IgG4-Related Disease

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Editorial Board

Contents

Editorial
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John H. Stone,[1] John K. C. Chan,[2] Vikram Deshpande,[3] Kazuichi Okazaki,[4] Hisanori Umehara,[5] and Yoh Zen[6]

[1] *Rheumatology Unit, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Yawkey 2, 55 Fruit Street, Boston, MA 02114, USA*

[2] *Department of Pathology, Queen Elizabeth Hospital, Wylie Road, Kowloon, Hong Kong*

[3] *Department of Pathology, Massachusetts General Hospital, Warren 2, 55 Fruit Street, Boston, MA 02114, USA*

[4] *Department of Gastroenterology and Hepatology, Kansai Medical University, Japan*

[5] *Division of Hematology and Immunology, Department of Internal Medicine, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Japan*

[6] *Histopathology Section, Institute of Liver Studies, King's College Hospital, Denmark Hill, London SE5 9RS, UK*

Correspondence should be addressed to John H. Stone; jhstone@partners.org

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Over the past decade and with increasing pace in the last few years, a "new" disease has emerged, gradually affecting a wide range of medical specialties and explaining a host of conditions previously regarded as separate entities. This newly recognized condition is IgG4-related disease (IgG4-RD), a potentially multiorgan disorder that is characterized by elevated serum IgG4 concentrations in the majority of cases. IgG4-RD was recognized in modern times in Japan through a series of seminal observations that occurred during the 1990s and the first few years of this century [1–5], but it is clear in reviewing the medical literature that IgG4-RD has been present and reported upon in various guises going back at least to the 1800s [6–11].

In addition to the frequent elevations of serum IgG4 concentrations, certain major pathologic hallmarks are generally present to one degree or another across all organ systems, providing the principal foundation for the belief that the disparate organ manifestations associated with this diagnosis are in fact part of the same systemic disease. These pathologic features include a lymphoplasmacytic infiltrate with a high percentage of plasma cells within the lesion staining for IgG4; a peculiar pattern of fibrosis known as "storiform" fibrosis; a tendency to affect veins in a manner that leads to obliterative phlebitis; and mild to moderate tissue eosinophilia [12].

IgG4-RD appears to sit at an intersection between different inflammatory pathways. Many but not all patients have substantial allergic or atopic histories, and early indications are that a "modified" Th2 response is critical to this condition [13]. Other patients also develop tumefactive lesions leading to misdiagnoses of cancer. Still others have clinical manifestations and serological findings that lead to erroneous classifications of their diagnoses as "connective tissue diseases." The full links between the various inflammatory players in this symphony of inflammation remain to be fully elucidated. It is likely that a broader understanding of the ways in which B and T cells, fibroblasts, plasma cells, immune complexes, and other elements interact in IgG4-RD will provide important insights into the nature of its individual inflammatory constituents and the broader immune system.

IgG4-RD is now recognized as a worldwide disease [14]. The international community convened in Boston in 2011 to compare notes, share experiences, and plan ways for moving ahead in understanding this condition. Building upon crucial earlier work in Japan, consensus papers pertaining to the nomenclature of this condition and to its pathological features have been published [12, 15]. Japanese investigators have also published diagnostic criteria for IgG4-RD [16].

In this special issue, we are pleased to present more than two dozen papers on IgG4-RD that address a number of facets

of this condition: from its clinical manifestations to its radiologic features; from its pathology hallmarks to its serologic characteristics; and from its diagnostic challenges to early indications of treatment success. These papers capture the essence of IgG4-RD in 2012 and represent the current state-of-the-art against which future advances will be compared.

John H. Stone
John K. C. Chan
Vikram Deshpande
Kazuichi Okazaki
Hisanori Umehara
Yoh Zen

References

[1] K. Kawaguchi, M. Koike, K. Tsuruta, A. Okamoto, I. Tabata, and N. Fujita, "Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas," *Human Pathology*, vol. 22, no. 4, pp. 387–395, 1991.

[2] K. Yoshida, F. Toki, T. Takeuchi, S. I. Watanabe, K. Shiratori, and N. Hayashi, "Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis," *Digestive Diseases and Sciences*, vol. 40, no. 7, pp. 1561–1568, 1995.

[3] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[4] T. Kamisawa, N. Funata, Y. Hayashi et al., "A new clinicopathological entity of IgG4-related autoimmune disease," *Journal of Gastroenterology*, vol. 38, no. 10, pp. 982–984, 2003.

[5] M. Yamamoto, H. Takahashi, S. Sugai, and K. Imai, "Clinical and pathological characteristics of Mikulicz's disease (IgG4-related plasmacytic exocrinopathy)," *Autoimmunity Reviews*, vol. 4, no. 4, pp. 195–200, 2005.

[6] J. Mikulicz, "Über eine eigenartige symmetrische erkränkung der thränen- und mundspeicheldrüsen," in *Beiträge zur Chirurgie Festschrift Gewidmet*, T. Billroth, Ed., pp. 610–630, Ferdinand Enke, Stuttgart, Germany, 1892.

[7] H. Küttner, "Über entzündliche tumoren der submaxillar-speicheldrüse," *Beiträge zur Klinischen Chirurgie*, vol. 15, pp. 815–834, 1896.

[8] B. Riedel, "Die chronische zur Bildung eisenharter Tumoren führende Entzündung der Schildrüse," *Verhandlung der deutsche Gesellschaft für Chirurgie*, vol. 25, p. 101, 1896.

[9] H. Sarles, J. C. Sarles, R. Muratore, and C. Guien, "Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease?" *The American Journal of Digestive Diseases*, vol. 6, no. 7, pp. 688–698, 1961.

[10] D. E. Comings, K. B. Skubi, J. van Eyes, and A. G. Motulsky, "Familial multifocal fibrosclerosis. Findings suggesting that retroperitoneal fibrosis, mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, and pseudotumor of the orbit may be different manifestations of a single disease," *Annals of Internal Medicine*, vol. 66, no. 5, pp. 884–892, 1967.

[11] S. Nakano, I. Takeda, K. Kitamura et al., "Vanishing tumor of the abdomen in patient with Sjogren's syndrome," *American Journal of Digestive Diseases*, vol. 23, no. 5, pp. 75–79, 1978.

[12] V. Deshpande, Y. Zen, J. K. Chan et al., "Consensus statement on the pathology of IgG4-related disease," *Modern Pathology*, vol. 25, no. 9, pp. 1181–1192, 2012.

[13] Y. Zen, T. Fujii, K. Harada et al., "Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis," *Hepatology*, vol. 45, no. 6, pp. 1538–1546, 2007.

[14] J. H. Stone, Y. Zen, and V. Deshpande, "IgG4-related disease," *The New England Journal of Medicine*, vol. 366, no. 6, pp. 539–551, 2012.

[15] J. H. Stone, A. Khosroshahi, V. Deshpande et al., "Recommendations for the nomenclature of IgG4-related disease and its individual organ system manifestations," *Arthritis and Rheumatism*, vol. 64, no. 10, pp. 3061–3067, 2012.

[16] H. Umehara, K. Okazaki, Y. Masaki et al., "Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD)," *Modern Rheumatology*, vol. 22, no. 1, pp. 21–30, 2012.

Clinical Study

Pathologies Associated with Serum IgG4 Elevation

Mikael Ebbo,[1, 2] **Aurélie Grados,**[1, 2] **Emmanuelle Bernit,**[2] **Frederic Vély,**[1, 3, 4]
José Boucraut,[3] **Jean-Robert Harlé,**[1, 2] **Laurent Daniel,**[1, 5] **and Nicolas Schleinitz**[1, 2, 4]

[1] *Université de la Méditerranée Aix-Marseille II, France*

[2] *Service de Médecine Interne, Hôpital de la Conception, Assistance Publique-Hôpitaux de Marseille, 13385 Marseille Cedex 5, France*

[3] *Laboratoire d'Immunologie, Hôpital de la Conception, Assistance Publique-Hôpitaux de Marseille, 13385 Marseille Cedex 5, France*

[4] *Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, Case 906, Campus de Luminy,*
  13288 Marseille Cedex 09, France

[5] *Service d'Anatomie Pathologique et de Neuropathologie, Hôpital de la Timone, Assistance Publique—Hôpitaux de Marseille,*
  13385 Marseille Cedex 5, France

Correspondence should be addressed to Mikael Ebbo, mikael.ebbo@ap-hm.fr

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Statement of Purpose. IgG4-related disease (IgG4-RD) is usually associated to an increase of serum IgG4 levels. However other conditions have also been associated to high serum IgG4 levels. *Methods*. All IgG subclasses analyses performed in our hospital over a one-year period were analyzed. When IgG4 level were over 1.35 g/L, the patient's clinical observation was analyzed and both final diagnosis and reason leading to IgG subclasses analysis were recorded. Only polyclonal increases of IgG4 were considered. *Summary of the Results*. On 646 IgG subclass analysis performed, 59 patients had serum IgG4 over 1.35 g/L. The final diagnosis associated to serum IgG4 increase was very variable. Most patients (25%) presented with repeated infections, 13.5% with autoimmune diseases, and 10% with IgG4-RD. Other patients presented with cancer, primary immune deficiencies, idiopathic interstitial lung disease, cystic fibrosis, histiocytosis, or systemic vasculitis and 13.5% presented with various pathologies or no diagnosis. Mean IgG4 levels and IgG4/IgG ratio were higher in IgG4-RD than in other pathologies associated to elevated IgG4 levels. *Conclusions*. Our study confirms that elevation of serum IgG4 is not specific to IgG4-RD. Before retaining IgG4-RD diagnosis in cases of serum IgG4 above 1.35 g/L, several other pathological conditions should be excluded.

1. Introduction

Immunoglobulin G4 (IgG4) represents the less abundant of the four IgG subclasses in human serum accounting for 3 to 6% of the total IgG [1].

IgG4 has been associated with several pathological conditions. Most of these associations suggest a protective effect of IgG4, such as in allergen-specific immunotherapy [2] and protection from inflammatory manifestations during parasitosis [3]. In few situations, IgG4 is associated with a direct pathogenic effect, such as in pemphigus. During this blistering dermatosis, antidesmosome autoantibodies belong to the IgG4 subclass [4]. However, total IgG4 serum levels are not raised in these conditions.

In 2001, Hamano et al. report a quantitative serum IgG4 elevation during sclerosing (or "autoimmune") pancreatitis

above the cutoff value of 135 mg/dL in 95% of patients with autoimmune pancreatitis [1]. This entity was first described in 1961 by Sarles and colleagues in patients with lymphoplasmacytic infiltrate and fibrosis of the pancreas associated to polyclonal hypergammaglobulinemia [5]. Polyclonal hypergammaglobulinemia raised initially the issue of the possible auto-immune nature of the disease, but this hypothesis has not been confirmed to date. Indeed, no specific autoantibody has been associated with auto-immune pancreatitis. Serum IgG4 elevation becomes from this date a biological marker of sclerosing (or "auto-immune") pancreatitis. Other fibroinflammatory organ involvements with similar histopathological characteristics have since been reported, associated or not with pancreatic involvement, in a context of serum IgG4 elevation [6], leading to the concept of an IgG4-related disease [7]. To date, serum

TABLE 1: Clinical reasons leading to IgG subclass measurement in patients with serum IgG4 elevation $n = 59$.

	% (n)
Hypogammaglobulinemia or PID suspicion	35.6% (21)
Hypergammaglobulinemia	25.4% (15)
IgG4-RD suspicion	23.7% (14)
Other	15.3% (9)

PID: primary immune deficiency; IgG4-RD: IgG4-related disease.

TABLE 2: Final diagnosis in patients with elevated serum IgG4 level (>1.35 g/L).

	n (%)	Mean IgG4 levels (g/L) (extremes)
Repeated infections	15 (25.4%)	2.31 (1.37–4.3)
Autoimmune diseases	8 (13.6%)	3.62 (1.38–11.3)
No final diagnosis	8 (13.6%)	1.94 (1.37–2.97)
IgG4-RD	6 (10.1%)	**12.64 (2.48–20.6)**
Possible IgG4-RD	5 (8.5%)	2.23 (1.56–3.37)
Cancer	4 (6.8%)	2.00 (1.71–2.32)
Primary immune deficiency	4 (6.8%)	1.80 (1.44–2.24)
Interstitial pneumonitis	3 (5%)	5.54 (1.51–12.7)
Cystic fibrosis	2 (3.4%)	4.49 (3.36–5.62)
Erdheim Chester disease	2 (3.4%)	3.05 (2.15–3.94)
Vasculitis	2 (3.4%)	3.68 (3.06–4.30)

IgG4-RD: IgG4-related disease.
Apart from the final diagnostic retained, allergic and atopic manifestations were found in 10 patients (16.9%).

IgG4 elevation is considered as a diagnosis criteria for IgG4-related disease [8–10]. However, serum IgG4 elevation is not necessary for the diagnosis, as proposed by the diagnosis criteria, and IgG4 elevation is not specific of the disease [11]. Serum IgG4 elevation has also been reported in various pathological situations: multicentric Castleman's disease [12], Wegener's granulomatosis [13], Churg-Strauss syndrome [14], or pancreatic adenocarcinoma [15].

Few works have systematically studied diagnosis associated with a serum IgG4 elevation [11, 16, 17]. In order to better know diagnosis associated with this biological situation, we studied retrospectively all IgG4 subclass measurements achieved during a one-year period at the University Hospital of Marseille, France.

2. Materials and Methods

All results for IgG subclasses evaluation performed from January 1st, 2009 to December 31th, 2009 at the laboratory of Immunology of our University Hospital of Marseille, France, were analyzed. Serum total IgG and IgG subclasses (1 to 4) levels were measured by immunonephelometry (Siemens Nephelometer Analyser II) with reagents from Siemens (NAS IgG1, NAS IgG2, N latex IgG3, and N Latex IgG4). All results with serum IgG4 polyclonal increase above the cutoff value of 1.35 g/L were considered for the study.

The patients' medical records were retrospectively analyzed and demographic, clinical, paraclinical, and evolutive characteristics were recorded. The reason leading the physician to the prescription of IgG subclass evaluation was also recorded. Patients were classified according to the disease associated to IgG4 elevation. Mean values in different groups were compared using the Mann-Whitney test. Results were considered significant for $P < 0.05$. Figures and statistics were realized using GraphPad Prism v. 4.0.

3. Results

3.1. Patients' Characteristics. A total of 646 IgG subclass analyses were recorded at the laboratory of Immunology during the one-year period. An IgG4 level above 1.35 g/L was found on 75 samples that corresponded to 60 different patients (some of them took 2 or more samples during the year). Among these 60 patients, data could be collected for 59 patients.

Among the 59 patients analyzed, 30 were men and 29 were women (*sex ratio* 1/1). Mean age at IgG subclass measurement was 47.2 years (range: 4–85 years). Mean serum IgG level was 18.50 g/L (range: 7.38–40.4 g/L). Thirty-eight patients (64%) presented an elevated serum IgG level (>14 g/L). By definition, a serum IgG4 level above 1.35 g/L was present in all 59 patients. Mean serum IgG4 level was 4.35 g/L (range: 1.37–20.6 g/L).

The reasons leading the physicians to perform IgG subclass analysis are presented in Table 1. Suspicion of immune deficiency (with or without hypogammaglobulinemia) was the first indication for IgG subclass measurement in 21 patients (35.6%). Polyclonal hypergammaglobulinemia was the reason leading to serum IgG subclass measurement in 15 patients (25.4%), IgG4-related disease (IgG4-RD) suspicion in 14 patients (23.7%) with one or more compatible organ involvement. In 9 patients (15.3%), the reason was unclear and classified as other.

3.2. Diagnosis Associated with Serum IgG4 over 1.35 g/L. Final diagnosis in patients who presented with elevated levels of serum IgG4 was divided into different categories presented in Table 2. Most represented categories were repeated infections with 15 patients (25.4%). Site of infections (pneumonia, sinusitis, skin and soft tissues infections, osteitis, and pericarditis) and microorganisms implicated (community-acquired bacteria, *Staphylococcus* sp., herpes virus group (HPV, HSV, and EBV), *Nocardia*, *Toxoplasma gondii*, *Enterobius vermicularis*) were variable in this group. In 8 patients (13.6%), final diagnosis was an autoimmune disease: systemic lupus erythematosus (SLE) in 4 patients (with secondary antiphospholipid syndrome in 2), Sjögren Syndrome (SS) in 2 patients, Biermer disease in 1 patient, and systemic sclerosis in 1 patient. An IgG4-related disease (IgG4-RD) was the final diagnosis in 6 patients (10.1%). Histological documentation was available in all cases of IgG4-RD with characteristic histopathological features with lymphocytic and plasmacytic polyclonal inflammatory infiltrate (with predominant IgG4 positive plasma cells when

FIGURE 1: Serum IgG4 levels in different final diagnostic categories of patients with serum IgG4 elevation. IgG4-RD = IgG4-related disease. Horizontal bars represent median values observed in each group. Results obtained in each group were compared to results obtained in IgG4-RD group (Mann-Whitney test): $**P < 0.005$; $*P < 0.05$; ns: not significant.

immunohistological study where available, $n = 5$) and fibrosis. Organ involvements included sclerosing pancreatitis in 4 patients, tubulointerstitial nephritis in 5 patients, polyadenopathy in 5 patients, sialadenitis in 3 patients, sclerosing cholangitis in 2 patients, dacryoadenitis in 1 patient, hypophysitis in one patient, and inflammatory pseudotumors (hepatic in one case, orbital in another case). Four of these patients with IgG4-RD were under treatment (corticosteroids and/or immunosuppressive therapy) at the time of IgG subclass measurement. In 5 patients (8.5%), an IgG4-RD was considered as possible. These patients presented with one or more compatible organ involvements but without histopathological documentation. Sclerosing cholangitis was presented in 4 cases (with renal involvement in one of these patients), sclerosing pancreatitis in the other case. In 4 patients (6.8%) the final diagnosis was a cancer: ampullary carcinoma, angioimmunoblastic T-cell lymphoma, pancreatic carcinoma, and bronchopulmonary carcinoma. Primary immune deficiency (PID) was the final diagnosis in 4 cases (6.8%): Wiskott-Aldrich syndrome, chronic granulomatous disease, common variable immunodeficiency, and complex humoral immune deficiency. Hypogammaglobulinemia on blood electrophoresis was found in 3 of these patients. Idiopathic interstitial pneumonitis was the final diagnosis in 3 patients (5%) (with pulmonary fibrosis in 2 cases). Cystic fibrosis, Erdheim-Chester disease and vasculitis (hepatitis C-virus-associated type-II-mixed cryoglobulinemia in one case and microscopic polyangiitis in another case) were, respectively, diagnosed in 2 patients (3.4%). In 8 patients (13.6%), no final diagnosis could be retained.

Apart from the diagnosis category considered, allergic and atopic manifestations (allergic rhinoconjunctivitis, nasal polyps and allergic chronic rhinosinusitis, asthma and bronchial hyperreactivity, urticarial skin lesions, and angioedema, hypereosinophilia and/or IgE elevation) were found in the record of only 10 patients (16.9%).

3.3. Serum IgG4 Levels Are Significantly Higher in IgG4-RD Than in Other Pathologies. Serum IgG4 levels found in patients of each diagnosis category are presented in Figure 1. Serum IgG4 elevation observed in IgG4-RD group was significantly more important than in the groups "repeated infections" ($P = 0.0021$), "auto-immune diseases" ($P = 0.0127$), "absence of diagnosis" ($P = 0.0027$), "possible IgG4-RD" ($P = 0.0173$), "cancer" ($P = 0.0095$), and "primary immune deficiency" ($P = 0.0095$). Difference with the group "idiopathic interstitial pneumonitis" was not statistically significant ($P = 0.1667$). Statistical analysis with the groups "cystic fibrosis," "Erdheim-Chester disease," and "vasculitis" was not possible because of the too small size of these groups ($n < 3$).

An analysis of the serum IgG4/serum IgG *ratio* was performed. Serum IgG4/serum IgG *ratios* observed in patients of each diagnosis category are presented in Figure 2. Elevation of serum IgG4/serum IgG *ratio* observed in IgG4-RD group was significantly more important than in the groups "repeated infections" ($P = 0.0217$), "auto-immune diseases" ($P = 0.0127$), "absence of diagnosis" ($P = 0.0426$), and "cancer" ($P = 0.0381$). Differences with the groups "possible IgG4-RD" ($P = 0.0519$), "primary immune deficiency" ($P = 0.1143$), and "idiopathic interstitial pneumonitis" ($P = 0.2619$) were not statistically significant. Statistical analysis with the groups "cystic fibrosis," "Erdheim-Chester disease," and "vasculitis" was not possible because of the too small size of these groups ($n < 3$).

4. Discussion

Serum IgG4 elevation above 1.35 g/L has been shown to be a predictive marker of type 1 AIP [1] and IgG4-related sclerosing cholangitis [39]. Usefulness of serum IgG4 in diagnosis of AIP has been evaluated for sensibility and specificity [40] and the cutoff value of 1.35 g/L (135 mg/dL) proposed by Hamano in 2001 is widely accepted in literature. To better distinguish AIP from pancreatic cancer more elevated IgG4 cutoff values (140 mg/dL [21] or 280 mg/dL [18]) have been proposed.

In IgG4-RD, the Japan G4 team has retained the elevation of serum IgG4 above 1.35 g/L as an individual diagnosis criteria, [10]. In fact, according to these criteria the presence of a clinical or radiological organ involvement with IgG4 above 1.35 g/L is sufficient to retain the diagnosis of possible IgG4-RD [10].

However, IgG4 elevation has been reported in several well-characterized pathologies as pancreatic carcinoma (10% for a cut-off of 140 mg/dL, 1% for a cut-off of 280 mg/dL [41]), allergic diseases [2], parasitic infections [3], and systemic diseases [11]. The different pathological situations associated to serum IgG4 elevation in literature are reported in Table 3.

TABLE 3: Pathologies (excepted IgG4-RD organ involvements) associated to serum IgG4 elevation in medical literature.

		Number of cases and references
Cancer	Pancreatic adenocarcinoma	13 cases [18], 5 cases [19], 1 case [20], 8 cases [16, 17], 2 cases [1], 11 cases [21], 1 case [22]
	Bile duct cancer/cholangiocarcinoma	3 cases [16, 17], 4 cases [22], 17 + 20 cases [23]
	Intraductal papillary mucinous neoplasm	1 case [16, 17]
Autoimmune diseases	Systemic lupus erythematosus	1 case [11], 2 cases [16, 17], 4 cases [our study], 1 case [24]
	Antiphospholipid syndrome	1 case [11], 2 cases [our study]
	Autoimmune hepatitis	1 case [16, 17]
	Rheumatoid arthritis	5 cases [11], 2 cases [25]
	Systemic sclerosis	3 cases [11], 1 case [our study]
	Sjögren's syndrome	3 cases [11], 2 patients [our study]
	Polymyositis/dermatomyositis	1 case [11]
ANCA-related vasculitis	**Churg-Strauss syndrome***	4 cases [11], 4 cases [14]
	Microscopic polyangiitis	1 case [11], 1 case [our study]
	Nonspecified	1 case [16, 17]
Infections	Parasitic infections	2 cases [our study], specific IgG4 antibody elevation [3, 26–29]
	Bacterial infections	10 cases [our study]
	Viral infections	3 cases [our study]
Others	**Multicentric Castleman's disease***	7 cases [11], 1 case [30], 1 case [16], 1 case [31], 5 cases [12]
	Eosinophilic disorders (fasciitis, pneumonia, and hypereosinophilic syndrome)	1 case each (fasciitis and pneumonia) [11], 2 cases of hypereosinophilic syndrome [16, 17]
	Chronic hepatitis	1 case [11], 2 cases (auto-immune) [32]
	Liver cirrhosis	2 cases [11], frequent cause of serum IgG4 elevation [33]
	Bronchial asthma	1 case [11], 3 cases [34]
	Idiopathic pulmonary fibrosis/interstitial pneumonia	1 case [11], 4 cases [16, 17], 3 cases [our study]
	Primary sclerosing cholangitis	1 case [16, 17], 3 cases [35], 12 cases [36], 33 cases [37]
	Chronic and idiopathic/acute pancreatitis	1 and 2 cases, respectively [16, 17], 4 and 5 cases, respectively [18]
	Behcet's disease	1 case [16, 17]
	Cystic fibrosis	7 cases [38], 2 cases [our study]

Pathologies in bold with * represent pathologies with especially frequent and high serum IgG4 elevation.

Thus, exclusion criteria, including pathologies other than IgG4-RD, should be added to the diagnosis criteria as proposed by recent CDC criteria for IgG4-RD [10]. To better characterize pathologies associated with serum IgG4 above 1.35 g/L which could be discussed in the differential diagnosis of IgG4-RD, we retrospectively studied all IgG subclass measurements performed over a one-year period at our university hospital. Recruitment was largely including pediatric and adult patients without restricted specialized medical field.

Only 10% of 59 patients with elevated serum IgG4 (>1.35 g/L) were diagnosed with IgG4-RD. Other diagnoses associated were infections, auto-immune diseases, cancers, primary immune deficiencies, idiopathic interstitial pneumonitis, or vasculitis. Only patients with IgG subclass evaluation and IgG4 above 1.35 g/L were included in this retrospective study without any control group. Thus, the specificity or the sensitivity of serum IgG4 elevation for diagnosis of IgG4-RD could not be assessed.

Mean serum IgG4 value was found significantly more important in the IgG4-RD patients. However, IgG4 values observed were overlapping with values observed in some other groups (Figure 1).

As already reported, IgG4 elevation was found to be associated with cystic fibrosis [38], vasculitis [11, 14], and cancer [15]. More surprisingly, it was also associated with auto-immune diseases and patients with repeated infections or primary-immune deficiencies. We also show that IgG4 elevation can be associated to hypogammaglobulinemia in few patients with other IgG subclass deficiencies.

