Serum soluble interleukin-2 receptor is a useful biomarker for disease activity but not for differential diagnosis in IgG4-related disease and primary Sjögren's syndrome adults from a defined population

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Competing interests: see page S-162.

ABSTRACT

Objective. *To identify biomarkers for disease activity in IgG4-related disease (IgG4-RD) and primary Sjögren's syndrome (pSS).*

Methods. *Forty-three consecutive treatment-naïve patients with IgG4-RD, 62 patients with pSS, and 5 patients with sicca syndrome were enrolled. IgG4-RD and pSS disease activity was assessed based on the IgG4-RD responder index (IgG4-RD RI) and EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), respectively. The associations of biomarkers with disease activity were examined.*

Results. *Comparison of the three diseases identified the serum levels of IgG, IgG4, IgG4/IgG ratio, IgE, and soluble interleukin-2 receptor (sIL-2R) for IgG4-RD and the serum levels of IgM and sIL-2R and lymphocyte counts for pSS as potential biomarkers of disease activity. Among these, serum sIL-2R levels correlate with baseline IgG4-RD RI scores and the number of affected organs in IgG4-RD (ϱ=0.74, p<0.0001 and ϱ=0.75, p<0.0001, respectively). Serum sIL-2R levels also correlate with ESSDAI scores and the number of affected organs in pSS (ϱ=0.67, p<0.0001 and ϱ=0.41, p<0.0001, respectively). Receiver operating characteristic curve analysis suggested serum sIL-2R levels as an efficient biomarker to distinguish the presence of extra-dacryosialadenitis involvements in IgG4-RD with a cut-off value of 424 U/mL (AUC=0.93, p<0.0001), and in pSS with 452 U/mL (AUC=0.89, p<0.0001). Serum sIL-2R levels decreased significantly after treatment in patients with IgG4-RD and pSS.*

Conclusion. *Serum sIL-2R levels are a potentially valuable biomarker for evaluating disease activity and treatment response in IgG4-RD and pSS.*

Introduction

IgG4-related disease (IgG4-RD) is a fibroinflammatory disease characterised by elevated levels of serum IgG4 and infiltration of $IgG4^+$ plasma cells at affected sites (1, 2). IgG4-RD can involve various organs systemically, such as the pancreas, kidney, aorta, lungs, retroperitoneum, lymph nodes and skin, as well as lacrimal and salivary glands (1, 2). The evaluation of disease activity is crucial for early, appropriate intervention, which can prevent irreversible organ damage (3, 4), and for the assessment of treatment response. Monitoring disease activity is also essential in the management of the disease; while glucocorticoids appear to be a promising treatment for IgG4-RD, relapse occurs in 30–50% of patients after tapering the glucocorticoid dose (5, 6). Hence, there is an urgent need to identify biomarkers associated with disease activity.

Primary Sjögren's syndrome (pSS) is an autoimmune disease with lymphocytic infiltration into lacrimal and salivary glands, which causes glandular atrophy leading to dry mouth and eyes (7, 8). A subgroup of patients presents extra-dacryosialadenitis involvements with higher mortality and morbidity (9, 10). Therefore, biomarkers that reflect systemic disease activity of pSS are also necessary.

IgG4-RD and pSS are different diseases and are clinically distinguishable. However, they have common symptoms of inflammation of the lachrymal and salivary glands (2). Recent advances in understanding the pathogenesis of both diseases have also clarified the importance of follicular helper T (Tfh) cells (11, 12). Biomarkers associated with Tfh cells such as circulating activated Tfh cells (11-20), plasmablasts (21, 22) and serum CXCL13 (23, 24), are reported to reflect disease activity

in IgG4-RD and pSS; however, those biomarkers cannot be measured in routine clinical laboratories. This has facilitated a search for more clinically useful biomarkers. The objective of this study was to identify clinically measurable biomarkers to assess disease activity in IgG4-RD and pSS.

Materials and methods

Study design

We retrospectively reviewed the medical records of consecutive patients with IgG4-RD, pSS, and sicca syndrome in Keio University Hospital between January 2008 and March 2017. Patients who had not received treatment at diagnosis, with disease duration not exceeding 5 years (25), and with laboratory data available were included.

