TSH levels (44). Anti-21-hydroxylase (OH) positivity, which was observed in pSS (17.5%), is suggested to be a potential link between blunted adrenal function and B cell hyperactivity (45). Additionally, Karsh *et al*. observed that approximately one-third of patients with pSS and thyroid dysfunction had abnormal responses to TRH stimula-

tion (46). Although some studies reported an improvement in fatigue after levothyroxine replacement in patients with subclinical and overt hypothyroidism (47), no studies have been available in patients with pSS.

Our study had several limitations. First, because of the cross-sectional study design, our results suggested only a possible association between a high burden of fatigue and S-Hypo, not a causal association. Second, the number of patients in each group was relatively small, which may have lowered the statistical power of the logistic regression analyses. Third, since heterophilic antibodies such as RF, anti-TSH, or anti-T4 are able to affect the immunoassays (48), preanalytical interfering factors may have existed in measuring thyroid-related hormones. In our study, RF was significantly associated with anti-TG, but not anti-TPO or TRAb. Fourth, the Korean Thyroid Association (KTA) recently introduced Korean-specific diagnostic criteria for S-Hypo, defined as normal free T4 and a TSH level ≥6.8 mIU/L in 2023 (49). The criteria are much stricter, compared to those applied in previous studies, and can complicate comparisons with results from other studies. In our study, only two patients met the recent Korean criteria and were included in fatigue-dominant group. Lastly, there is wide variation in the epidemiology of AITD and thyroid-related antibodies depending on age, sex, and geographic zones (50). Therefore, our results may not be generalisable to other populations, and further multi-ethnic, multinational studies are warranted to confirm our results.

In conclusion, our study reveals that approximately one-third of Korean pSS patients had at least one thyroid-related autoantibody, even in the absence of overt thyroid dysfunction. Additionally, we present novel evidence showing that TRAb positivity is related to high systemic disease activity and lymphopenia and indicating that fatigue-dominancy is mutually associated with S-Hypo. Although further validation through large-scale studies is warranted, these findings offer valuable insights into the intricate interplay between pSS and latent thyroid autoimmunity.

Acknowledgement

We thank all the patients who participated in the current study.