Cystic fibrosis has already been associated with serum IgG4 elevation during colonization and infection by *Pseudomonas aeruginosa* (present in our two patients) [42] and immediate-type hypersensitivity manifestations [38]. These observations, taken together with the observation of IgG4 elevation in a group of patients presenting with repeated infections, raise the question of the role of chronic infectious stimulation in IgG4 elevation. Cancer was associated with serum IgG4 elevation in 6.7% of patients in our study. Of note, a recent work found a more higher standardized incidence ratio for malignancies in IgG4-RD than in the general population [43]. However, none of our patients with
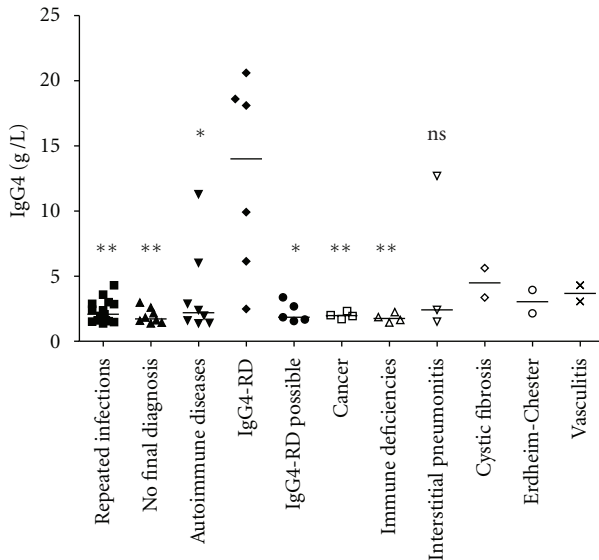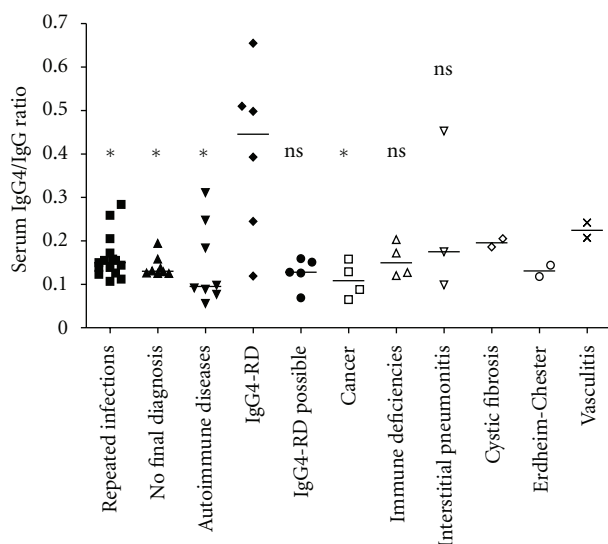
Figure 2: Serum IgG4/serum IgG ratios in different final diagnostic categories of patients with serum IgG4 elevation. IgG4-RD = IgG4-related disease. Horizontal bars represent median values observed in each group. Results obtained in each group were compared to results obtained in IgG4-RD group (Mann-Whitney test): $*P < 0.05$; ns: not significant.

cancer presented either clinical or histological evidence for IgG4-RD.

In 3 cases, a diagnosis of idiopathic interstitial lung disease was retained. Different intrathoracic involvements have been reported during IgG4-RD including interstitial lung disease [44]. Because of the absence of histological documentation obtained in these three patients, we cannot exclude an IgG4-RD with isolated or predominant lung involvement, especially in patient with highest serum IgG4 level.

Allergic manifestations were noted in only 10 patients (16.7%) in our study and could therefore not account for IgG4 elevation in this population.

Our retrospective study clearly confirms that serum IgG4 elevation above 1.35 g/L is not specific for the diagnosis of IgG4-RD and can be observed in several clinical situations. The serum IgG4 elevation is more important in IgG4-RD but associated with an important variability within this group. Further studies are needed to define the sensibility and specificity of IgG4 values for the diagnosis of IgG4-RD. Until more specific biomarkers for IgG4-RD are made available, it must be kept in mind that several pathologies should be evoked end excluded in case of IgG4 elevation, before IgG4-RD diagnosis is retained.

5. Conclusion

IgG4-related disease (IgG4-RD) is characterized by one or several fibroinflammatory organ involvements with typical pathological findings. A serum IgG4 elevation above 1.35 g/L is currently retained as an important biomarker of the disease, included in the diagnosis criteria. We confirm in this retrospective study, analyzing systematically the diagnosis

associated with serum IgG4 above 1.35 g/L in a large unselected cohort of patients evaluated for IgG subclass, that IgG4 elevation is not specific of IgG4-RD. However, the patients with defined IgG4-RD presented with the most elevated serum IgG4 levels. Thus, several different pathologies should be excluded before IgG4-RD is retained in the context of serum IgG4 elevation.

References

[1] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[2] R. C. Aalberse, F. Van Milligen, K. Y. Tan, and S. O. Stapel, "Allergen-specific IgG4 in atopic disease," *Allergy*, vol. 48, no. 8, pp. 559–569, 1993.

[3] R. Hussain, R. W. Poindexter, and E. A. Ottesen, "Control of allergic reactivity in human filariasis: predominant localization of blocking antibody to the IgG4 subclass," *Journal of Immunology*, vol. 148, no. 9, pp. 2731–2737, 1992.

[4] Y. Shirakata, S. Shiraishi, K. Sayama, and Y. Miki, "Subclass characteristics of IgG autoantibodies in bullous pemphigoid and pemphigus," *Journal of Dermatology*, vol. 17, no. 11, pp. 661–666, 1990.

[5] H. Sarles, J. C. Sarles, R. Muratore, and C. Guien, "Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease?" *The American Journal of Digestive Diseases*, vol. 6, no. 7, pp. 688–698, 1961.

[6] T. Saeki, A. Saito, T. Hiura et al., "Lymphoplasmacytic infiltration of multiple organs with immunoreactivity for IgG4: IgG4-related systemic disease," *Internal Medicine*, vol. 45, no. 3, pp. 163–167, 2006.

[7] A. Khosroshahi and J. H. Stone, "IgG4-related systemic disease: the age of discovery," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 72–73, 2011.

[8] K. Okazaki, "Proposal of the concept and diagnostic criteria of IgG4-related disease," in *Annual Reports of Research Committee of Intractable Diseases Supported By Ministry of Health, Labour and Welfare of Japan*, 2009.

[9] K. Okazaki, K. Uchida, M. Koyabu, H. Miyoshi, and M. Takaoka, "Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease," *Journal of Gastroenterology*, vol. 46, no. 3, pp. 277–288, 2011.

[10] H. Umehara, K. Okazaki, Y. Masaki, M. Kawano, M. Yamamoto, T. Saeki et al., "Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD)," *Modern Rheumatology*, vol. 22, no. 1, pp. 21–30, 2012.

[11] M. Yamamoto, T. Tabeya, Y. Naishiro, H. Yajima, K. Ishigami, Y. Shimizu et al., "Value of serum IgG4 in the diagnosis of IgG4-related disease and in differentiation from rheumatic diseases and other diseases," *Modern Rheumatology*, vol. 22, no. 3, pp. 419–425, 2012.

[12] Y. Sato, M. Kojima, K. Takata et al., "Multicentric Castleman's disease with abundant IgG4-positive cells: a clinical and pathological analysis of six cases," *Journal of Clinical Pathology*, vol. 63, no. 12, pp. 1084–1089, 2010.

[13] L. J. Liu, M. Chen, F. Yu, M. H. Zhao, and H. Y. Wang, "IgG subclass distribution, affinity of anti-myeloperoxidase antibodies in sera from patients with Wegener's granulomatosis and microscopic polyangiitis," *Nephrology*, vol. 13, no. 7, pp. 629–635, 2008.

[14] M. Yamamoto, H. Takahashi, C. Suzuki et al., "Analysis of serum IgG subclasses in churg-strauss syndrome-The meaning of elevated serum levels of IgG4," *Internal Medicine*, vol. 49, no. 14, pp. 1365–1370, 2010.

[15] R. P. Sah and S. T. Chari, "Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 108–113, 2011.

[16] T. Tabata, T. Kamisawa, K. Takuma et al., "Serum IgG4 concentrations and IgG4-related sclerosing disease," *Clinica Chimica Acta*, vol. 408, no. 1-2, pp. 25–28, 2009.

[17] T. Tabata, T. Kamisawa, K. Takuma et al., "Serial changes of elevated serum IgG4 levels in IgG4-related systemic disease," *Internal Medicine*, vol. 50, no. 2, pp. 69–75, 2011.

[18] A. Ghazale, S. T. Chari, T. C. Smyrk et al., "Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1646–1653, 2007.

[19] A. Raina, A. M. Krasinskas, J. B. Greer et al., "Serum immunoglobulin G fraction 4 levels in pancreatic cancer: elevations not associated with autoimmune pancreatitis," *Archives of Pathology and Laboratory Medicine*, vol. 132, no. 1, pp. 48–53, 2008.

[20] E. K. Choi, M. H. Kim, T. Y. Lee et al., "The sensitivity and specificity of serum immunoglobulin G and immunoglobulin G4 levels in the diagnosis of autoimmune chronic pancreatitis: Korean experience," *Pancreas*, vol. 35, no. 2, pp. 156–161, 2007.

[21] L. Frulloni, C. Lunardi, R. Simone et al., "Identification of a novel antibody associated with autoimmune pancreatitis," *New England Journal of Medicine*, vol. 361, no. 22, pp. 2135–2142, 2009.

[22] T. Nakazawa, I. Naitoh, K. Hayashi, F. Okumura, K. Miyabe, M. Yoshida et al., "Diagnostic criteria for IgG4-related sclerosing cholangitis based on cholangiographic classification," *Journal of Gastroenterology*, vol. 47, no. 1, pp. 79–87, 2012.

[23] A. M. Oseini, R. Chaiteerakij, A. M. Shire et al., "Utility of serum immunoglobulin G4 in distinguishing immunoglobulin G4-associated cholangitis from cholangiocarcinoma," *Hepatology*, vol. 54, pp. 940–948, 2011.

[24] G. G. Lin and J. M. Li, "IgG subclass serum levels in systemic lupus erythematosus patients," *Clinical Rheumatology*, vol. 28, no. 11, pp. 1315–1318, 2009.

[25] G. Lin and J. Li, "Elevation of serum IgG subclass concentration in patients with rheumatoid arthritis," *Rheumatology International*, vol. 30, no. 6, pp. 837–840, 2010.

[26] M. Satoh, H. Toma, Y. Sato et al., "Production of a high level of specific IgG4 antibody associated with resistance to albendazole treatment in HLA-DRB1*0901-positive patients with strongyloidiasis," *American Journal of Tropical Medicine and Hygiene*, vol. 61, no. 4, pp. 668–671, 1999.

[27] R. M. Genta and J. P. Lillibridge, "Prominence of IgG4 antibodies in the human response to Strongyloides stercoralis infection," *Journal of Infectious Diseases*, vol. 160, no. 4, pp. 692–699, 1989.

[28] P. Hagan, U. J. Blumenthal, D. Dunn, A. J. G. Simpson, and H. A. Wilkins, "Human IgE, IgG4 and resistance to reinfection with *Schistosomahaematobium*," *Nature*, vol. 349, no. 6306, pp. 243–245, 1991.

[29] G. E. Kwan-Lim, K. P. Forsyth, and R. M. Maizels, "Filarial-specific IgG4 response correlates with active Wuchereria bancrofti infection," *Journal of Immunology*, vol. 145, no. 12, pp. 4298–4305, 1990.

[30] M. Yamamoto, H. Takahashi, K. Hasebe et al., "The analysis of interleukin-6 in patients with systemic IgG4-related plasmacytic syndrome—expansion of SIPS to the territory of

Castleman's disease," *Rheumatology*, vol. 48, no. 7, pp. 860–862, 2009.

[31] F. Ishida, K. Kitano, H. Kobayashi, H. Saito, and K. Kiyosawa, "Elevated IgG4 levels in a case with multicentric Castleman's disease," *British Journal of Haematology*, vol. 99, no. 4, pp. 981–982, 1997.

[32] T. Umemura, Y. Zen, H. Hamano et al., "Clinical significance of immunoglobulin G4-associated autoimmune hepatitis," *Journal of Gastroenterology*, vol. 46, supplement 1, pp. 48–55, 2011.

[33] L. Dorn, A. Finkenstedt, M. Schranz, W. Prokop, A. Griesmacher, W. Vogel et al., "Immunoglobulin subclass 4 for the diagnosis of immunoglobulin subclass 4-associated diseases in an unselected liver and pancreas clinic population," *HPB*, vol. 14, pp. 122–125, 2012.

[34] C. De Moraes Lui, L. C. Oliveira, C. L. Diogo, M. Kirschfink, and A. S. Grumach, "Immunoglobulin G subclass concentrations and infections in children and adolescents with severe asthma," *Pediatric Allergy and Immunology*, vol. 13, no. 3, pp. 195–202, 2002.

[35] M. Koyabu, K. Uchida, N. Fukata et al., "Primary sclerosing cholangitis with elevated serum IgG4 levels and/or infiltration of abundant IgG4-positive plasma cells," *Journal of Gastroenterology*, vol. 45, no. 1, pp. 122–129, 2010.

[36] F. D. Mendes, R. Jorgensen, J. Keach et al., "Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis," *American Journal of Gastroenterology*, vol. 101, no. 9, pp. 2070–2075, 2006.

[37] E. Björnsson, S. Chari, M. Silveira et al., "Primary sclerosing cholangitis associated with elevated immunoglobulinG4: clinical characteristics and response to therapy," *American Journal of Therapeutics*, vol. 18, no. 3, pp. 198–205, 2011.

[38] F. Shakib, D. R. Stanworth, C. A. Smalley, and G. A. Brown, "Elevated serum IgG4 levels in cystic fibrosis patients," *Clinical Allergy*, vol. 6, no. 3, pp. 237–240, 1976.

[39] Y. A. Alderlieste, B. D. J. Van Den Elzen, E. A. J. Rauws, and U. Beuers, "Immunoglobulin G4-associated cholangitis: one variant of immunoglobulin G4-related systemic disease," *Digestion*, vol. 79, no. 4, pp. 220–228, 2009.

[40] A. M. Morselli-Labate and R. Pezzilli, "Usefulness of serum IgG4 in the diagnosis and follow up of autoimmune pancreatitis: a systematic literature review and meta-analysis," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 1, pp. 15–36, 2009.

[41] R. P. Sah and S. T. Chari, "Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 108–113, 2011.

[42] R. B. Moss, "The role of IgG subclass antibodies in chronic infection: the case of cystic fibrosis," *New England and regional allergy proceedings*, vol. 9, no. 1, pp. 57–61, 1988.

[43] M. Yamamoto, H. Takahashi, T. Tabeya, C. Suzuki, Y. Naishiro, K. Ishigami et al., "Risk of malignancies in IgG4-related disease," *Modern Rheumatology*, vol. 22, no. 3, pp. 414–418, 2012.

[44] J. H. Ryu, H. Sekiguchi, and E. S. Yi, "Pulmonary manifestations of IgG4-related sclerosing disease," *European Respiratory Journal*, vol. 39, no. 1, pp. 180–186, 2012.

Clinical Study

Immunohistochemical Characteristics of IgG4-Related Tubulointerstitial Nephritis: Detailed Analysis of 20 Japanese Cases

Mitsuhiro Kawano,[1] Ichiro Mizushima,[1] Yutaka Yamaguchi,[2]
Naofumi Imai,[3] Hitoshi Nakashima,[4] Shinichi Nishi,[5] Satoshi Hisano,[6]
Nobuaki Yamanaka,[7] Motohisa Yamamoto,[8] Hiroki Takahashi,[8]
Hisanori Umehara,[9] Takao Saito,[4] and Takako Saeki[10]

[1]*Division of Rheumatology, Department of Internal Medicine, Kanazawa University Hospital, Kanazawa, Ishikawa 920-8641, Japan*
[2]*Yamaguchi's Pathology Laboratory, Matsudo, Chiba 270-2231, Japan*
[3]*Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences,*
Niigata 951-8510, Japan
[4]*Division of Nephrology and Rheumatology, Department of Internal Medicine, Faculty of Medicine, Fukuoka University,*
Fukuoka 814-0180, Japan
[5]*Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan*
[6]*Department of Pathology, Faculty of Medicine, Fukuoka University, Fukuoka 814-0180, Japan*
[7]*Tokyo Kidney Research Institute, Tokyo 113-0023, Japan*
[8]*First Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo 060-8543, Japan*
[9]*Department of Hematology and Immunology, Kanazawa Medical University,*
Kanazawa 920-0293, Ishikawa, Japan
[10]*Department of Internal Medicine, Nagaoka Red Cross Hospital, Nagaoka 940-2085, Japan*

Correspondence should be addressed to Mitsuhiro Kawano, sk33166@gmail.com

Although tubulointerstitial nephritis with IgG4+ plasma cell (PC) infiltration is a hallmark of IgG4-related kidney disease (IgG4-RKD), only a few studies are available about the minimum number of IgG4+ PC needed for diagnosis along with IgG4+/IgG+ PC ratio in the kidney. In addition, the significance of the deposition of IgG or complement as a reflection of humoral immunity involvement is still uncertain. In this study, we analyzed 20 Japanese patients with IgG4-RKD to evaluate the number of IgG4+ PCs along with IgG4+/IgG+ PC ratio and involvement of humoral immunity. The average number of IgG4+ PCs was 43.8/hpf and the average IgG4+/IgG+ or IgG4+/CD138+ ratio was 53%. IgG and C3 granular deposits on the tubular basement membrane (TBM) were detected by immunofluorescence microscopy in 13% and 47% of patients, respectively. Nine patients had a variety of glomerular lesions, and 7 of them had immunoglobulin or complement deposition in the glomerulus. In conclusion, we confirmed that infiltrating IgG4+ PCs > 10/hpf and/or IgG4/IgG (CD138)+ PCs > 40% was appropriate as an item of the diagnostic criteria for IgG4-RKD. A relatively high frequency of diverse glomerular lesions with immunoglobulin or complement deposits and deposits in TBM may be evidence of immune complex involvement in IgG4-related disease.

1. Introduction

The main histopathological finding in the kidney of IgG4-RD is tubulointerstitial nephritis (TIN) [1–3], which may result in renal failure [4]. IgG4-related TIN is composed of dense lymphoplasmacytic infiltrates with fibrosis and copious IgG4+ plasma cell infiltration, which are common features shared by other involved organs [5], and these

common pathologic features in the kidney have clearly been described by previous studies [1–3]. However, the minimum number of IgG4+ plasma cells needed for diagnosis has been differently reported in each affected organ [6–9], and only a few studies are available about the actual number of IgG4+ plasma cells evaluated along with IgG4+/IgG+ plasma cell ratio in IgG4-related kidney disease (IgG4-RKD) [2].

In addition to this issue, case reports or case series of a variety of glomerular disease concurrent with TIN have been accumulated [10–26]. These glomerular lesions are frequently accompanied by immunoglobulin or complement deposits suggesting that immune complexes might be involved in the pathogenesis of some cases with IgG4-RKD [2, 3]. However, the significance of these glomerular lesions as a reflection of humoral immunity involvement is still uncertain, and whether these glomerular lesions represent some IgG4-related kidney lesions with common etiopathological background or unrelated lesions merely concurrent with IgG4-TIN is still controversial.

In this study, we analyzed 20 Japanese patients with IgG4-RKD that were collected in our previous study aimed at establishing diagnostic criteria for IgG4-RKD [27], to address these pathological issues about the number of IgG4+ plasma cells along with IgG4+/IgG+ plasma cell ratio and involvement of humoral immunity in Japanese IgG4-RKD patients.

2. Methods

2.1. Patients. Between 2004 and 2011, we found 41 patients with IgG4-RKD in Kanazawa University Hospital, Nagaoka Red Cross Hospital, Niigata University Hospital, Sapporo Medical University Hospital, and Fukuoka University Hospital, of whom 28 underwent renal biopsy. In the remaining 13 patients with IgG4-RKD without renal biopsy, 4 had only pelvic lesion and 9 had typical radiologic findings such as multiple low-density lesions on enhanced CT, high serum IgG4 levels, and other organ involvement with biopsy proven IgG4+ plasma cell infiltration. In addition, these 9 patients had radiographic improvement after successful corticosteroid treatment. Of these 28 patients, 20 who received renal needle biopsy were included in this study because they had sufficient data to determine the number of IgG4-positive cells, IgG4/IgG or IgG4/CD138 ratio, and immunofluorescence microscopy or electron microscopy. Five patients with glomerular lesions (2 Henoch-Schönlein purpura [28, 29]; 2 membranous glomerulonephritis [4, 30]; 1 membranoproliferative glomerulonephritis [23]) were reported as case reports previously. Ten patients with crescentic glomerulonephritis or antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis (1 Churg-Strauss syndrome; 1 Wegener's granulomatosis; 4 microscopic polyangiitis; 4 renal limited ANCA vasculitis) were also included in the study of infiltrating IgG4+ plasma cells as a control because IgG4+ plasma cell infiltration in some patients with ANCA associated vasculitis has been shown in previous studies [2, 31, 32]. Written informed consent for use of all data and samples was obtained from each patient. The diagnosis of IgG4-RKD was made based on the histopathologic findings

of one or more organs, characteristic diagnostic imaging findings, elevated serum IgG4 levels, and other organ involvement typical for IgG4-RD. This study was approved by each institutional ethics board and the ethics board of the Japanese Society of Nephrology. The research was conducted in compliance with the Declaration of Helsinki.

2.2. Clinical Features. The clinical picture including allergic symptoms and those resulting from other organ involvement of IgG4-RD was noted. Serum IgG, IgG4, IgE, complement, and creatinine levels were obtained from the clinical data file. Urinary abnormalities including proteinuria, hematuria, and casturia were collected.

2.3. Imaging. Computed tomography (CT) with or without enhancement with contrast medium was performed before corticosteroid therapy to make the diagnosis of kidney involvement. Other modalities including gallium scintigraphy, magnetic resonance imaging, and fluorodeoxyglucose positron emission tomography were also employed to identify renal and extra-renal lesions.

2.4. Histology and Immunostaining. Bouin's fluid-fixed or formalin-fixed and paraffin-embedded renal specimens of patients with IgG4-RKD were analyzed, and tubulointerstitial nephritis with or without glomerular lesions was evaluated. These specimens were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), periodic acid methenamine silver (PAM), and Masson's trichrome for light microscopy (LM). Immunofluorescence microscopy was performed against IgG, IgA, IgM, C3, C1q, and fibrinogen. Immunostaining for infiltrating plasma cells was performed using mouse monoclonal antibody against human IgG4 (Zymed Laboratory, San Francisco, CA, USA, or The Binding Site, Birmingham, UK), antihuman IgG (Dako, Glostrup, Denmark), and/or antihuman CD138 (AbD serotec, Oxford, UK). IgG4+ plasma cells were counted in five different high power fields (hpf) ($\times$400 magnification with an eyepiece with a field number of 22) with intensive infiltration, and the average IgG4+ plasma cell count was calculated. Average of IgG4+/IgG+ or IgG4+/CD138+ plasma cell ratio of at least two different hpf (2–5 hpf) was calculated.

2.5. Statistical Analysis. Mann-Whitney U test or Fisher's exact probability test was employed for the statistical analyses. A value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical and Laboratory Features. The patients were 18 men and 2 women with an average age 64 years (range: 55 to 83). Table 1 shows clinical and laboratory features of the patients with IgG4-related TIN. Six patients had elevated serum creatinine levels (>2 mg/dL). The mean serum IgG level was 3479 mg/dL (range 1679–5380 mg/dL), and the mean serum IgG4 level was 923 mg/dL (range 408–1860 mg/dL) with all patients having elevated serum IgG4 levels. Hypocomplementemia was detected in 13 patients. Serum IgE level was evaluated in 11 of 12 patients tested.

TABLE 1: Clinical and laboratory features of IgG4-related tubulointerstitial nephritis.

Pt. no.	Age/gender	U-Prot	Cr	IgG	IgG4	IgE	CH50	C3	C4	Other organ involvement
1	76/F	—	0.59	2,990	769	267	60	110	27	Sa, Lu
2	70/M	0.26 g/day	0.90	3,496	623	NA	<12	52	2	Pa
3	59/M	—	1.10	2,319	734	542	>66.0	106	24	Sa, Pa, Pr, RP
4	63/M	0.2 g/gCr	1.20	1,756	408	513	51	98	16	Sa, Pa, Lu, Ao
5	58/M	0.2 g/gCr	1.20	3,170	1,204	3,960	<10	33	7	Sa, LN, Lu
6	58/M	—	1.30	1,960	1,280	456	34	81	16	Li, Ne
7	75/M	0.21 g/day	1.34	5,380	587	NA	<14	41	<5	Sa, LN, Lu
8	68/M	0.1 g/day	1.37	2,995	670	2,323	10	41	2	Sa
9	75/M	0.22 g/day	2.34	1,679	890	631	52	81	29	Sa
10	55/M	0.5 g/day	2.10	5,040	1,780	NA	49	74	36	Sa, Pa
11	69/M	0.25 g/day	2.36	4,001	1,340	NA	10	55	2	Pa
12	80/M	0.4 g/day	1.60	4,657	660	NA	<12	35	<1	Pa
13	68/M	—	1.90	3,830	736	NA	3	33	1	Sa, LN
14	79/M	—	0.60	4,756	409	457	8	41	3	Jo
15	69/M	1.0 g/gCr	7.26	4,661	1,120	335	5	10	7	La, Sa, LN, Pa, Lu, Pr
16	72/M	0.22 g/day	0.80	4,359	1,100	537	<12	55	3	LN
17	75/F	3.0 g/gCr	2.25	3,695	486	1,226	2	18	2	Sa, LN, Lu
18	83/M	2.3 g/day	1.48	3,144	944	32.1	16	56	6	—
19	60/M	0.5 g/gCr	1.59	1,952	886	575	56	86	21	La, Sa
20	78/M	1.4 g/day	6.17	3,731	1,860	NA	27.3	57	28	Pa

Note: Conversion factor for Cr: mg/dL to μmol/L, $\times 88.4$.

Abbreviations: Ao: aorta; CH50, serum CH50 (U/mL); Cr: serum creatinine (mg/dL); C3: serum C3 (mg/dL); C4: serum C4 (mg/dL); IgG: serum immunoglobulin G (mg/dL); IgG4: serum immunoglobulin G4 (mg/dL); IgE: serum immunoglobulin E (IU/mL); Jo: joint; La: lacrimal gland; Li: liver; LN: lymph node; Lu: lung; NA: not available; Ne: nerve; Pa: pancreas; Pr: prostate; RP: retroperitoneum; Sa: salivary gland; U-Prot: proteinuria.

All patients except one had other organ involvement, and the clinical picture in relation to systemic organ involvement contributed to making the diagnosis of IgG4-RD. Frequently, involved organs were the salivary gland, pancreas, and lung. Twelve patients had sialadenitis, and 7 autoimmune pancreatitis type 1.

