Hypergammaglobulinaemia predicts glandular and extra-glandular damage in primary Sjögren's syndrome: results from the KISS cohort study

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ABSTRACT

Objective. *To investigate whether temporal changes in immunoglobulin (Ig) levels and persistent hypergammaglobulinaemia cause glandular and extra-glandular damage in patients with primary Sjögren's syndrome (pSS).*

Methods. *Cumulative demographics and clinical and serological data from pSS patients in the Korean Initiative pSS cohort were evaluated. Persistent hypergammaglobulinaemia was defined as mean IgG levels of ≥1600 mg/dL over 3 years. Salivary gland damage was assessed by measuring salivary flow impairment, and lacrimal gland damage was assessed by examining ocular structural abnormalities. Solid organ damage included neurological and pleuropulmonary damage, renal impairment and lymphoproliferative disease. Independent predictors of glandular and extra-glandular damage in the third year were identified by logistic regression.*

Results. *Of 256 patients with pSS (median age, 55 years; 98% female), 47% had hypergammaglobulinaemia at baseline. IgG levels fell during the first 2 years in patients with hypergammaglobulinaemia at baseline, but not in those with normal IgG levels. Changes in IgG levels were associated with hydroxychloroquine and glucocorticoids. In the third year of follow-up, salivary flow impairment and solid organ damage were present in 71% and 9% of patients, respectively. After adjusting for age and medication use, persistent hypergammaglobulinaemia was associated with salivary flow impairment and solid organ damage in the third year. Patients in whom IgG fell by more than 80 mg/dL from baseline over 2 years showed less solid organ damage.*

Conclusion. *Persistent hypergammaglobulinaemia was associated with salivary gland and solid organ damage. Decreased IgG may attenuate progression to solid organ dysfunction.*

Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease that mainly affects the exocrine glands, causing oral and ocular dryness (1). The hallmark histopathological finding is the presence of a focal lymphoid infiltrate in salivary gland tissue, which reflects the autoimmune process and local B cell hyperactivity (2). The pathogenesis of pSS is complex, and is associated with the both innate and adaptive immunity; triggering the innate immune system, which results in production of type I interferon (IFN), plays an important role at the early stage of the disease, whereas the adaptive immune system makes a later contribution through autoimmune B cell activation (3).

B cell hyperactivity is reflected by serological manifestations; the presence of rheumatoid factor, autoantibodies specific for Ro/SSA and La/SSB, hypergammaglobulinaemia and cryoglobulinaemia (1-3). Antibodies, often referred to as immunoglobulins (Ig), are secreted after cross-linking of the B cell receptor (4). Thus, Ig encompass a number of autoantibodies, including rheumatoid factor, antinuclear antibodies, antibodies specific for extractable cellular antigens and antibodies that target organ-specific antigens in salivary duct cells (3, 5).

The biological domain of the European League Against Rheumatism (EULAR) SS Disease Activity Index (ESSDAI), which determines the disease activity of pSS, includes hypergammaglobulinaemia, hypocomplementaemia and cryoglobulinaemia (6). These are prognostic factors for systemic involvement in pSS, which includes skin vasculitis

and renal, pulmonary, neurological and haematological involvement (7). In addition, high baseline IgG and low C4 levels predict progression to SS among individuals who do not meet the pSS classification criteria (8).

Although hypergammaglobulinaemia is the most common serologic finding in pSS (it is observed in approximately half of patients in cohort studies) (9, 10), serological activity is not a manifestation that indicates use of systemic immunomodulatory or immunosuppressive agents (11). For that reason, some rheumatologists are often sceptical about regular follow-up measurement of Ig levels. Indeed, Ig levels were not measured serially in many cases in a retrospective cohort study (12).

Changes in Ig levels over time, which represent B cell activity, have not been studied in patients with pSS. Moreover, it is not known whether cumulative hypergammaglobulinaemia or decreased Ig levels are associated with organ dysfunction in pSS. Here, we evaluated temporal changes in Ig levels, taking into account the effects of medication. As B cell hyperactivity is a key prognostic factor of pSS, and is linked to autoimmune-mediated organ dysfunction, we analysed whether persistent hypergammaglobulinaemia causes glandular and extra-glandular damage during 3 years.

Methods

Patients

The Korean Initiative of primary Sjögren's syndrome (KISS) cohort is a nationwide prospective cohort that includes all clinical data and samples from pSS patients who fulfilled the 2002 American-European Consensus group classification criteria (13) and/or the 2012 American College of Rheumatology (ACR) criteria (14). Participants were recruited from October 2013 to November 2017 and underwent annual follow-up thereafter. The database included 498 patients with pSS from 11 hospitals. All data were collected and managed by the Clinical Research and Trial Management System (iCReaT; Korea National Institutes of Health, Korea Centers for Disease Control and Prevention [CDC]). The study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC13ONMI0646). The study is registered with the Korea CDC Clinical Research Information Service (KCT0001099). Written informed consent was obtained from all participants. The study enrolled patients who fulfilled the 2016 ACR/ EULAR classification criteria for SS (15, 16). Patients with autoimmune diseases other than pSS (at baseline) were excluded.

