Review

Clinicopathological Analysis between IgG4-related and Non-IgG4-related Diseases Occurring in Various Organs and Tissues: Re-evaluation of Comprehensive Diagnostic Criteria and Usefulness of Interleukin-6 Immunostaining

Xipeng Wang^{1,2*}, Yuyu Sun¹, Wei Liu¹, Weiguo Zhang²

¹Department of Orthopaedic Surgery, Affiliated Hospital 2 of Nantong University, Nantong 226001, China;

²Department of Orthopaedic the center hospital of Wuhan, Wuhan 430010, China

*Corresponding Author: Xipeng Wang MD, PhD, Department of Orthopaedic Surgery, Affiliated Hospital 2 of Nantong University, Nantong 226001, China. E-mail: tigerwxp@foxmail.com

Received February 28, 2019; Accepted March 17, 2019

Comprehensive diagnostic criteria for IgG4-related diseases are widely accepted. However, clinicopathological features of probable and possible Ig4-related and non-IgG4-related diseases, which are subdivided by comprehensive diagnostic criteria, are not fully understood. IgG4-related disease (IgG4-RD) is a recently identified systemic fibro-sclerotic disease of unknown etiology. It exhibits multiple organ involvement, causes symmetrical or asymmetrical organomegaly, and forms nodular or hypertrophic lesions concurrently or metachronously. IgG4-RD has several typical characteristics such as predominant occurrence in middle-aged men; presence of antinuclear antibodies; high incidence of allergic diseases; eosinophilia; elevation of serum gammaglobulins, IgG, IgG4, and IgE; and good response to steroid therapy. Histologically, IgG4-RD is characterized by lymphoplasmacytic infiltration, lymphoid follicle formation, eosinophil infiltration, IgG4-positive plasma cell infiltration, storiform fibrosis (SF), and obliterative phlebitis (OP)(1,2). Useful diagnostic tool to distinguish between IgG4-related and non-IgG4-related diseases and prediction of therapeutic responsiveness for IgG4-related disease is required. The purpose of this study is to clarify these diagnostic problems.

Methods: The clinicopathological features and expression of interleukin-6 and glycoprotein 130 were analyzed in 94 and 34 cases of IgG4-related and non-IgG4-related diseases, respectively.

Results: Probable IgG4-related cases showed decreased plasma cell number, increased eosinophils and mast cell numbers, and marked fibrosis. Marked fibrosis and inflammatory cell infiltration were not observed in possible IgG4-related cases. Although most non-IgG4-related cases matched the comprehensive diagnostic criteria, completely or partially, could be easily differentiated based on histological findings, multicentric Castleman's disease exhibited findings closely similar to those of IgG4-related disease. Immunohistochemical expression of interleukin-6 in IgG4-related disease was significantly lower than that in multicentric Castleman's disease. Comprehensive diagnostic criteria could be effectively applied to IgG4-related disease occurring in various organs and tissues and was applied to biopsied and surgically resected specimens, although histological information was limited in the biopsied specimen. Relapsed cases after steroid therapy showed a higher incidence of multiple organ involvement and lower incidences of storiform fibrosis and obliterative phlebitis than the non-relapsed cases.

Conclusion: Probable IgG4-related disease was considered as the advanced phase of definite IgG4-related disease. Possible IgG4-related disease had to undergo repeat biopsy or surgical excision of other organs. Careful consideration of clinical information and immunostaining for interleukin-6 may be crucial to distinguish between IgG4-related and multicentric Castleman's diseases. It is useful to assess the fibrosis grade to evaluate the disease progression and the therapeutic responsiveness for IgG4-related disease occurring in various organs and tissues.

Key Words: *immunity, IgG4-related disease, comprehensive diagnostic criteria, fibrosis grade, IL-6*

Introduction

IgG4-related disease (IgG4-RD) is a recently identified systemic fibro-sclerotic disease of unknown etiology. It exhibits multiple organ involvement, causes symmetrical or asymmetrical organomegaly, and forms nodular or hypertrophic lesions concurrently or metachronously.¹ IgG4-RD has several typical characteristics such as predominant occurrence in middle-aged men; presence of antinuclear antibodies; high incidence of allergic diseases; eosinophilia; elevation of serum gammaglobulins, IgG, IgG4, and IgE; and good response to steroid therapy.¹ Histologically, IgG4-RD is characterized by lymphoplasmacytic infiltration, lymphoid follicle formation, eosinophil infiltration, IgG4-positive plasma cell infiltration, storiform fibrosis (SF), and obliterative phlebitis (OP).²

In 2012, Umehara et al. proposed the following comprehensive diagnostic criteria (CDC) for IgG4-RD of various organs and tissues: 1) clinical examination shows characteristic diffuse/ localized swellings or masses in single or multiple organs; 2) hematological examination shows elevated serum IgG4 (S-IgG4) concentrations (≥ 135 mg/dL); and 3) histological examination shows a) marked lymphocyte and plasma cell infiltration and fibrosis and b) infiltration of IgG4-positive plasma cells, particularly with IgG4-positive plasma cell/IgG-positive plasma cell ratios (IgG4/IgG ratios) $>40\%$ and >10 IgG4-positive plasma cells/ high power field (IgG4/HPF). Definite IgG4-RD is defined as the presence of 1, 2, and 3; probable IgG4-RD as the presence of 1 and 3; and possible IgG4-RD as the presence of 1 and 2. Differential diagnosis is necessary from other disorders, including sarcoidosis, Castleman's disease, Wegener's granulomatosis, lymphoma, and cancer.³

CDC for IgG4-RD has been widely accepted. Although the histological diagnostic scheme of IgG4-RD was proposed by a set of cut-off points that is specific to each organ,² clinicopathological features of probable, possible, and non-IgG4-RDs are not clear. Therefore, Okazaki et al.⁴ reported that organ-specific criteria^{5,6} should be used concurrently in the probable or possible cases. In addition, clinicopathological analysis of IgG4-RD occurring in various organs and tissues and clinicopathological analysis of single or multiple lesions in IgG4-RD have not been sufficiently examined. There are increasing opportunities to diagnose IgG4-RD based on biopsied specimens, since a minimally invasive procedure is required. Moriyama et al.⁷ reported the diagnostic utility of labial salivary gland biopsy in IgG4-RD. However, diagnostic usefulness of biopsied specimens is not examined in other various organs and tissues.