IgG4-RD was diagnosed according to the 2011 comprehensive IgG4-related disease diagnostic criteria (26). Diagnosis of IgG4-RD was biopsy-proven in 41 patients (95%). Patients with pSS fulfilled the 2002 American-European Consensus Group criteria (27). Sicca syndrome was defined as xerostomia with normal levels of serum IgG and IgG4; negative anti-nuclear, anti-Ro/SSA, and anti-La/SSB antibodies; and no lymphocytic infiltration in labial salivary glands assessed by lip biopsy. Patients having other autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, and myositis were excluded. No patients had past history of cervical radiotherapy and none received immunosuppressive treatment including glucocorticoids at the time of assessment. No patients reported past or current acute viral or bacterial infections, sarcoidosis, amyloidosis, human-immunodeficiency virus infection, or hepatitis C virus infection.

This study was approved by the ethics committee of our institution. Written informed consent from patients was waived in accordance with the regulation in Japan. All investigations were conducted according to the principles in the Declaration of Helsinki.

Data collection

Clinical data including age, sex, and disease duration were obtained for all patients. Laboratory findings were the positive results of anti-nuclear, anti-Ro/SSA and anti-La/SSB antibodies; C-reactive protein (normal range: 0–0.35 mg/dL), lactate dehydrogenase (normal range: 120–220 U/L), IgG (normal range: 870–1700 mg/dL), IgA (normal range: 110–410 mg/dL), IgM (normal range: 46–260 mg/dL), IgG4 (normal range: 5–117 mg/dL), IgE (normal range: 0–170 IU/mL), soluble interleukin (IL)-2 receptor (sIL-2R, normal range: 142–500 U/mL), CH50 (normal range: 31.6–57.6 U/mL), and counts of white blood cells, lymphocytes, and eosinophils. The respective kits used to measure IgG, IgA, and IgM, IgG4, total IgE, and sIL-2R were N-assay TIA IgG-SH (Nittobo Co., Ltd, Tokyo, Japan), BN II (Siemens AG, Efrurt, Germany), ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden), and cell-free N IL-2R (Kyowa Medics Co., Ltd, Tokyo, Japan). Serum laboratory analysis was performed at the time of the diagnosis or just before the initiation of immunosuppressive treatment in clinical practice. The amount of serum required to measure sIL-2R value is 300μl. Anti-nuclear antibody (ANA)-positive status was defined by a cut-off titre of 1:40 because a test for anti-dsDNA antibodies should be considered in samples with a homogeneous pattern at any titre including low titres (28). We also investigated the positivity rates of ANA with a titre ≥1:160 as ANA by a cut-off titre of 1:160 increases the positive predictive values of ANA for anti-ENA and anti-dsDNA antibodies. Organ involvement was determined by physical examination, radiological examinations (computed tomography, magnetic resonance imaging and/or fluorodeoxyglucose-positron emission tomography), and/or histological findings.

Assessment of disease activity

Disease activity in patients with IgG4-RD and pSS was assessed based on the IgG4-RD responder index (IgG4-RD RI) score (29) and European League Against Rheumatism (EULAR) Sjögren's syndrome disease activity index (ESSDAI) (30), respectively. The extra-dacryosialadenitis involvements were assessed based on the IgG4-RD RI and ESSDAI.

Statistical analyses

Group-wise comparisons were performed using the Mann-Whitney U-test. Fisher's exact test was used to compare categorical variables. Correlations were analysed using Spearman's correlation coefficient. Receiver operating characteristic curve (ROC) was used to estimate the presence of extra-dacryosialadenitis involvements. Differences before and after immunosuppressive treatment were determined using the Wilcoxon rank sum test for paired samples. A p-value <0.05 was considered significant. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad, La Jolla, California, USA).

Results

Patients' characteristics

A total of 110 untreated patients were enrolled, which included 43 with IgG4-RD, 62 with pSS, and 5 with sicca syndrome. Baseline characteristics of the patients are shown in Table I. The median age and disease duration did not differ among the three groups. The proportion of females was higher in patients with pSS (95%) than in patients with IgG4-RD (65%) and sicca syndrome (60%). ANA positivity was higher in patients with pSS (79%) than in patients with IgG4-RD (21.4%) and sicca syndrome (0%). The positivity of ANA with a titre of ≥1:160 was 12 % (5/43) in IgG4-RD, 52% (32/62) in pSS, and 0% (0/5) in sicca syndrome. In terms of serum sIL-2R levels, 53% (23/43) in IgG4-RD, 27% (17/62) in pSS, and 0% (0/5) in sicca syndrome showed above upper limit of serum sIL-2R of healthy subjects (>500 U/mL). There was no correlation between the serum levels of sIL-2R and C-reactive protein in patients with IgG4-RD (ϱ=-0.06, p=0.70), while the weak correlation was observed in patients with pSS (ϱ=0.35, p=0.005).