The KISS cohort was allowed to enter "not tested" for all study parameters according to individual hospital settings and patient requirements. To observe changes in Ig levels, patients with three consecutive measurements were included in the analysis.

Design

This longitudinal cohort study was conducted in patients with pSS in whom Ig levels were measured serially. Patients were divided into two groups: those with and those without hypergammaglobulinaemia at baseline. Changes in Ig levels were monitored for 3 years. In addition, the association between persistent hypergammaglobulinaemia from baseline to the second year and glandular or extra-glandular organ dysfunction in the third year was examined, as was the association between cumulative changes in IgG levels from baseline to the second year and the prevalence of glandular and extra-glandular dysfunction in the third year.

Data collection

The KISS cohort data includes demographics, current use of medications, comorbidities, extra-glandular manifestations and laboratory tests. The results of minor salivary glands biopsies taken at the time of diagnosis were retrieved; a positive result was defined as a focus score ≥1 (17). The Xerostomia Inventory (XI), Ocular Surface Disease Index (OSDI), the EULAR SS Patient-Reported Index (ESSPRI), ESSDAI, the SS disease damage index (SSDDI) and the patient's and physician's global assessment results were assessed directly by rheumatologists or obtained from patient questionnaires completed during the annual follow-up visits (18-22). The ClinESSDAI, which excludes the biological domain of the ESSDAI, was calculated (23). Autoantibody profiles, including rheumatoid factor, anti-Ro/SSA, anti-La/SSB, anti-centromere, anti-double strand DNA (anti-dsDNA) and anti-ribonucleoprotein (RNP) were retrieved from baseline data. Positivity for anti-Ro/SSA, anti-La/SSB, anti-RNP and anti-dsDNA antibodies was determined by line immunoassay. The titre of rheumatoid factor, and IgG, IgA and IgM antibodies, was measured in an immunoturbidimetric assay. Annual complete cell counts (CBC), Ig levels and concomitant medications were also reviewed.

The most frequently measured Ig was IgG, followed by IgA and IgM. High IgG was defined as ≥1600 mg/dL (6). Hypogammaglobulinaemia was defined as <500 mg/dL. High IgA was defined as ≥400 mg/dL and high IgM as ≥200 mg/dL. Persistent hypergammaglobulinaemia was defined as a mean value of ≥1600 mg/dL over 3 years. For all patients, the cumulative decrease in IgG was defined as a reduction of more than the median change (Δ) in IgG level from baseline to the second year.

Outcomes

Salivary gland dysfunction was assessed by measuring salivary flow impairment (an unstimulated salivary flow rate <0.1 mL/min). The unstimulated salivary flow rate was measured for 5 minutes, which was then extrapolated to 15 minutes. Prior to the measurement, participants were instructed to discontinue the use of parasympathomimetics for 12 hours, and artificial saliva for 3 hours, prior to saliva collection. In addition, participants were asked to refrain from drinking anything within 90 minutes of saliva collection. Lacrimal gland damage was assessed by examining ocular structural abnormalities such as corneal ulcers, cataracts and chronic blepharitis (24). Ophthalmological examination of both eyes was conducted by ophthalmologists at each hospital.

The presence of extra-glandular manifestations was assessed by ESSDAI (excluding the biological and glandular domains). Solid organ damage was defined as neurological or pleuropul-