References

1. AMADOR-PATARROYO MJ, ARBELAEZ JG, MANTILLA RD *et al*.: Sjögren's syndrome at the crossroad of polyautoimmunity. *J Autoimmun* 2012; 39: 199-205. https://doi.org/10.1016/j.jaut.2012.05.008
2. SUN X, LU L, LI Y, YANG R, SHAN L, WANG Y: Increased risk of thyroid disease in patients with Sjögren's syndrome: a systematic review and meta-analysis. *PeerJ* 2019; 7: e6737. https://doi.org/10.7717/peerj.6737
3. BALDINI C, FERRO F, MOSCA M, FALLAHI P, ANTONELLI A: The association of Sjögren syndrome and autoimmune thyroid disorders. *Front Endocrinol* (Lausanne) 2018; 9: 121. https://doi.org/10.3389/fendo.2018.00121
4. WANG Q, SHANGGUAN J, ZHANG Y, PAN Y, YUAN Y, QUE W: The prevalence of thyroid autoantibodies in autoimmune connective tissue diseases: a systematic review and meta-analysis. *Expert Rev Clin Immunol* 2020; 16: 923-30. https://doi.org/10.1080/1744666x.2020.1811089
5. PUNZI L, BETTERLE C: Chronic autoimmune thyroiditis and rheumatic manifestations. *Joint Bone Spine* 2004; 71: 275-83. https://doi.org/10.1016/j.jbspin.2003.06.005
6. D'ARBONNEAU F, ANSART S, LE BERRE R, DUEYMES M, YOUINOU P, PENNEC YL: Thyroid dysfunction in primary Sjögren's syndrome: a long-term follow-up study. *Arthritis Rheum* 2003; 49: 804-9. https://doi.org/10.1002/art.11460
7. ANAYA JM, RESTREPO-JIMENEZ P, RODRIGUEZ Y *et al*.: Sjögren's syndrome and Autoimmune Thyroid Disease: Two Sides of the Same Coin. *Clin Rev Allergy Immunol* 2019; 56: 362-74. https://doi.org/10.1007/s12016-018-8709-9
8. CARAMASCHI P, BIASI D, CAIMMI C *et al*.: The co-occurrence of Hashimoto thyroiditis in primary Sjögren's syndrome defines a subset of patients with milder clinical phenotype. *Rheumatol Int* 2013; 33: 1271-5. https://doi.org/10.1007/s00296-012-2570-6
9. ZHANG Y, LI M, ZHANG L *et al*.: Association between comorbidities and extraglandular manifestations in primary Sjögren's syndrome: a multicenter cross-sectional study. *Clin Rheumatol* 2020; 39: 2677-88. https://doi.org/10.1007/s10067-020-04992-x
10. GIRON-PILLADO M, CRUZ-BAUTISTA I, SAAVEDRA-GONZALEZ V *et al*.: Autoimmune thyroid disease in primary Sjögren's syndrome: real-life screening practice and clinical outcomes. *Curr Rheumatol Rev* 2022; 18: 272-7. https://doi.org/10.2174/1573397118666220127105546
11. OZGEN AG, KESER G, ERDEM N *et al*.: Hypothalamus-hypophysis-thyroid axis, triiodothyronine and antithyroid antibodies in patients with primary and secondary Sjögren's syndrome. *Clin Rheumatol* 2001; 20: 44-8. https://doi.org/10.1007/s100670170102
12. ZEHER M, HORVATH IF, SZANTO A, SZODORAY P: Autoimmune thyroid diseases in a large group of Hungarian patients with primary Sjögren's syndrome. *Thyroid* 2009; 19: 39-45. https://doi.org/10.1089/thy.2007.0398
13. NAKAMURA H, USA T, MOTOMURA M *et al*.: Prevalence of interrelated autoantibodies in thyroid diseases and autoimmune disorders. *J Endocrinol Invest* 2008; 31: 861-65. https://doi.org/10.1007/bf03346432
14. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 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. *Arthritis Rheumatol* 2017; 69: 35-45. https://doi.org/10.1002/art.39859
15. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome. *Ann Rheum Dis* 2010; 69: 1103-9. https://doi.org/10.1136/ard.2009.110619
16. SEROR R, RAVAUD P, MARIETTE X *et al*.: EULAR Sjögren's syndrome Patient Reported Index (ESSPRI): development of a consensus patient index for primary Sjögren's syndrome. *Ann Rheum Dis* 2011; 70: 968-72. https://doi.org/10.1136/ard.2010.143743
17. SEROR R, MEINERS P, BARON G *et al*.: Development of the ClinESSDAI: a clinical score without biological domain. A tool for biological studies. *Ann Rheum Dis* 2016; 75: 1945-50. https://doi.org/10.1136/annrheumdis-2015-208504
18. KIM SH, AHN J, OCK M *et al*.: The EQ-5D-5L valuation study in Korea. *Qual Life Res* 2016; 25: 1845-52. https://doi.org/10.1007/s11136-015-1205-2
19. COOPER DS, BIONDI B: Subclinical thyroid disease. *Lancet* 2012; 379: 1142-54. https://doi.org/10.1016/S0140-6736(11)60276-6
20. KIM YA, PARK YJ: Prevalence and risk factors of subclinical thyroid disease. *Endocrinol Metab* (Seoul) 2014; 29: 20-29. https://doi.org/10.3803/EnM.2014.29.1.20
21. ANDERSON JR, GRAY KG, BECK JS, KINNEAR WF: Precipitating autoantibodies in Sjögren's disease. *Lancet* 1961; 2: 456-60. https://doi.org/10.1016/s0140-6736(61)92430-8
22. PEREZ B, KRAUS A, LOPEZ G, CIFUENTES M, ALARCON-SEGOVIA D: Autoimmune thyroid disease in primary Sjögren's syndrome. *Am J Med* 1995; 99: 480-84. https://doi.org/10.1016/s0002-9343(99)80223-x
23. COLAFRANCESCO S, CELIA AI, BALDINI C *et al*.: Clinical and histological features of patients with primary Sjögren's syndrome and autoimmune thyroiditis: a national multicentre cross-sectional study. *Clin Exp Rheumatol* 2023; 41: 2389-96. https://doi.org/10.55563/clinexprheumatol/eh36vs
24. KOSZARNY A, MAJDAN M, DRYGLEWSKA M, TABARKIEWICZ J: Prevalence of selected organ-specific autoantibodies in rheumatoid arthritis and primary Sjögren's syndrome patients. *Reumatologia* 2015; 53: 61-68. https://doi.org/10.5114/reum.2015.51504
25. MAVRAGANI CP, FRAGOULIS GE, MOUTSOPOULOS HM: Endocrine alterations in primary Sjögren's syndrome: an overview. *J Autoimmun* 2012; 39: 354-58. https://doi.org/10.1016/j.jaut.2012.05.011