3.2. Histology and Immunostaining. Table 2 shows histologic features of 20 patients with IgG4-related TIN. Dense lymphoplasmacytic infiltration with fibrosis in the interstitium was a common feature, but one patient did not have obvious fibrosis. In immunohistochemistry, the average number of IgG4 positive plasma cells was 43.8/hpf (range 10–156/hpf), and average IgG4+/IgG+ or IgG4+/CD138+ ratio was 53% (range 18–90%). All patients fulfilled the histologic part of our diagnostic criteria for IgG4-related kidney disease, namely, infiltrating IgG4-positive plasma cells >10/hpf and/or IgG4/IgG (CD138)-positive plasma cells >40% [27]. IgG and C3 granular deposits on the tubular basement membrane (TBM) were detected by immunofluorescence microscopy in 2 (13%) and 7 (47%) of 15 patients for whom pathological reports about TBM staining were available. Granular C1q deposits on TBM were detected by IF in 2 (13%) of 15 patients. Of these, C3 granular deposits in the tubular basement membranes without accompanying IgG were thought to be a nonspecific feature because of possible production of C3 by tubular epithelial cells. Electron dense deposits were detected by electron microscopy (EM) in 6 (40%) of 15 patients. Glomerular lesions concurred with

IgG4-related TIN in 9 patients, in all of whom other immune complex-mediated glomerulopathies such as lupus nephritis, Sjögren's syndrome, and cryoglobulinemia were ruled out by appropriate clinical, biochemical, serological, and other testing. The most frequently observed glomerular lesion was membranous glomerulonephritis, and three patients had this lesion (Figure 1). These patients did not have any mesangial or subendothelial dense deposits suggesting secondary membranous glomerulonephritis such as lupus nephritis. Similarly, they did not have clinical features suggesting secondary forms of membranous glomerulonephritis such as hepatitis B or C. Two patients had Henoch-Schönlein purpura nephritis (Figure 2) with typical purpuric skin lesions, the histopathology of which was composed of typical leukocytoclastic vasculitis with neutrophils and rare IgG4+ plasma cells. In addition, one patient showed IgA positive staining in the skin, while IgA immunostaining was not performed in the other patient. The remaining glomerular lesions were IgA nephropathy (Figure 3), membranoproliferative glomerulonephritis, and focal and segmental endocapillary hypercellularity.

3.3. Comparison between IgG4-Related TIN with and without Glomerular Lesions. Table 3 shows a comparison between IgG4-related TIN with or without glomerular lesions. The mean age of the glomerular lesion positive group (GL group) was higher than that of the glomerular lesion negative group (nonGL group) (73.8 ± 7.2 versus 66.0 ± 7.7 y; $P < 0.05$). Serum C3 levels of the GL group tended to be lower than

(a)

(b)

(c)

(d)
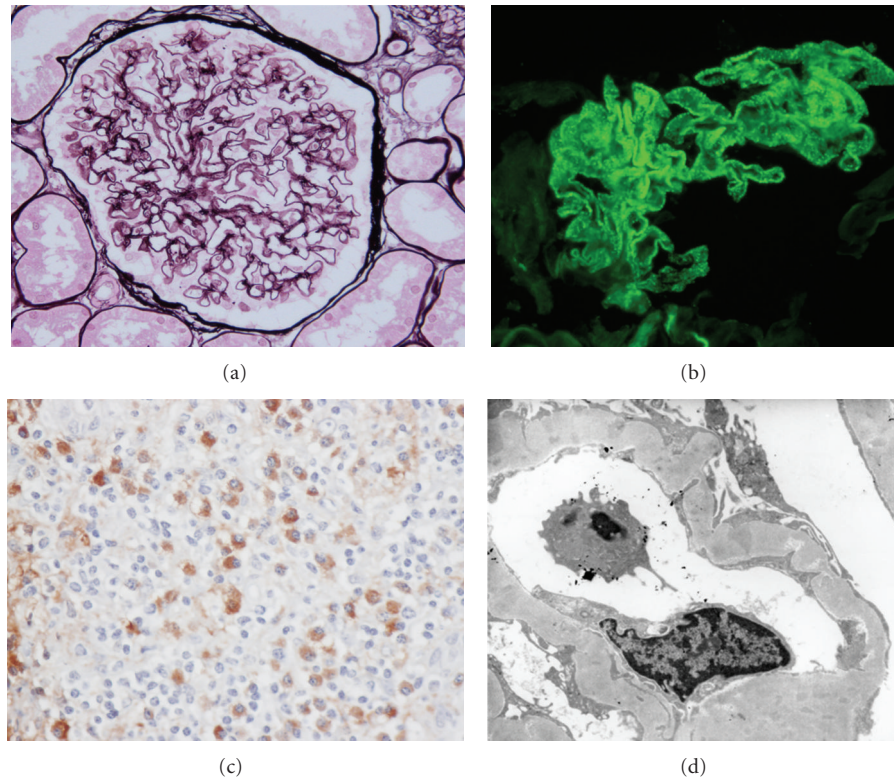
FIGURE 1: IgG4-related tubulointerstitial nephritis with membranous glomerulonephritis. (a) Periodic acid methenamine silver (PAM) staining reveals spike and bubbling formation (PAM ×400). (b) Immunofluorescence staining for IgG reveals granular deposits along the glomerular capillary walls (×400). (c) Many IgG4+ plasma cells are seen in the interstitium (IgG4 ×400). (d) Electron microscopy (EM) shows subepithelial deposits and variable reabsorption of these deposits with thickened glomerular basement membrane. (Ehrenreich-Churg stage II–IV).

those of the nonGL group (43 ± 23 versus 70 ± 27), but the difference was not statistically significant. The average number of IgG4 positive plasma cells, average IgG4+/IgG+ or IgG4+/CD138+ ratio, frequency of IgG, C3, C1q, and electron dense deposits on the TBM were not significantly different between the two groups.

3.4. IgG4-Positive Plasma-Cell-Rich ANCA-Associated Vasculitis. We analyzed 10 patients with ANCA-associated vasculitis immunohistochemically. Of these, 6 patients had more than 30/hpf plasma cell infiltration in the interstitium. Using IgG4 immunostaining, we found four patients with ANCA-associated vasculitis who fulfilled the immunohistochemical item of the diagnostic criteria of IgG4-related kidney disease (Figures 4(a) and 4(b)). Table 4 shows a summary of these four patients, all of whom had infiltrating IgG4-positive plasma cells >10/hpf and IgG4/CD138-positive plasma cells >40%. In contrast, in 2 patients only a small part of the infiltrating plasma cells were IgG4 positive (Figures 4(c) and 4(d)).

4. Discussion

In this study, we showed data about IgG4 positive plasma cell number per high power field (hpf) and IgG4+/IgG+

or IgG4+/CD138+ plasma cell ratios in the kidneys in some Japanese patients with IgG4-RKD. In addition, we compared IgG4-RKD patients with glomerular lesions with those without them clinically.

The number of IgG4+ plasma cells varies in affected organs and according to the biopsy method used (percutaneous needle biopsy or open surgical biopsy) [6–9]. As the kidney is suited for percutaneous needle biopsy and this method is most commonly chosen, obtained samples are relatively small and insufficient material is obtained in some cases. Therefore, to choose the most appropriate cutoff level in IgG4-RKD, the accumulation of studies focused on the infiltrating number of IgG4+ cells in the kidneys is needed. Our result supported the previously proposed cutoff value of >10/hpf [2]. On the other hand, 15 of 20 patients fulfilled the criterion of IgG4+/IgG+ plasma cell ratio > 40%, while the remaining 5 patients showed a ratio less than or equal to 40%. Thus, the quantitative assessment of infiltrating IgG4-positive plasma cells seems to supplement the IgG4+/IgG+ (CD138+) plasma cell ratio if this ratio is less than or equal to 40%.

Raissian et al. showed that 25 of 30 patients (83%) had TBM immune complex deposits by immunofluorescence microscopy (IF) or electron microscopy (EM) [2]. In contrast, we found that 47% of patients had C3 deposits in TBM

(a)

(b)

(c)

(d)

FIGURE 2: IgG4-related tubulointerstitial nephritis with Henoch-Schönlein purpura nephritis. (a) Periodic acid-Schiff (PAS) staining reveals severe tubulointerstitial nephritis (PAS ×100). (b) Global endocapillary proliferation is evident (PAS ×400). (c) Immunofluorescence staining for C3 reveals mesangial and capillary wall deposits (×400). (d) Many IgG4+ plasma cells are seen in the interstitium (IgG4 ×400).

TABLE 2: Histologic features of IgG4-related tubulointerstitial nephritis.

Pt. no.	Age/gender	IgG4 IHC (cells per hpf)	IgG4/IgG	Glomerular Lesion	IF TBM IgG	IF TBM C3	IF TBM C1q	IF GL IgG	IF GL IgA	IF GL C3	IF GL C1q	EM TBM	EM GL
1	76/F	50	81%	−	−	+	−	−	−	−	−	−	−
2	70/M	19	38%	−	NA	NA	NA	NA	NA	NA	NA	+	−
3	59/M	57	54%	−	−	−	−	−	−	−	−	NA	−
4	63/M	37	46%	−	−	+	+	−	−	−	−	−	−
5	58/M	21	81%	−	NA	NA	NA	−	−	−	NA	NA	−
6	58/M	156	77%	−	−	−	−	−	−	−	−	−	−
7	75/M	25	18%	−	−	−	−	NA	NA	NA	NA	+	−
8	68/M	17	40%	−	−	−	−	+	−	+	−	−	−
9	75/M	28	64%	−	+	+	−	−	−	−	−	±	−
10	55/M	49	55%	−	−	−	−	±	−	−	−	−	−
11	69/M	30	51%	−	−	−	−	+	−	−	2+	+	−
12	80/M	10	90%	MPGN	NA	NA	NA	2+	−	2+	+	NA	+
13	68/M	28	38%	IgA GN	−	+	−	−	2+	±	±	NA	NA
14	79/M	42	41%	EC	−	+	+	−	−	−	−	+	−
15	69/M	73	57%	EC	−	−	−	−	−	+	−	−	−
16	72/M	51	58%	HSPN	NA	NA	NA	2+	+	±	−	NA	+
17	75/F	62	40%	HSPN	−	−	−	−	+	2+	−	−	+
18	83/M	25	43%	MGN	+	+	−	+	−	+	−	+	+
19	60/M	68	42%	MGN	−	−	−	3+	−	−	−	−	+
20	78/M	28	45%	MGN	−	+	−	−	−	−	−	−	+

Abbreviations: EC: endocapillary hypercellularity; EM: electron microscopy; GL: glomeruli; hpf: high-power field; HSPN: Henoch-Schönlein purpura nephritis; IF: immunofluorescence; IgA GN: IgA nephropathy; IHC: immunohistochemistry; MGN: membranous glomerulonephritis; MPGN: membranoproliferative glomerulonephritis; NA: not available; Pt.: patient; TBM: tubular basement membranes.

(a)

(b)

(c)

(d)
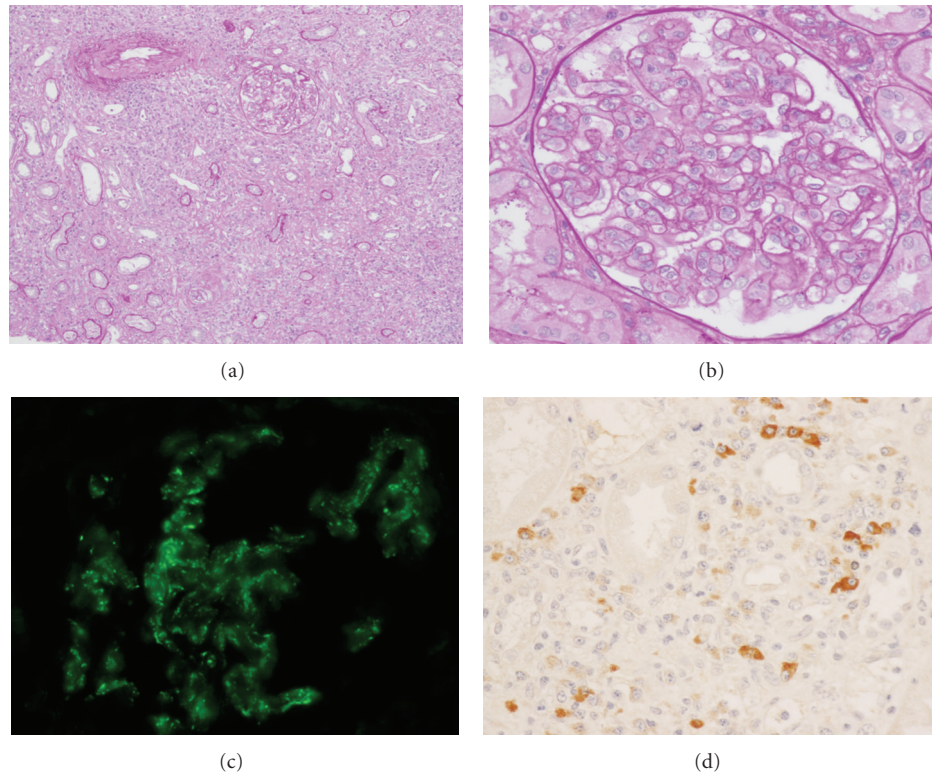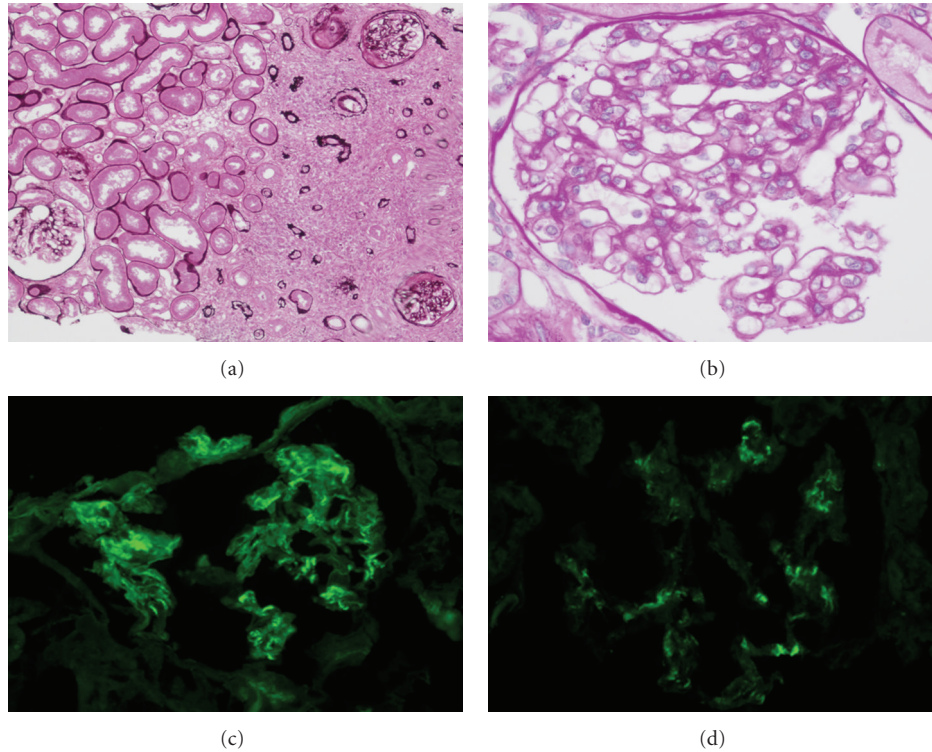
FIGURE 3: IgG4-related tubulointerstitial nephritis with IgA nephropathy. (a) Periodic acid-Schiff (PAS) staining reveals severe tubulointerstitial nephritis (PAS x100). Regional lesion distribution is evident. (b) Segmental mesangial proliferation is seen (PAS ×400). (c) Immunofluorescence staining for IgA reveals bright mesangial deposits (×400). (d) Immunofluorescence staining for C3 reveals weak mesangial staining for C3 (×400).

TABLE 3: Laboratory difference between IgG4-TIN patients with glomerular lesions and those without glomerular lesions.

	IgG4-TIN with GL	IgG4-TIN without GL	P value
Number of patients	9	11	
Age (years), mean ± SD	73.8 ± 7.2	66.0 ± 7.7	0.036
Serum creatinine (mg/dL)	2.6 ± 2.4	1.4 ± 0.6	0.239
Serum IgG (mg/dL)	3865 ± 903	3162 ± 1251	0.16
Serum IgG4 (mg/dL)	909 ± 434	935 ± 413	0.909
SerumC3 (mg/dL)	43 ± 23	70 ± 27	0.068
Low C4	7/9	5/11	0.197
Low CH50	7/9	5/11	0.197
IgG4 IHC (cells per hpf)	43.0 ± 21.8	44.5 ± 39.4	0.493
IgG4/IgG (%)	50.4 ± 16.5	54.1 ± 20.8	0.518
IF TBM IgG	1/7	1/9	>0.999
IF TBM C3	4/7	3/9	0.615
IF TBM C1q	1/7	1/9	>0.999
EM TBM	2/6	4/9	>0.999

Note: Conversion factor for creatinine: mg/dL to μmol/L, ×88.4.
Abbreviations: EM: electron microscopy; GL: glomerular lesions; hpf: high-power field; IF: immunofluorescence; IHC: immunohistochemistry; TBM: tubular basement membranes; TIN: tubulointerstitial nephritis.

TABLE 4: IgG4-positive plasma-cell-rich ANCA-related vasculitis.

Pt. no.	Age/gender	Diagnosis	PC infiltration	IgG4/hpf	IgG4/CD138 ratio (%)
1	75/F	CSS	++	19	47
2	59/M	mPA	++	22	52
3	79/F	mPA	+++	34	78
4	67/F	RLV	++	19	69

Abbreviations: CSS: Churg-Strauss syndrome; hpf: high-power field; mPA: microscopic polyangiitis; PC: plasma cell; RLV: renal limited vasculitis.

by IF and 13% of them had IgG deposits in TBM by IF. The difference in the frequency of TBM deposits might be due to a population difference, or IF sample size which might be smaller in our study. Although the frequency is different, the fact that more than 40% of patients were shown to have TBM deposits implies a close relationship between TBM deposits and IgG4-RKD. TBM deposits may thus show some immune complex involvement in IgG4-related disease.

Glomerular diseases sometimes concur with tubulointerstitial nephritis in patients with IgG4-related disease [4, 10, 12, 20, 23, 24, 28–30]. These include IgA nephropathy, Henoch-Schönlein purpura nephritis, endocapillary proliferative nephritis, crescentic glomerulonephritis, and membranous glomerulonephritis (MGN). Of these, MGN is

(a)

(b)

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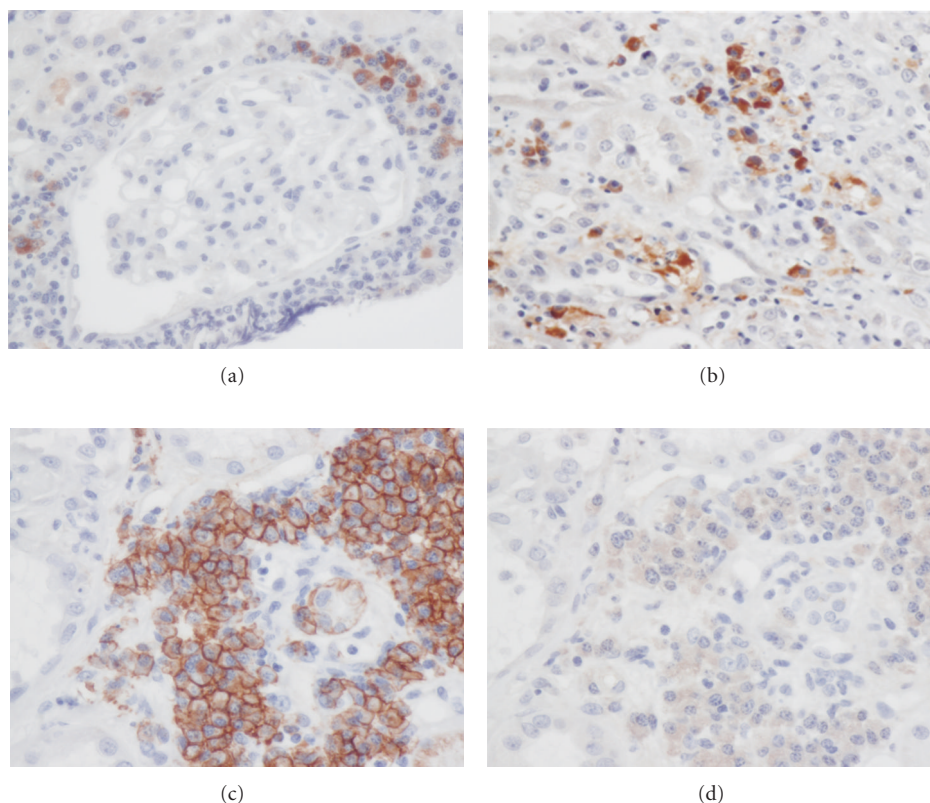
Figure 4: Anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis. (a) IgG4+ plasma cells surround a glomerulus (IgG4 immunostaining ×400). (b) Accumulation of many IgG4+ plasma cells is seen in the interstitium (IgG4 immunostaining ×400). (c) Many CD138+ cells are seen in the interstitium (CD138 immunostaining ×400). (d) These plasma cells are IgG4 negative (IgG4 immunostaining x400).

the most frequently reported glomerular pathology [10, 12, 30, 33–35].

Interestingly, the first IgG4-RKD case reported by Uchiyama-Tanaka et al. had tubulointerstitial nephritis with MGN, and subepithelial and intramembranous electron-dense deposits disappeared after successful corticosteroid therapy [10]. In contrast, Watson et al. reported a second patient with IgG4-related TIN with MGN, the steroid responsiveness of which differed markedly and whose proteinuria persisted despite 7-months treatment [12]. Although laboratory and immunohistochemical features were not significantly different between IgG4-related TIN with or without glomerular lesions in this study, further studies will be necessary including some focused on the responsiveness to treatment.

MGN detected during the clinical course of IgG4-RD is classified into two groups based on the presence or absence of simultaneous overlapping of TIN. Cravedi et al. reported a patient with IgG4-RD of the pancreas with salivary gland involvement who developed proteinuria after the cessation of successful steroid therapy [34]. The renal biopsy revealed pure MGN without IgG4+ plasma cell rich TIN. Palmisano et al. also reported a pure MGN development in a patient with IgG4-related chronic periaortitis [35]. These two cases had in common MGN development without IgG4+ plasma

cell infiltration in the clinical course of typical IgG4-RD. Although these cases seem to be pure MGN, careful judgment is needed because regional lesion distribution is a feature of IgG4-TIN, and sometimes only unaffected samples are obtained by percutaneous needle biopsy.

Although case reports of Henoch-Schönlein purpura (HSP) nephritis associated with IgG4-RD are very rare and only our two cases are so far known [28, 29], occasional development of anaphylactoid purpura in patients with IgG4-RD has been experienced (personal communication). As involvement of an allergic background is commonly presumed in both diseases, we should carefully evaluate the association of HSP with IgG4-RD when IgG4-RD patients have purpura.

In conclusion, we confirmed that infiltrating IgG4-positive plasma cells >10/hpf and/or IgG4/IgG (CD138)-positive plasma cells >40% was appropriate as an item of the diagnostic criteria for IgG4-RKD. Relatively high frequency of a variety of glomerular lesions concurrent with characteristic IgG4+ plasma-cell-rich lymphoplasmacytic infiltration with fibrosis seemed to show evidence of immune complex involvement in IgG4-related disease. However, as the number of analyzed cases in this study is small and some bias exists in case selection, worldwide study is needed to clarify the accurate frequency of the glomerular lesions in IgG4-RKD

and pathophysiological significance of immune deposits in TBM and in the glomerular lesion.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

References

[1] T. Saeki, S. Nishi, N. Imai et al., "Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis," *Kidney International*, vol. 78, no. 10, pp. 1016–1023, 2010.

[2] Y. Raissian, S. H. Nasr, C. P. Larsen et al., "Diagnosis of IgG4-related tubulointerstitial nephritis," *Journal of the American Society of Nephrology*, vol. 22, no. 7, pp. 1343–1352, 2011.

[3] Y. Yamaguchi, Y. Kanetsuna, K. Honda, N. Yamanaka, M. Kawano, and M. Nagata, "Characteristic tubulointerstitial nephritis in IgG4-related disease," *Human Pathology*, vol. 43, no. 4, pp. 536–549, 2012.

[4] Y. Saida, N. Homma, H. Hama et al., "A case of IgG4-related tubulointerstitial nephritis showing the progression of renal dysfunction after a cure for autoimmune pancreatitis," *Japanese Journal of Nephrology*, vol. 52, no. 1, pp. 73–79, 2010.

[5] Y. Zen and Y. Nakanuma, "IgG4-related disease: a cross-sectional study of 114 cases," *American Journal of Surgical Pathology*, vol. 34, no. 12, pp. 1812–1819, 2010.

[6] K. M. Lindstrom, J. B. Cousar, and M. B. S. Lopes, "IgG4-related meningeal disease: clinico-pathological features and proposal for diagnostic criteria," *Acta Neuropathologica*, vol. 120, no. 6, pp. 765–776, 2010.

[7] W. Cheuk, H. K. L. Yuen, and J. K. C. Chan, "Chronic sclerosing dacryoadenitis: part of the spectrum of IgG4-related sclerosing disease?" *American Journal of Surgical Pathology*, vol. 31, no. 4, pp. 643–645, 2007.

[8] S. T. Chari, T. C. Smyrk, M. J. Levy et al., "Diagnosis of autoimmune pancreatitis: the mayo clinic experience," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 8, pp. 1010–1016, 2006.

[9] Y. Zen, M. Onodera, D. Inoue et al., "Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4," *American Journal of Surgical Pathology*, vol. 33, no. 12, pp. 1833–1839, 2009.

[10] Y. Uchiyama-Tanaka, Y. Mori, T. Kimura et al., "Acute tubulointerstitial nephritis associated with autoimmune-related pancreatitis," *American Journal of Kidney Diseases*, vol. 43, no. 3, pp. e18–e25, 2004.

[11] S. I. Takeda, J. Haratake, T. Kasai, C. Takaeda, and E. Takazakura, "IgG4-associated idiopathic tubulointerstitial nephritis complicating autoimmune pancreatitis," *Nephrology Dialysis Transplantation*, vol. 19, no. 2, pp. 474–476, 2004.

[12] S. J. W. Watson, D. A. S. Jenkins, and C. O. S. Bellamy, "Nephropathy in IgG4-related systemic disease," *American Journal of Surgical Pathology*, vol. 30, no. 11, pp. 1472–1477, 2006.

[13] L. Rudmik, K. Trpkov, C. Nash et al., "Autoimmune pancreatitis associated with renal lesions mimicking metastatic tumours," *Canadian Medical Association Journal*, vol. 175, no. 4, pp. 367–369, 2006.

[14] H. Nakamura, H. Wada, T. Origuchi et al., "A case of IgG4-related autoimmune disease with multiple organ involvement," *Scandinavian Journal of Rheumatology*, vol. 35, no. 1, pp. 69–71, 2006.

[15] V. Deshpande, S. Chicano, D. Finkelberg et al., "Autoimmune pancreatitis: a systemic immune complex mediated disease," *American Journal of Surgical Pathology*, vol. 30, no. 2, pp. 1537–1545, 2006.