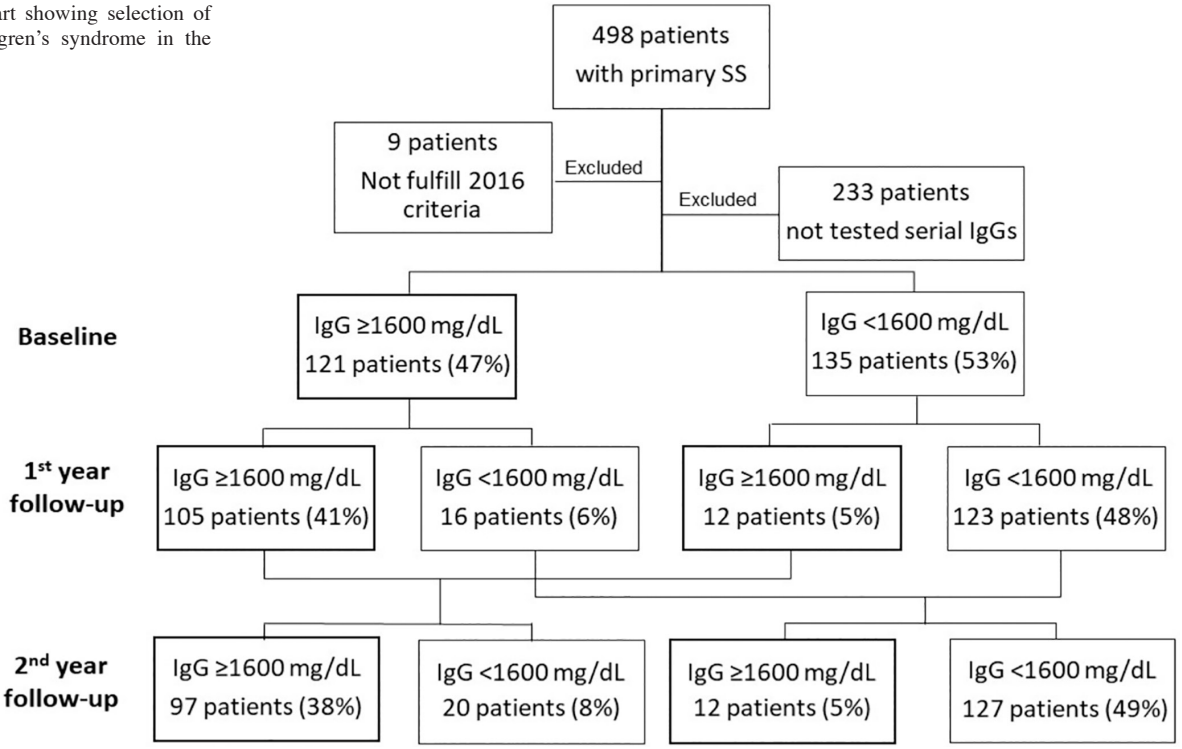

Fig. 1. Flow chart showing selection of patients with Sjögren's syndrome in the KISS cohort.

monary damage, renal impairment and lymphoproliferative disease, as determined by the SSDDI (24).

Statistical analysis

Cases without serial Ig measurements were handled using listwise deletion. The missing values were denoted as missing completely at random, as assessed by Little's test. To avoid possible selection bias, the prevalence of organ dysfunction in the third year was compared between patients in whom Ig was measured and in whom it was not. The Shapiro-Wilk test was used to test continuous outcome variables for departures from a normal distribution. The analysed parameters were not normally distributed; therefore, continuous variables are expressed as the median (interquartile range [IQR]), while categorical data are expressed as absolute numbers and percentages (%). The Mann-Whitney U-test was used to compare groups of continuous variables, while the χ^2 test and Fisher's exact test were used to compare categorical variables as appropriate.

Year-on-year changes in Ig levels were compared using the Wilcoxon signed-rank test. Changes in autoantibodies were compared using the exact McNemar test. Whether ΔIgG from baseline to each follow-up visit was affected by systemic treatments such as hydroxychloroquine (HCQ), methotrexate (MTX) and oral glucocorticoids was analysed by analysis of covariance (ANCOVA), with the baseline IgG level as a covariate. Results are presented as least square (LS) mean differences and standard error.

The correlations between objective and subjective oral, ocular and serologic parameters were examined using Spearman's correlation coefficient analysis. The effects of clinical variables related to oral, ocular or solid organ damage were estimated using binary logistic regression analyses. Model assumptions were checked using residual analysis.

All computed *p*-values were two-sided and values <0.05 were considered statistically significant. All data were analysed using SAS software (v. 9.4; SAS, Cary, NC, USA) and graphs were drawn using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

Results

Baseline characteristics

Among the KISS cohort, 51.4% (256 patients) had three consecutive IgG measurements as of May 2020 (Fig. 1). Of these, 252 were female. The median age at the enrolment was 55 years ([IQR], 46–60) and the median disease duration was 1.2 years (IQR, 0.1–4.3). The median level of IgG was 1583 mg/dL (IQR, 1343–1955 mg/dL); 47% of patients had high IgG (≥1600 mg/dL) at baseline. No patient had an IgG level <500 mg/dL. The median IgA and IgM levels were 262 mg/dL (IQR, 198–345 mg/dL) and 118 mg/dL (IQR, 86–155 mg/dL), respectively. Patients with high IgA and IgM accounted for 14% and 10% of cases, respectively. Baseline characteristics are shown in Table I.

Patients with high IgG at baseline were younger and had higher rates of ANA, anti-Ro/SSA, anti-La/SSB and rheumatoid factor. Among patients who underwent minor salivary gland biopsy (n=115), the proportion showing a positive result (focus score ≥1) was not different, regardless of whether they had high IgG or not at baseline. Focus score data were available for 65 patients; patients with high baseline IgG levels had a higher focus score than those with normal IgG levels at baseline (5 [3–8] *vs*. 2 [1–4], respectively; *p*=0.001). More patients with high IgG levels were positive for anti-dsDNA antibodies than patients with normal IgG levels

(p=0.052). Patients positive for autoantibodies other than anti-Ro/SSA or La/SSB did not have other autoimmune diseases. Patients with anti-centromere antibodies had a higher prevalence of Raynaud's phenomenon than those without (9/23 [39%] *vs*. 33/211 [16%], respectively; p=0.010), and Hashimoto's thyroiditis (6/23 [26%] *vs*. 23/211 [11%], respectively; p=0.047).

Patients with high IgG at baseline tended to be prescribed more HCQ and glucocorticoids (p=0.066 and p=0.067, respectively) (Table I).

Table I. Baseline characteristics of patients with primary SS according to persistent hypergammaglobulinaemia.