26. KIM WG, KIM WB, WOO G *et al*.: Thyroid stimulating hormone reference range and prevalence of thyroid dysfunction in the Korean population: Korea National Health and Nutrition Examination Survey 2013 to 2015. *Endocrinol Metab* (Seoul) 2017; 32: 106-14. https://doi.org/10.3803/enm.2017.32.1.106
27. CHUNG JH: Update on thyroid hormone levels and thyroid dysfunction in the korean population based on data from the Korea National Health and Nutrition Examination Survey VI (2013 to 2015). *Endocrinol Metab* (Seoul) 2020; 35: 7-13. https://doi.org/10.3803/enm.2020.35.1.7
28. MARIA NI, BRKIC Z, WARIS M *et al*.: MxA as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjögren's syndrome. *Ann Rheum Dis* 2014; 73: 1052-9. https://doi.org/10.1136/annrheumdis-2012-202552
29. NISHIKAWA A, SUZUKI K, KASSAI Y *et al*.: Identification of definitive serum biomarkers associated with disease activity in primary Sjögren's syndrome. *Arthritis Res Ther* 2016; 18: 106. https://doi.org/10.1186/s13075-016-1006-1
30. LIN JD, WANG YH, FANG WF *et al*.: Serum BAFF and thyroid autoantibodies in autoimmune thyroid disease. *Clin Chim Acta* 2016; 462: 96-102. https://doi.org/10.1016/j.cca.2016.09.004
31. CHENG CW, FANG WF, TANG KT, LIN JD: Serum interferon levels associated with the disease activity in women with overt Graves' disease. *Cytokine* 2021; 138: 155353. https://doi.org/10.1016/j.cyto.2020.155353
32. WENZEK C, BOELEN A, WESTENDORF AM, ENGEL DR, MOELLER LC, FþHRER D: The interplay of thyroid hormones and the immune system – where we stand and why we need to know about it. *Eur J Endocrinol* 2022; 186: R65-77. https://doi.org/10.1530/eje-21-1171
33. VIEIRA IH, RODRIGUES D, PAIVA I: The mysterious universe of the TSH receptor. *Front Endocrinol* (Lausanne) 2022; 13: 944715. https://doi.org/10.3389/fendo.2022.944715
34. KAHALY GJ, BANG H, BERG W, DITTMAR M: Alpha-fodrin as a putative autoantigen in Graves' ophthalmopathy. *Clin Exp Immunol* 2005; 140: 166-72. https://doi.org/10.1111/j.1365-2249.2004.02750.x
35. SOHN SY, AHN JY, LEE MK *et al*.: Risk of non-thyroidal autoimmune diseases in patients with Graves' disease: a nationwide retrospective cohort study. *Rheumatology* (Oxford) 2024 Jan 5. https://doi.org/10.1093/rheumatology/kead708
36. FESSLER J, FASCHING P, RAICHT A *et al*.: Lymphopenia in primary Sjögren's syndrome is associated with premature aging of naïve CD4+ T cells. *Rheumatology* (Oxford) 2021; 60: 588-97. https://doi.org/10.1093/rheumatology/keaa105
37. ZEHER M, SZODORAY P, GYIMESI E, SZONDY Z: Correlation of increased susceptibility to apoptosis of CD4+ T cells with lymphocyte activation and activity of disease in patients with primary Sjögren's syndrome. *Arthritis Rheum* 1999; 42: 1673-81. https://doi.org/10.1002/1529-0131(199908)42:8<1673::aid-anr16>3.0.co;2-1