The steroid is a first-line therapy in IgG4-RD, and it is performed following pathological diagnosis. Novel therapeutic approaches are proposed in the field of IgG4-RD.⁸ It is necessary to

find the factors which influence on the therapeutic responsiveness to the IgG4-RD. Some cases of non-IgG4-RD such as autoimmune diseases, multicentric Castleman's disease (MCD), or malignant tumors exhibit overproduction of interleukin -6 (IL-6), leading to IgG4-positive plasm cell infiltration and serum IgG4 elevation.⁹ In particular, MCD is challenging to distinguish from IgG4-RD because of histological similarity.¹⁰ Therefore, immunohistochemical analysis for IL-6 and glycoprotein 130 (gp130), which transmits an IL-6 signal into the cell together with the IL-6 receptor,¹¹ was performed in order to evaluate whether it is possible to distinguish between IgG4-RD and MCD on the tissue sections.

Materials and methods

1. Specimens analyzed

A total of 94 IgG4-RD cases and 34 non-IgG4-RD cases were retrospectively evaluated. Biopsied and surgically resected specimens of IgG4-RD and non-IgG4-RD were collected from the cases of Second Affiliated Hospital of Nantong University ($n = 30$) and from cases consulted from 2008 to 2018 ($n = 98$). This study was approved by the institutional ethics committee of Nantong University. The male:female ratio was 62:32 among IgG4-RD cases and 24:10 among non-IgG4-RD cases, and patients in the former group were older than those in the latter group (63.0 ± 12.1 years vs 53.0 ± 18.8 years; $p = 0.0072$). The age of all the patients are above 30 years were in either group.

Although non-IgG4-RD groups are clinically suspected as IgG4-RD because of organ enlargement or serum IgG4 elevation, it is possible to distinguish between the two based on their clinical and histopathological findings. In this study, non-IgG4-RD is defined as the disease that fulfills condition 1) and satisfies conditions 2) or 3) or both of the CDC for IgG4-RD.

There were a total of 177 lesions in the IgG4-RD group and 37 lesions in the non-IgG4-RD group. Ninety-four cases diagnosed with the biopsied specimen and 34 cases diagnosed with surgically resected specimen were evaluated. The non-IgG4-RD cases included 14 Castleman's disease (6 multicentric, 8 unicentric), 7 malignant lymphoma (6 MALToma, 1 angioimmunoblastic T-cell lymphoma), 3 non-specific reactive lymphadenopathy, 2 chronic sialoadenitis, 2 Warthin's tumor, 1 Crow-Fukase disease, 1 Kimura's disease, 1 interstitial nephritis, 1 membranous nephropathy, 1 retroperitoneal fibrosis, and 1 inflammatory myofibroblastic tumor.

All tissue samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE), toluidine blue (pH 7.0) to count numbers of mast cells, and elastica van Gieson to detect OP.

2. Immunohistochemistry

Immunohistochemical reactions were performed on 4–5 μm -thick formalin-fixed and paraffin-embedded sections. All stains were performed automatically (BenchMark GX, Ventana Medical System, Tucson, Arizona, USA) using the standard immunoperoxidase method, and 3,3'-diaminobenzidine was used as a chromogen. Slides were immunostained using either an anti-human IgG4 monoclonal antibody (clone HP6025, 1:400 dilutions; Binding Site, Birmingham, UK), an anti-human IgG polyclonal antibody (1:5,000 dilution; DAKO, Carpinteria, CA, USA), an anti-human IL-6 monoclonal antibody (clone 10C12, 1:50 dilution; Leica Microsystems, Wetzlar, Germany), or an anti-human gp130

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: weda-h@weda-h.org

How to cite this article: Wang XP, Sun YY, Cui ZM, Liu W. Clinicopathological Analysis between IgG4-related and Non-IgG4-related Diseases Occurring in Various Organs and Tissues: Re-evaluation of Comprehensive Diagnostic Criteria and Usefulness of Interleukin-6 Immunostaining. *J ADV HEALTH* 2019; 1(2): 122-131.

polyclonal antibody (1:500 dilution; Santa Cruz Biotechnology, San Diego, CA, USA) with incubation for 30 min at 37°C. Antigen retrieval with proteinase K (85 µg/ml, DAKO) for 6 min was performed for the anti-human IgG polyclonal antibody.

3. Analyzed factors and assessment of light microscopic findings

Clinicopathological features in IgG4-RD and nonIgG4-RD were analyzed with the following factors: 1) age and sex, 2) sites involved, 3) S-IgG4 level, 4) histological features, 5) IgG4/IgG ratios and IgG4/HPF, 6) lymphocyte-infiltration grade and infiltration of eosinophils and mast cells, 7) fibrosis grade, and 8) presence of SF and OP. Clinicopathological features in definite IgG4-RD were analyzed with the following factors: 9) biopsied and surgically resected specimens, 10) single and multiple organ involvement, and 11) relapse after steroid therapy. In addition, 12) clinicopathological and immunohistochemical comparison in definite IgG4-RD and MCD were also analyzed. All histological features were measured under 400x magnification (objective, Plan Apo 40x/0.95, ocular; CFI 10x/22; Nikon ECLIPSE Microscope E600, Nikon Instech Co., Ltd., Tokyo). To evaluate IgG4/IgG ratios, IgG4/HPF, infiltration of eosinophils and mast cells, 5 HPFs were selected from prominent inflammatory areas and number of cells were counted under microscopy. The average number of IgG4-positive plasma cells and IgG-positive plasma cells/5HPF and the total number of eosinophils and mast cells /5 HPFs were calculated. Plasma cells that could be clearly identified by the presence of a nucleus and cytoplasm were counted. The status of fibrosis was graded according to four categories ranging from 0 to 3, where 0 = no fibrosis, 1 = slight fibrosis, 2 = septal fibrosis, and 3 = dense fibrosis or sclerosis (Fig. 1a-d). The lymphocyte infiltration was defined using the following grading scheme: 0 = very slight infiltration of lymphocytes, 1 = mild infiltration of lymphocytes, 2 = lymphocyte aggregation without germinal centers, and 3 = lymphoid follicles with germinal centers (Fig. 2a-d). IL-6 and gp130 were assessed by cytoplasmic staining of plasma cells.