	All patients (n=256)	Patients with high IgG (n=121)	Patients with normal IgG (n=135)	p-value
Age, year	55 (46–60)	53 (44–58)	56 (47–62)	**0.007**
Female, n (%)	252 (98.4)	121 (100)	131 (97.0)	0.124
Disease duration, mo.	19 (3–51)	21 (5–54)	18 (1–50)	0.140
Ever smoked, n (%)	11 (4.3)	3 (2.5)	8 (5.9)	0.175
Comorbidity, n (%)				
Diabetes Mellitus	8 (3.1)	2 (1.7)	6 (4.4)	0.287
Hashimoto's thyroiditis	30 (11.7)	13 (10.7)	17 (12.6)	0.646
Grave's disease	5 (2.0)	2 (1.7)	3 (2.2)	>0.999
Fibromyalgia	12 (4.7)	5 (4.1)	7 (5.2)	0.691
Autoantibody profiles				
ANA, n (%)	228/243 (93.8)	108/111 (97.3)	120/132 (90.9)	**0.039**
Anti-Ro/SSA, n (%)	217/252 (86.1)	115/118 (97.5)	102/134 (76.1)	**<0.001**
Anti-La/SSB, n (%)	123/251 (49.0)	85/117 (72.7)	38/134 (28.4)	**<0.001**
Anti-centromere, n (%)	23/234 (9.8)	7/105 (6.7)	16/129 (12.4)	0.186
Anti-RNP, n (%)	5/142 (3.5)	3/63 (4.8)	2/79 (2.5)	0.655
Anti-dsDNA, n (%)	7/234 (3.0)	6/109 (5.5)	1/125 (0.8)	0.052
Rheumatoid factor, n (%)	160/245 (65.3)	100/114 (87.7)	60/131 (45.8)	**<0.001**
- titre, IU/mL	44 (11–100)	80 (38–151)	18 (3–52)	<0.001
Positive MSG biopsy*, n (%)	100/115 (87.0)	45/50 (90.0)	55/65 (84.6)	0.395
Medications, n (%)				
Hydroxychloroquine	176 (68.8)	90 (74.4)	86 (63.7)	0.066
Methotrexate	8 (3.1)	4 (3.3)	4 (3.0)	>0.999
Azathioprine	6 (2.3)	3 (2.5)	3 (2.2)	>0.999
Biologics	1 (0.4)	1 (0.8)	0 (0)	0.473
Glucocorticoid	93 (36.3)	51 (42.2)	42 (31.1)	0.067
Pilocarpine	214 (85.6)	98 (81.0)	116 (85.9)	0.287
N-acetylcysteine	23 (9.0)	9 (7.4)	14 (10.4)	0.413
Rebamipide	52 (20.3)	26 (21.5)	26 (19.3)	0.658

*Focus score ≥1.
ANA: antinuclear antibodies; dsDNA: double stranded DNA; MSG: minor salivary gland; RNP: ribonucleoprotein; SSA: Sjögren's syndrome A; SSB: Sjögren's syndrome B.

Changes in Ig levels

IgG levels decreased during the follow-up period; there was a year-on-year decline at the first and second follow-up (median ΔIgG= -39 mg/dL and -33 mg/dL; log-rank p<0.001 and 0.001, respectively), but not at the third follow-up. IgM showed a similar pattern. Serum IgA levels decreased significantly only from the first to the second-year follow-up (Fig. 2A). Positivity for anti-Ro/SSA, centromere and RNP antibodies, and rheumatoid factor did not change significantly over 3 years. However, anti-La/SSB antibodies emerged in 9% of patients but had disappeared in 4% of these patients at the first-year follow-up (p=0.048). Anti-dsDNA antibodies emerged in 6% of patients at the first-year follow-up, and patients positive for anti-dsDNA antibodies at baseline remained positive (p<0.001) (Supplementary Table S1).

Of the patients with high IgG at the baseline, 105 patients (87%) maintained high IgG levels at the first-year follow-up, while 16 (13%) showed decreased IgG levels (within the normal range). Among patients with normal IgG at the baseline, 123 patients (91%) maintained normal levels while 12 (9%) showed an increase to 1600 mg/dL or more at the first-year follow-up. At the second-year follow-up, 83% of patients with hypergammaglobulinaemia showed high IgG levels whereas 91% of those with normal IgG maintained normal levels (Fig. 1).

Decreased IgG levels were observed in patients with high baseline IgG during the first 2 years (LS mean $\Delta IgG_{2nd\text{-}year\ -\ baseline}$ = -180.8 [95% CI, -227.1–-134.5]), but not in patients with normal baseline IgG (Fig. 2B).

IgG levels correlated positively with the ESSDAI, ClinESSDAI, PhGA and ESR, and negatively with the white blood cell (WBC) count, haemoglobin level, unstimulated salivary flow and C4. However, they did not correlate with ESSPRI, PGA, XI, OSDI, Schirmer I test results and CRP levels (Fig. 2C). These correlations persisted over the 3 years of follow-up.

Effect of systemic treatments on IgG levels

To determine the effect of drugs on IgG levels, we analysed the association between ΔIgG at baseline to the second-year follow-up and medications (HCQ, MTX, oral glucocorticoids, pilocarpine, N-acetylcysteine (NAC) and rebamipide) commonly used to treat pSS. Treatment with HCQ or oral glucocorticoids was associated with decreased IgG levels, whereas treatment with MTX, pilocarpine, rebamipide or NAC was not (Table II).