While 88% of IgG4-RD and 100% of pSS had dacryosialadenitis, the proportion of patients having extra-dacryosialadenitis involvements were 67% in IgG4-RD and 23% in pSS. The median number of involved organs were 3 in IgG4-RD, and 2 in pSS. Details of the extra-dacryosialadenitis involvements

Table I. Baseline characteristics of patients before the initiation of immunosuppressive treatments.

	IgG4-RD (n=43)	pSS (n=62)	Sicca syndrome (n=5)	IgG4-RD vs sicca syndrome	pSS *vs*. sicca syndrome	IgG4-RD *vs*. pSS
Demographics						
Age (years), median (range)	60 (26-84)	61 (21-81)	60 (47-67)	0.84	0.66	0.61
Sex (female, %)	65 (28/43)	95 (59/62)	60.0 (3/5)	0.24	0.0002	0.0001
Disease duration (months) , median (range)	1 (1-60)	22 (1-60)	24 (1-42)	0.80	0.74	0.07
ANA (positive, %)	21 (9/42)	79 (49/62)	0 (0/5)	0.57	**0.0009**	**<0.0001**
Anti-Ro/SSA (positive, %)	0 (0/40)	79 (49/62)	0 (0/5)	1.000	**0.0009**	**<0.0001**
Anti-La/SSB (positive, %)	0 (0/40)	48 (30/62)	0 (0/5)	1.000	0.06	**<0.0001**
IgG4-RD RI, median (range)	12 (3-21)	-	-	-	-	-
ESSDAI, median (range)	-	1 (0-29)	-	-	-	-
The number of organ involvements, median (range)	3 (1-6)	2 (1-5)	-	-	-	**0.003**
Extra-dacryosialadenitis involvements (%)	67 (29/43)	23 (14/62)	-	-	-	**<0.0001**
Blood biomarkers, median (range)						
White blood cells (cells/μL)	5900 (3700-19800)	4850 (2800-12000)	6900 (4500-9800)	0.35	0.05	**0.0006**
Lymphocytes (cells/μL)	1766 (650-4059)	1458 (390-3038)	1885 (1580-2463)	0.13	**0.03**	0.08
Eosinophils (cells/μL)	258 (0-1568)	112 (0-964)	235 (72-311)	0.28	0.33	**0.0002**
CRP (mg/dL)	0.04 (0.01-1.1)	0.03 (0.01-2.3)	0.06 (0.02-0.5)	0.34	0.11	0.28
LDH (IU/L)	169 (112-303)	174 (31-420)	156 (134-205)	0.63	0.36	0.62
IgG (mg/dL)	1719 (1135-4431)	1652 (848-5599)	1272 (1033-1562)	**0.004**	0.06	0.17
IgA (mg/dL)	161 (71-426)	266 (116-545)	203 (80-478)	0.25	0.27	**<0.0001**
IgM (mg/dL)	74 (26-219)	90 (43-318)	59 (37-99)	0.55	**0.02**	**0.02**
IgG4 (mg/dL)	495 (38-1870)	31 (3-115)	43 (27-85)	**<0.0001**	0.15	**<0.0001**
IgG4/IgG ratio	0.26 0.02	0.04 **0.0002**	**0.01 <0.0001**			
IgE (IU/dL)	370 (5-3300)	50 (5-3700)	110 (31-370)	**0.04**	0.28	**<0.0001**
sIL-2R (U/mL)	568 (143-4040)	388 (136-2037)	247 (198-336)	**0.01**	**0.01**	**0.01**
CH50 (U/mL)	52 (10-60)	53 (39-60)	55 (48-60)	0.23	0.54	0.09

IgG4-RD: IgG4-related disease; pSS: primary Sjögren's syndrome; ANA: anti-nuclear antibody; IgG4-RD RI: IgG4-related Disease Responder Index; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; CRP: C-reactive protein; LDH: lactate dehydrogenase; sIL-2R: soluble interleukin-2 receptor.

in the patients with IgG4-RD and pSS are provided in supplementary Table I.