Figure 1a-d. Fibrosis grade. (a) The lymph node showing diffuse infiltration by lymphocytes and plasma cells without fibrosis, Grade 0. (b) The submandibular gland showing slight periductal (arrow) and perivascular fibrosis (arrow head), Grade 1. (c) The parenchyma of the submandibular gland is separated by thick collagen bundles, Grade 2. (d) The parenchyma of the submandibular gland is replaced by dense fibrosis, Grade 3. a-d, hematoxylin-eosin stain; a, b 200x; c 40x; d 100x.

Figure 2a-d. Lymphocyte infiltration grade. (a) There is very slight infiltration of lymphocytes in the submandibular gland, Grade 0. (b) The pancreas showing mild infiltration of lymphocytes around the acinar cells, Grade 1. (c) Aggregation of lymphocytes without an agerminal center in the minor salivary gland, Grade 2. Acinar cells showing atrophic change. (d) The submandibular gland showing hyperplastic lymphoid follicle with a germinal center, Grade 3. a-d, hematoxylin-eosin stain; a, b, c 200x; d 40x

4. Statistical analysis

All statistical analyses were performed using PRISM ver.6 (GraphPad Software, La Jolla, CA, USA). Comparisons were made using the Mann-Whitney U test, and comparisons for a positive rate of SF, OP, IL-6, and gp-130 were made using chi-square test. The receiver operating characteristic curves were constructed and compared with area under the curve to determine the cut-off values for the positive rate of IL-6 and gp130. $P < 0.05$ was considered as statistically significant.

Results

1. Age and sex

The IgG4-RD cases were subsequently divided into the following three groups: definite IgG4-RD ($n = 83$), probable IgG4-RD ($n = 5$), and possible IgG4-RD ($n = 6$) according to CDC for IgG4-RD (Table 1). The male:female ratio was 62:32 among IgG4-RD cases and 24:10 among non-IgG4-RD cases, and patients in the former group were older than those in the latter group (63.0 ± 12.1 years vs 53.0 ± 18.8 years; $p = 0.0072$). Definite IgG4-RD, probable IgG4-RD, and non-IgG4-RD showed male predominance (Table 1). No patients under the age of 30 years were included in either group.

2. Sites

The distribution of the 177 lesions in the IgG4-RD group was as follows. The maximum number of involved organs were six (submandibular gland, parotid gland, lacrimal gland, lymph node, paranasal sinus and prostatic gland; submandibular gland, lacrimal gland, lymph node, lung, retroperitoneum, and ureter). The number of affected organs and cases was 5 ($n = 3$), 4 ($n = 7$), 3 ($n = 14$), 2 ($n = 12$), and 1 ($n = 56$). Exocrine glands, including major salivary glands (31.1%), lacrimal gland (16.4%), minor salivary glands (6.8%), and pancreas (6.8%), were affected in all IgG4-RD lesions.

Table 1. Clinicopathological findings of IgG4-related disease and non-IgG4-related disease

	IgG4-RD (n = 94)			
	Definite (n = 83)	Probable (n = 5)	Possible (n = 6)	Non-IgG4-RD (n = 34)
Age (y/o)	62.7 ± 11.7**	56.8 ± 18.4	71.5 ± 6.7*	53.0 ± 18.8
Sex (M : F)	57:26	3:2	2:4	24:10
S-IgG4 (mg/dL)	903.9 ± 649.4***	87.0 ± 44.7*	835.2 ± 400.1	418.8 ± 504.9
IgG4/HPF	139.9 ± 71.0***	79.2 ± 49.5	9.6 ± 10.9**	75.4 ± 95.6
Lymphocyte-infiltration grade	2.2 ± 0.8	2.2 ± 1.3	1.1 ± 1.1*	2.4 ± 1.0
Eosinophils/5 HPFs	27.4 ± 118.1*	101.0 ± 190.0*	0.6 ± 1.6	14.2 ± 42.4
Mastcells/5 HPFs	15.2 ± 25.8*	93.8 ± 183.5	9.8 ± 18.7	30.0 ± 47.3
Fibrosis grade	1.9 ± 1.0****	2.2 ± 1.3*	1.7 ± 1.0*	0.7 ± 1.1
Storiform fibrosis n (%)	22 (26.5%)**	1 (20.0%)	1 (16.7%)	1 (2.5%)
Obliterative phlebitis n (%)	27(32.5%***	2 (40.0%)*	1 (16.7%)	1 (2.5%)

Abbreviations: n, number; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; IgG, immunoglobulin G; RD, related disease; y/o, years old; M, male; F, female; S, serum; HPF, high powered field

The distribution of the 37 lesions in the non-IgG4-RD group were as follows: 54.1% in the lymph nodes (n = 20); 10.8% in the major salivary glands (n = 4); 8.1% in the lung (n = 3) and lachrymal gland (n = 3); 5.4% in the kidney (n = 2); 2.7% in the pancreas (n = 1), prostatic gland (n = 1), bone marrow (n = 1), skin (n = 1), and retroperitoneum (n = 1). The maximum number of involved organs was two; two cases of MCD showed the involvement of two organs (lung and lymph node). Other 33 lesions in the non-IgG4-RD group affected a single organ.

3. S-IgG4 level

S-IgG4 was significantly higher in the IgG4-RD group (855.5 ± 640.7 mg/dL, n = 94) than in the non-IgG4-RD group (418.8 ± 504.9 mg/dL, n = 34) (p = 0.0013); definite IgG4-RD exhibited significantly higher levels than non-IgG4-RD (903 ± 649.4 mg/dL, p < 0.001) (Table 1).

4. Histological features

Tissue specimens of definite IgG4-RD showed abundant lymphoplasmacytic infiltration (Fig. 3a and Fig. 3b), SF, and OP (Fig. 3c and Fig. 3d). Definite IgG4-RD also had dense fibrosis and septal fibrosis accompanied by SF. At the periphery of the lesions, periductal fibrosis and perivascular fibrosis were observed. Acinar glands were surrounded by fine fibrosis, particularly in the case of the pancreas, salivary glands, and lacrimal gland. Immunohistochemically, many of the infiltrating plasma cells were positive for IgG and IgG4 (Fig. 3e and Fig. 3f). Compared with definite IgG4-RD, probable IgG4-RD showed noticeably decrease of IgG4-positive plasma cell infiltration, an increase of eosinophil and mast cell infiltration, and marked fibrosis. Possible IgG4-RD exhibited slight inflammatory cell infiltration (10 > lymphocytes and plasma cells/HPF) (Table 1).