[16] K. Shimoyama, N. Ogawa, T. Sawaki et al., "A case of Mikulicz's disease complicated with interstitial nephritis successfully treated by high-dose corticosteroid," *Modern Rheumatology*, vol. 16, no. 3, pp. 176–182, 2006.

[17] M. Murashima, J. Tomaszewski, and J. D. Glickman, "Chronic tubulointerstitial nephritis presenting as multiple renal nodules and pancreatic insufficiency," *American Journal of Kidney Diseases*, vol. 49, no. 1, pp. e7–e10, 2007.

[18] L. D. Cornell, S. L. Chicano, V. Deshpande et al., "Pseudotumors due to IgG4 immune-complex tubulointerstitial nephritis associated with autoimmune pancreatocentric disease," *American Journal of Surgical Pathology*, vol. 31, no. 10, pp. 1586–1597, 2007.

[19] K. Yoneda, K. Murata, K. Katayama et al., "Tubulointerstitial nephritis associated with IgG4-related autoimmune disease," *American Journal of Kidney Diseases*, vol. 50, no. 3, pp. 455–462, 2007.

[20] K. Katano, Y. Hayatsu, T. Matsuda et al., "Endocapillary proliferative glomerulonephritis with crescent formation and concurrent tubulo-interstitial nephritis complicating retroperitoneal fibrosis with a high serum level of IgG4," *Clinical Nephrology*, vol. 68, no. 5, pp. 308–314, 2007.

[21] N. Mise, Y. Tomizawa, A. Fujii, Y. Yamaguchi, and T. Sugimoto, "A case of tubulointerstitial nephritis in IgG4-related systemic disease with markedly enlarged kidneys," *NDT Plus*, vol. 2, no. 3, pp. 233–235, 2009.

[22] A. Aoki, K. Sato, M. Itabashi et al., "A case of Mikulicz's disease complicated with severe interstitial nephritis associated with IgG4," *Clinical and Experimental Nephrology*, vol. 13, no. 4, pp. 367–372, 2009.

[23] J. Morimoto, Y. Hasegawa, H. Fukushima et al., "Membrano-proliferative glomerulonephritis-like glomerular disease and concurrent tubulointerstitial nephritis complicating IgG4-related autoimmune pancreatitis," *Internal Medicine*, vol. 48, no. 3, pp. 157–162, 2009.

[24] I. Naitoh, T. Nakazawa, H. Ohara et al., "Autoimmune pancreatitis associated with various extrapancreatic lesions during a long-term clinical course successfully treated with azathioprine and corticosteroid maintenance therapy," *Internal Medicine*, vol. 48, no. 23, pp. 2003–2007, 2009.

[25] Y. Tsubata, F. Akiyama, T. Oya et al., "IgG4-related chronic tubulointerstitial nephritis without autoimmune pancreatitis and the time course of renal function," *Internal Medicine*, vol. 49, no. 15, pp. 1593–1598, 2010.

[26] F. Kim, K. Yamada, D. Inoue et al., "IgG4-related tubulointerstitial nephritis and hepatic inflammatory pseudotumor without hypocomplementemia," *Internal Medicine*, vol. 50, no. 11, pp. 1239–1244, 2011.

[27] M. Kawano, T. Saeki, H. Nakashima et al., "Proposal for diagnostic criteria for IgG4-related kidney disease," *Clinical and Experimental Nephrology*, vol. 15, no. 5, pp. 615–626, 2011.

[28] R. Tamai, Y. Hasegawa, S. Hisano et al., "A case of IgG4-related tubulointerstitial nephritis concurrent with Henoch-Schonlein purpura nephritis," *Allergy, Asthma and Clinical Immunology*, vol. 7, article 5, 2011.

[29] K. Ito, K. Yamada, I. Mizushima et al., "Henoch-Schönlein purpura nephritis in a patient with IgG4-related disease: a possible association ," *Clinical Nephrology*. In press.

[30] T. Saeki, N. Imai, T. Ito, H. Yamazaki, and S. Nishi, "Membranous nephropathy associated with IgG4-related systemic disease and without autoimmune pancreatitis," *Clinical Nephrology*, vol. 71, no. 2, pp. 173–178, 2009.

[31] M. Yamamoto, H. Takahashi, C. Suzuki et al., "Analysis of serum IgG subclasses in churg-strauss syndrome—the meaning of elevated serum levels of IgG4," *Internal Medicine*, vol. 49, no. 14, pp. 1365–1370, 2010.

[32] D. C. Houghton and M. L. Troxell, "An abundance of IgG4+ plasma cells is not specific for IgG4-related tubulointerstitial nephritis," *Modern Pathology*, vol. 24, no. 11, pp. 1480–1487, 2011.

[33] F. C. Fervenza, G. Downer, L. H. Beck Jr., and S. Sethi, "IgG4-related tubulointerstitial nephritis with membranous nephropathy," *American Journal of Kidney Diseases*, vol. 58, no. 2, pp. 320–324, 2011.

[34] P. Cravedi, M. Abbate, E. Gagliardini et al., "Membranous nephropathy associated with IgG4-related disease," *American Journal of Kidney Diseases*, vol. 58, no. 2, pp. 272–275, 2011.

[35] A. Palmisano, D. Corradi, M. L. Carnevali et al., "Chronic periaortitis associated with membranous nephropathy: clues to common pathogenetic mechanisms," *Clinical Nephrology*, vol. 74, no. 6, pp. 485–490, 2010.

Research Article

Prevalence of IgG4-Related Disease in Japan Based on Nationwide Survey in 2009

Kazushige Uchida,[1] Atsushi Masamune,[2] Tooru Shimosegawa,[2] and Kazuichi Okazaki[1]

[1] *Division of Gastroenterology and Hepatology, The Third Department of Internal Medicine, Kansai Medical University,*
 2-3-1 Shinmachi, Hirakata, Osaka 573-1197, Japan
[2] *Department of Gastroenterology, Tohoku University Graduate School of Medicine, Seiryo-Cho, Obaku, Sendai 980-8514, Japan*

Correspondence should be addressed to Kazuichi Okazaki, okazaki@hirakata.kmu.ac.jp

The number of patients with autoimmune pancreatitis who visited hospitals in Japan in 2007 was approximately 2709 (95% confidence interval; range 2540–3040). Because IgG4-related disease is a new clinical entity, there are no data with regard to its prevalence. To estimate the number of patients with IgG4-related disease in Japan, we randomly selected hospitals using stratification and asked them how many patients they had with IgG4-related disease in 2009. The number of patients with Mikulicz's disease, IgG4-related retroperitoneal fibrosis, IgG4-related renal disease, IgG4-related pulmonary disease, and IgG4-related lymphadenopathy who visited hospitals in Japan in 2009 was approximately 4304 (95% confidence interval; range 3360–5048), 272 (95% confidence interval; range 264–306), 57 (95% confidence interval; range 47–66), 354 (95% confidence interval; range 283–424), and 203 (95% confidence interval; range 187–240), respectively. The total number of patients with IgG4-related disease without autoimmune pancreatitis in Japan was approximately 5190 (95% confidence interval; range 4141–6084). The male : female ratio was 1 : 0.77, and the average of age of disease onset was 58.8 years. The total number of patients with IgG4-related disease in Japan in 2009, including autoimmune pancreatitis, was approximately 8000.

1. Introduction

IgG4-related disease (IgG4-RD) has recently been proposed as a new disease entity, and a number of case reports and studies evaluating the clinical characteristics of IgG4-RD have appeared in the literature. In 1995, Yoshida et al. proposed autoimmune pancreatitis (AIP) [1]. Hamano et al. reported that these patients showed elevated serum IgG4 [2]. Recently, autoimmune pancreatitis has been distinguished variously as type 1 and type 2 [3]. Type 1 AIP is characterized by IgG4. On the other hand, type 2 AIP is characterized by neutrophil infiltration. Type 1 AIP is commonly complicated with other organ involvement (OOI) [4, 5]. Kamisawa et al. proposed IgG4-related sclerosing disease [6]. This concept is based on sclerosing fibrosis. Systemic IgG4-related plasmacytic syndrome (SIPS) and IgG4-positive multiorgan lymphoproliferative syndrome (IgG4-MOLPS) were proposed based on lymphoproliferation [7, 8]. The Research Program

for Intractable Disease by the Ministry of Health, Labor and Welfare (MHLW) has agreed to use the term "IgG4-related disease (IgG4-RD)" [9]. The most common OOIs are the well-known Mikulicz's disease, IgG4-related retroperitoneal fibrosis, IgG4-related renal disease, IgG4-related pulmonary disease, and IgG4-related lymphadenopathy. However, there has been no epidemiological report regarding the prevalence of IgG4-RD, even in a restricted area. We conducted a national survey for IgG4-RD, based on a national survey for AIP in 2009.

2. Methods

In 2006, the Japan Pancreas Society first proposed the diagnostic criteria for AIP [10, 11]. In 2007, using these criteria, a second nationwide survey for AIP was conducted and estimated the prevalence of AIP in Japan [12]. Briefly, following the guidelines of the Nationwide Epidemiological
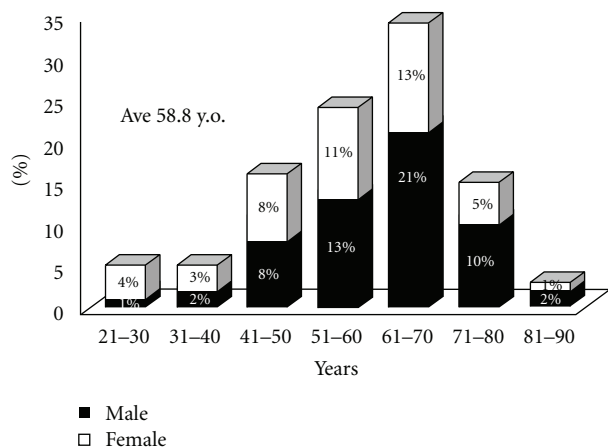
FIGURE 1: Sex and age of onset of IgG4-related disease. The male : female ratio was 1 : 0.77, and the average of age of disease onset was 58.8 years.

	Estimated patients	Lower limits	Upper limits
Dept. of Respiratory Medicine	21.8	13.6	30
Rheumatology	151.4	136.3	166.6
Internal Medicine	726.7	663.8	789.6
Otolaryngology	354.2	322.7	385.9
Ophthalmology	297.9	277.8	448.4
Total	1552.1	1283.7	1820.5

FIGURE 2: Mikulicz' disease without autoimmune pancreatitis. The number of patients with Mikulicz's disease without autoimmune pancreatitis, who visited hospitals in Japan in 2009, was approximately 4304 (95% confidence interval; range 3360–5048).

Survey Manual issued by the Research Committee on the Epidemiology of Intractable Diseases, [13] hospitals using stratification were randomly selected according to the number of beds in each; the more beds a hospital had, the greater the probability that it would be selected. Furthermore, 37 departments in hospitals in a particular stratum which were considered to have recorded AIP cases for research purposes and 317 departments in university hospitals were stratified separately. Next, the following departments were selected: Internal Medicine, Gastroenterology, Surgery, and Gastroenterological Surgery. From these selections, 2972 departments were nominated. A questionnaire was sent to the selected departments along with the diagnostic criteria for AIP, and respondents were asked to provide the number and sex of AIP patients who had visited the hospital in 2007. This survey included questions about sex, age of disease onset, and the diagnostic basis for AIP. According to this national survey of AIP, the number of AIP patients who visited a hospital in Japan in 2007 was estimated to be 2790 (95% confidence interval; range 2540–3040). AIP in the Japanese population was estimated as 0.82 per 100,000. The number of newly contracted patients in one year was 1,120 patients (95% confidence interval; range 1,000–1,240). Japanese vital statics of the Ministry of Health, Labor and Welfare for 2007 showed the total population to be 127,771,000 people (adult population, 104,197,000). 36.05% of the autoimmune pancreatitis patients were in the 250 hospitals who responded to the autoimmune pancreatitis nationwide survey in Japan. It was assumed that 2.773 times the number of presumptive patients in these 250 hospitals would be the number of estimated patients for the whole country. There were many experiences of autoimmune pancreatitis in these 250 hospitals. It seemed that patients with IgG4-related disease gathered a lot in these 250 hospitals. A questionnaire was sent to selected departments (Respiratory Medicine, Rheumatology, Internal Medicine (except Gastroenterology), Otolaryngology, Ophthalmology, and Urology) in these 250 hospitals, and respondents were asked to provide the number of IgG4-RD patients who had visited the hospital in 2009. This survey included questions about sex, age of disease onset, and the diagnostic basis for IgG4-RD.

3. Results

In these patients, the male : female ratio was 1 : 0.77. Figure 1 shows the distribution of the age of these patients at disease onset. The average of age of disease onset was 58.8 years. The peak was in the age range 61–70 years, and the disease-onset age in approximately one-third of the patients (33%) was 61–70 years. Interestingly, the number of patients with a disease-onset age of less than 40 years was dramatically lower, as most of the patients (90%) started to show IgG4-RD after the age of 40.

A total of 301 (26.8%) of 1250 departments responded to the questionnaire (Table 1). Based on these results, the number of patients with Mikulicz's disease without autoimmune pancreatitis who visited hospitals in Japan in 2009, was approximately 4304 (95% confidence interval; range 3360–5048) (Figure 2). The number of patients with IgG4-related retroperitoneal fibrosis without autoimmune pancreatitis who visited hospitals was approximately 272 (95% confidence interval; range 246–303) (Figure 3). The number of patients with IgG4-related renal disease without autoimmune pancreatitis, who visited hospitals was approximately 57 (95% confidence interval; range 47–66) (Figure 4). The number of patients with IgG4-related pulmonary disease without autoimmune pancreatitis who visited hospitals was approximately 354 (95% confidence interval; range 283–424) (Figure 5). The number of patients with IgG4-related lymphadenopathy without autoimmune pancreatitis who visited hospitals was approximately 203 (95% confidence interval;

TABLE 1: Stratification and selection of hospitals and survey results.

Stratification	Hospitals nominated	Department nominated	Departments replying	Reply rate (%)
University hospital	49	245	58	23.7
Particular hospital[a]	55	275	96	34.9
≥500 beds	72	360	99	27.5
400–499 beds	33	165	38	23.0
300–399 beds	27	135	33	23.0
200–299 beds	12	60	10	16.7
100–199 beds	1	5	0	0
≤99 beds	1	5	0	0
Total	250	1250	301	26.6

[a] Hospitals considered to have collected AIP cases for research purposes.
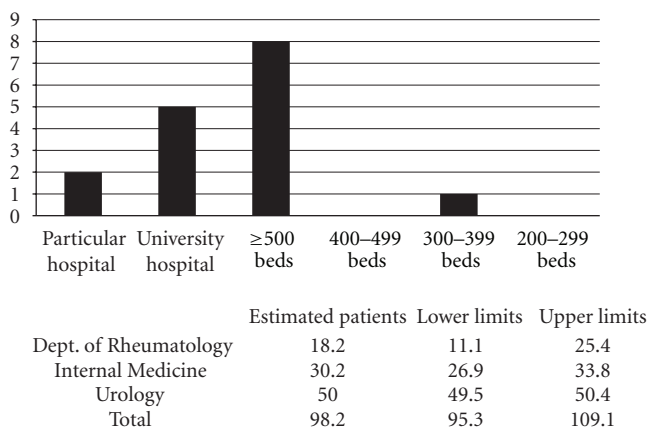
	Estimated patients	Lower limits	Upper limits
Dept. of Rheumatology	18.2	11.1	25.4
Internal Medicine	30.2	26.9	33.8
Urology	50	49.5	50.4
Total	98.2	95.3	109.1

FIGURE 3: IgG4-related retroperitoneal fibrosis without autoimmune pancreatitis. The number of patients with IgG4-related retroperitoneal fibrosis without autoimmune pancreatitis, who visited hospitals in Japan in 2009 was approximately 272 (95% confidence interval; range 246–303).

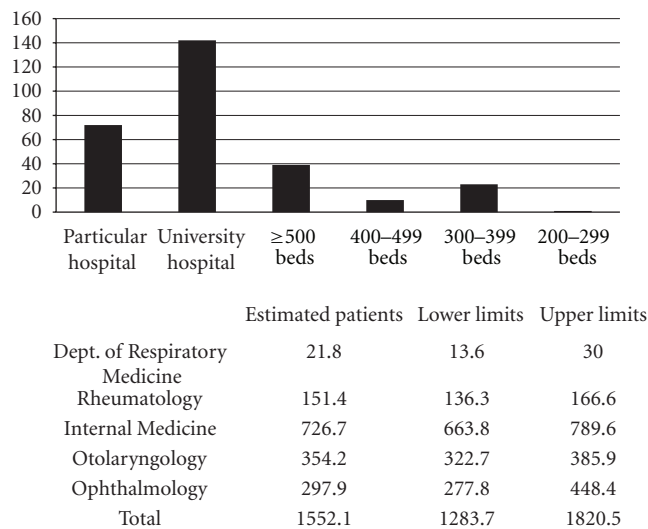|	Estimated patients	Lower limits	Upper limits
Dept. of Rheumatology	20.4	17.1	23.8

FIGURE 4: IgG4-related renal disease without autoimmune pancreatitis. The number of patients with IgG4-related renal disease without autoimmune pancreatitis, who visited hospitals in Japan in 2009, was approximately 57 (95% confidence interval; range 47–66).

range 187–240) (Figure 6). The total number of patients with IgG4-related disease without autoimmune pancreatitis in Japan was estimated to be approximately 5190 (95% confidence interval; range 4141–6084).

4. Discussion

This is one of several nationwide surveys conducted to elucidate the number of AIP patients in Japan and also the first such survey to be conducted worldwide. It is difficult to ascertain the number of patients with IgG4-RD, the awareness of this disease is low, and its symptoms are varied. Another national survey in Japan was reported by Umehara et al. [9]. They have estimated the number of individuals with IgG4-RD throughout Japan by using the number of patients in Ishikawa Prefecture as an example. Populations in Ishikawa Prefecture contains 1.16 million people, with little population inflow/outflow. In Ishikawa Prefecture, there are two University Hospitals, Kanazawa Medical University Hospital and Kanazawa University Hospital. Assuming that new patients with IgG4-RD would visit one of the two university
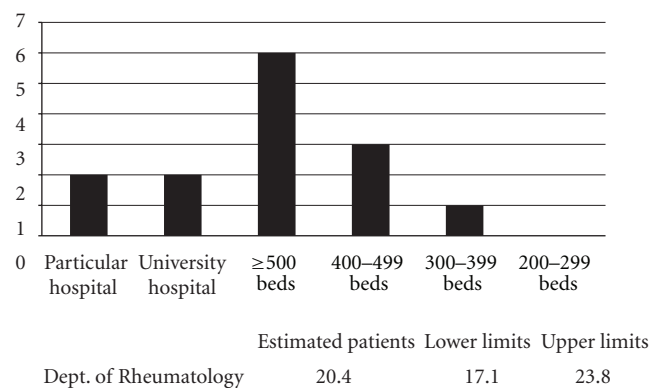
hospitals, it was estimated that the incidence of this disease throughout Japan is 0.28–1.08/100,000 population with 336–1,300 patients newly diagnosed per year from 2003 to 2009. Since the median age of onset of IgG4-RD is 58 years and the clinical symptoms are relatively mild, with slow progression and good response to steroid therapy, life expectancy after diagnosis has been estimated at 20 years. Thus, it has been estimated that there are approximately 6,700 to 26,000 patients in Japan who have developed IgG4-RD over the past 20 years. From our national survey, the total number of patients with IgG4-RD without autoimmune pancreatitis in Japan was approximately 5190 (95% confidence interval; range 4141–6084). The number of AIP patients who visited a hospital in Japan was estimated to be 2790 patients. Therefore, the total number of patients with IgG4-related disease including autoimmune pancreatitis in Japan in 2009 was approximately 8000. Our estimate is somewhat lower than another national survey from Umehara et al. There are several possibilities of reasons for this matter. In this survey, we estimated the number of IgG4-RD patients based on the hospitals' treatment of AIP. On the other hand, Umehara et al.'s result was estimated from the two university hospitals

	Estimated patients	Lower limits	Upper limits
Dept. of Respiratory Medicine	127.6	102.2	152.9

Figure 5: IgG4-related pulmonary disease without autoimmune pancreatitis. The number of patients with IgG4-related pulmonary disease without autoimmune pancreatitis, who visited hospitals in Japan in 2009, was approximately 354 (95% confidence interval; range 283–424).

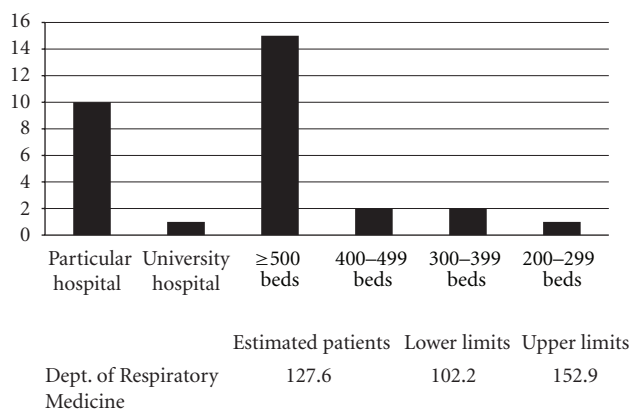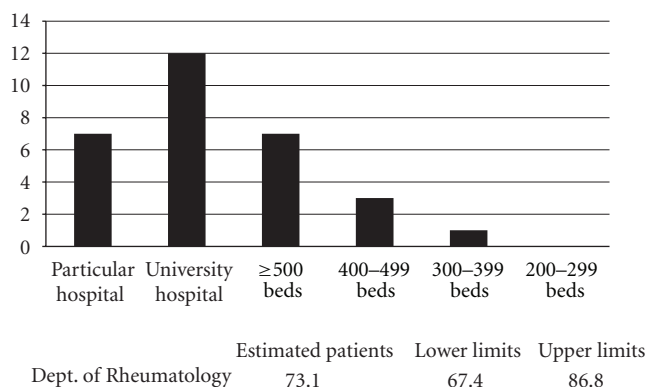|	Estimated patients	Lower limits	Upper limits
Dept. of Rheumatology	73.1	67.4	86.8

Figure 6: IgG4-related lymphadenopathy without autoimmune pancreatitis. The number of patients with IgG4-related lymphadenopathy without autoimmune pancreatitis, who visited hospitals in Japan in 2009, was approximately 203 (95% confidence interval; range 187–240).

(Department of Rheumatology) in Ishikawa Prefecture. From these two national surveys, it is suggested that total number of patients with IgG4-related disease including autoimmune pancreatitis in Japan was approximately about 10,000 and the average of age of disease onset was 58 years.

It has been reported the ratio of male patients in autoimmune pancreatitis [5]. In this survey, the male : female ratio was 1 : 0.77. Most patients have Mikulicz's disease without autoimmune pancreatitis. In Mikulicz's disease, the male : female ratio was 1.30 : 1. This is the reason that the deference is few at male and female ratio compared with autoimmune pancreatitis. Answer rate of this survey is not so high. One reason is guessed that IgG4-RD is not familiarized, therefore the patients with IgG4-RD are concentrated on the hospital that answered this survey. In 2011, the comprehensive diagnostic criteria for IgG4-RD are established by all Japan G4 team [14]. It will be necessary to familiarize general physicians with this new disease concept.

References

[1] K. Yoshida, F. Toki, T. Takeuchi, S. I. Watanabe, K. Shiratori, and N. Hayashi, "Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis," *Digestive Diseases and Sciences*, vol. 40, no. 7, pp. 1561–1568, 1995.

[2] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[3] T. Shimosegawa, S. T. Chari, L. Frulloni et al., "International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the international association of pancreatology," *Pancreas*, vol. 40, no. 3, pp. 352–358, 2011.

[4] K. Uchida, K. Okazaki, Y. Konishi et al., "Clinical analysis of autoimmune-related pancreatitis," *American Journal of Gastroenterology*, vol. 95, no. 10, pp. 2788–2794, 2000.

[5] K. Okazaki, K. Uchida, M. Koyabu, H. Miyoshi, and M. Takaoka, "Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease," *Journal of Gastroenterology*, vol. 46, no. 3, pp. 277–288, 2011.

[6] T. Kamisawa, H. Nakajima, N. Egawa, N. Funata, K. Tsuruta, and A. Okamoto, "IgG4-related sclerosing disease incorporating sclerosing pancreatitis, cholangitis, sialadenitis and retroperitoneal fibrosis with lymphadenopathy," *Pancreatology*, vol. 6, no. 1-2, pp. 132–137, 2006.

[7] M. Yamamoto, H. Takahashi, M. Ohara et al., "A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease," *Modern Rheumatology*, vol. 16, no. 6, pp. 335–340, 2006.

[8] Y. Masaki, L. Dong, N. Kurose et al., "Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders," *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1310–1315, 2009.

[9] H. Umehara, K. Okazaki, Y. Masaki et al., "A novel clinical entity, IgG4-related disease (IgG4RD)—general concept and details," *Modern Rheumatology*, vol. 22, no. 1, pp. 1–14, 2012.

[10] Members of the Criteria Committee for Autoimmune Pancreatitis of the Japan Pancreas Society, "Diagnostic criteria for autoimmune pancreatitis by the Japan Pancreas Society," *Journal of The Japan Pancreas Society*, vol. 17, no. 6, pp. 585–587, 2002.

[11] K. Okazaki, S. Kawa, T. Kamisawa et al., "Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 626–631, 2006.

[12] K. Satoh, T. Shimosegawa, A. Masamune et al., "Nationwide epidemiological survey of acute pancreatitis in Japan," *Pancreas*, vol. 40, no. 4, pp. 503–507, 2011.

[13] Y. Ohno, *The Nationwide Epidemiological Survey Manual For Investigating the Number of Patients and Clinico-Epidemiological Features of Intractable Diseases*, Japanese Ministry of Health and Welfare, Tokyo, Japan, 1998.

[14] H. Umehara, K. Okazaki, Y. Masaki et al., "Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD)," *Modern Rheumatology*, vol. 22, no. 1, pp. 21–30, 2012.