Patients treated with HCQ showed a decline of IgG for the first 2 years (LS mean $\Delta IgG_{2nd\text{-}year\ -\ baseline,}$ -127.3 [95% CI, -163.7– -90.9]), whereas those without HCQ showed no significant change (LS mean $\Delta IgG_{2nd\text{-}year\ -\ baseline,}$ -48.7 [95% CI, -97.8–0.3]) (Fig. 2D). The LS mean differences in ΔIgG from baseline to the second- and third-year follow-up between patients treated with HCQ and those not treated were significant after adjusting for baseline IgG levels (difference between LS means of two treatment arms: 78.6 [95% CI, 17.5–139.7] and 80.1 [95% CI, 7.7–152.6], respectively).

In patients treated with and without oral glucocorticoids, IgG levels decreased from baseline to each follow-up visit;

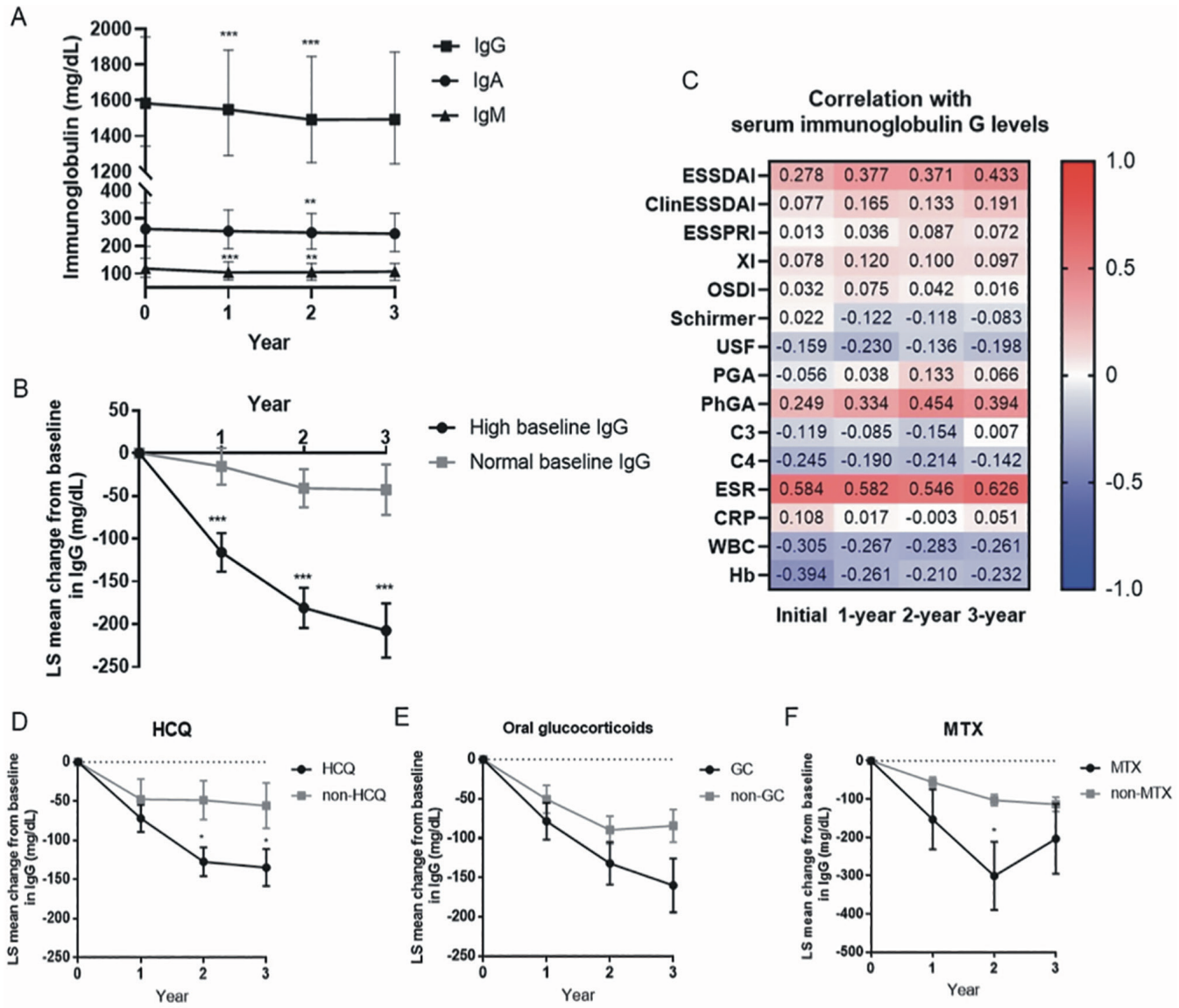

Fig. 2. Immunoglobulin (Ig) levels over time and the correlation between serum IgG and clinical parameters related to Sjögren's syndrome activity.
(**A**) Changes in IgG, IgA and IgM during the 3-year follow-up. The median (symbol) and interquartile range (bar) of each Ig class is presented. The asterisk (*) denotes a significant change from the previous year (Wilcoxon signed-rank test). (**B**) The changes in IgG level from baseline to each yearly follow-up are presented as the LS mean (symbol) and standard error (bar), according to the presence of baseline hypergammaglobulinaemia (IgG ≥1600 mg/dL). (**C**) Exact correlation coefficients derived from Spearman's correlation analysis are depicted on a heatmap. Cumulative changes in IgG from baseline to each follow-up year according to the medication (adjusted according to baseline IgG levels) are presented as the LS mean (symbol) and standard error (bar) and according to (**D**) HCQ, (E) oral glucocorticoid and (**F**) methotrexate (MTX) use.
***p<0.001; **p<0.01; *p<0.05.
ESSDAI: EULAR Sjögren's syndrome disease activity index; ESSPRI: EULAR Sjögren's syndrome Patient-Reported Index; LS mean: lean square mean; OSDI: ocular surface disease index; PGA: patient's global assessment; PhGA: physician's global assessment; USF: unstimulated salivary flow rate; WBC: white blood cell count; XI: Xerostomia Inventory.

there was no difference in the degree of decline between groups (Fig. 2E). IgG levels also decreased in patients treated (or not) with MTX, and the degree of decline was greater in patients treated with MTX from baseline to the second-year follow-up (difference between LS means: 197.7; 95% CI, 19.8–375.7; p=0.030) (Fig. 2F).

The autoantibody profile that presented at baseline usually remained unaltered, regardless of medication. Anti-La/SSB and anti-dsDNA antibodies emerged in patients who were not treated with HCQ or oral glucocorticoids (Suppl. Table S2).

Association between persistent hypergammaglobulinaemia and glandular and extra-glandular damage

The follow-up rate in the third year was 88%; 20 patients were lost to follow-up and 11 patients did not attend the third-year follow-up visit. Among them, 96 patients (43%) had persistent hypergammaglobulinaemia (mean IgG level over 3 years ≥1600 mg/dL) and 129 patients (57%) maintained normal mean IgG levels over 3 years. Extra-glandular manifestations, excluding the glandular and biological domains from the ESSDAI, were present in 47% of patients; haematological involvement