The non-IgG4-RD cases completely or partially matched CDC guidelines for IgG4-RD. Some of the non-IgG4-RD cases could be easily differentiated from the others based on the histological findings only; however, some cases exhibited similar clinical and histological findings to IgG4-RD. In particular, it was difficult to distinguish MCD from IgG4-RD because of their histological similarity, a final diagnosis was reached based on clinical information.

Figure 3a-f. Pathological findings. (a) Indefinite IgG4-related disease, chronic inflammatory cells markedly infiltrate into the submandibular glands. Hyperplastic lymphoid follicles are present (arrows), and fibrosclerotic changes are seen in the interfollicular area (arrow head). (b) Interstitial infiltration of lymphocytes and plasma cells is seen in the submandibular gland. (c) Scattered lymphocytes and plasma cells are seen in the dense fibrotic area. Collagen fibers exhibit storiform fibrosis (arrow). (d) Chronic inflammatory cells infiltrate into the venous walls and form obliterative phlebitis (arrow). (e) Dense infiltration of IgG-positive plasma cells is seen. (f) Many of the IgG-positive plasma cells are positive for IgG4. a-c, hematoxylin-eosin stain; d, elastic van Gieson stain, e, IgG; f, IgG4; a 40×; b, e, f 200×; c, d 100×.

5. IgG4/IgG ratios and IgG4/HPF

IgG4/IgG ratios were the highest in definite IgG4-RD and were significantly different between definite IgG4-RD (p < 0.0001),

probable IgG4-RD ($p < 0.01$), possible IgG4-RD ($p < 0.05$), and non-IgG4-RD (Table 1). In the IgG4-RD groups, definite IgG4-RD showed the highest mean number of IgG4/HPF, with a statistically

significant difference (Table 1).

Table 2 summarizes the average of the IgG4/IgG ratios and IgG4/HPF in each of the organs or tissues.

Table 2. Average of IgG4/IgG ratios and IgG4/HPF of IgG4-related disease in each site

	IgG4/IgG% (n)			IgG4/HPF (n)		
	Definite	Probable	Possible	Definite	Probable	Possible
Major salivary gland(n = 34)	76.8(29)	74.8(2)	13.8(3)	154.8(29)	66.5(2)	9.9(3)
Lacrymal gland(n = 17)	77.3(16)	74.7(1)		135.0(16)	52.6(1)	
Minor salivary gland(n = 12)	83.1(10)		16.4(2)	112.7(10)		13.6(2)
Kidney(n = 10)	70.4(10)			139.7(10)		
Pancreas(n = 3)	73.6(3)			47.1(3)		
Lung(n = 3)	80.1(3)			151.9(3)		
Lymph node(n = 3)	83.8(3)			166.3(3)		
Retroperitoneum(n = 3)	92.8(2)	44.1(1)		99.9(2)	44.8(1)	
Intestine(n = 2)	91.5(1)		4.0(1)	136.0(1)		0.8(1)
Bile duct(n = 1)	48.6(1)			92.6(1)		
Mediastinum(n = 1)	85.1(1)			37.8(1)		
Liver(n = 1)	74.1(1)			77.8(1)		
Prostatic gland(n = 1)	100.0(1)			170.2(1)		
Paranasal sinus(n = 1)	66.6(1)			114.6(1)		
Pericardium(n = 1)	86.9(1)			37.8(1)		
Skin(n = 1)		51.2(1)			165.6(1)	
Total average	79.4(83)	61.2(5)	11.4(6)	111.6(83)	82.4(5)	8.1(6)

Abbreviations: IgG, immunoglobulin G; HPF, high powered field; n, number

The average of the IgG4/IgG ratios in each of the sites were 79.4% in the definite IgG4-RD group, 61.2% in the probable IgG4-RD group (range: 48.6%–100% and 44.1%–74.8%, respectively) and 11.4% in the possible IgG4-RD group (range: 4.0%–16.4%). The average number of IgG4/HPF in each of the sites was 111.6 in the definite IgG4-RD group (range: 37.8–166.3) and 82.4 in the probable IgG4-RD group (range: 44.8–165.6). The average of possible IgG4-RD was equal to 8.1 (range: 0.8–13.6). The IgG4/IgG ratios of retroperitoneum, bile duct, and skin indicated lower levels. The IgG4/HPF of pancreas, retroperitoneum, mediastinum, and pericardium indicated lower levels. No significant differences in IgG4/IgG ratios and IgG4/HPF were observed between organs.

The average of the IgG4/IgG ratios in each of the sites were 79.4% in the definite IgG4-RD group, 61.2% in the probable IgG4-RD group (range: 48.6%–100% and 44.1%–74.8%, respectively) and 11.4% in the possible IgG4-RD group (range: 4.0%–16.4%). The average number of IgG4/HPF in each of the sites was 111.6 in the definite IgG4-RD group (range: 37.8–166.3) and 82.4 in the probable IgG4-RD group (range: 44.8–165.6). The average of possible IgG4-RD was equal to 8.1 (range: 0.8–13.6). The IgG4/IgG ratios of retroperitoneum, bile duct, and skin indicated lower levels. The IgG4/HPF of pancreas, retroperitoneum, mediastinum, and pericardium indicated lower levels. No significant differences in IgG4/IgG ratios and IgG4/HPF were observed between organs.

The averages of the IgG4/IgG ratios and IgG4/HPF in the non-IgG4-RD group are summarized in Table 3. IgG4/IgG ratios in MCD, malignant lymphoma (MALToma), Crow-Fukase disease,

and Kimura's disease were >40%. Average of IgG4/HPF in non-IgG4-RD exceeded >10, and an increase in IgG4-positive plasma cells was particularly noticeable in MCD cases.