Review Article

Autoantibodies in Autoimmune Pancreatitis

Daniel S. Smyk,[1] Eirini I. Rigopoulou,[2] Andreas L. Koutsoumpas,[1] Stephen Kriese,[1] Andrew K. Burroughs,[3] and Dimitrios P. Bogdanos[1, 2, 4]

[1] *Institute of Liver Studies, King's College London School of Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK*
[2] *Department of Medicine, University of Thessaly Medical School, Viopolis, 41110 Larissa, Greece*
[3] *The Sheila Sherlock Liver Centre and University Department of Surgery, Royal Free Hospital, London NW3 2QG, UK*
[4] *Research Group of Cell Immunotherapy and Molecular Immunodiagnostics, Institute of Biomedical Research & Technology, Centre for Research and Technology-Thessaly (CE.RE.TE.TH), 41222 Larissa, Greece*

Correspondence should be addressed to Dimitrios P. Bogdanos, dimitrios.bogdanos@kcl.ac.uk

Autoimmune pancreatitis (AIP) was first used to describe cases of pancreatitis with narrowing of the pancreatic duct, enlargement of the pancreas, hyper-γ-globulinaemia, and antinuclear antibody (ANA) positivity serologically. The main differential diagnosis, is pancreatic cancer, which can be ruled out through radiological, serological, and histological investigations. The targets of ANA in patients with autoimmune pancreatitis do not appear to be similar to those found in other rheumatological diseases, as dsDNA, SS-A, and SS-B are not frequently recognized by AIP-related ANA. Other disease-specific autoantibodies, such as, antimitochondrial, antineutrophil cytoplasmic antibodies or diabetes-specific autoantibodies are virtually absent. Further studies have focused on the identification of pancreas-specific autoantigens and reported significant reactivity to lactoferrin, carbonic anhydrase, pancreas secretory trypsin inhibitor, amylase-alpha, heat-shock protein, and plasminogen-binding protein. This paper discusses the findings of these investigations and their relevance to the diagnosis, management, and pathogenesis of autoimmune pancreatitis.

1. Introduction

Autoimmune pancreatitis (AIP) is a form of chronic pancreatitis with raised levels of serum IgG4, responsiveness to immunosuppressive therapy, and no apparent underlying cause such as chronic alcoholic pancreatitis [1–8]. Although first described in 1961 as a case of pancreatitis with autoimmune features [9], the term AIP was first used to describe a case involving diffuse enlargement of the pancreas, irregular narrowing of the pancreatic duct and serological markers of hyper-γ-globulinaemia, as well as antinuclear antibody (ANA) positivity by indirect immunofluorescence (IIF) [10]. AIP is subclassified in two types: IgG4-related (type 1) and non-IgG4-related (type 2). Type 1 is more prevalent in Asia, whereas type 2 appears to have a higher prevalence in Europe, followed by the USA then Asia [7]. In this paper, AIP will refer to type 1. Patients with AIP are normally responsive to immunosuppressive therapy [1–7, 9–11].

AIP predominantly affects males of middle age [12–14], with the most common presenting symptom being obstructive cholestasis [15, 16]. Laboratory investigations usually reveal hyperbilirubinaemia, raised ALP and transaminases, and occasionally raised carbohydrate antigen 19-9 (CA19-9) [7]. Approximately half of cases have elevated levels of pancreatic enzymes [17]. Elevated IgG4 (>135 mg/dL) is the hallmark of AIP, being elevated in more than 90% of patients [18]. The elevation of IgG4 has been confirmed in several studies [19–21]. The major differential diagnosis is pancreatic cancer, which is usually ruled out through radiological, serological, or histological investigation [22, 23]. Diffuse pancreatic enlargement with a capsule-like rim and narrowed pancreatic duct is suggestive of AIP over cancer, as are delayed enhancement and downstream dilation of the pancreatic duct [24]. Diffuse, solitary or multiple areas of signal hypersensitivity on diffusion-weighted MRI are characteristics of AIP, as opposed to solitary signals in pancreatic cancer [24]. Also, IgG4 levels greater than

280 mg/dl are typically found in AIP as opposed to pancreatic cancer [17], as is IgG4+ immunostaining of the duodenal papilla [25, 26].

Although a variety of diagnostic criteria have been proposed [27–29], a firm diagnosis of AIP is based on radiological, serological, and histological findings. Typical radiological features include diffuse pancreatic enlargement with a narrowed pancreatic duct [7, 29]. These features, in the presence of a raised IgG4, are diagnostic of AIP [7, 29]. Features of autoimmune disease may also be present and include the presence of a variety of autoantibodies and/or raised IgG [7, 29]. Immunostaining for IgG4 with ≥10 IgG4+ plasma cells per high-power field supports a diagnosis of AIP [30]. A purely histological diagnosis of AIP requires the presence of four features; (1) prominent lymphoplasmacytic infiltration with fibrosis but no neutrophil infiltration, (2) prominent IgG4+ plasma cell infiltration, (3) storiform/swirling fibrosis, and (4) obliterative phlebitis [7]. Histopathological diagnosis is particularly important for retrospective reviews of cases without sufficient serological and/or radiological data. It is currently unknown whether findings of a needle biopsy are sufficient for the diagnosis.

In addition to its role in the diagnosis of AIP, IgG4 also appears to relate to the disease severity and clinical course. Matsubayashi et al. [4] evaluated the clinical course of AIP in relation to IgG4 levels, in a cohort of 27 AIP patients. Elevated IgG4 was present in 74% of the patients, compared to normal IgG4 in 26% [4]. Jaundice was more prevalent in patients with raised IgG4 (80% versus 14.3%), as was weight loss (40% versus 14.3%) [4]. However, abdominal/back pain was reported more in the group with low IgG4 (71.4% versus 50%), as was fatigue (42.9% versus 40%) [4]. Although steroid therapy was successful in both groups, 85.7% of those with raised IgG4 required maintenance therapy compared to 33.3% of those with normal IgG4 [4]. Despite clinical improvement after immunosuppression being observed in other studies [5, 31–34], no consensus has been reached in regards to treatment requirements/alterations in those with higher IgG4 levels compared with those with lower levels.

Although pancreatic involvement is the major feature of AIP, lesions may also be present in various other organs [3, 35, 36], including (but not limited to) the lachrymal and salivary glands [3], lungs [37], retroperitoneum [19], and prostate [1, 38]. Sclerosing cholangitis is the most common extrapancreatic lesion and is practically associated with the IgG4 form of the disease (IgG4 sclerosing cholangitis) [24]. These lesions are histologically similar to those found in the pancreas [24], clearly indicating that AIP is a pancreatic manifestation of a systemic IgG4 disease [24, 39, 40].

A number of autoantibodies have been detected in AIP patients. The presence of autoantibodies supports the prevailing notion that AIP is an immune-mediated and/or autoimmune disease. This paper describes the current data surrounding the presence and role of autoantibodies in AIP.

2. Pathogenesis of AIP: The Role of Autoantibodies

Several genetic susceptibility factors for AIP have been identified [41–44], and the disease is now believed to be autoimmune. The autoimmune hypothesis surrounding IgG4-related disease has initiated a series of studies investigating the specificity of autoantibody responses in patients with AIP leading to the identification of several autoantigens/autoantibodies. Several autoantibodies have been found in AIP patients (see Table 1 for a list of the major autoantibodies found), some more prevalent than others. These autoantibodies can be subdivided into two broad categories consisting of nonorgan and organ-specific autoantibodies. Organ-specific autoantibodies have attracted special attention because of their potential pathogenetic relevance to the initiation of the disease, but most of them are not highly prevalent in AIP or are seen in low titers. The broad variety of antibodies include antilactoferrin (anti-LF) [45, 46], anticarbonic anhydrase II (anti-CA-IIAb) [47, 48], anticarbonic anhydrase IV (anti-CA-IVAb) [49], antipancreas secretory trypsin inhibitor (anti-PSTI) [50], antitrypsinogens [51], antiamylase alpha [52], anti-heat shock protein 10 (anti-HSP10) [53], and antiplasminogen-binding protein peptide (anti-PBP) [54]. None of these autoantibodies have been considered disease specific, and this may be one of the distinctive features of AIP compared to other gastrointestinal and liver autoimmune diseases characterized by disease specific autoantibodies, including antimitochondrial antibodies (AMA) in patients with primary biliary cirrhosis (PBC) or anti-liver kidney microsomal-1 (anti-LKM1) antibodies in type 2 autoimmune hepatitis. Up to 40% of patients has detectable rheumatoid factor (RF) and ANA [5, 6]. Yamamoto et al. have reported ANA and RF positivity in 44% and 16%, respectively in their cohort of AIP patients [55]. Autoantibodies, such as, anti SS-A/Ro and SS-B/La or disease specific antimitochondrial antibodies are not commonly found [7]. Okazaki et al. [7] have attempted to provide theoretical support to the thesis that these self antigens may play a critical role in the pathogenesis of AIP. According to their scenario, an initial response to self-antigen (such as LF, CA-II, CA-IV, PSTI, amylase alpha, and PBP) is induced by decreased naïve T regulatory cells (or Tregs) and leads to the induction of a proinflammatory Th1 response and the release of IFN-γ, IL-1β, IL-2, and TNF-α [7]. Secondary to the initial Th1-predominant reaction, a Th2 response would lead the progression of the disease, which would include the production of IgG and IgG4, together with autoantibodies [7]. IL-10 and TGF-β may regulate IgG4 and fibrosis, and the classical complement pathway may be activated by the IgG1 immune complex [7]. A puzzling feature of the fine specificity of the autoantibodies found in patients in AIP is that they do not necessarily belong to the IgG4 subclass as at least those against PSTI mainly belong to the IgG1 subclass [50]. It is also puzzling as to why only the pancreas in involved in many cases, since many of the indicated autoantibodies are ubiquitous. As well, it would be expected that the prevalence of AIP would be much higher (especially as a concomitant condition with other

TABLE 1: Main disease-related autoantibody specificities in autoimmune pancreatitis. Several autoantibodies have been detected in the sera of patients with autoimmune pancreatitis (AIP). Although none have been established to be disease specific, it appears that a loss of tolerance to several pancreatic antigens may be involved in the initiation of AIP. The prevalence of antibodies against carbonic anhydrase, lactoferrin, heat-shock protein 10, amylase alpha, plasminogen-binding protein, and pancreatic secretory trypsin inhibitor antigens, key references of studies investigating autoantibodies against these antigens as well as the origin of the cohorts are provided. Information regarding conventional autoantibodies, such as, antinuclear and smooth muscle autoantibodies is provided within the text.

Autoantibody/antigen	Number of AIP sera tested	Patient origin	Frequency in confirmed AIP (%)	Frequency in controls	Reference
Anticarbonic Anhydrase-II	17	Japanese	59	—	[45]
	54	Japanese	28	1.9 healthy controls, 10.5 chronic alcoholic pancreatitis, 64 Sjogren's	[47]
	33	Japanese	33	62 Sjögren's	[62]
	48	European	12.5	0 healthy controls	[63]
Antilactoferrin	17	Japanese	76	—	[45]
	48	European	20.8	0	[63]
Anticarbonic Anhydrase-IV	—	Japanese	27 (protein), 30 (peptide)	0 healthy controls, 45 (protein) Sjögren's, 20 (peptide) Sjögren's	[49]
Heat-shock protein 10	—	Japanese	92	81 type 1 diabetes mellitus, 8 chronic alcoholic pancreatitis, 1.4 healthy controls	[53]
Amylase-2α	15	Japanese	100	88 type 1 diabetes mellitus, 6 type 2 diabetes mellitus, 0 chronic alcoholic pancreatitis and pancreatic cancer	[52]
Plasminogen-binding protein antibodies	20	European	95 (93 in second series)	10 pancreatic cancer (1 in second series), 0 chronic alcoholic pancreatitis and intraductal papillary mucinous neoplasm	[54]
Antitrypsinogens	19	German	79 on ELISA	10 non-AIP chronic cholangitis and healthy controls	[51]
Antipancreatic secretory trypsin inhibitor	26	Japanese	42.3 on western blot, 30.8 on ELISA	0	[50]

autoimmune diseases) if the disease is characterized by a loss of tolerance to a variety of autoantigens.

Zen et al. found that Th1 cells are predominant in the periphery of AIP patients, while Th2 cells were predominant in the affected organ [56]. That study also found that there was an overproduction of Th2 and increased CD4+CD25+FoxP3 Tregs in the organs of AIP patients [56]. In view of the fact that Tregs are involved in the production of IL-10, the hypothesis that type 1 AIP is characterized by an IL-10 mediated IgG4 class switching has been formulated [56]. As well, increased immune complexes are present in AIP, which is linked to increased IgG1 and low C3/C4, with a normal mannose-binding lectin [57]. These findings are in support of the hypothesis that the classical pathway of complement activation is involved in the pathogenesis of AIP [57].

Kawa et al. have tested their cohort of 44 AIP patients for the presence of autoantibodies and RF [58]. Thirteen out of 44 patients were RF positive. ANA at a titre of more than $1:40$ were present in 54.5% (14/44) of the patients, 17 (38.6%) of them having ANA $> 1:80$ by IIF [58]. Anti-dsDNA antibodies were present in only 2/44 (4.5%) patients with AIP. SS-A and SS-B autoantibodies were totally absent [58]. Twenty one per cent of the patients had smooth muscle autoantibodies (SMAs) at a titre of more than $1:20$, while only 2 had antimitochondrial antibodies [58]. Thyroglobulin and thyroid peroxidase autoantibodies were present in 7/41 (17.1%) and 5/41 (12.2%), respectively [58]. Overall, autoantibodies of any specificity were present in 79.5% (35/44) [58]. These data suggested that autoantibody markers are frequently present in patients with AIP, the most frequent autoantibody specificity being that against nuclear antigens. However, the target antigens of the ANA and SMA reactivities remain elusive, and SMA is not found in the majority of AIP cases. dsDNA may be a frequent target of autoantibody responses in autoimmune rheumatological diseases, but this appears unlikely in the case of AIP. The presence of a variety of autoantibody reactivities and several

antigen specificities of the observed autoantibodies has led authors to speculate that the loss of tolerance seen in AIP is unlikely to be antigen driven. The investigation of the fine specificity of autoantibody reactivities in twin pairs, including individuals affected with AIP, may help us understand the origin of these responses and the immunopathogenesis of the disease. As well, twin studies may help elucidate to what degree environmental and genetic factors play a role in the disease development. Such studies have been useful in the understanding of other autoimmune conditions [59–61].

2.1. Antibodies to Carbonic Anhydrase and Lactoferrin. Anti-CA-IIAb and anti-LF antibodies are most frequently detected in AIP (54% and 73%, resp.) [45]. Aparisi et al. [47] investigated the role of CA-IIAb and IgG4 for the diagnosis of autoimmune pancreatitis. ELISA analysis for CA-IIAb followed by confirmatory western blot was performed in 227 subjects, comprised of 54 with idiopathic chronic pancreatitis (ICP), 54 age and sex-matched healthy controls, 86 with chronic alcoholic hepatitis and 33 with Sjögren's syndrome [47]. Increased serum CA-IIAb were present in 28% of ICP patients compared to 1.9% of healthy controls and 10.5% with chronic alcoholic pancreatitis [47]. Thus, the presence of CA-IIAb appears (at least in some extent) to relate to a state of pancreatic inflammation, irrespective of the stimuli responsible for the maintenance of pancreatic destruction. The finding of 64% of Sjögren's syndrome patients being seropositive for CA-IIAb clearly demonstrates that this autoantibody lacks disease specificity and cannot be used as a diagnostic marker for the confirmation of AIP in patients with a clinical suspicion of the disease [47]. When the analysis included the evaluation of IgG4, their levels were elevated in 15% ICP, 1.9% healthy controls, 8% chronic alcoholic pancreatitis, and 0% Sjögren's syndrome [47], suggesting that IgG4 is better at discriminating better AIP from Sjögren's syndrome with detectable CA-IIAb. Interestingly, all 4 ICP patients with increased CA-IIAb also had raised IgG4, cholestasis/jaundice at presentation, concomitant autoimmune disease, lymphoplasmacytic infiltration on histology, and prominent IgG4 infiltration [47]. Two of the 4 ICP patients had a positive response to corticosteroid therapy [47]. Similar results were obtained in another study which found increased CA-IIAb in 33% of ICP patients, in addition to those with Sjögren's syndrome [62]. Hardt et al. [63] found raised CA-IIAb and anti-LF in 22.9% ICP, which was also the case in 29.2% of type 1 diabetes mellitus (T1DM) patients. Other studies have also found raised CA-IIAb and/or anti-LF antibodies in AIP patients [45, 46]. At the experimental level in murine models of autoimmune sialadenitis and cholangitis, immunization with CA-II or LF induced the formation of systemic lesions (pancreatitis, sialadenitis, cholangitis, and interstitial nephritis) similar to IgG4-related disease [64, 65]. ANA and SMA have also been found in those studies, with 76% having ANA and 18% having SMA in one study [45], and 50% with ANA and 12% with SMA in another [46]. The relevance of these findings to the pathogenesis of AIP remains elusive in view of the lack of organ specificity of the observed reactivities.

Investigation as to the role of other carbonic anhydrase isoenzymes, such as, CA-IV, IX, and XII (which are all normally expressed in pancreatic ductal cells) has been conducted in a study by Nishimori et al. [49]. Increased levels of CA-IV protein and peptide were found in patients with confirmed AIP (4/15 and 6/20, resp.), probable AIP (6/14 and 3/14), and Sjögren's syndrome (9/20 and 8/40) compared to none being detected in 26 healthy controls [49]. There was no difference noted between normal controls and pathological controls consisting of patients with chronic alcoholic pancreatitis and pancreatic cancer [49]. There was a significant correlation noted between the presence of serum antibodies to CA-IV and serum gamma-globulin and IgG levels in AIP patients [49]. There were no positive results in any groups in relation to CA-IX or CA-XII [49]. Although data suggests that antibodies to CA are present in patients with AIP, it should also be noted that these antibodies have also been found to a high degree in other conditions. If reactivity to CA is characteristic of AIP, it would be expected that other conditions with reactivity to CA (such as Sjögren's) would have a higher incidence of concomitant AIP and vice versa. This would also be the case for other autoantibody specificities being present in patients with AIP, such as, CA-IIAb. Whether these autoantibodies are just indicators of immune dysregulation and can be considered the end result of polyclonal activation characterizing autoimmune disorders. The association of AIP and autoimmune rheumatological conditions, such as, Sjögren's syndrome and SLE is rare, and this may reflect the lack of a significant presence of Sjögren's syndrome and SLE-related autoantibodies, like, the SS-A/Ro and SS-B/La and anti-dsDNA antibodies, respectively.

Significant homology between human CA-II and alpha CA of *Helicobacter pylori* (*H. pylori*) has been noted [66], and reactivity against a pancreatic homologue of Helicobacter has been demonstrated [54], suggesting that *H. pylori* infection may be involved in triggering AIP and AIP-related sclerosing cholangitis *via* mechanisms, such as, molecular mimicry, in individuals with a genetic predisposition [54, 66]. Molecular mimicry involving *H. pylori* and self antigens has been proposed to account for the immunopathogenesis of other cholestatic liver diseases, such as, primary biliary cirrhosis [67–69].

2.2. Antibodies to Amylase-2α and HSP-10: A Link with Type 1 Diabetes Mellitus? Several studies have noted antibodies to amylase in AIP patients. Wiley and Pietropaolo [70] note that autoantibodies and autoreactive T cells in CD-28-deficient NOD mice (which develop AIP) recognized pancreatic amylase. Another study found that the administration of tolerogenic amylase-coupled fixed spleen cells reduced the severity of the disease [71]. Endo et al. have suggested that autoantibodies directed against amylase-2α may be a specific marker for AIP and T1DM. By ELISA, that group demonstrated that only AIP patients had reactivity to amylase-2α, compared to no reactivity in chronic alcoholic pancreatitis and pancreatic cancer [52]. Reactivity to amylase-2α was also observed in 88% T1DM, 21% acute-onset T1DM, and

6% T2DM [52]. Takizawa et al. [53] obtained 10 positive clones when the sera from AIP patients was screened through a human pancreas cDNA library. Seven of the 10 clones were amylase-2α, with 1 of the remaining 3 being identical to HSP-10 [53]. That same group developed an ELISA for detecting HSP-10 and found antibodies to HSP-10 in 92% of an AIP cohort and in 81% of patients with T1DM [53]. Antibodies to HSP-10 were present in only 8% of chronic alcoholic pancreatitis patients as controls and in 1.4% of healthy controls [53]. Larger studies are needed to establish the prevalence of antibodies against amylase-2α, as the pancreatic specificity of this antigen is of interest in the pathogenesis of AIP and T1DM.

2.3. Antibodies to Plasminogen-Binding Protein.

Frulloni et al. [54] screened a random peptide library with IgG from 20 patients with confirmed AIP. Peptide AIP1-7 was recognized by the serum of 18 out of 20 (90%) AIP patients, 4 of 40 (10%) patients with pancreatic cancer, and in none of the healthy controls. This peptide demonstrated homology with the PBP of *H. pylori* and with ubiquitin-protein ligase E3 component n-recognin 2, which is highly expressed in the pancreatic acinar cells [54]. Antibodies against PBP were present in 95% of AIP patients, as well as in 10% of patients with pancreatic cancer [54]. No antibodies were present in patients with chronic alcoholic pancreatitis or those with intraductal papillary mucinous neoplasm [54]. A second series had similar results with 93% of AIP patients and 1% of patients with pancreatic cancer having antibodies to the PBP peptide. Original antibody testing was performed using DELFIA, a time-resolved fluorescent assay. One of the limitations of the study was that the concentration used was relatively high (20μg), and the sera were tested in 1 in 50 dilution, raising the possibility that the observed reactions were due to hyperglobulinaemia characterizing AIP. However, this appeared unlikely, as the authors clearly demonstrated that sera from patients with autoimmune-rheumatic diseases were totally unreactive. Subsequent experiments were based on ELISA and western blotting and confirmed the presence of anti-PBP antibodies. These findings have led Frulloni et al. to suggest that pancreatic acinar cells may be the target of autoimmune attack in AIP, but that antibodies to PBP could not be used to differentiate AIP from pancreatic cancer [54]. Anti-PBP antibodies warrant further investigation, as cross reactivity with ubiquitin-protein ligase E3 component n-recognin 2 may account for pancreatic specificity. These findings must be interpreted with caution, as the prevalence of anti-PBP antibodies has not been investigated in great detail. In fact, larger multicenter studies are needed to confirm the significance of the diagnostic and clinical relevance of anti-PBP antibodies in AIP patients.

2.4. Anti-Pancreatic Secretory Trypsin Inhibitor.

Asada et al. [50] have considered PSTI as a potential target autoantigen in AIP. They based their hypothesis on data suggesting that endogenous trypsin inhibitor and mutations in PSTI are closely associated with the pathogenesis of hereditary pancreatitis and idiopathic chronic pancreatitis [55]. These

Investigators [50] noted that 42.3% of AIP patients had antibodies to PSTI by immunoblotting and 30.8% by ELISA, compared to none of the controls. Both assays were developed *in house* for the purpose of this study. The serum dilution used for the ELISA testing was 1 : 40, a dilution which is generally considered inadequate for proper antibody detection. Also, the mean absorbance values of the ELISA testing were relatively low (0.27 ± 0.19). However, the authors were able to demonstrate the presence of anti-PSTI antibodies by immunoblotting in 1 : 1000, indicating that AIP patients react with PSTI [50]. The same group of researchers investigated the immune responses of mice injected with polyinosinic polycytidylic acid, which accelerates the development of pancreatitis [72]. The severity of the pancreatitis in the mice was graded histologically, followed by immunohistological examination and analysis of serum autoantibodies by ELISA [72]. Histologically, there was a rich infiltration of B cells and CD138 plasmacytes in the pancreatic tissue [72]. A variety of autoantibodies were present in these mice, including anti-PSTI (91.7% of mice) [72]. This finding is intriguing based on the above-mentioned data, as it is indicative of PSTI being an autoantigen in an animal model of the disease, as well as in humans. In fact, anti-PSTI were more prevalent than anti-CA-IIAb (33.3%) and anti-LF (45.8%) [72]. No antibodies were found against glutamic acid decarboxylase, suggesting that the loss of tolerance seen in AIP is antigen driven [72]. The epitope of the anti-PSTI antibodies was determined to be the site of PSTI which is involved in the suppression of trypsin activity [72]. This is of interest as the group led by Löhr (see above) found that the serum of AIP patients contained high titers of autoantibodies against trypsinogens, including those to PSTI [51]. Further investigation is needed to identify the prevalence of anti-PSTI antibodies in AIP patients. The same Investigators tested serum samples for various other autoantibodies. Among their 26 patients with AIP, 19 (73.1%) had anti-LF antibodies and 18 (69.2%) had ANA. Anti-CA-IIAb and RF was found in 14 (53.8%) and 6 (23.1%), respectively, while SMA were present in only 4 (15.4%) of the AIP patients. Asada et al. [50] have also tested for reactivity to insulin-dependent diabetes and found that just 1 (3.8%) of the patients had antibodies against antiglutamic acid decarboxylase and anti-islet cell antibody each. None of the patients had detectable AMA. They concluded that the diagnostic sensitivity increases from 73.1 to 76.9% by a combination of anti-LF and anti-PSTI antibodies. No data have been provided for the specificity of these autoantibodies as the study did not include tests in pathological and healthy controls, and this is one of its limitations.

Further support for the organ-specific autoimmune attack in AIP has been presented by Löhr et al. [51], which examined the expression of proteins involved in the inflammatory process in a murine AIP model, as well as in the pancreatic tissue of 12 AIP patients and 8 patients with non-AIP chronic pancreatitis. That group identified 272 upregulated genes involved with immunoglobulin, chemokine and chemokine receptor production [51]. As well, 86 genes encoding pancreatic proteases

were downregulated, and trypsin-positive acinar cells were virtually absent [51]. The sera of AIP patients contained high titers of antibodies against the trypsin inhibitor PST1, and similar results were found in the murine AIP model [51]. The same study has found elevated titers of autoantibodies against trypsinogens PRSS1 and PRSS2 but not against PRSS3. ELISA testing based on recombinant antigens revealed significantly increased levels at 1/600 serum dilution, particularly against trypsinogen PRSS1 in AIP patients compared to non-AIP chronic pancreatitis patients or normal controls. These results have led the authors to suggest that a loss of tolerance to and production of antibodies against trypsinogens (and likely other pancreatic antigens) are probably involved in the pathogenesis of AIP and may therefore provide useful diagnostic targets. Their data clearly support the notion that the autoimmune attack in AIP is not only directed to the ductal cell constituents but also against the acinar cell components, such as, the trypsinogens (PRSS1 and PRSS2) and PSTI, but these data require external validation.

3. Conclusion

A variety of autoantibodies have been found in the sera of patients with AIP. Presently, none of these autoantibodies appear to be disease specific. It is possible that a loss of tolerance to a variety of pancreatic specific and nonpancreatic-specific antigens may be involved in the initiation of AIP. However, this does not explain the pancreatic specificity of the disease, as they may also be present in other conditions, such as, Sjögren's syndrome, as well as in pancreatic cancer. It would be expected that a loss of tolerance to an ubiquitously expressed antigen would result in a higher prevalence of AIP in conditions that are characterized by specific auto-antibodies, which is not the case. With that said, the extra-pancreatic lesions encountered in AIP may be indicative of loss of tolerance to an ubiquitous antigen. Organ-specific immunological targets in AIP may include PSTI, amylase-2α, or other currently unknown pancreatic antigens. Further studies are needed to clarify whether loss of tolerance to these antigens plays a role in the immunopathogenesis of AIP. Additionally, molecular mimicry with *H. pylori* antigens and possibly other microbial antigens may also be involved in the loss of tolerance, especially in regards to PBP which shares homologous regions with pancreatic ubiquitin-protein ligase E3 component n-recognin 2. Further investigation is needed to characterize autoantibodies present in AIP, in addition to their clinical and diagnostic significance.