Biomarkers for IgG4-RD

Serum laboratory findings were compared between patients with IgG4-RD and sicca syndrome (Table I). Compared to patients with sicca syndrome, those with IgG4-RD displayed significantly higher serum levels of IgG (1719 mg/dL *vs*. 1272 mg/dL, p=0.004), IgG4 (495 mg/dL *vs*. 43 mg/dL, p<0.0001), IgG4/IgG ratio (0.26 *vs*. 0.02, p<0.0001), IgE (370 IU/dL *vs*. 110 IU/dL, p=0.04), and sIL-2R (568 *vs*. 247 U/mL, p=0.01). Positive correlation with baseline IgG4-RD RI scores was evident for sIL-2R (ϱ=0.74, p<0.0001), IgG (ϱ=0.66, p<0.0001), IgG4 (ϱ=0.61, p<0.0001) and IgG4/IgG ratio (ϱ=0.48, p=0.001) (Fig. 1A). Positive correlation with the number of affected organs in IgG4-RD was evident for sIL-2R (ϱ=0.75, p<0.0001), IgG (ϱ=0.65, p<0.0001), IgG4 (ϱ=0.58, p<0.0001) and IgG4/IgG ratio (ϱ=0.45, p=0.003) (Fig. 1B). Serum sIL-2R levels were positively correlated with serum IgG4/IgG ratio in patients with IgG4-RD (ϱ=0.53, p=0.0003), while there was no correlation between the serum levels of sIL-2R and blood eosinophil counts (ϱ=0.18, p=0.27) or serum IgE levels (ϱ=0.13, p=0.43).

Association with the presence of extra-dacryosialadenitis involvements in IgG4-RD

The association between serum levels of sIL-2R, IgG, IgG4, IgG4/IgG ratio and the presence of extra-dacryosialadenitis involvements was examined. IgG4-RD patients with extra-dacryosialadenitis involvements, compared to those without, showed higher levels of serum sIL-2R (768 U/mL *vs*. 279 U/mL, p<0.0001), IgG (1923 mg/dL *vs*. 1500 mg/dL, p=0.001), IgG4 (731 mg/dL *vs*. 271 mg/dL, p<0.0001) and IgG4/IgG ratio (0.32 *vs*. 0.16, p=0.002) (Fig. 2A). The respective cut-off values of serum sIL-2R, IgG, IgG4 and IgG4/IgG ratio indicating the presence of extra-dacryosialadenitis involvements were 424 U/mL (sensitivity, 83%; specificity, 100%; area under the ROC curve [AUC], 0.93; p<0.0001), 1901 mg/dL (sensitivity, 53%; specificity, 100%; AUC, 0.81; p=0.002), 507 mg/dL (sensitivity, 70%; specificity, 100%; AUC, 0.87; p=0.0001) and 0.24 (sensitivity, 77%; specificity, 69%; AUC, 0.80; p=0.002) (Fig. 2B), thus suggesting serum sIL-2R as the most sensitive biomarker in IgG4-RD.

Biomarkers for pSS

Compared to patients with sicca syndrome, those with pSS displayed significantly lower circulating lymphocyte counts (1488 cells/μL *vs*. 1885 cells/μL, p=0.03), but significantly higher serum IgM (90 mg/dL *vs*. 59 mg/dL, p=0.02) and serum sIL-2R (388 *vs*. 247 U/mL, p=0.01). Only sIL-2R was positively correlated with baseline ESSDAI scores (ϱ=0.67, p<0.0001; Fig. 3A). When the analysis focused on 11 patients with moderate or high activity (ESSDAI ≥5), the results were similar, with positive correlation of sIL-2R levels with ESSDAI scores