Table 3. Average of IgG4/IgG ratios and IgG4/HPF in non-IgG4-related disease

	IgG4/IgG ratio(%)	IgG4/HPF
Castleman's disease(14)	29.4 ± 21.7	116.6 ± 130.3
Multicentric(6)	41.2 ± 21.7	181.7 ± 130.3
Unicentric(8)	20.5 ± 16.4	67.9 ± 72.5
Malignant lymphoma(7)	44.5 ± 31.5	64.2 ± 68.2
MALToma(6)	47.6 ± 31.5	65.6 ± 68.2
AILT(1)	26.0	55.8
NSRL(3)	18.4 ± 15.6	46.4 ± 45.5
Chronic sialadenitis(2)	12.7 ± 17.9	13.3 ± 18.8
Warthin's tumor(2)	28.3 ± 3.0	37.0 ± 36.2
Crow-Fukase disease(1)	45.7	34.0
Kimura's disease(1)	40.9	64.4
Interstitial nephritis(1)	32.6	22.8
Membranous nephropathy(1)	17.3	26.0
Retroperitoneal fibrosis(1)	24.5	27.8
IMT(1)	24.6	67.0

mucosa associated lymphoid tissue; AILT, angioimmunoblastic T-cell lymphoma; NSRL, non-specific reactive lymphadenopathy; IMT, inflammatory myofibroblastic tumor

6. Lymphocyte-infiltration grade and infiltration of eosinophils and mast cells

There were no significant differences in lymphocyte -infiltration grade among definite IgG4-RD, probable IgG4-RD, and non-IgG4- RD groups; the lymphocyte-infiltration grade was the lowest impossible IgG4-RD (Table 1). Lymphocyte- infiltration grade of minor salivary glands and lymph nodes were higher

than those of other organs (Table 4). Significant differences in the number of eosinophils were observed among definite IgG4-RD (27.4 ± 118.1 , $p < 0.05$), probable IgG4-RD (101.0 ± 190.0 , $p < 0.05$), and non-IgG4-RD groups. Significant differences in the number of mast cells were observed between definite IgG4-RD (15.2 ± 25.8 , $p < 0.05$) and nonIgG4-RD groups. The number of eosinophils and mast cells in probable IgG4-RD indicated the highest levels (Table 1).

Table 4. Average of lymphocyte-infiltration grade and fibrosis grade of IgG4-related disease in each site

	Lymphocyte-infiltration grade(n)			Fibrosis grade(n)		
	Definite	Probable	Possible	Definite	Probable	Possible
Major salivary gland(n = 34)	2.7(29)	2.5(2)	1.7(3)	2.5(29)	3.0(2)	2.3(3)
Lacrymal gland(n = 17)	2.4(16)	3.0(1)		1.4(16)	0.0(1)	
Minor salivary gland(n = 12)	1.8(10)		2.0(2)	1.5(10)		1.5(2)
Kidney(n = 10)	1.3(10)			1.5(10)		
Pancreas(n = 3)	1.7(3)			3.0(3)		
Lung(n = 3)	2.3(3)			1.3(3)		
Lymph node(n = 3)	3.0(3)			0.0(3)		
Retroperitoneum(n = 3)	1.5(2)	0.0(1)		3.0(2)	3.0(1)	
Intestine(n = 2)	2.0(1)		0.0(1)	0.0(1)		0.0(1)
Bile duct(n = 1)	1.0(1)			3.0(1)		
Mediastinum(n = 1)	1.0(1)			1.0(1)		
Liver(n = 1)	1.0(1)			3.0(1)		
Prostatic gland(n = 1)	2.0(1)			1.0(1)		
Paranasal sinus(n = 1)	1.0(1)			3.0(1)		
Pericardium(n = 1)	1.0(1)			3.0(1)		
Skin(n = 1)		3.0(1)			2.0(1)	
Total average	1.7(83)	2.2(5)	1.2(6)	1.9(83)	2.2(5)	1.3(6)

Abbreviation: n, number

7. Fibrosis grade

The fibrosis grade was significantly lower (0.7 ± 1.1) in the non-IgG4-RD group than in the definite IgG4-RD (1.9 ± 1.0 , $p < 0.0001$), probable IgG4-RD (2.2 ± 1.3 , $p < 0.05$), and possible IgG4-RD (1.7 ± 1.0 , $p < 0.05$). Fibrosis grade was the highest improbable IgG4-RD (Table 1)

The incidence of SF and OP were seen to gradually increase with fibrosis grade (Fig. 4a and Fig. 4b). The fibrosis grade of the major salivary glands, pancreas, retroperitoneum, bile duct, liver, paranasal sinus, and pericardium were higher than that of other organs (Table 4).

8. SF and OP

SF was observed in 22 cases (26.5%, $p < 0.01$) of definite IgG4-RD but was in probable IgG4-RD (20.0%), possible IgG4-RD (16.7%), and non-IgG4-RD (2.5%) (Table 1). OP was observed in 27 cases of definite IgG4-RD (32.5%, $p < 0.001$). It was observed in two cases of probable IgG4-RD (40.0%, $p < 0.01$), one case of possible IgG4-RD (16.7%), and one case of non-IgG4-RD (2.5%) groups (Table 1).

High incidence of SF and OP was observed in the major salivary glands, pancreas, and retroperitoneum (Table 5). SF and OP were

seen in one of 6 case of MCD.

Figure 4a, b. Correlation between fibrosis grade and lymphocyte infiltration grade. Storiform fibrosis and obliterative phlebitis gradually increased with fibrosis grade. Abbreviations: n, number; SF, storiform fibrosis; OP, obliterative phlebitis.

9. Biopsied and surgically resected specimens

There were no apparent differences in IgG4/IgG ratios and IgG4/HPFs between biopsied and surgically-resected specimens; biopsied specimens of definite IgG4-RD exhibited significantly a lower fibrosis grade, lymphocyte-infiltration grade, SF, and OP compared with surgically resected specimens (Table 6). All cases of probable IgG4-RD were surgically-resected specimens; all cases of possible IgG4-RD were biopsied specimens.