Abbreviations

AIP: Autoimmune pancreatitis
ANA: Antinuclear antibody
anti-PBP: Antiplasminogen-binding protein peptide
anti-PSTI: Antipancreas secretory trypsin inhibitor
CA: Carbonic anhydrase
H. Pylori: *Helicobacter pylori*
HSP-10: Heat-shock protein 10
ICP: Idiopathic chronic pancreatitis
LF: lactoferrin;
T1DM: Type 1 diabetes mellitus
Th: T-helper
TGF-β: Transforming growth factorbeta
Treg: Regulatory T-cells.

References

[1] S. Kawa, K. Okazaki, T. Kamisawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: II. Extrapancreatic lesions, differential diagnosis," *Journal of Gastroenterology*, vol. 45, no. 4, pp. 355–369, 2010.

[2] M. Kawano, T. Saeki, H. Nakashima et al., "Proposal for diagnostic criteria for IgG4-related kidney disease," *Clinical and Experimental Nephrology*, vol. 15, no. 5, pp. 615–626, 2011.

[3] A. Khosroshahi and J. H. Stone, "A clinical overview of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 57–66, 2011.

[4] H. Matsubayashi, H. Sawai, H. Kimura et al., "Characteristics of autoimmune pancreatitis based on serum IgG4 level," *Digestive and Liver Disease*, vol. 43, no. 9, pp. 731–735, 2011.

[5] K. Okazaki, "Autoimmune pancreatitis: etiology, pathogenesis, clinical findings and treatment. The Japanese experience," *Journal of the Pancreas*, vol. 6, no. 1, pp. 89–96, 2005.

[6] K. Okazaki, K. Uchida, and T. Fukui, "Recent advances in autoimmune pancreatitis: concept, diagnosis, and pathogenesis," *Journal of Gastroenterology*, vol. 43, no. 6, pp. 409–418, 2008.

[7] K. Okazaki, K. Uchida, M. Koyabu, H. Miyoshi, and M. Takaoka, "Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease," *Journal of Gastroenterology*, vol. 46, no. 3, pp. 277–288, 2011.

[8] Y. Zen, D. P. Bogdanos, and S. Kawa, "Type 1 autoimmune pancreatitis," *Orphanet Journal of Rare Diseases*, vol. 6, article 82, 2011.

[9] H. Sarles, J. C. Sarles, R. Muratore, and C. Guien, "Chronic inflammatory sclerosis of the pancreas-an autonomous pancreatic disease?" *The American Journal of Digestive Diseases*, vol. 6, no. 7, pp. 688–698, 1961.

[10] K. Yoshida, F. Toki, T. Takeuchi, S. Watanabe, K. Shiratori, and N. Hayashi, "Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis," *Digestive Diseases and Sciences*, vol. 40, no. 7, pp. 1561–1568, 1995.

[11] I. Nishimori, A. Tamakoshi, S. Kawa et al., "Influence of steroid therapy on the course of diabetes mellitus in patients with autoimmune pancreatitis: findings from a nationwide survey in Japan," *Pancreas*, vol. 32, no. 3, pp. 244–248, 2006.

[12] I. Nishimori, A. Tamakoshi, and M. Otsuki, "Prevalence of autoimmune pancreatitis in Japan from a nationwide survey in 2002," *Journal of Gastroenterology*, vol. 42, supplement 18, pp. 6–8, 2007.

[13] D. H. Park, M. H. Kim, and S. T. Chari, "Recent advances in autoimmune pancreatitis," *Gut*, vol. 58, no. 12, pp. 1680–1689, 2009.

[14] J. K. Ryu, J. B. Chung, S. W. Park et al., "Review of 67 patients with autoimmune pancreatitis in Korea: a multicenter nationwide study," *Pancreas*, vol. 37, no. 4, pp. 377–385, 2008.

[15] N. Hyodo and T. Hyodo, "Ultrasonographic evaluation in patients with autoimmune-related pancreatitis," *Journal of Gastroenterology*, vol. 38, no. 12, pp. 1155–1161, 2003.

[16] T. Nishino, F. Toki, H. Oyama et al., "Biliary tract involvement in autoimmune pancreatitis," *Pancreas*, vol. 30, no. 1, pp. 76–82, 2005.

[17] A. Ghazale, S. T. Chari, T. C. Smyrk et al., "Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1646–1653, 2007.

[18] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[19] H. Hamano, S. Kawa, Y. Ochi et al., "Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis," *The Lancet*, vol. 359, no. 9315, pp. 1403–1404, 2002.

[20] K. Hirano, Y. Komatsu, N. Yamamoto et al., "Pancreatic mass lesions associated with raised concentration of IgG4," *American Journal of Gastroenterology*, vol. 99, no. 10, pp. 2038–2040, 2004.

[21] R. Pezzilli and R. Corinaldesi, "IgG4 as a serological marker of autoimmune pancreatitis: the latest news," *Journal of the Pancreas*, vol. 5, no. 6, pp. 531–533, 2004.

[22] S. C. Abraham, M. Cruz-Correa, P. Argani, E. E. Furth, R. H. Hruban, and J. K. Boitnott, "Lymphoplasmacytic chronic cholecystitis and biliary tract disease in patients with lymphoplasmacytic sclerosing pancreatitis," *American Journal of Surgical Pathology*, vol. 27, no. 4, pp. 441–451, 2003.

[23] S. M. Weber, O. Cubukcu-Dimopulo, J. A. Palesty et al., "Lymphoplasmacytic sclerosing pancreatitis: inflammatory mimic of pancreatic carcinoma," *Journal of Gastrointestinal Surgery*, vol. 7, no. 1, pp. 129–139, 2003.

[24] K. Takuma, T. Kamisawa, and Y. Igarashi, "Autoimmune pancreatitis and IgG4-related sclerosing cholangitis," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 80–87, 2011.

[25] T. Kamisawa, Y. Tu, N. Egawa, K. Tsuruta, and A. Okamoto, "A new diagnostic endoscopic tool for autoimmune pancreatitis," *Gastrointestinal Endoscopy*, vol. 68, no. 2, pp. 358–361, 2008.

[26] T. Kamisawa, Y. Tu, H. Nakajima, N. Egawa, K. Tsuruta, and A. Okamoto, "Usefulness of biopsying the major duodenal papilla to diagnose autoimmune pancreatitis: a prospective study using IgG4-immunostaining," *World Journal of Gastroenterology*, vol. 12, no. 13, pp. 2031–2033, 2006.

[27] K. P. Kim, M. H. Kim, J. C. Kim, S. S. Lee, D. W. Seo, and S. K. Lee, "Diagnostic criteria for autoimmune chronic pancreatitis revisited," *World Journal of Gastroenterology*, vol. 12, no. 16, pp. 2487–2496, 2006.

[28] M. Otsuki, J. B. Chung, K. Okazaki et al., "Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea symposium on autoimmune pancreatitis," *Journal of Gastroenterology*, vol. 43, no. 6, pp. 403–408, 2008.

[29] T. Shimosegawa, S. T. Chari, L. Frulloni et al., "International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the international association of pancreatology," *Pancreas*, vol. 40, no. 3, pp. 352–358, 2011.

[30] L. Zhang, K. Notohara, M. J. Levy, S. T. Chari, and T. C. Smyrk, "IgG4-positive plasma cell infiltration in the diagnosis of autoimmune pancreatitis," *Modern Pathology*, vol. 20, no. 1, pp. 23–28, 2007.

[31] T. Ito, I. Nishimori, N. Inoue et al., "Treatment for autoimmune pancreatitis: consensus on the treatment for patients with autoimmune pancreatitis in Japan," *Journal of Gastroenterology*, vol. 42, supplement 18, pp. 50–58, 2007.

[32] T. Kamisawa, K. Okazaki, S. Kawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: III. Treatment and prognosis of AIP," *Journal of Gastroenterology*, vol. 45, no. 5, pp. 471–477, 2010.

[33] T. Kamisawa, T. Shimosegawa, K. Okazaki et al., "Standard steroid treatment for autoimmune pancreatitis," *Gut*, vol. 58, no. 11, pp. 1504–1507, 2009.

[34] R. Pannala and S. T. Chari, "Corticosteroid treatment for autoimmune pancreatitis," *Gut*, vol. 58, no. 11, pp. 1438–1439, 2009.

[35] Y. Masaki, L. Dong, N. Kurose et al., "Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders," *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1310–1315, 2009.

[36] M. Yamamoto, H. Takahashi, M. Ohara et al., "A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease," *Modern Rheumatology*, vol. 16, no. 6, pp. 335–340, 2006.

[37] T. Taniguchi, M. Ko, S. Seko et al., "Interstitial pneumonia associated with autoimmune pancreatitis," *Gut*, vol. 53, no. 5, pp. 770–771, 2004.

[38] Y. Yoshimura, S. Takeda, Y. Ieki, E. Takazakura, H. Koizumi, and K. Takagawa, "IgG-4 associated prostatitis complicating autoimmune pancreatitis," *Internal Medicine*, vol. 45, no. 15, pp. 897–901, 2006.

[39] T. Kamisawa, N. Funata, Y. Hayashi et al., "Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis," *Gut*, vol. 52, no. 5, pp. 683–687, 2003.

[40] T. C. Smyrk, "Pathological features of IgG4-related sclerosing disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 74–79, 2011.

[41] M. C. Chang, Y. T. Chang, Y. W. Tien et al., "T-cell regulatory gene CTLA-4 polymorphism/haplotype association with autoimmune pancreatitis," *Clinical Chemistry*, vol. 53, no. 9, pp. 1700–1705, 2007.

[42] S. Kawa, M. Ota, K. Yoshizawa et al., "HLA DRB10405-DQB1 0401 haplotype is associated with autoimmune pancreatitis in the Japanese population," *Gastroenterology*, vol. 122, no. 5, pp. 1264–1269, 2002.

[43] T. Umemura, M. Ota, H. Hamano, Y. Katsuyama, K. Kiyosawa, and S. Kawa, "Genetic association of Fc receptor-like 3 polymorphisms with autoimmune pancreatitis in Japanese patients," *Gut*, vol. 55, no. 9, pp. 1367–1368, 2006.

[44] T. Umemura, M. Ota, H. Hamano et al., "Association of autoimmune pancreatitis with cytotoxic T-lymphocyte antigen 4 gene polymorphisms in Japanese patients," *American Journal of Gastroenterology*, vol. 103, no. 3, pp. 588–594, 2008.

[45] K. Okazaki, K. Uchida, M. Ohana et al., "Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response," *Gastroenterology*, vol. 118, no. 3, pp. 573–581, 2000.

[46] K. Uchida, K. Okazaki, Y. Konishi et al., "Clinical analysis of autoimmune-related pancreatitis," *American Journal of Gastroenterology*, vol. 95, no. 10, pp. 2788–2794, 2000.

[47] L. Aparisi, A. Farre, L. Gomez-Cambronero et al., "Antibodies to carbonic anhydrase and IgG4 levels in idiopathic chronic pancreatitis: relevance for diagnosis of autoimmune pancreatitis," *Gut*, vol. 54, no. 5, pp. 703–709, 2005.

[48] H. Nishi, A. Tojo, M. L. Onozato et al., "Anti-carbonic anhydrase II antibody in autoimmune pancreatitis and tubulointerstitial nephritis," *Nephrology Dialysis Transplantation*, vol. 22, no. 4, pp. 1273–1275, 2007.

[49] I. Nishimori, E. Miyaji, K. Morimoto, K. Nagao, M. Kamada, and S. Onishi, "Serum antibodies to carbonic anhydrase IV in

patients with autoimmune pancreatitis," *Gut*, vol. 54, no. 2, pp. 274–281, 2005.

[50] M. Asada, A. Nishio, K. Uchida et al., "Identification of a novel autoantibody against pancreatic secretory trypsin inhibitor in patients with autoimmune pancreatitis," *Pancreas*, vol. 33, no. 1, pp. 20–26, 2006.

[51] J. M. Löhr, R. Faissner, D. Koczan et al., "Autoantibodies against the exocrine pancreas in autoimmune pancreatitis: gene and protein expression profiling and immunoassays identify pancreatic enzymes as a major target of the inflammatory process," *American Journal of Gastroenterology*, vol. 105, no. 9, pp. 2060–2071, 2010.

[52] T. Endo, S. Takizawa, S. Tanaka et al., "Amylase α-2A autoantibodies novel marker of autoimmune pancreatitis and fulminant type 1 diabetes," *Diabetes*, vol. 58, no. 3, pp. 732–737, 2009.

[53] S. Takizawa, T. Endo, X. Wanjia, S. Tanaka, M. Takahashi, and T. Kobayashi, "HSP 10 is a new autoantigen in both autoimmune pancreatitis and fulminant type 1 diabetes," *Biochemical and Biophysical Research Communications*, vol. 386, no. 1, pp. 192–196, 2009.

[54] L. Frulloni, C. Lunardi, R. Simone et al., "Identification of a novel antibody associated with autoimmune pancreatitis," *The New England Journal of Medicine*, vol. 361, no. 22, pp. 2135–2142, 2009.

[55] T. Yamamoto, Y. Nakamura, J. Nishide et al., "Molecular cloning and nucleotide sequence of human pancreatic secretory trypsin inhibitor (PSTI) cDNA," *Biochemical and Biophysical Research Communications*, vol. 132, no. 2, pp. 605–612, 1985.

[56] Y. Zen, T. Fujii, K. Harada et al., "Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis," *Hepatology*, vol. 45, no. 6, pp. 1538–1546, 2007.

[57] T. Kamisawa, K. Takuma, N. Egawa, K. Tsuruta, and T. Sasaki, "Autoimmune pancreatitis and IgG4-related sclerosing disease," *Nature Reviews Gastroenterology and Hepatology*, vol. 7, no. 7, pp. 401–409, 2010.

[58] S. Kawa, H. Hamano, and K. Kiyosawa, *Pancreatitis*, Mackay Elsevier Academic Press, St Louis, MO, USA, 2006.

[59] D. P. Bogdanos, D. S. Smyk, E. I. Rigopoulou et al., "Twin studies in autoimmune disease: genetics, gender and environment," *Journal of Autoimmunity*, vol. 38, no. 2-3, pp. J156–J169, 2012.

[60] D. Smyk, E. I. Rigopoulou, H. Baum, A. K. Burroughs, D. Vergani, and D. P. Bogdanos, "Autoimmunity and environment: am i at risk?" *Clinical Reviews in Allergy and Immunology*, vol. 42, no. 2, pp. 199–212, 2012.

[61] D. S. Smyk, E. I. Rigopoulou, A. Lleo et al., "Immunopathogenesis of primary biliary cirrhosis: an old wives' tale," *Immunity & Ageing*, vol. 8, article 12, 2011.

[62] J. Kino-Ohsaki, I. Nishimori, M. Morita et al., "Serum antibodies to carbonic anhydrase I and II in patients with idiopathic chronic pancreatitis and Sjogren's syndrome," *Gastroenterology*, vol. 110, no. 5, pp. 1579–1586, 1996.

[63] P. D. Hardt, N. Ewald, K. Bröckling et al., "Distinct autoantibodies against exocrine pancreatic antigens in European patients with type 1 diabetes mellitus and non-alcoholic chronic pancreatitis," *Journal of the Pancreas*, vol. 9, no. 6, pp. 683–689, 2008.

[64] I. Nishimori, T. Bratanova, I. Toshkov et al., "Induction of experimental autoimmune sialoadenitis by immunization of PL/J mice with carbonic anhydrase II," *Journal of Immunology*, vol. 154, no. 9, pp. 4865–4873, 1995.

[65] Y. Ueno, M. Ishii, S. Takahashi, T. Igarashi, T. Toyota, and N. F. LaRusso, "Different susceptibility of mice to immune-mediated cholangitis induced by immunization with carbonic anhydrase II," *Laboratory Investigation*, vol. 78, no. 5, pp. 629–637, 1998.

[66] F. Guarneri, C. Guarneri, and S. Benvenga, "Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry?" *Journal of Cellular and Molecular Medicine*, vol. 9, no. 3, pp. 741–744, 2005.

[67] D. P. Bogdanos and D. Vergani, "Bacteria and primary biliary cirrhosis," *Clinical Reviews in Allergy and Immunology*, vol. 36, no. 1, pp. 30–39, 2009.

[68] D. P. Bogdanos, H. Baum, F. Gunsar et al., "Extensive homology between the major immunodominant mitochondrial antigen in primary biliary cirrhosis and Helicobacter pylori does not lead to immunological cross-reactivity," *Scandinavian Journal of Gastroenterology*, vol. 39, no. 10, pp. 981–987, 2004.

[69] A. Koutsoumpas, M. Mytilinaiou, D. Polymeros, G. N. Dalekos, and D. P. Bogdanos, "Anti-helicobacter pylori antibody responses specific for VacA do not trigger primary biliary cirrhosis-specific antimitochondrial antibodies," *European Journal of Gastroenterology and Hepatology*, vol. 21, no. 10, p. 1220, 2009.

[70] J. W. Wiley and M. Pietropaolo, "Autoimmune pancreatitis: the emerging role of serologic biomarkers," *Diabetes*, vol. 58, no. 3, pp. 520–522, 2009.

[71] C. Meagher, Q. Tang, B. T. Fife et al., "Spontaneous development of a pancreatic exocrine disease in CD28-deficient NOD mice," *Journal of Immunology*, vol. 180, no. 12, pp. 7793–7803, 2008.

[72] M. Asada, A. Nishio, T. Akamatsu et al., "Analysis of humoral immune response in experimental autoimmune pancreatitis in mice," *Pancreas*, vol. 39, no. 2, pp. 224–231, 2010.

Review Article

IgG4-Related Lymphadenopathy

Yasuharu Sato and Tadashi Yoshino

Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences,
2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

Correspondence should be addressed to Yasuharu Sato, satou-y@cc.okayama-u.ac.jp

Lymphadenopathy is frequently observed in patients with immunoglobulin G4-related disease (IgG4-RD) and sometimes appears as the first manifestation of the disease. The diagnosis of IgG4-related lymphadenopathy is complicated owing to a great histological diversity, with at least 5 histological subtypes. Indeed, lymph node biopsy may be performed under the suspicion that the lymphadenopathy is a malignant lymphoma or other lymphoproliferative disorder. The diagnosis of IgG4-RD is characterized by both elevated serum IgG4 (>135 mg/dL) and histopathological features, including a dense lymphoplasmacytic infiltrate rich in IgG4$^+$ plasma cells (IgG4$^+$/IgG$^+$ plasma cell ratio >40%). However, patients with hyper-interleukin (IL-) 6 syndromes such as multicentric Castleman's disease, rheumatoid arthritis, and other immune-mediated conditions frequently show lymph node involvement and often fulfill the diagnostic criteria for IgG4-RD. Owing to these factors, IgG4-RD cannot be differentiated from hyper-IL-6 syndromes on the basis of histological findings alone. Laboratory analyses are crucial to differentiate between the 2 diseases. Hyper-IL-6 syndromes are characterized by elevated serum levels of IgG, IgA, IgM, and C-reactive protein (CRP); thrombocytosis; anemia; hypoalbuminemia; hypocholesterolemia. In contrast, IgG4-RD does not share any of these characteristics. Therefore, the diagnosis of IgG4-RD requires not only pathological findings but also clinical and laboratory analyses.

1. Introduction

Immunoglobulin G4-related disease (IgG4-RD) frequently involves lymph nodes in a localized or systemic fashion [1–3]. Indeed, approximately 80% of patients with autoimmune pancreatitis (IgG4-related pancreatitis) has lymphadenopathy, most commonly involving the mediastinal and intraabdominal lymph nodes [4]. Moreover, lymphadenopathy sometimes appears as the first manifestation of IgG4-RD [1–3].

IgG4-RD is an inflammatory condition characterized by a dense lymphoplasmacytic infiltrate rich in IgG4$^+$ plasma cells; an IgG4$^+$/IgG$^+$ plasma cell ratio of >40% is an important diagnostic criterion for the disease [3, 5]. Patients with IgG4-related lymphadenopathy occasionally show systemic lymphadenopathy and elevated serum levels of IgG4 and IgE, and less often show low titers of various autoantibodies [1–3, 5, 6]. Therefore, the disease often shares clinical characteristics with malignant lymphoma,

multicentric Castleman's disease, and immune-mediated conditions [1–3, 7, 8]. However, the patients often show an excellent response to steroid therapy and do not show the B symptoms of fever, fatigue, weight loss, and night sweats. Moreover, no monoclonal immunoglobulin gene rearrangement is observed [1, 3].

Recently, several studies dealing with the morphological and immunohistological findings of IgG4-related lymphadenopathy have been performed [1–3]. Furthermore, these studies have shown that lymphadenopathies are histologically distinct from the effects of IgG4-RD on other organs (i.e., storiform fibrosis and obliterative phlebitis are usually absent) [1–3]. From this histological diversity, we consider the presence of 5 subtypes of IgG4-related lymphadenopathy (Table 1): multicentric Castleman's disease-like, reactive follicular hyperplasia-like, interfollicular expansion and immunoblastosis, progressively transformed germinal center (PTGC-) type, and inflammatory pseudotumor-like IgG4-related lymphadenopathy [1–3].

Table 1: Histological subtypes of IgG4-related lymphadenopathy.

	Histological type	Distribution of IgG4$^+$ plasma cells	Lymphadenopathy
I	Multicentric Castleman's disease-like	Interfollicular	Systemic
II	Reactive follicular hyperplasia-like	Interfollicular	Localized
III	Interfollicular expansion and immunoblastosis	Interfollicular	Systemic
IV	PTGC-type	Intragerminal center	Localized/systemic
V	Inflammatory pseudotumor (IPT-) like	Interfollicular	Localized

PTGC; progressively transformed germinal centers.

Table 2: Distinction between IgG4-related disease and hyper-IL-6 syndromes.

	IgG4-related disease	Hyper-IL-6 syndromes
Serum immunoglobulin	IgG↑(IgG4↑), IgE↑	IgG↑(IgG1~IgG4↑), IgA↑, IgM -/↑, IgE↑
Serum IgG4/IgG ratio	Elevated	Normal (~sightly elevated)
Serum IL-6	Normal (~sightly elevated)	Elevated
Serum CRP	Normal (~sightly elevated)	Elevated
Thrombocytosis	No	Yes
Anemia	No	Yes
Hypoalbuminemia	No	Yes
Hypocholesterolemia	No	Yes

Hyper IL-6 syndromes; multicentric Castleman's disease, rheumatoid arthritis, and other immune-mediated conditions.

2. Clinical and Pathological Features of IgG4-Related Lymphadenopathy

2.1. Type I: Multicentric Castleman's Disease-Like. This type is frequently characterized by systemic lymphadenopathy [1–3]. Histologically, the lymph node shows interfollicular expansion with normal to hyperplastic germinal centers, penetrated by blood vessels. Abundant plasma cells and scattered eosinophils are apparent in the interfollicular zone (Figure 1). Although these features are similar to the features of multicentric Castleman's disease (MCD), MCD is usually characterized by the presence of small and regressive germinal centers and no eosinophil infiltration [8]. However, pathological diagnosis is difficult, because MCD sometimes fulfills the diagnostic criteria for IgG4-RD, namely, abundant IgG4$^+$ plasma cell infiltration (i.e., IgG4$^+$/IgG$^+$ plasma cell ratio >40%) and elevated serum IgG4 levels [8]. Therefore, the 2 diseases cannot be differentiated on the basis of histological findings alone, and laboratory analyses are critical for a definitive diagnosis (Table 2).

2.2. Type II: Reactive Follicular Hyperplasia-Like. The lymph nodes usually exhibit reactive follicular hyperplasia, and sinuses are intact. The reactive follicles comprise a germinal center surrounded by a discrete mantle zone. The interfollicular zone contains a small to moderate number of mature plasma cells, with small lymphocytes and eosinophils (Figure 2). This type is frequently found in the regional lymph nodes of IgG4-RD [1, 2].

2.3. Type III: Interfollicular Expansion and Immunoblastosis. This type is also frequently characterized by systemic lymphadenopathy [1–3]. Histologically, the lymph nodes show marked interfollicular expansion with prominent high endothelial venules and patent sinuses. The lymphoid follicles are usually normal to atrophic. A mixed infiltrate of small lymphocytes, immunoblasts, immature plasma cells, mature plasma cells, and scattered eosinophils is observed (Figure 3). The morphological features overlap with those of atypical lymphoplasmacytic and immunoblastic proliferation (ALPIBP), which is a characteristic lymphadenopathy observed in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and other autoimmune diseases [9].

This type is somewhat similar to angioimmunoblastic T-cell lymphoma. However, it is noteworthy that these lesions lack clusters of clear cells and definite cytologic atypia typical of the lymphoma. Moreover, CD21$^+$ follicular dendritic cell proliferation, the presence of CD10$^+$ T-cells, and T-cell receptor gene rearrangement are not observed [1–3].

2.4. Type IV: Progressively Transformed Germinal Centers (PTGC)-Type. PTGC is a benign condition of unknown origin characterized by reactive follicular hyperplasia in the lymph nodes [10, 11]. Recently, we were the first to report cases of patients with IgG4-RD in PTGC of lymph nodes (PTGC-type IgG4-related lymphadenopathy) [3]. In this type, the lymph nodes demonstrate numerous lymphoid follicles with hyperplastic germinal centers and a distinct mantle zone but no expansion of the interfollicular zone. PTGCs are also apparent, appearing as round to oval structures with diameters 2 or 3 times the diameter of the other reactive follicles. They are predominantly composed of small lymphocytes, centrocytes, centroblasts, and numerous mature plasma cells and plasmacytoid cells. The interfollicular zone shows infiltration by numerous eosinophils, whereas

(a)

(b)

(c)

(d)

FIGURE 1: IgG4-related lymphadenopathy (type I). (a) The lymph node shows interfollicular expansion with normal to hyperplastic germinal centers. (b) The germinal centers are penetrated by blood vessels. (c) A large number of mature plasma cells with small lymphocytes are seen. (d) Immunostaining shows numerous IgG4+ cells in the interfollicular zone.