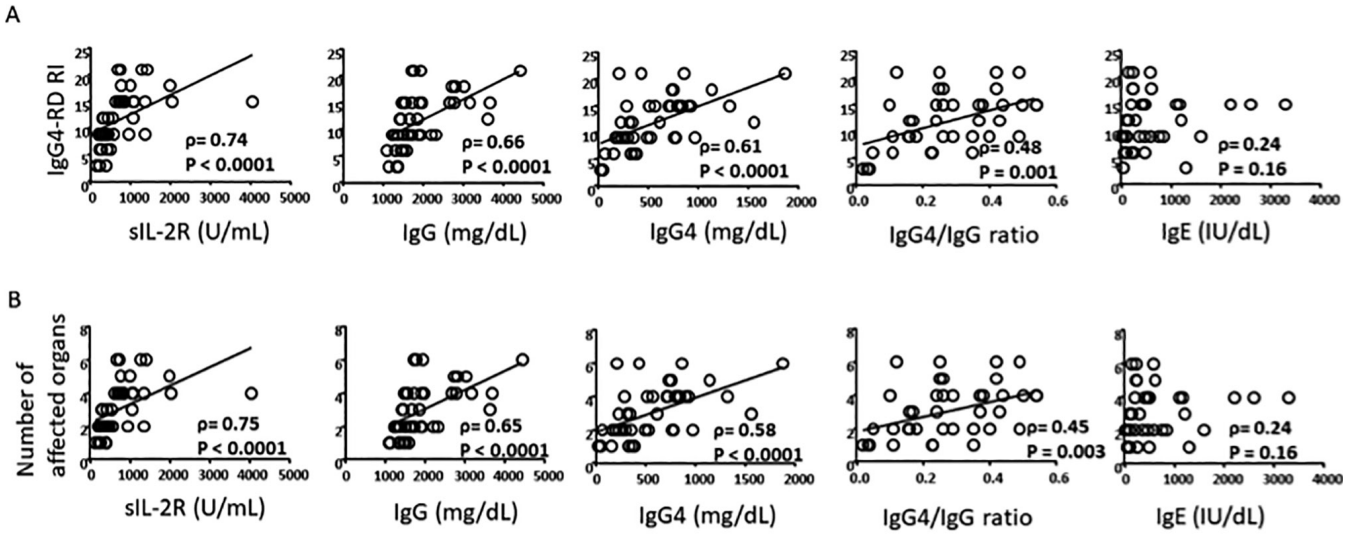

Fig. 1. Serum levels of sIL-2R, IgG, IgG4 and IgG4/IgG ratio positively correlated with disease activity or the number of affected organs in IgG4-related disease. (**A**) Correlation between blood biomarkers and disease activity assessed by IgG4-related disease responder index (IgG4-RD RI) score. (**B**) Correlation between blood biomarkers and the number of affected organs.

(ϱ=0.65, p=0.03) and no correlation of circulating lymphocyte counts (ϱ=-0.25, p=0.45) and serum IgM levels (ϱ=0.44, p=0.18). The level of sIL-2R was positively correlated with the number of affected organs in pSS (ϱ=0.41, p=0.0008; Fig. 3B).

Association with the presence of extra-dacryosialadenitis involvements in pSS

The levels of serum sIL-2R were significantly higher in pSS patients with extra-dacryosialadenitis involvements than in those without involvements (629 U/mL *vs*. 336 U/mL, p<0.0001; Fig. 4A). The cut-off value of serum sIL-2R indicating the presence of extra-dacryosialadenitis involvements was 452 U/mL (sensitivity=86%, specificity=81%, AUC=0.89, p<0.0001; Fig. 4B).

Serum sIL-2R levels following treatment in IgG4-RD and pSS

In 14 patients with IgG4-RD, serum sIL-2R levels were longitudinally measured within 12 months after treatment with prednisolone. The mean duration of treatment was 7.9 months, and the mean initial dose of prednisolone was 33mg/day. The median level of serum sIL-2R was significantly decreased from 394 U/mL to 216 U/mL (p<0.0001) along with the improvement in IgG4-RD RI score from 9 to 0 (p<0.0001) (Fig. 5A).

In 6 patients with pSS, serum sIL-2R levels were longitudinally measured within 12 months after prednisolone ± other immunosuppressant treatment. The mean duration of treatment was 4.8 months, and the mean initial dose of prednisolone was 36mg/day. The details of immunosuppressant were cyclophosphamide in 2 cases, tacrolimus in 1 case, azathioprine in 1 case, and RCHOP chemotherapy in 1 case. A significant decrease was noted from 645 U/mL to 317 U/mL (p=0.03) with disease improvement (Fig. 5B).

Serum sIL-2R was re-elevated from 175 U/mL to 227 U/mL at disease relapse (submandibular gland re-swelling) in one patient and in two patients with the emergence of new pancreatic lesions (from 429 U/mL to 690 U/mL, and from 213 U/mL to 265 U/mL).

Comparison between IgG4-RD and pSS

While serum sIL-2R level reflected disease activity and the presence of extra-dacryosialadenitis involvements in both diseases, serum sIL-2R levels were significantly higher in IgG4-RD than in pSS (568 U/mL *vs*. 388 U/mL, p=0.01), although they were not statistically different between IgG4-RD with extra-dacryosialadenitis involvements and pSS with extra-dacryosialadenitis involvements (768 U/mL *vs*. 629 U/mL, p=0.21). However, the diseases did display significant differences, with circulating white blood cell counts, eosinophil counts, and serum levels of IgG4, IgG4/IgG ratio and IgE being significantly higher, and serum levels of IgM and IgA significantly lower in IgG4-RD than in pSS (Table I).

Discussion

Of the biomarkers examined, serum sIL-2R was found to be the most useful in IgG4-RD and pSS. Positive associations were evident between serum sIL-2R and disease activity, number of affected organs, and presence of extra-dacryosialadenitis involvements. The biomarker reflected the response to glucocorticoid in both diseases.