38. FLORES-CHAVEZ A, KOSTOV B, SOLANS R *et al*.: Severe, life-threatening phenotype of primary Sjögren's syndrome: clinical characterisation and outcomes in 1580 patients (GEAS-SS Registry). *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S121-29
39. BRITO-ZERON P, KOSTOV B, SOLANS R *et al*.: Systemic activity and mortality in primary Sjögren syndrome: predicting survival using the EULAR-SS Disease Activity Index (ESSDAI) in 1045 patients. *Ann Rheum Dis* 2016; 75: 348-55. https://doi.org/10.1136/annrheumdis-2014-206418
40. NISHISHINYA MB, PEREDA CA, MUNOZ-FERNANDEZ S *et al*.: Identification of lymphoma predictors in patients with primary Sjögren's syndrome: a systematic literature review and meta-analysis. *Rheumatol Int* 2015; 35: 17-26. https://doi.org/10.1007/s00296-014-3051-x
41. MÈLAND E, MIYAMOTO ST, HAMMENFORS D, VALIM V, JONSSON MV: Understanding fatigue in Sjögren's syndrome: outcome measures, biomarkers and possible interventions. *Front Immunol* 2021; 12: 703079. https://doi.org/10.3389/fimmu.2021.703079
42. LONGHINO S, CHATZIS LG, DAL POZZOLO R *et al*.: Sjögren's syndrome: one year in review 2023. *Clin Exp Rheumatol* 2023; 41: 2343-56. https://doi.org/10.55563/clinexprheumatol/255qsx
43. HACKETT KL, DAVIES K, TARN J *et al*.: Pain and depression are associated with both physical and mental fatigue independently of comorbidities and medications in primary Sjögren's syndrome. *RMD Open* 2019; 5: e000885. https://doi.org/10.1136/rmdopen-2018-000885
44. JOHNSON EO, VLACHOYIANNOPOULOS PG, SKOPOULI FN, TZIOUFAS AG, MOUTSOPOULOS HM: Hypofunction of the stress axis in Sjögren's syndrome. *J Rheumatol* 1998; 25: 1508-14
45. MAVRAGANI CP, SCHINI M, GRAVANI F, KALTSAS G, MOUTSOPOULOS HM: Brief report: Adrenal autoimmunity in primary Sjögren's syndrome. *Arthritis Rheum* 2012; 64: 4066-71. https://doi.org/10.1002/art.34679
46. KARSH J, PAVLIDIS N, WEINTRAUB BD, MOUTSOPOULOS HM: Thyroid disease in Sjögren's syndrome. *Arthritis Rheum* 1980; 23: 1326-9. https://doi.org/10.1002/art.1780231118
47. SAMUELS MH, SCHUFF KG, CARLSON NE, CARELLO P, JANOWSKY JS: Health status, mood, and cognition in experimentally induced subclinical hypothyroidism. *J Clin Endocrinol Metab* 2007; 92: 2545-51. https://doi.org/10.1210/jc.2007-0011
48. FAVRESSE J, BURLACU MC, MAITER D, GRUSON D: Interferences with thyroid function immunoassays: clinical implications and detection algorithm. *Endocr Rev* 2018; 39: 830-50. https://doi.org/10.1210/er.2018-00119
49. CHUNG HK, KU EJ, YOO WS *et al*.: 2023 Korean Thyroid Association management guidelines for patients with subclinical hypothyroidism. *Int J Thyroidol* 2023; 16(1): 32-50. https://doi.org/10.11106/ijt.2023.16.1.32
50. MA C, LI D, YIN Y *et al*.: Establishing thresholds and effects of gender, age, and season for thyroglobulin and thyroid peroxidase antibodies by mining real-world big data. *Clin Biochem* 2019; 74: 36-41. https://doi.org/10.1016/j.clinbiochem.2019.08.011