(a)

(b)

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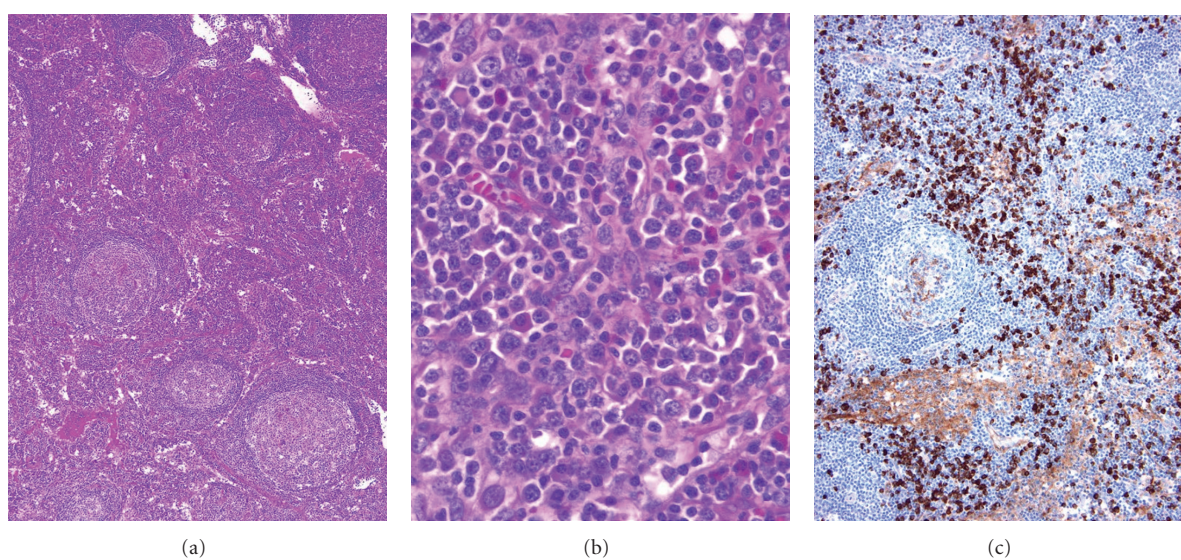
FIGURE 2: IgG4-related lymphadenopathy (type II). (a) The lymph node shows reactive follicular hyperplasia with intact sinuses. (b) A small to moderate number of mature plasma cells with small lymphocytes and eosinophils are present. (c) Immunostaining shows numerous IgG4+ cells in the interfollicular zone.
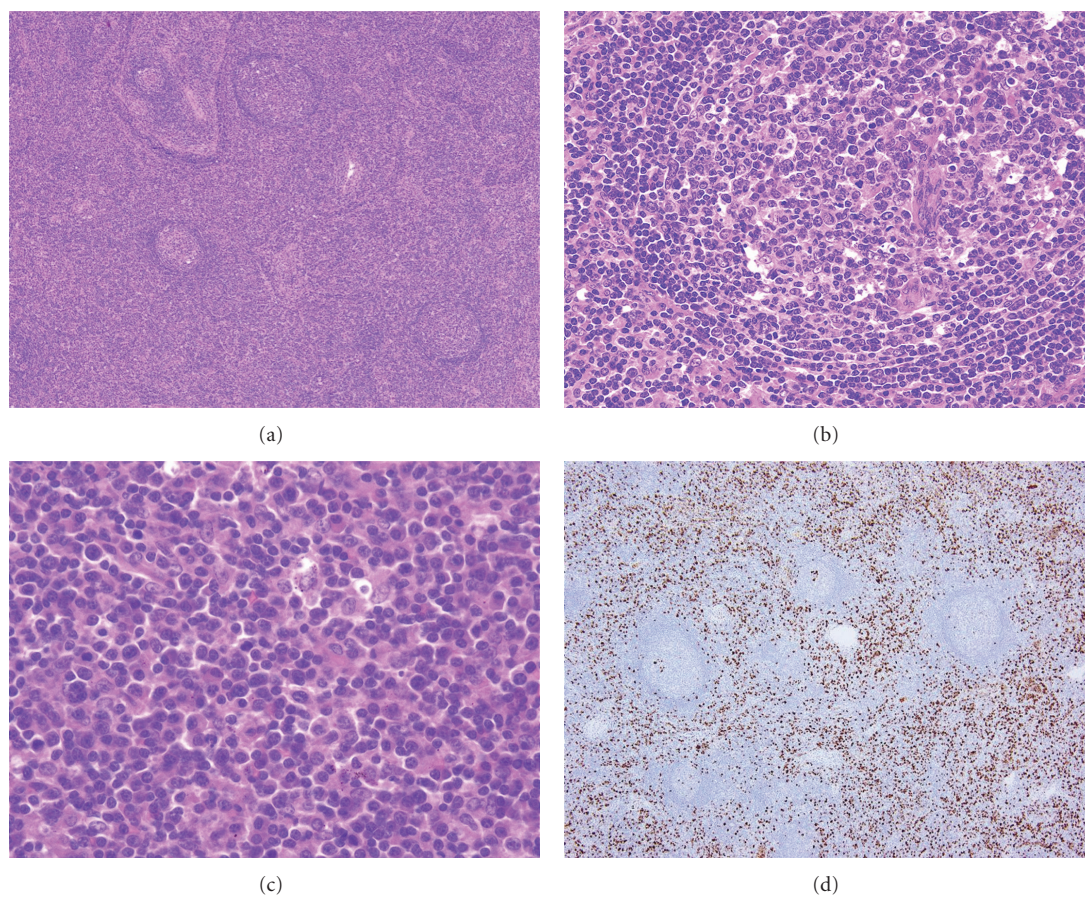
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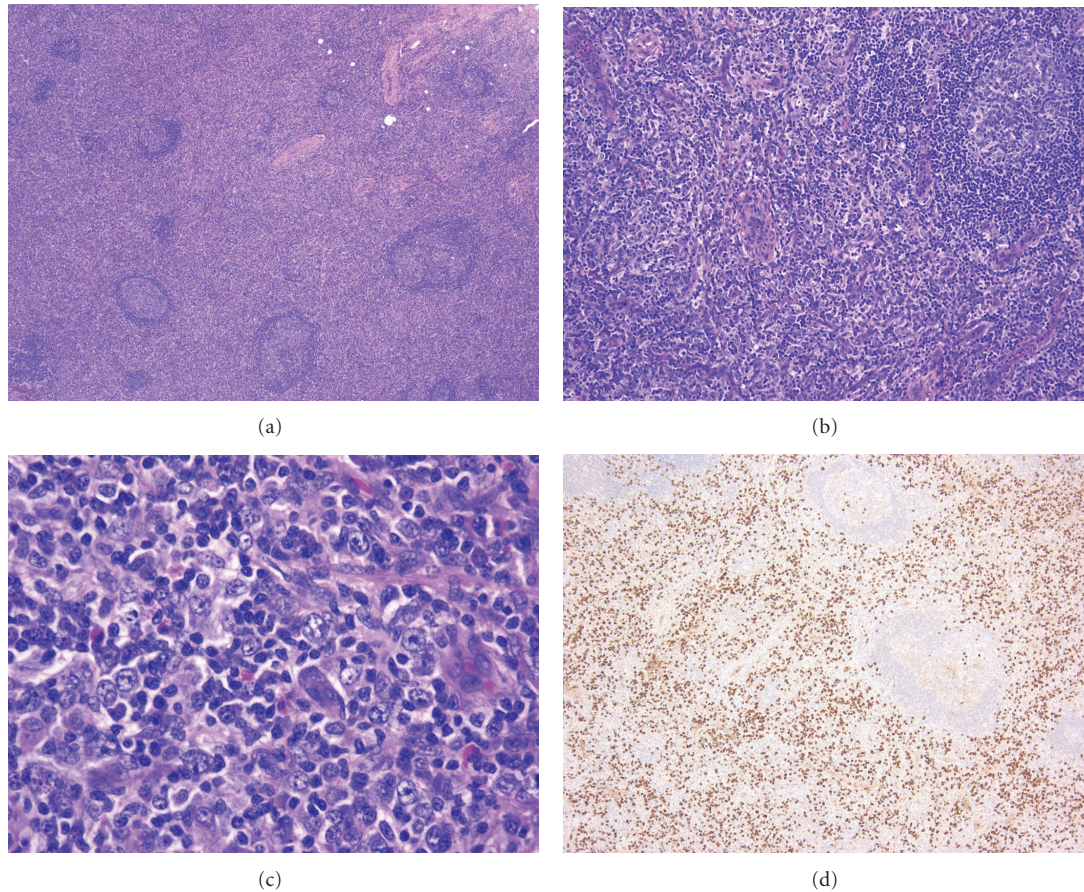
FIGURE 3: IgG4-related lymphadenopathy (type III). (a) The lymph node shows interfollicular expansion with normal to small germinal centers. (b) Hypervascular proliferation is seen in the interfollicular zone. (c) A mixed infiltrate of small lymphocytes, immunoblasts, immature plasma cells, mature plasma cells, and scattered eosinophils is observed (d) Numerous IgG4$^+$ cells are present in the interfollicular zone.

T zones are indistinct (Figure 4). Interestingly, a unique feature of this type is the localization of the majority of IgG4$^+$ plasma cells in the germinal centers, with only a small number present in the interfollicular zone [12]. However, in a few cases of this type, IgG4$^+$ plasma cells are detected in both the germinal centers and interfollicular zone [12].

Patients with this type have a uniform clinicopathology. The patients initially present with asymptomatic localized submandibular lymphadenopathy, with half of them showing progression to extranodal IgG4-RD, systemic disease, or both during the follow-up period [12].

2.5. Type V: Inflammatory Pseudotumor (IPT)-Like. In this type, the lymph nodes show asymptomatic localized lymphadenopathy [13]. Histologically, most of the lymph node is occupied by hyalinized fibrous tissue. A few residual lymphoid follicles with hyperplastic germinal centers and a focally dense lymphoid infiltrate are observed in the lymph node. Small lymphocytes, plasma cells, and eosinophils infiltrate the dense sclerotic tissue (Figure 5). This type is rare; we have encountered only 2 cases, and no other cases have been reported thus far [1, 13].

These histological findings are somewhat similar to those characteristic of nodal IPT. Nodal IPT has been histologically classified into 3 stages (i.e., Stage I, II, and III) [14, 15]. IPT-like IgG4-related lymphadenopathy is similar to lymphadenopathy in patients with stage III nodal IPT [1, 13]. However, IPT-like IgG4-related lymphadenopathy and nodal IPT are clinically different, because patients with nodal IPT usually show symptoms that are suggestive of lymphoid malignancy (e.g., fever, fatigue, weight loss, and night sweats) [14, 15]. In contrast, patients with IPT-like IgG4-related lymphadenopathy show no symptoms suggestive of lymphoid malignancy [13]. Moreover, nodal IPT is positive for smooth muscle actin [14, 15], which further differentiates it from IPT-like IgG4-related lymphadenopathy [13].

3. Differential Diagnosis between IgG4-RD and Hyper-Interleukin (IL-) 6 Syndromes

Hyper-IL-6 syndromes such as MCD, RA, and other immune-mediated conditions are characterized by elevated serum IL-6 levels [16, 17]. Moreover, IL-6 itself functions to raise the serum levels of IgG4 and other IgG subclasses

(a)

(b)

(c)

(d)

(e)

(f)
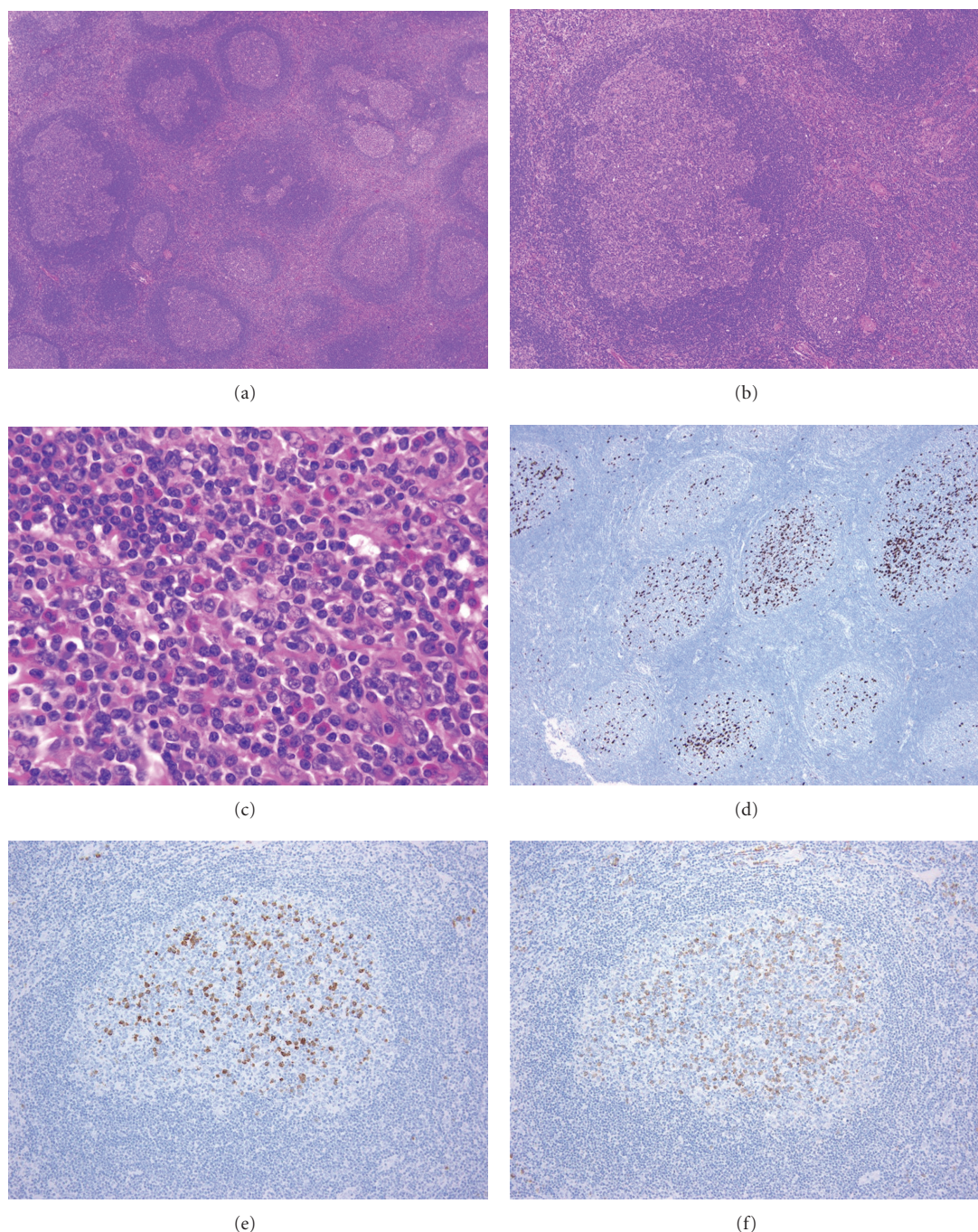
FIGURE 4: IgG4-related lymphadenopathy (type IV). (a) The lymph node shows marked follicular hyperplasia with PTGC. (b) The PTGCs appear as round to oval structures 2-3 times the diameter of the other reactive follicles. (c) Numerous eosinophils infiltrate the interfollicular zone. (d) The majority of IgG4+ plasma cells reside in the germinal centers, with a small number present in the interfollicular zone. (e), (f): The IgG4+/IgG+ plasma cell ratio is >40% (e: IgG4-immunostain, f: IgG-immunostain).

[18, 19]. In fact, MCD, RA, and other immune-mediated conditions sometimes fulfill the histological diagnostic criteria for IgG4-RD (Figures 6 and 7) and are characterized by elevated serum IgG4 levels [8, 20–23]. This complicates diagnosis, owing to the fact that hyper-IL-6 syndromes frequently involve lymph nodes. Because of this, laboratory analyses are crucial to differentiate between the 2 diseases [8].

Unlike IgG4-RD, hyper-IL-6 syndromes are characterized by elevated serum levels of IgG, IgA, IgM, and C-reactive protein (CRP); thrombocytosis; anemia; hypoalbuminemia; hypocholesterolemia (Table 2). These abnormalities are closely related to high IL-6 levels [8, 17, 20]. On the other hand, elevated serum IgE is often typical of IgG4-RD [1, 3, 5]. However, IL-6 plays a critical role in IL-4-driven IgE

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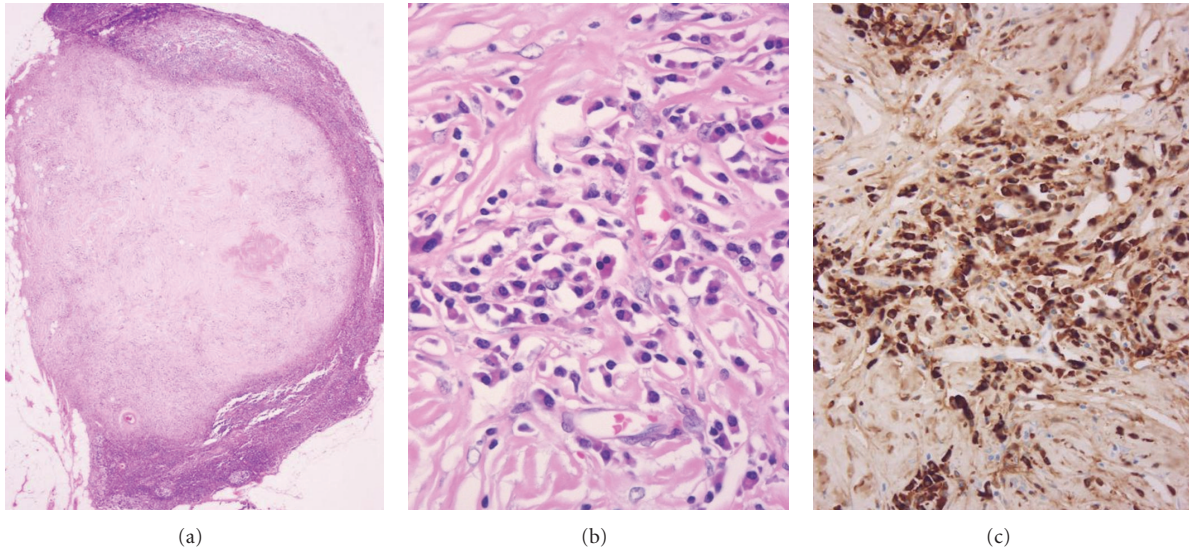
FIGURE 5: IgG4-related lymphadenopathy (type V). This is a regional lymph node with IgG4-related cholangitis. (a) The majority of the lymph node is replaced by hyalinized fibrous tissue. (b) Mature plasma cells infiltrate the hyalinized fibrous tissue. (c) The mature plasma cells are IgG4$^+$.

| (a) | (b) | (c) |
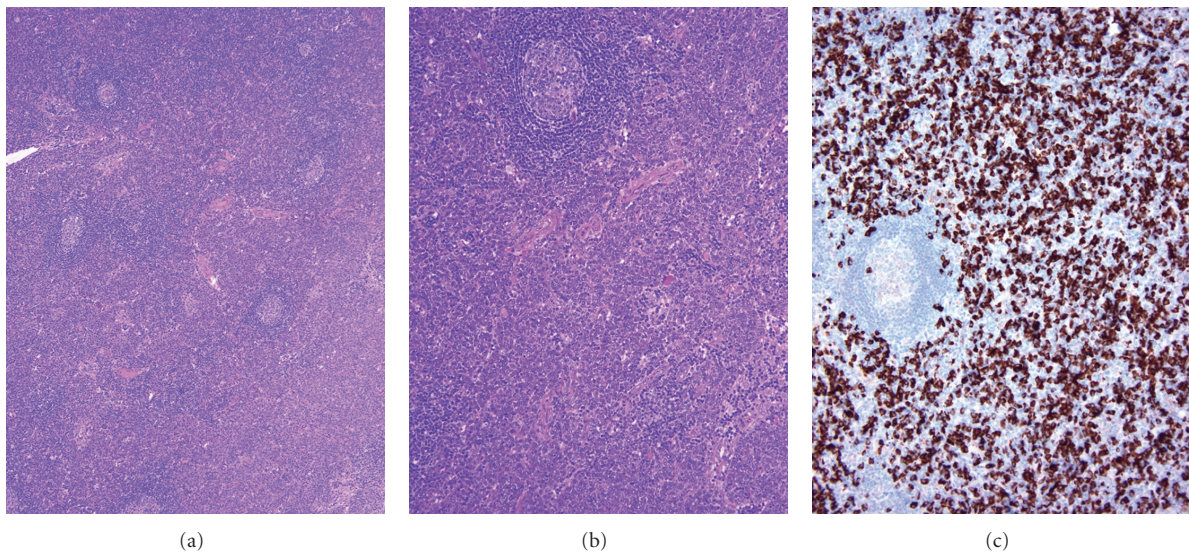
FIGURE 6: Multicentric Castleman's disease with abundant IgG4$^+$ cells. (a) Atrophic germinal centers and interfollicular expansion are seen. (b) Sheets of proliferating mature plasma cells are present in the interfollicular zone. (c) The majority of mature plasma cells are positive for IgG4 (IgG4$^+$/IgG$^+$ plasma cell ratio >70%).

synthesis [24]. As such, hyper-IL-6 syndromes may also be characterized by elevated serum IgE levels, rendering serum IgE level less useful as a biomarker for a differential diagnosis of the 2 diseases [8, 22].

4. Conclusion

Unlike IgG4-RD that involves other organs, IgG4-related lymphadenopathy shows histological diversity, with 5 distinct subtypes. Moreover, recently, Takahashi et al. reported a unique case of IgG4-related lymphadenopathy with epithelioid granuloma [25]. This histological diversity complicates the diagnosis of IgG4-related lymphadenopathy, especially considering the similarities of the different histological subtypes to the histological characteristics of other organs involved in IgG4-RD.

Indeed, hyper-IL-6 syndromes can often fulfill the diagnostic criteria for IgG4-RD. Therefore, IgG4-RD, and especially IgG4-related lymphadenopathy, cannot be differentiated on the basis of histological findings alone. The diagnosis of IgG4-RD needs to be based not only on

(a)　　　　　(b)　　　　　(c)
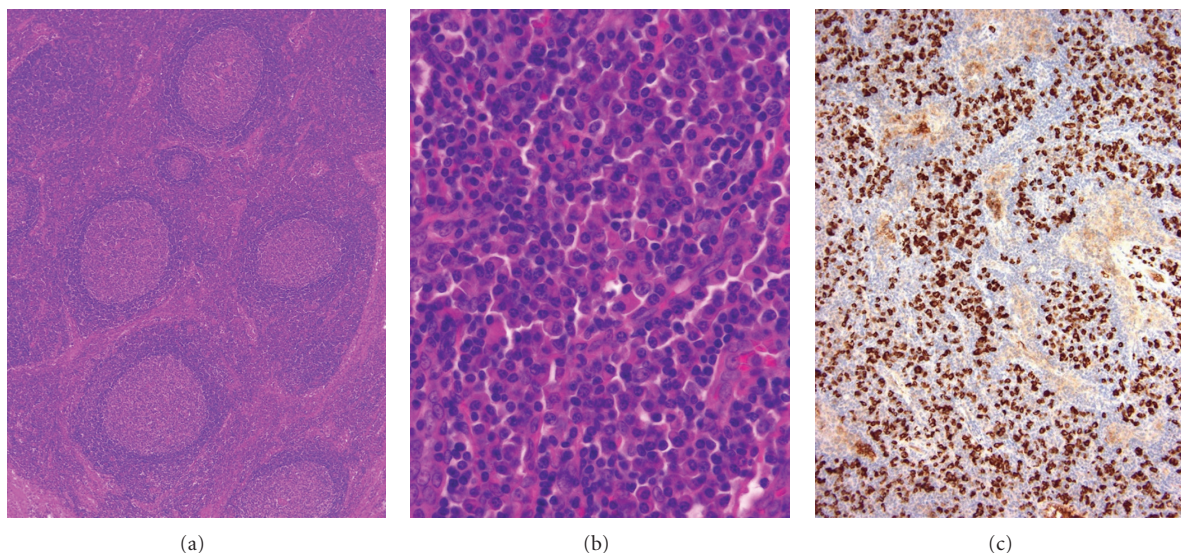
FIGURE 7: Rheumatic lymphadenopathy with abundant IgG4$^+$ cells. (a), (b) The lymph node shows marked follicular hyperplasia and interfollicular plasmacytosis with small lymphocytes; eosinophil infiltration is absent. (c) The majority of mature plasma cells are positive for IgG4 (IgG4$^+$/IgG$^+$ plasma cell ratio >60%).

pathological findings but also on clinical and laboratory findings.

Acknowledgment

References

[1] Y. Sato, K. Notohara, M. Kojima, K. Takata, Y. Masaki, and T. Yoshino, "IgG4-related disease: historical overview and pathology of hematological disorders," *Pathology International*, vol. 60, no. 4, pp. 247–258, 2010.

[2] W. Cheuk, H. K. L. Yuen, S. Y. Y. Chu, E. K. W. Chiu, L. K. Lam, and J. K. C. Chan, "Lymphadenopathy of IgG4-related sclerosing disease," *American Journal of Surgical Pathology*, vol. 32, no. 5, pp. 671–681, 2008.

[3] Y. Sato, M. Kojima, K. Takata et al., "Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease," *Modern Pathology*, vol. 22, no. 4, pp. 589–599, 2009.

[4] H. Hamano, N. Arakura, T. Muraki, Y. Ozaki, K. Kiyosawa, and S. Kawa, "Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis," *Journal of Gastroenterology*, vol. 41, no. 12, pp. 1197–1205, 2006.

[5] Y. Masaki, N. Kurose, and H. Umehara, "IgG4-related disease: a novel lymphoproliferative disorder discovered and established in Japan in the 21st century," *Journal of Clinical and Experimental Hematopathology*, vol. 51, no. 1, pp. 13–20, 2011.

[6] Y. Sato, K. I. Ohshima, K. Ichimura et al., "Ocular adnexal IgG4-related disease has uniform clinicopathology," *Pathology International*, vol. 58, no. 8, pp. 465–470, 2008.

[7] Y. Sato, K. Takata, K. Ichimura et al., "IgG4-producing marginal zone B-cell lymphoma," *International Journal of Hematology*, vol. 88, no. 4, pp. 428–433, 2008.

[8] Y. Sato, M. Kojima, K. Takata et al., "Multicentric Castleman's disease with abundant IgG4-positive cells: a clinical and pathological analysis of six cases," *Journal of Clinical Pathology*, vol. 63, no. 12, pp. 1084–1089, 2010.

[9] C. H. Koo, B. N. Nathwani, and C. D. Winberg, "Atypical lymphoplasmacytic and immunoblastic proliferation in lymph nodes of patients with autoimmune disease (autoimmune-disease-associated lymphadenopathy)," *Medicine*, vol. 63, no. 5, pp. 274–290, 1984.

[10] K. Lennert and H. K. Müller–Hermelink, "Lymphocyten and ihre funktionsformen morphologie, organization und immunologische bedeutung," *Verhandlungen der Anatomischen Gesellschaft*, vol. 69, pp. 19–62, 1975.