In IgG4-RD, evaluation of disease activity is important, since appropriate treatment that is initiated before irreversible progression of fibrosis improves the functional prognosis of the affected organs (3, 4). Although experimental biomarkers, such as increased numbers of circulating activated Tfh cells and plasmablasts, are reported to reflect disease activity in IgG4-RD (11, 13-17, 21-22), it is difficult to monitor those biomarkers in routine clinical practice. Decreased levels of serum complement may serve as another biomarker during follow-up of IgG4-related tubulointerstitial nephritis (31). However, its potential for other organs has not been proven. Recently,

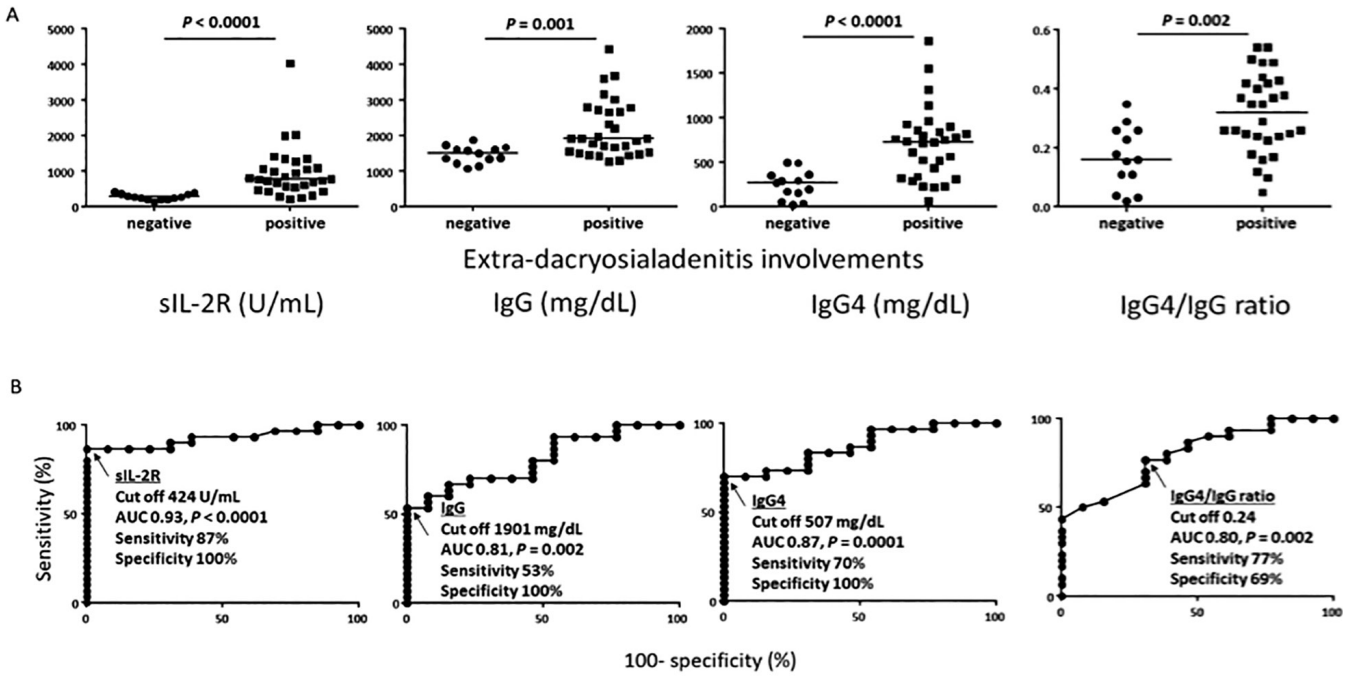

Fig. 2. Serum levels of sIL-2R were significantly higher in IgG4-related disease patients with extradacryosialadenitis involvements compared to those without, resulting in the good performance to estimate the presence of extra-dacryosialadenitis involvements.
(**A**) Levels of serum sIL-2R, IgG, IgG4 and IgG4/IgG ratio in IgG4-related disease patients with or without extra-dacryosialadenitis involvements.
(**B**) Receiver operating characteristic (ROC) curve analyses to estimate the presence of extra-dacryosialadenitis involvements.