Table 5. Average of storiform fibrosis and obliterative phlebitis of IgG4-related disease in each site

	SF%(n)			OP%(n)		
	Definite	Probable	Possible	Definite	Probable	Possible
Majorsalivary gland(n = 34)	42.9(29)	0.0(2)	33.3(3)	50.0(29)	50.0(2)	33.3(3)
Lacrymal gland(n = 17)	6.2(16)	0.0(1)		18.8(16)	0.0(1)	
Minor salivary gland(n = 12)	0.0(10)		0.0(2)	0.0(10)		0.0(2)
Kidney(n = 10)	20.0(10)			20.0(10)		
Pancreas(n = 3)	33.3(3)			66.7(3)		
Lung(n = 3)	33.3(3)			33.3(3)		
Lymph node(n = 3)	0.0(3)			0.0(3)		
Retroperitoneum(n = 3)	50.0(2)	0.0(1)		50.0(2)	100.0(1)	
Intestine(n = 2)	0.0(1)		0.0(1)	0.0(1)		0.0(1)
Bile duct(n = 1)	100.0(1)			100.0(1)		
Mediastinum(n = 1)	0.0(1)			0.0(1)		
Liver(n = 1)	100.0(1)			0.0(1)		
Prostatic gland(n = 1)	0.0(1)			0.0(1)		
Paranasal sinus(n = 1)	100.0(1)			100.0(1)		
Pericardium(n = 1)	100.0(1)			100.0(1)		
Skin(n = 1)		100.0(1)			0.0(1)	
Total average	39.0(83)	20.0(5)	11.1(6)	35.9(83)	40.0(5)	11.1(6)

Abbreviations: SF, storiform fibrosis; OP, obliterative phlebitis; n, number

Table 6. Clinicopathological findings of definite IgG4-related disease in biopsied and surgically resected specimens

	DefiniteIgG4-RD(n=83)		
	Biopsy (n = 63)	Surgical (n = 20)	P
Age (y/o)	61.9 ± 1.4	65.1 ± 2.6	NS
Sex (M : F)	45:18	12:8	
S-IgG4 (mg/dL)	920.9 ± 83.4	926.5 ± 229.0	NS
IgG4/IgG ratios (%)	76.1 ± 1.8	82.5 ± 3.2	NS
IgG4/HPF	129.5 ± 9.2	147.5 ± 4.1	NS
Lymphocyte-infiltration grade	2.0 ± 0.1	2.5 ± 0.1	0.0203
Eosinophils/5 HPFs	32.4 ± 17.7	12.6 ± 3.6	NS
Mast cells/5 HPFs	15.3 ± 3.8	14.9 ± 6.5	NS
Fibrosis grade	1.7 ± 0.1	2.5 ± 0.1	0.0033
Storiform fibrosis n (%)	13 (20.6%)	9 (45.0%)	0.0315
Obliterative phlebitis n (%)	16 (19.3%)	11 (55.0%)	0.0138

Abbreviations: IgG, immunoglobulin G; RD, related disease; n, number; NS, not significant; y/o, years old; M, male; F, female; S, serum; HPF, high powered field

10. Single or multiple organ involvement

Indefinite IgG4-RD, there were no significant differences in all parameters between cases involving a single organ (n = 51) and those involving multiple organs (n = 32)(Table 7). However, S-IgG4 levels of cases involving multiple organs tended to be higher than those of cases involving a single organ (1,085 ± 115.5 mg/dL vs. 820.3 ± 112.9 mg/dL, p = 0.6126).

All 5 cases of probable IgG4-RD exhibited single organ involvement; all 6 cases of possible IgG4-RD showed multiple organ involvement.

11. Relapse after steroid therapy

Improved S-IgG4, S-IgG, and serum IgG4/IgG ratios (S-IgG4/IgG ratios) and disappearance of organ enlargement were observed in 15 cases of definite IgG4-RD subjected to steroid therapy. The mean duration of steroid therapy was 45.4 months (range, 19–84 months), and the average dose was 33 mg/day (range, 20–45 mg/day). However, 6 of 15 cases had prolonged S-IgG4 elevation, which gradually increased after the steroid therapy, and 3 of 6 cases exhibited submandibular gland and lacrimal gland re-enlargement (average, 20.7 months; range, 7–47 months). S-IgG4 and S-IgG levels were decreased significantly after the steroid therapy in nine

Table 7. Clinicopathological findings between cases involving a single organ and multiple organs in definite IgG4-related disease

	Definite IgG4-RD (n=83)	
	Single organ (n = 51)	Multiple organs (n = 32)
Age (y/o)	62.8 ± 1.9	62.6 ± 1.7
Sex (M : F)	34:17	23:9
S-IgG4 (mg/dL)	820.3 ± 112.9	1,085.0 ± 115.5
IgG4/IgG ratios (%)	77.3 ± 13.8	78.4 ± 16.7
IgG4/HPF	126.8 ± 68.5	145.1 ± 74.5
Lymphocyte-infiltration grade	2.2 ± 0.1	2.1 ± 0.2
Eosinophils/5 HPFs	36.7 ± 22.1	13.8 ± 5.0
Mast cells/5 HPFs	15.8 ± 4.3	14.5 ± 5.2
Fibrosis grade	2.1 ± 0.1	1.7 ± 0.2
Storiform fibrosis n (%)	10 (19.6%)	12 (37.5%)
Obliterative phlebitis n (%)	15 (29.4%)	12 (37.5%)

Abbreviations: IgG, immunoglobulin G; RD, related disease; n, number; y/o, years old; M, male; F, female; S, serum; HPF, high powered field

nonrelapsed cases (Table 8, $p < 0.0001$, and $p < 0.001$). Six relapsed cases also showed decreased S-IgG4 and S-IgG without significant differences 1 year after steroid therapy (Table 8). Nine non-relapsed cases included 6 cases involving a single organ and 3 cases involving multiple organs; six relapsed cases included 1 case with single organ involvement and 5 cases with multiple organ

involvement. Among the non-relapsed cases, SF was found in 3 of 9 cases (33.3%) and OP was found in 4 of 9 cases (44.4%). SF and OP were not found in relapsed cases. IgG4/IgG ratios, IgG4/HPF, eosinophils/5 HPFs, mast cells/5 HPFs, fibrosis grade, and lymphocyte-infiltration grade in the non-relapsed cases were not significantly different from the relapsed cases.