was the most common (31%), followed by articular involvement (9%), cutaneous involvement (6%) and constitutional symptoms (5%). There was no difference between patients with and without persistent hypergammaglobulinaemia with respect to the presence of each domain of extra-glandular manifestations in the third-year follow-up; the exceptions were the haematological and lymphadenopathy domains. Lymphadenopathy was observed only in patients with persistent hypergammaglobulinaemia (four cases) (p=0.032). Haematological abnormalities were more common in patients with persistent hypergammaglobulinaemia than in those with normal mean IgG levels (38 cases [40%] versus 32 cases [24.8%], respectively; p=0.018). Overall, the presence of persistent hypergammaglobulinemia was associated with the prevalence of extra-glandular manifestations in the third year, after adjusting for age and medication use (OR, 1.905; 95% CI, 1.097–3.308) (Table III).

Of the 225 patients who completed the third-year follow-up, 159 (71%) presented with salivary flow impairment and 18 (8%) lost teeth completely or almost completely. The persistent hypergammaglobulinaemia was associated with salivary flow impairment (OR, 2.417; 95% CI, 1.258-4.642). Pilocarpine was used more often in patients with salivary flow impairment. Among all autoantibodies measured at baseline, anti-La/SSB and centromere antibodies were associated with salivary flow impairment at the third-year follow-up (Suppl. Table S3). Ocular structural abnormalities were found in 76% of patients in the third year, and chronic blepharitis accounted for 88% of these. Ocular structural abnormalities were associated with higher age at baseline (OR, 1.067; 95% CI, 1.031–1.104), but not with persistent hypergammaglobulinaemia (Table III). Of the autoantibodies measured at baseline, anti-Ro/SSA was associated with ocular structural abnormalities (Suppl. Table S3).

Solid organ damage, including neurological damage, pleuropulmonary damage and renal impairment was present in 9% of patients at the third-year follow-up. No lymphoproliferative disease was reported. Solid organ damage was associated with persistent hypergammaglobulinaemia (OR, 2.881; 95% CI, 1.056–7.857) and age at baseline (OR, 1.058; 95% CI, 1.005–1.113). The use of HCQ was associated with less solid organ damage (OR, 0.262; 95% CI, 0.097–0.707) (Table III). The presence of anti-La/SSB antibodies at baseline was associated with solid organ damage (OR, 3.2; 95% CI, 1.006–10.177) (Suppl. Table S3).

Next, we asked whether the decrease in IgG from baseline to the second year was associated with the prevalence of glandular and extra-glandular dysfunction in the third year. In 62% of patients with baseline hypergammaglobulinaemia, IgG levels decreased by more than 80 mg/dL from baseline to the second-year follow-up; solid organ damage in the third year was less common in these patients than in those without a decrease in IgG (odds ratio, 0.237; 95% CI, 0.068–0.830). By contrast, changes in IgG levels in patients with normal baseline IgG levels did not affect clinical outcomes in the third year (Table IV).

Table II. Effect of medications on changes on immunoglobulin G levels*.