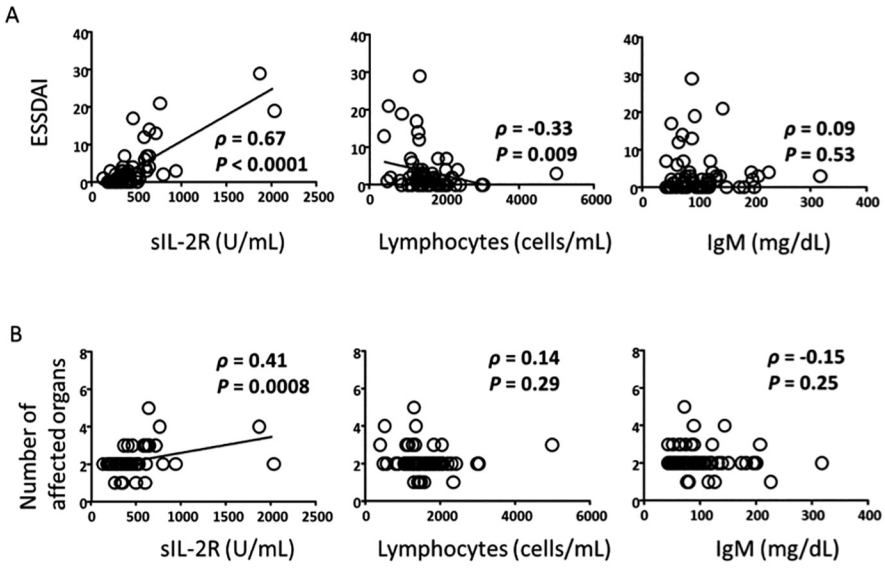

Fig. 3. Serum levels of sIL-2R positively correlated with disease activity or the number of affected organs in primary Sjogren's syndrome.
(**A**) Correlation between blood biomarkers and disease activity assessed by EULAR Sjögren's syndrome disease activity index (ESSDAI). (**B**) Correlation between blood biomarkers and the number of affected organs.

elevated levels of serum sIL-2R and its significant reduction after glucocorticoid treatment were reported in small case series of autoimmune pancreatitis (32). Another report indicated that serum sIL-2R levels reflect local lesion activity of affected organs evaluated by fluorodeoxyglucose-positron emission tomography/computed tomography in IgG4-RD (33). In our present study, serum sIL-2R, which is easily determined, was strongly correlated with disease activity and treatment response, and indicated the presence of extra-dacryosialadenitis involvements. These results suggest that serum sIL-2R levels are useful for assessing disease activity in IgG4-RD.

Our study also demonstrates the correlation of sIL-2R levels with the activity of pSS. pSS is a an autoimmune disease that affects lacrimal and glandular lesions. However, some patients suffer from extra-dacryosialadenitis involvements and are at higher risk of mortality and morbidity (9, 10). Levels of serum sIL-2R have been correlated with the degree of lacrimal dysfunction (34), and high levels of serum sIL-2R reportedly denote the presence of extra-dacryosialadenitis involvements in patients with pSS (35). Our results are consistent with these prior findings; we found for the first time that serum sIL-2R levels reflected systemic disease activity assessed by ESSDAI, a recently established clinical disease activity score, and indicated the presence of extra-dacryosialadenitis involvements. Monitoring serum sIL-2R levels may provide an early and sensitive index for the development of lymphoma in pSS, since serum sIL-2R is a tumour marker for lymphoma (36).

sIL-2R is a 55-kDa protein released from activated T cells, B cells, and dendritic cells (37-39). *In vivo* inter-

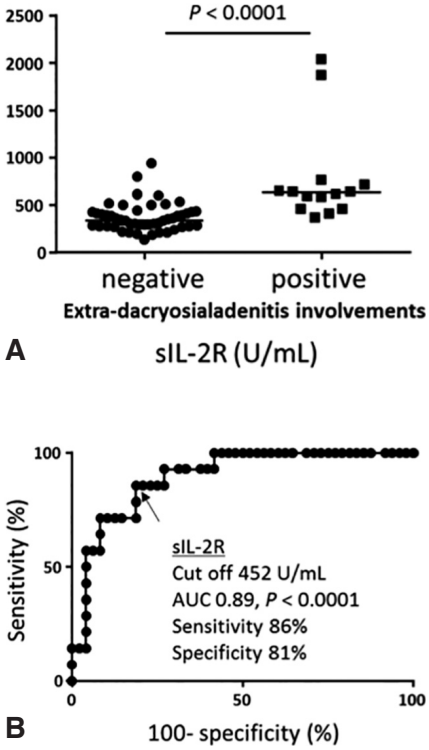

Fig. 4. Serum levels of sIL-2R were significantly higher in primary Sjögren's syndrome patients with extra-dacryosialadenitis involvements compared to those without, resulting in the good performance to estimate the presence of extra-dacryosialadenitis involvements.
(**A**) Levels of serum sIL-2R in primary Sjögren's syndrome patients with or without extra-dacryosialadenitis involvements.
(**B**) Receiver operating characteristic curve (ROC) analyses to estimate the presence of extra-dacryosialadenitis involvements.