Table 8. Serum IgG, IgG4 and their ratios before and after steroid therapy for non-relapsed and relapsed definite IgG4-related disease

	Non-relapsed (n=9)		Relapsed (n=6)	
	Before	After	Before	After
S-IgG (mg/dL)	2,427.4****	991.4	1,883.3	1,385.7
S-IgG4 (mg/dL)	917.4***	184.0	649.5	393.0
S-IgG4/IgG ratios (%)	35.0	19.3	33.3	25.9

Abbreviations: n, number; ***, $p < 0.001$; ****, $p < 0.0001$; Before, before steroid therapy; After, 1 year after steroid therapy; S, serum; HPF, high powered field

12. Clinicopathological and immunohistochemical comparison of definite IgG4-RD and MCD

Age, IgG4/IgG ratios, and fibrosis grade were significantly higher in definite IgG4-RD cases when compared with the MCD cases (Table 9).

The threshold for IL-6 and gp130 was selected to be more than 20% of the positive rates for plasma cells based on the best

diagnostic efficacy achieved through equilibrium between sensitivity and specificity. The immunohistochemical positivity for IL-6 was 21.4% in definite IgG4-RD cases ($n = 70$) and 83.3% in MCD cases ($n = 6$). A significant difference was observed between definite IgG4-RD and MCD ($p = 0.001$). Gp130 showed a higher positive rate for both definite IgG4-RD (79.2%, $n = 48$) and MCD (100.0%, $n = 5$), but this difference was not statistically significant ($p = 0.2572$) (Fig. 5a, and Fig. 5b).

Table 9. Difference of clinicopathological findings between definite IgG4-related disease and multicentric Castleman's disease

	Definite IgG4-RD (n = 83)	MCD (n = 6)
Age (y/o)	62.7 ± 11.7**	47.0 ± 14.6
Sex (M : F)	57:26	5:1
S-IgG4 (mg/dL)	903.9 ± 649.4	349.8 ± 120.0
IgG4/IgG ratios (%)	77.7 ± 14.8***	41.2 ± 23.5
IgG4/HPF	139.9 ± 71.0	181.7 ± 167.1
Lymphocyte-infiltration grade	2.2 ± 0.8	2.7 ± 0.8
Eosinophils/5 HPFs	27.4 ± 118.1	8.5 ± 14.2
Mast cells/5 HPFs	15.2 ± 25.8	50.6 ± 77.8
Fibrosis grade	1.9 ± 1.0**	0.7 ± 1.2
Storiform fibrosis n (%)	22 (26.5%)	1 (16.7%)
Obliterative phlebitis n (%)	27 (32.5%)	1 (16.7%)

Abbreviations: IgG, immunoglobulin G; RD, related disease; n, number; MCD, multicentric Castleman's disease; y/o, years old; M, male; F, female; S, serum; HPF, high powered field

Figure 5a, b. Immunohistochemical findings for interleukin-6. (a) Plasma cells are not staining in the case of definite IgG4-related disease of the pancreas. (b) In the interfollicular area, plasma cells are positive in the case of Castleman's disease of the lymph node. a, b IL-6; a, b 200 $\times$.

Discussion

CDC is the most pragmatic diagnostic criteria for IgG4-RD so far. However, clinicopathological features of probable and possible IgG4-RD using CDC has not yet been fully understood. Furthermore, other various diagnostic problems to be solved of CDC for IgG4-RD were also evaluated.

Probable IgG4-RD was characterized by IgG4/IgG ratios $>40\%$, >10 IgG4/HPF (but lower than definite IgG4-RD), lower levels of S-IgG4, marked fibrosis, and increased numbers of eosinophil and mast cell infiltration. Masaki et al.¹² reported that IgG4/HPF in the fibrotic parts of IgG4-RD was lower than in the non-fibrotic parts. The lower levels of S-IgG4 improbable IgG4-RD may be related to marked fibrosis and decreased IgG4/HPF. Eosinophils and mast cells are also known to be the major effector cells for tissue fibrosis and remodeling.¹³ They release various cytokines, including TGF- β , IL-13, IL-14, and vascular endothelial growth factor, which regulate fibroblastic function.¹³ The histological findings of probable IgG4-RD were similar to those of definite IgG4-RD, except for the presence of marked fibrosis, decreased number of plasma cells, and increased number of eosinophils and mast cells. Probable IgG4-RD may be considered as the advanced phase of definite IgG4-RD. Possible IgG4-RD cases showed high S-IgG4, whereas the IgG4/IgG and IgG4/HPF ratios were lower. Additionally, marked fibrosis, lymphoplasmacytic infiltration, SF, and OP was not observed. Although Umehara et al and Okazaki et al^{3,4} have recommended the application of organ-specific criteria for IgG4-RD, a repetition of the biopsy or surgical excision of other organs should be considered in such cases.

In the biopsied specimens, the IgG4/IgG ratios and IgG4/HPF of various organs and tissues could be evaluated. Compared with the surgically resected specimens, biopsied specimens showed significantly lower fibrosis grade and lymphocyte-infiltration grade, SF, and OP. Therefore, it is considered that the CDC is applied to a biopsied specimen as well as a surgically resected specimen. However, the former is smaller and does not reflect the entirety of the lesion, thus increasing the tendency for underestimation of the pathological diagnosis.

The number of affected organs and tissues differed between the IgG4-RD and non-IgG4-RD cases. The IgG4-RD cases revealed multiple organs involvement when compared with the non-IgG4-RD cases; in addition, selective exocrine organ involvement was also noted. Fibrosis grade is useful for the quantitative histological evaluation of IgG4-RD occurring invariable sites and tissues. As shown in Tables 4 and 5, deep-seated organs exhibiting higher fibrosis grades tended to show an increased incidence of SF and OP, suggesting that fibrosis is associated with the occurrence of SF and OP. The reason for this

phenomenon is thought to be related to the anatomical site not easily noticed organ enlargement.

Steroid therapy for IgG4-RD has been widely recognized as a treatment measure, and it seems to be increasing opportunities to diagnose treated IgG4-RD cases. This study identified S-IgG4, S-IgG, S-IgG4/IgG ratios, number of involved organs, SF, and OP as good indicators for the prediction of the relapse of definite IgG4-RD after steroid therapy. Mizushima et al¹⁴ reported clinical and histological changes associated with steroid therapy in IgG4-related tubulointerstitial nephritis. They also reported that a decrease in IgG4-positive plasma cells was observed after steroid therapy, although interstitial fibrosis and small lymphocytic infiltration did not improve. Therefore, new therapeutic strategies for IgG4-RD is required by using these prediction factors.