	ΔImmunoglobulin $G_{2nd\ year\ -\ baseline}$		
	Parameter estimate	R^2	p-value
Methotrexate (MTX)	-54.723	0.010	0.105
Hydroxychloroquine (HCQ)	-37.393	0.034	**0.003**
Oral glucocorticoids	-31.694	0.019	**0.028**
Pilocarpine	-18.807	0.004	0.281
Rebamipide	-16.343	0.004	0.337
N-Acetylcysteine	-26.495	0.007	0.187
HCQ + oral glucocorticoids	-15.104	0.032	**0.004**
MTX + oral glucocorticoids	-20.605	0.010	0.113
HCQ + MTX	-34.471	0.020	**0.024**
HCQ + MTX + oral glucocorticoids	-18.329	0.028	**0.008**

*The effect of each drug on ΔImmunoglobulin G was calculated based on the number of years of use.

Table III. Multiple logistic analysis of factors prognostic for glandular and extra-glandular damage at the third-year follow-up.

Dependent variable	Independent variable	Odds ratio (95% CI)	p-value
Extra-glandular manifestations			
	Age at baseline, per year	1.001 (0.975–1.028)	0.936
	Mean IgG ≥1600 mg/dL (*vs*. <1600 mg/dL)	1.905 (1.097–3.308)	**0.022**
	HCQ use (*vs*. no use)	1.012 (0.562–1.822)	0.969
	MTX (*vs*. no use)	3.051 (0.569–16.367)	0.193
	Oral glucocorticoids (*vs*. no use)	1.567 (1.883–2.780)	0.125
Salivary flow impairment			
	Age at baseline, per year	1.021 (0.991–1.053)	0.173
	Mean IgG ≥1600 mg/dL (*vs*. <1600 mg/dL)	2.417 (1.258–4.642)	**0.008**
	HCQ use (*vs*. no use)	1.387 (0.733–2.626)	0.315
	Pilocarpine (*vs*. no use)	2.857 (1.282–6.368)	**0.010**
	Oral glucocorticoids (*vs*. no use)	1.416 (0.734–2.729)	0.299
Ocular structural abnormalities			
	Age at baseline, per year	1.067 (1.031–1.104)	**<0.001**
	Mean IgG ≥1600 mg/dL (*vs*. <1600 mg/dL)	0.921 (0.472–1.796)	0.809
	HCQ use (*vs*. no use)	0.488 (0.228–1.043)	0.064
	Pilocarpine (*vs*. no use)	1.530 (0.660–3.549)	0.322
	Oral glucocorticoids (*vs*. no use)	1.851 (0.892–3.844)	0.099
Solid organ damage			
	Age at baseline, per year	1.058 (1.005–1.113)	**0.033**
	Mean IgG ≥1600 mg/dL (*vs*. <1600 mg/dL)	2.881 (1.056–7.857)	**0.039**
	HCQ use (*vs*. no use)	0.262 (0.097–0.707)	**0.008**
	MTX (*vs*. no use)	0.921 (0.086–9.908)	0.946
	Oral glucocorticoids (*vs*. no use)	1.982 (0.728–5.394)	0.181

HCQ: hydroxychloroquine; IgG: immunoglobulin G; MTX: methotrexate.

Table IV. Association between changes of IgG level from baseline to the 2-year follow-up, and pSS outcome in the third year.

Outcomes in 3rd year follow-up	High baseline IgG (n=106)			Normal baseline IgG (n=119)		
	ΔIgG <-80 mg/dL (n=65)	ΔIgG ≥-80 mg/dL (n=41)	Odds ratio (95% CI)	ΔIgG <-80 mg/dL (n=48)	ΔIgG ≥-80 mg/dL (n=71)	Odds ratio (95% CI)
Salivary flow impairment	46 (70.8)	35 (85.4)	0.415 (0.150–1.148)	28 (58.3)	49 (69.0)	0.629 (0.293–1.348)
Ocular structural abnormalities*	50 (76.9)	32 (78.1)	0.938 (0.367–2.395)	36 (75.0)	53 (74.7)	1.019 (0.438–2.370)
Extra-glandular manifestations†	36 (55.4)	25 (61.0)	0.795 (0.359–1.760)	16 (33.3)	28 (39.4)	0.768 (0.357–1.652)
Solid organ damage‡	4 (6.2)	9 (22.0)	0.237 (0.068–0.830)	2 (4.2)	5 (7.0)	0.574 (0.107–3.087)

*Ocular structural abnormalities include corneal ulcers, cataract and chronic blepharitis.
† Extra-glandular manifestations exclude the glandular and biological domains from the ESSDAI.
‡ Solid organ damage includes neurological damage, pleuropulmonary damage, renal impairment and lymphoproliferative disease.

Discussion

This study of a nationwide observational database capturing real-world clinical practice investigated the prevalence of hypergammaglobulinaemia in patients with pSS, along with changes in IgG levels during follow-up. We identified an association between persistent hypergammaglobulinaemia and salivary flow impairment and solid organ damage in the consequent years. Hypergammaglobulinaemia was the most common extra-glandular manifestation, present in approximately half of the patients in this cohort. IgG levels decreased over 2 years in patients with baseline hypergammaglobulinaemia, and were affected by HCQ and oral glucocorticoids. A cumulative decrease in IgG levels was linked with less solid organ damage in patients with baseline hypergammaglobulinaemia.