action of sIL-2R with immune cells including Tfh cells has recently been reported (39). IL-2 is a cytokine that modulates T effector cell differentiation and negatively regulates Tfh cell differentiation (38). Activated dendritic cells produce sIL-2R to antagonise IL-2 function, facilitating Tfh cell differentiation (39). Tfh cells are important in the pathogenesis of both IgG4-RD and pSS (11-20). Increased Tfh cells induce the production of IgG4 and plasmablast differentiation, with correlation to IgG4-RD RI scores in IgG4-RD (13). A significant correlation between the increased number of circulating activated Tfh cells and serum sIL-2R levels in IgG4-RD has been reported (13). Circulating Tfh cells are also increased in pSS, particularly in patients with extra-dacryosialadenitis involvements (18), and the number of circulating Tfh cells correlates with ESSDAI scores (18, 19). Thus, our findings that serum sIL-2R levels correlated with IgG4-RD RI in IgG4-RD and ESSDAI in pSS suggest that sIL-2R secreted from activated dendritic cells play a role in facilitating Tfh cell differentiation in both diseases. We would propose that above a cut-off of 450 U/mL in serum sIL-2R, a patient should have scanning to identify organ disease elsewhere as shown in ROC analysis, although the normal range of sIL-2R in our experience is less than 500 U/mL. Thus, future studies to determine the appropriate threshold serum sIL-2R level are warranted.

We observed that serum sIL-2R was similarly elevated in IgG4-RD and pSS along with disease activity in our study. However, the two diseases differed in terms of the presence of autoantibodies, circulating eosinophil counts, and the levels of serum IgG4, IgE, IgA, and IgM. These findings indicate a different regulation of immunoglobulin production and autoreactivity by the network between T cells, B cells, and dendritic cells in IgG4-RD and pSS. Skewing toward IgG4 and IgE class-switching and elevated circulating eosinophil counts may be induced by T follicular helper type 2 cytokines including IL-4, -5, and -21 in IgG4-RD (11, 13, 15, 40, 41), while polyclonal elevation of immunoglobulins including IgA and IgM are observed in pSS (42). Further studies are needed to clarify these findings. As, serum IgG4 levels have been reported as being increased also in a subgroup of pSS patients (43), the elevated serum sIL-2R levels in pSS patients may be confounded by elevated serum IgG4. However, in our present study, all pSS patients showed normal level of serum IgG4 (range; 3-115 mg/dL), suggesting that sIL-2R levels are increased independently of serum IgG4 levels in pSS patients.

We note that our study has limitations. This is a retrospective, observational study with a small sample size due to the rarity of the disease, although the number of patients who were examined serum sIL-2R levels were largest so far. Since the number of sicca syndrome patients is quite limited in our study, it may have affected the results

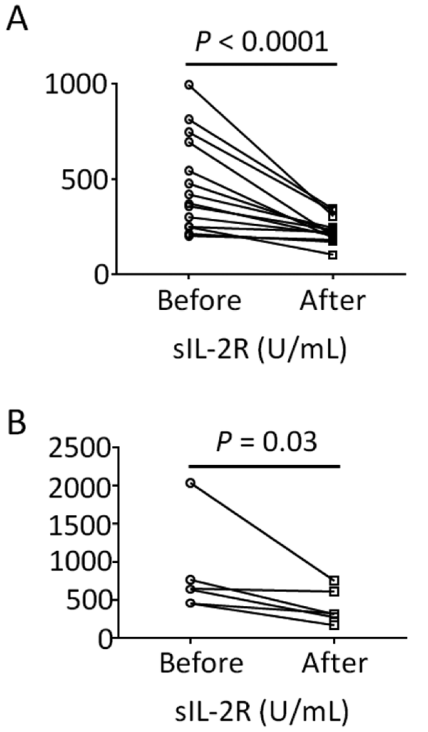

Fig. 5. Serum levels of sIL-2R in IgG4-related disease (**A**) and primary Sjögren's syndrome (**B**) were significantly decreased after immunosuppressive treatment.

of statistical analysis. Further prospective studies in larger cohorts of patients are needed to confirm our results. In addition, serum sIL-2R level itself ddi not distinguish IgG4-RD from pSS in the differential diagnosis because there was no significant difference in serum sIL-2R level between IgG4-RD and pSS patients with extra-dacryosialadenitis involvements.

In conclusion, our findings underscore the usefulness of serum sIL-2R level in evaluating disease activity and detecting systemic organ involvements in both IgG4-RD and pSS. This biomarker may be also useful for monitoring treatment response.

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