The pathological features of MCD were very similar to those of IgG4-RD. However, useful an immunohistochemical tool to distinguish MCD from IgG4-RD has not yet known. Therefore, the final diagnosis of MCD had been based on clinical information.⁵ MCD is known to exhibit overproduction of IL-6, leading to IgG4-positive plasma cell infiltration and serum IgG4 elevation. In this study, the positive expression of IL-6 in definite IgG4-RD cases was significantly lower than that in MCD; the production of IL-6 may be decreased in IgG4-RD patients. Hence, IL-6 immunostaining may be useful as a diagnostic tool to distinguish between IgG4-RD and MCD.

Nakajima et al.¹⁵ reported that impaired expression of innate immune response-related genes (including defensin) was associated with the development of IgG4-RD. Defensin families play an important role in the innate immune response against bacteria, fungi, protozoa, and viruses. Conversely, Ahn et al¹⁶ reported that defensin-1 induces the secretion of IL-6 in rheumatoid arthritis. IL-6 is one of the cytokines that control humoral immunity and is produced by various cells, including B-cells, T-cells, fibroblasts, monocytes, endothelial cells, and mesangial cells.¹⁷ It induces the differentiation of B-cells to plasma cells and naïve T-helper cells to Th17 cells; it also inhibits the differentiation of Treg-induced by TGF- β , and regulates the balance between Th17 and Treg immune reactions.¹⁸ Th17 plays a role in the defense against bacterial infections by enhancing neutrophil migration, whereas Treg is known to produce regulatory cytokines, such as IL-10 and TGF- β , and facilitate IgG4 production, as well as fibrosis.¹⁹ Therefore, reduced expression of neutrophil-derived innate immune response related genes (including defensin) may decrease IL-6 secretion in IgG4-RD patients, leading to Treg dominant immune responses that may disturb the Treg/Th17 balance.¹⁸

In conclusion, the clinicopathological features of IgG4-RD and non-IgG4-RD occurring in various organs and tissues were analyzed. The diagnostic problems of CDC in IgG4-RD, the prediction of therapeutic responsible factors, and the usefulness of IL-6 immunostaining for MCD were also discussed. However, the number of cases in the present study is limited, warranting the need for further studies to clarify the clinicopathological features of IgG4-RD and non-IgG4-RD.

This work was partially supported by a Grant-inAid for Scientific Research (C) of the Ministry of Culture and Science of Japan (25460465) and Health and Labour Sciences Research grants for Intractable Diseases from the Minister of Labour and Welfare of Japan. The author would like to thank Drs. Hiroshi Minato, Eriko Kinoshita-Kobayashi, Satoko Nakada, Takayuki Nojima, Yasufumi Masaki, and Hisanori Umehara for their expert assistance and advice. The author would also like to thank the members

of MHLW for providing the cases and clinical data.

Acknowledgments

We thank Lee Seaman of Seaman Medical, Inc. (Bellingham, WA, USA) for providing professional English-language editing of this article.

Conflict of interest statement

The authors declare they have no conflicts of interest relevant to this manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Umehara H, Okazaki K, Masaki Y, et al: A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details. *Mod Rheumatol* 2012; 22:1-14.
2. Deshpande V, Zen Y, Chan JK, et al. Consensus statement on the pathology of IgG4-related disease. *Mod Pathol* 2012; 25: 1181-92.
3. Umehara H, Okazaki K, Masaki Y, et al. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 2012; 22: 21-30.
4. Okazaki K, Umehara H. Are classification criteria for IgG4-RD The concept of IgG4-Related Disease and proposal of comprehensive diagnostic criteria in Japan. *Int J Rheumatol* 2012; 2012: 357071.
5. Shimosegawa T, Chari ST, Frulloni L, et al. International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the international association of pancreatology. *Pancreas* 2011; 40: 352-8.
6. Kawano M, Saeki T, Nakashima H, et al. Proposal for diagnostic criteria for IgG4-related kidney disease. *Clin Exp Nephrol* 2011; 15: 615-26.
7. Moriyama M, Ohta M, Furukawa S, et al. The diagnostic utility of labial salivary gland biopsy in IgG4-related disease. *Mod Rheumatol* 2016; 3: 1-5.
8. Lang D, Zwerina J, Pieringer H. IgG4-related disease: current challenges and future prospects. *Ther Clin Risk Manag* 2016; 12: 189-99.
9. Nishimoto N, Terao K, Mima T, et al. Mechanisms and pathologic significance in the increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood* 2008; 112: 3959-64.
10. Sato Y, Kojima M, Takata K, et al. Multicentric Castleman's disease with abundant IgG4-positive cells: a clinical and pathological analysis of six cases. *J Clin Pathol* 2010; 63: 1084-9.
11. Rose-John S, Scheller J, Elson G et al: Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J Leukoc Biol* 2006; 80: 227-36.
12. Masaki Y, Kurose N, Yamamoto M, et al. Cutoff values of serum IgG4 and histopathological IgG4+ plasma cells for diagnosis of patients with IgG4-related disease. *Int J Rheumatol* 2012; 2012: 580814.
13. Levi-Schaffer F, Weg VB. Mast cells, eosinophils and fibrosis. *Clin Exp Allergy* 1997; 27: 64-70.
14. Mizushima I, Yamada K, Fujii H, et al. Clinical and histological changes associated with corticosteroid therapy in IgG4-related tubulointerstitial nephritis. *Mod Rheumatol* 2012; 22: 859-70.
15. Nakajima A, Masaki Y, Nakamura T, et al. Decreased expression of innate immunity-related genes in peripheral blood mononuclear cells from patients with IgG4-related disease. *PLoS One* 2015; 10: e0126582.
16. Ahn JK, Huang B, Bae EK, et al. The role of α -defensin-1 and related signal transduction mechanisms in the production of IL-6, IL-8 and MMPs in rheumatoid fibroblast-like synoviocytes. *Rheumatology* 2013; 52: 1368-76.
17. Hirano T, Akira S, Taga T, et al. Biological and clinical aspects of interleukin 6. *Immunol Today* 1990; 11: 443-9.
18. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. *Eur J Immunol* 2010; 40: 1830-5.
19. Zen Y, Fujii T, Harada K, et al. Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007; 45: 1538-46.