Hypergammaglobulinemia was more common in younger patients. A diagnosis of pSS at a young age is associated with a higher frequency of immunological markers, and active disease is in turn associated with an increased risk of systemic involvement (25, 26). However, age did not affect ΔIgG in the current study. In line with the existing literature, we found no association between Ig levels and patient-reported outcomes such as PGA, OSDI, XI and ESSPRI (7).

The level of IgG decreased most during the first 2 years of observation in patients with hypergammaglobulinaemia at baseline, whereas levels remained steady in those with normal IgG at baseline. The increased IgG level is attributed to a marked increase in the number of memory B cells and IgG (not IgA)-secreting plasma cells in exocrine glands (27), as well as to increased differentiation of IgG-secreting plasma cells associated with disturbed B cell subsets in the peripheral blood (28). Thus, the decrease of IgG in patients with hypergammaglobulinaemia implies that B cell activity in patients with B cell hyperactivity decreased over time. Moreover, persistent hypergammaglobulinemia (or sustained B cell hyperactivity) was linked to solid organ and salivary gland dysfunction, while a decrease in IgG was associated with less solid organ damage, in the following year. Various autoantibodies produced by B cells and plasma cells are presumed to play a pathologic role in pSS; for example, anti-Ro/SSA and anti-La/SSB antibodies may induce apoptosis of salivary gland epithelial cells by enhancing caspase-8 activity (29). Indeed, pSS patients who were anti-La/SSB antibody-positive at baseline had salivary flow impairment at the third-year follow-up. This result agrees with that of the previous study showing that anti-La/SSB-positive patients had a higher frequency of abnormal ocular and oral diagnostic tests (30). B cells also play an antibody-independent role, such as production of inflammatory cytokines, which is related to differentiation of B cells into effector B cells (31). Our data suggest that decreased B cell activity, reflected by IgG levels, may ameliorate salivary gland and solid organ dysfunction, and inflammation.

HCQ was the most frequently used immunomodulatory drug in this cohort, and it was associated with decreased IgG levels. A randomised placebo-controlled trial (RCT) reported that the efficacy of HCQ in pSS is limited (32); the trial showed that the ESSDAI, ESSPRI, unstimulated salivary flow measurements and Schirmer test did not improve in the HCQ-treated arm compared with the placebo arm over 24 weeks (33). However, HCQ has reduced systemic IFN-stimulated gene expression, and improved ESR, IgG and IgM levels (34). Demarchi *et al*. reported a lower incidence of extra-glandular manifestations in patients on HCQ therapy (35). Taken together, the data suggest that HCQ modulates B cell and IFN activity, which is represented by a decrease in IgG levels; HCQ may reduce extra-glandular manifestations and related organ damage several years later, although the decrease in IgG associated with HCQ was too small to draw definitive conclusions.

Oral glucocorticoids, the second most frequently used immunosuppressive drug in this cohort, were also associated with decreased IgG levels. Although the literature does not provide reliable evidence for the use of systemic glucocorticoids in pSS, glucocorticoids are commonly prescribed for those with glandular and extra-glandular manifestations (36). A small RCT showed that prednisolone decreased serum IgG and IgA levels, and the ESR, while increasing the WBC (37). These results can be explained by B cell sensitivity to glucocorticoid-induced apoptosis (38), and by the fact that glucocorticoids reduce expression of activation-induced cytidine deaminase, which is the principal regulator of Ig gene somatic hypermutation and class-switch recombination in B cells (39). MTX was also associated with a decrease in Ig levels, which is consistent with the findings of a previ-

ous study reporting a significant decrease in transitional B cell numbers and Ig levels in patients with juvenile rheumatoid arthritis receiving MTX (40).

This study has several limitations. First, the level of Ig was measured in three consecutive years in only half of all participants in the KISS cohort; therefore, there is a risk of selection bias. However, the outcomes of patients with three consecutive Ig measurements were no different from those without. In addition, the number of patients lost to follow-up was not linked to baseline IgG levels. Second, the period of use of each medication was estimated based on a 1-year follow-up; thus the actual duration of medication use was not assessed. Because adverse events associated with these medications were not recorded in this cohort, further research on the risk-benefits of long-term medication use in pSS is needed. Third, the number of patients treated with MTX, biologics or azathioprine was too small to evaluate.

To the best of our knowledge, this is the first study to monitor temporal changes in Ig levels in a prospective cohort of patients with pSS. We describe the natural evolution of serum Ig levels and the effects of changing Ig levels, as well as the effects of persistent hypergammaglobulinaemia, on the outcome of pSS.

In conclusion, we confirmed the impact of persistent hypergammaglobulinemia on salivary flow impairment and solid organ damage in a nationwide pSS cohort in Korea. We found that solid organ dysfunction is less evident in patients with a decrease in IgG levels over 2 years compared with that in those with persistent hypergammaglobulinaemia. Therefore, monitoring Ig levels in patients with pSS is necessary. Hypergammaglobulinaemia may be considered a component of active disease and a candidate target for treatment.

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