[11] B. M. Osborne and J. J. Butler, "Clinical implications of progressive transformation of germinal centers," *American Journal of Surgical Pathology*, vol. 8, no. 10, pp. 725–733, 1984.

[12] Y. Sato, D. Inoue, N. Asano, K. Takata, H. Asaoku, Y. Maeda et al., "Association between IgG4-related disease and progressively transformed germinal centers of lymph nodes," *Modern Pathology*. In press.

[13] Y. Sato, M. Kojima, K. Takata, H. Huang, E. Hayashi, and A. Manabe, "Immunoglobulin G4-related lymphadenopathy with inflammatory pseudotumor-like features," *Medical Molecular Morphology*, vol. 44, no. 3, pp. 179–182, 2011.

[14] C. A. Moran, S. Suster, and S. L. Abbondanzo, "Inflammatory pseudotumor of lymph nodes: a study of 25 cases with emphasis on morphological heterogeneity," *Human Pathology*, vol. 28, no. 3, pp. 332–338, 1997.

[15] M. Kojima, S. Nakamura, K. Shimizu et al., "Inflammatory pseudotumor of lymph nodes: clinicopathologic and immunohistological study of 11 Japanese cases," *International Journal of Surgical Pathology*, vol. 9, no. 3, pp. 207–214, 2001.

[16] G. Frizzera, "Atypical lymphoproliferative disorders," in *Neoplastic Hematopathology*, D. M. Knowles, Ed., pp. 569–622, Lippincott Williams & Wilkins, Baltimore, Md, USA, 2nd edition, 2000.

[17] N. Nishimoto, K. Terao, T. Mima, H. Nakahara, N. Takagi, and T. Kakehi, "Mechanisms and pathologic significances

in 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*, vol. 112, no. 10, pp. 3959–3964, 2008.

[18] J. N. Bertolini and E. M. Benson, "The role of human Interleukin-6 in B-cell isotype regulation and differentiation," *Cellular Immunology*, vol. 125, no. 1, pp. 197–209, 1990.

[19] Y. Kawano, T. Noma, K. Kou, I. Yoshizawa, and J. Yata, "Regulation of human IgG subclass production by cytokines: human IgG subclass production enhanced differentially by interleukin-6," *Immunology*, vol. 84, no. 2, pp. 278–284, 1995.

[20] M. Kojima, N. Nakamura, N. Tsukamoto et al., "Atypical lymphoplasmacytic and immunoblastic proliferation of autoimmune disease : clinicopathologic and immunohistochemical study of 9 cases," *Journal of Clinical and Experimental Hematopathology*, vol. 50, no. 2, pp. 113–119, 2010.

[21] J. D. Strehl, A. Hartmann, and A. Agaimy, "Numerous IgG4-positive plasma cells are ubiquitous in diverse localised non-specific chronic inflammatory conditions and need to be distinguished from IgG4-related systemic disorders," *Journal of Clinical Pathology*, vol. 64, no. 3, pp. 237–243, 2011.

[22] N. Asano and Y. Sato, "Rheumatoid lymphadenopathy with abundant IgG4^{+} plasma cells: a case mimicking IgG4-related disease," *Journal of Clinical and Experimental Hematopathology*. In press.

[23] M. Yamamoto, T. Tabeya, Y. Naishiro, H. Yajima, K. Ishigami, Y. Shimizu et al., "Value of serum IgG4 in the diagnosis of IgG4-related disease and in differentiation from rheumatic diseases and other diseases," *Modern Rheumatology*. In press.

[24] H. H. Jabara, D. J. Ahern, D. Vercelli, and R. S. Geha, "Hydrocortisone and IL-4 induce IgE isotype switching in human B cells," *Journal of Immunology*, vol. 147, no. 5, pp. 1557–1560, 1991.

[25] E. Takahashi, M. Kojima, M. Kobayashi, A. Kitamura, T. Yokoi, K. Hara et al., "Primary IgG4-related lymphadenopathy with prominent granulomatous inflammation and reactivation of Epstein-Barr virus," *Virchows Archiv*, vol. 460, no. 2, pp. 225–259, 2012.

Review Article

IgG4-Related Fibrotic Diseases from an Immunological Perspective: Regulators out of Control?

Laura C. Lighaam,[1] Rob C. Aalberse,[1] and Theo Rispens[1, 2]

[1] *Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, 1066 CX Amestrdam, The Netherlands*
[2] *Sanquin Blood Supply Foundation, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands*

Correspondence should be addressed to Theo Rispens, t.rispens@sanquin.nl

Patients with autoimmune pancreatitis have a striking polyclonal elevation of total IgG4 in serum. This observation has been confirmed and extended to other fibrotic conditions (that are therefore called IgG4-related disease) but as yet remains unexplained. The affected tissue contains many IgG4-producing plasma cells embedded in a fibrotic matrix originating from activated mesenchymal (stellate) cells. We propose that the process results from an unusual interaction between two regulatory systems: the regulatory arm of the immune system (including Bregs) and the tissue repair regulatory components orchestrated by the activated stellate cell. This interaction results in ongoing mutual activation, generating TGFbeta, IL10, and vitamin D. This environment suppresses most immune reactions but stimulates the development of IgG4-producing plasma cells.

1. IgG4 Production in IRD

IgG4-related disease (IRD, see Box 1) is a group of diseases with disparate symptoms, but sharing a common pathophysiology, which has only recently been recognized as a new disease entity [1]. IRD is characterized by massive infiltration of the affected organ by IgG4-positive plasma cells. This infiltration coincides with a disruption of the organization of the tissue and thus of tissue function. The extent of the plasmacytic tissue reaction in IRD is such that the first impression is often that of a tumor. While the prototypic site of IgG4 production in IRD is the pancreas, many other sites in the body can be involved, for example, the salivary and tear glands, reminding of Sjögren's syndrome. However, in IRD, the ducts usually remain largely intact, and secretion by the glands is less severely affected [2]. It is not at all unusual to find several organs to be involved simultaneously (for details, see Box 1).

A 5–50-time elevation of total IgG4 levels is found in patients with IRD. This results in a markedly increased IgG4/IgG ratio, both for serum immunoglobulin levels and for plasma cells in the affected tissue. It is not clear if the increased levels of IgG4 contribute to the pathology of IRD.

So far, convincing support for the hypothesis that (auto-) antibody activity of IgG4 is driving the pathology is lacking. Several candidate autoantibodies have been suggested in IRD, such as antibodies directed against pancreatic trypsin inhibitor, lactoferrin, and carbonic anhydrase, mainly in patients with pancreatic involvement [3]. These antibodies were mostly not of the IgG4 subclass. Since they are present in only a small part of the patients, their role in the pathophysiology of the disease is probably limited. In the absence of an obvious (auto) antigen driving the reaction, it is unclear how these responses are triggered, and, therefore, how IRD may develop.

Toll-like receptor and Nod-like receptor stimulation have also been implied in IRD, since PBMCs of IRD patients produce IL-10 and high levels of IgG4 in response to stimulation of these receptors in a BAFF-dependent manner [4, 5].

Recently, some IRD patients have been treated with Rituximab, a monoclonal antibody drug that targets CD20 [6]. Patients treated with Rituximab show a fast decline in serum IgG4 levels, while the decrease of other subclasses is less pronounced [7]. This is not due to a direct effect on the IgG4-producing plasma cells, because CD20 is present on B

IgG4-related disease (IRD) is a syndrome characterized by raised serum IgG4 levels. Clinically, tumor-like enlargements are observed, often in the retroperitoneal area or in one or more exocrine glands, most commonly in the pancreas, biliary tract, with more submandibular gland and lacrimal gland. The pathology involves a massive polyclonal lymphoplasmacytic Infiltration more than 30% of the plasma cells staining for IgG4, and fibrosis with a typical star-like or storiform appearance. The lymphocytes are mostly T helper cells, which presumably are follicular Th cells [28], and relatively few B cells. Furthermore, extensive neutrophil infiltration is absent in IRD. Manifestations of IRD are manifold. Descriptions of the full clinical and pathological spectrum of IRD can be found, for instance, in the reviews by Umehara [1] and by Khosroshahi and Stone [29]. An illustration of the scope of the spectrum is the finding that IRD is involved in many cases of retroperitoneal fibrosis, which may cause severe, potentially fatal, aortic pathology, including aortic aneurism [30].
The pancreatic variant of IRD is often referred to as autoimmune pancreatitis (AIP) type 1, which should be distinguished from the classical duct-destructive AIP, nowadays called AIP type 2 [31]. In AIP type 1, the glandular ducts are typically not infiltrated.
Similarly, if salivary and tear glands are affected, their secretion is less affected than in Sjögren's syndrome, because the ducts remain relatively undamaged. Extensive neutrophil infiltration is absent in IRD. Lymph nodes may be involved in IRD, raising suspicion of IL-6-hypersecreting multicentric Castleman's disease (MCD). However, IgG4-RD cases have been found to be negative for the herps virus associated with MCD, and IgG4-RD is not associated with fever. The complex connection between IRD and lymphadenopathy is well discussed by Sato et al. [32]. The emphasis in this opinion is on the pancreas and related tissues (biliary tree, salivary glands, and tear glands). In other locations, some aspects of the histopathology may differ, particularly the extent of the fibrosis.

Box 1: IgG4-related disease (IRD).

cells from the pre-B cell stage, but is lost upon differentiation into plasma cells. Therefore, the rapid decline of IgG4 levels upon B-cell depletion strongly suggests that the lifespan of the IgG4-secreting plasma cells is short, that is, less than a week. The large number of IgG4-secreting plasma cells before treatment must be caused by the continuous differentiation of IgG4-switched B cells into plasma cells.

Here, we will discuss two features related to IgG4 that may be involved in the preferential recruitment and retention of IgG4-switched B cells into the affected tissue in IRD. First, as explained below, IgG4 has been linked to "tolerogenic" immune responses. Second, there are indications of unusual Fab glycosylation in (part of) IgG4. Our hypothesis is that the B-cell receptors (BCRs) of some B cells are Fab glycosylated with an oligomannose glycan, which is recognized by an endogenous lectin found on the tissue-resident myofibroblast (stellate cell). This interaction may result in an ongoing mutual stimulation of two regulatory systems: the blood-derived immune regulators, including IgG4-committed B cells, and the tissue-resident damage-controlling stellate cell, resulting in the pathology observed in IRD.

2. IgG4: An Antibody Linked to Tolerogenic Conditions

IgG4 is a peculiar subclass of human immunoglobulins. It represents about 5% of total IgG in serum of healthy adults (0.5 g/L, normal range: 0.05–1.4 g/L). However, IgG4 antibody can represent up to 80% of total IgG antibody after chronic exposure to antigen [8, 9]. Since IgG4 antibodies do not activate complement and bind to Fc receptors with lower affinity [10], they do not activate the effector functions of the immune system in the same way the other subclasses do [11, 12]. Furthermore, IgG4 antibodies are able to exchange half molecules in $vivo$ [12, 13]. This process results in the generation of asymmetric antibodies with two different Fab arms. Since these antibodies can, in general, only bind to antigen with one Fab arm, IgG4 is not able to cross-link antigens and thus to form large immune complexes. IgG4 has even been shown to interfere with the complement-activating and immune-precipitating activities of human IgG1 antibodies [14].

All in all, the immunochemical properties of IgG4 antibodies point towards a dampening role in the effector phase of the immune response. This fits well with the requirements for IgG4 production. IgG4 responses require frequent and/or high antigen exposure and are observed in situations associated with tolerance induction, such as during immunotherapy. IgG4 responses are also often associated with IgE-mediated allergy, but IgG4 responses are distinct from IgE responses. Although both IgG4 and IgE need the Th2 cytokines IL-4 and/or IL-13 [15], production of IgE antibodies often occurs well before IgG4 antibodies appear (e.g., in novice beekeepers [8]). It is also common to find IgG4 antibodies in the absence of IgE antibodies, a process called the modified Th2 response [16]. One important regulatory component in the modified Th2 response is IL-10. Under the influence of this cytokine, the switch to IgE is inhibited, while switch to IgG4 is promoted [17].

In the case of prolonged and/or high-dose antigenic stimulation, immune regulatory circuits play an important role. They counteract the effects of antigenic stimulation and dampen the immune response, resulting in, amongst others, the decrease of $T_{effector}$ responses and of the production of human IgG1 antibodies. It is only then that the IgG4 response develops to its full extent. One of these regulatory signals is the above-mentioned cytokine IL-10. This explains why upon chronic exposure to antigen, IgG4 levels increase. It is likely that IL-10 needed for the development of an IgG4 immune response is in part produced by Tregs present in the lesions of IRD patients as demonstrated by in $situ$

Fibrosis is a reaction of a fibroblast to injury. Upon activation, the fibroblast becomes a myofibroblast, which produces (intracellular) myosin and starts secreting matrix proteins, particularly collagens [33]. This local fibrotic damage control program is often accompanied by an inflammatory reaction [34]. The inflammation generates an influx of external "damage controllers," including granulocytes, monocytes, lymphocytes, and the recently recognized monocyte-related fibrocyte [35, 36]. Depending on the nature and the time course of the damage the cellular composition of the infiltrate will vary markedly, which results in a broad spectrum of tissue changes. Macrophages are assumed to play a crucial role in the regulation of inflammation and fibrosis [37]. In IgG4-related fibrosis, the contribution of neutrophils is typically small. In the pancreas, the cell most prominently involved in fibrotic reactions is often referred to as "spindle cell," which is not a well-defined (myo-) fibroblast cell type. In 1998, a fibroblast-related cell with all characteristics of the hepatic stellate cell was identified in the pancreas [38]. The stellate cell is also known as "lipocyte," because of the presence of many lipid-containing vesicles that show a typical autofluorescence caused by the presence of vitamin A [39]. The presence of these lipid vesicles results in a characteristic low buoyant density, which can be used to isolate stellate cells. Hepatic stellate cells have been found to be closely associated with plasma cells in hepatic fibrosis [40]. Upon activation, the stellate cell releases much of its lipid vesicles, which makes it more difficult to distinguish it from other myofibroblast-related cells. For a recent review on the pancreatic stellate cell, see [41]. In IgG4-related fibrosis, the characteristic pattern is described as "storiform," a whirling pattern. This pattern presumably reflects the interaction of clusters of proliferating myofibroblasts [42]. One of the functions of myofibroblasts is to contract during a wound healing process. Such a contraction in the absence of a wound to heal may result in a whirling pattern. Plasma cells (of which typically more than 40% are IgG4 producing) are found within this fibrotic network, suggesting that this could be a niche for the IgG4 plasma cells in IRD.

Box 2: The role of the myofibroblast-type stellate cell in fibrosis, tissue repair and plasma cell differentiation.

hybridization [18], as well as increased levels of circulating Tregs [19]. Besides Tregs, another likely source for IL-10 is regulatory B cells (for a review on regulatory B cells, see [20]), some of which may later develop into IgG4-producing cells [21].

3. IgG4 Fab Glycosylation

There are indications that IgG4 may sometimes be unusually glycosylated in the Fab region: two sets of information point to a link between IgG4 and Fab glycosylation of the oligomannose type. First, a subject that has been studied for many years by Margni and coworkers is the association between oligomannose-type Fab glycosylation and nonprecipitating antibodies. They fractionated antigen-specific polyclonal antibodies based on their glycosylation pattern by ConA lectin chromatography, which preferentially binds oligomannose glycans. The bound fraction was unable to form an immune precipitate with antigen. The lack of immune precipitation was found to be due to asymmetric Fab glycosylation, that is, glycosylation of only one of the two antigen-binding domains. A possible mechanism explaining the formation of asymmetrically glycosylated antibodies is the aforementioned Fab arm exchange of IgG4. Fab arm exchange between glycosylated and nonglycosylated IgG4 would result in a nonprecipitating asymmetrically glycosylated antibody. Conditions that lead to enhanced production of asymmetrically glycosylated antibody (such as pregnancy) are similar to the tolerizing conditions that promote IgG4 production. These data suggest that IgG4 might be preferentially Fabglycosylated with oligomannose glycans.

The other set of information comes from a study on IgG4 antibody responses in infancy to a panel of food allergens [22]. In this study, a strong reactivity to a protein in banana was found, which was then characterized and found to be a lectin with a preference for oligomannose glycans: BanLec1 [23, 24]. IgG, including IgG4, is a glycoprotein. The obvious question was whether BanLec1 bound to a glycan on IgG4, or whether IgG4 reacted as a genuine antibody with a protein that happened to be a lectin. At that time, IgG glycosylation was generally assumed to be restricted to the Fc part and was of the complex glycan type. When we found that the binding of BanLec1 to IgG4 was restricted to the Fab part, we considered this to be a strong argument in favor of IgG4 binding as an antibody, rather than as a glycoprotein. However, these recent data make us uncertain about the interpretation of our earlier results, and research is currently carried out to further explore the glycosylation of IgG4.

4. Lectin-Driven B-Cell Activation

As already mentioned, because of the highly elevated levels of IgG4 in serum of IRD patients (typically more than 5 g/L), we consider it unlikely that the signal for activation of the IgG4+ B cell is a regular antigen. The above-mentioned indications of unusual glycosylation of the Fab of (part of) IgG4 suggest that, instead, an endogenous lectin may function as an alternative trigger of the BCR. The B cell would be activated by the lectin upon cross-linking of the BCR via its Fab glycan. In a way, the lectin would act as an endogenous superantigen, resulting in recruitment of IgG4-switched B cells in particular.

Support for an "oligomannose Fab glycan + endogenous lectin" scenario for IRD comes from the work of Stevenson

A hallmark of IRD is a substantially elevated serum level of IgG4, even if in some patients the level is in the normal range. The finding of large numbers of IgG4-positive plasma cells in the affected organ, makes it likely that this is the primary source of the increased IgG4 production. Yet, we want to address the quantitative aspect: does the histological analysis show a sufficient number of plasma cells to explain the IgG4 level in the serum? As detailed below, there may be cases where additional sites of IgG4 production are likely to be present. The following calculation depends on three estimates: (1) the daily production rate needed to maintain the IgG4 level in plasma, (2) the number of plasma cells in the affected organ and (3) the IgG4 production per plasma cell.

(1) The daily production rate of IgG for a 70 kg healthy adult is 2 g, which maintains a plasma level of 12 g/L (1200 mg/dL). The IgG4 level in IRD is on average 3 g/L, which is 2.6 g/L higher than the average normal level (0.4 g/L). Assuming a similar turnover, the increased IgG4 level requires a daily production of $2 \times 2.6/12 = 0.43$ gram "pathological" IgG4.

(2) The number of plasma cells (PCs) in the affected organ is not known, but an estimate can be made. In high-density areas of affected tissue, 100 $IgG4^+$ PCs per HPF (of 0.2 mm^2) is considered convincingly positive. This corresponds to 500 PCs/mm^2. Assuming a section thickness of 4 μm, this would correspond to a cell density of 125000 PCs/mm^3. However, the same PC (average diameter 12 μm) will be visible in 3 to 4 consecutive sections, so the actual density will be 37000 PCs/mm^3, or 37 million PCs/cm^3 tissue. Since the PCs are usually counted in areas selected for high PC numbers, this is likely to be an upper limit of the number of plasma cells per gram affected tissue.

(3) Ig production per PC has been estimated both from *in vitro* and from *in vivo* data. *In vitro*, a production rate of 1000 pg/PC/24 hrs has been reported [43], much higher than *in vivo*. The number of PCs in bone marrow, spleen, and mesenteric and inguinal lymph nodes (so, without the mucosal plasma cells and contributions of scattered plasma cells found all over the body) has been reported to be 25×10^9 [44], of which some 60% (15×10^9) produce IgG [45]. This would indicate a daily production rate of 2000×10^9 pg IgG/15×10^9 PCs, or 133 pg/PC/24 hrs.

(4) Combining the *in vivo* production rate with the plasma cell numbers, a tissue mass of 1 gram (containing 37×10^6 PCs) would produce $133 \times 37 \times 10^6 = 5 \times 10^9$ pg = 5 mg IgG4/day, which is 1.2% of the amount required to maintain an IgG4 level in plasma of 2.6 mg/mL, and the average level of "pathological" IgG4 is serum. This corresponds to 86 gram IgG4-rich tissue. Using the 7.5 times higher daily production rate derived from cultured cells, the value is 12 gram.

For a pancreas, which in pathological conditions may well be over 100 gram, the calculated required mass may seem to correspond reasonably well, considering that these calculations are based on imprecise estimates. However, the actual number of plasma cells in the affected organ is likely to be substantially lower than the number calculated from the counts in areas with high plasma cell density (which are the areas selected during the evaluation of the histological sections). Furthermore, IgG4 levels in some of the IRD patients are substantially higher than 3 g/L. Particularly in the latter patients, it is relevant to note that the IgG half-life shortens at high IgG levels. This obviously increases the number of plasma cells required. It is clear that we need better data, particularly on the number of IgG4 PC in a total affected tissue. Still, our calculations suggest that in some Patients, other tissue sources, without obvious pathology, might be important contributors to IgG4 production in IRD.

Box 3: A quantitative conundrum: the number of tissue-residing plasma cells is insufficient to explain the strongly elevated IgG4 level in plasma.
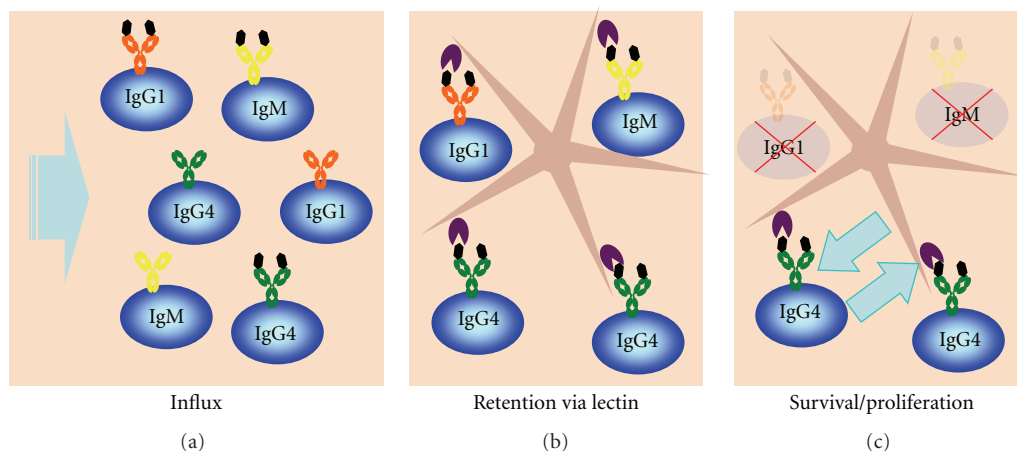
FIGURE 1: Proposed model of B-cell infiltration into affected tissue. (a) B cells from the circulation enter the inflamed tissue. (b) Differential glycosylation of IgG4-switched B cells allows retention and activation of this cell type via an as-yet-unidentified lectin on the stellate cell. (c) In this model, the local environment of the affected tissue will further promote survival/proliferation of IgG4-switched B cells due to a tolerogenic environment that may in part be created via signals from the IgG4 B cells themselves.

and coworkers on the activation of B cells in follicular lymphoma (FL). They found that the majority of FL cases involve a mutation resulting in the incorporation of a glycan acceptor site in the variable region of the Ig [25]. They showed that the binding of mannose-binding lectin to the FL cells triggers BCR-mediated signaling. These cells do not need to recognize antigen anymore to proliferate, giving them a major growth advantage. Furthermore, they found that the glycan attached to the Fab arm in these follicular lymphomas is terminated in oligomannose, an uncommon structure for human glycoproteins. Interestingly, cases of IRD are sometimes mistaken for FL due to similarities between these two diseases. In both IRD and FL, B-lymphocytes invade tissues and extensively proliferate there. However, in IRD the cells differentiate into plasma cells, whereas in FL they typically do not, making high serum levels of IgG4 a diagnostic marker to distinguish IRD from lymphoma. Furthermore, the IgG4 cells in IRD are of polyclonal origin, in contrast to the monoclonal B cells in FL.

5. A Model for IgG4 Plasma Cell Development in IRD

Based on the requirements for IgG4 production and the link between IgG4 and Fab oligomannose glycans, we propose a model in which B cells in circulation are entering into the inflamed tissue, where IgG4 cells are preferentially retained and differentiate in the tolerogenic environment of the lesion (Figure 1). The initial sequence is presumably a traumatic or infectious event that triggers a repair response. In case of pancreatitis, the local repair reaction in the tissue is orchestrated by the pancreatic stellate cell (see Box 2), which results in a storiform fibrotic reaction, one of the hallmarks of IRD. This results in the attraction and entrapment of circulating regulatory cells, including Tregs and Bregs, together creating a "tolerogenic" environment characterized amongst others by cytokines like IL-10 and IL-21, as well as vitamins A and/or D released from the activated stellate cells. In this environment, interaction of infiltrated IgG4-switched B cells with an as-yet-unidentified-lectin in combination with the tolerogenic conditions leads to differentiation of these B cells to plasma cells (but also see Box 3). On the other hand, proliferation/differentiation of other B cells is disfavored. Support in favor of this scheme comes from the IgG4 plasmacytosis seen in myofibroblastic tumors [26, 27]. This repair process should be self-limiting, but somehow this feedback is not working, and a feed-forward reaction is initiated. To stop this feed-forward loop, not only the B cells but also the stellate cells may need to receive signals to terminate their "repair mode" that sustains the local tolerogenic conditions. This could explain why anti-CD20 B cell depletion therapy with Rituximab needs to be perpetuated: it targets the B cells but leaves the pancreatic stellate cell unaffected. How can we regulate the regulators?

6. Perspectives

Some of the many questions that need to be answered are the following: (1) upon stimulation, is the stellate cell capable to initiate or increase the production of the hypothetical oligomannose-specific lectin, and of chemokine receptor ligands that attract Tfh and class-switched B cells? Possible involvement of BCR stimulation via oligomannose could be studied *in vitro*, for example, using BanLec-1, something that is currently being pursued in our lab; (2) what is the phenotype of the T cells (e.g., Tfh or Treg) and B cells (e.g., IL-10 producing and/or IgG4 switched; glycosylation status of BCR) within the affected organ?; (3) what is the relation between the lymphocytes in the tissue and those in the blood, particularly in relation to their chemokine receptors and (for the B cells) their Fab glycosylation profile?

References

[1] H. Umehara, K. Okazaki, Y. Masaki et al., "A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details," *Modern Rheumatology*, vol. 22, no. 1, pp. 1–14, 2012.

[2] H. Takahashi, M. Yamamoto, T. Tabeya et al., "The immuno-biology and clinical characteristics of IgG4 related diseases," *Journal of Autoimmunity*. In press.

[3] K. Okazaki, K. Uchida, M. Koyabu, H. Miyoshi, and M. Takaoka, "Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease," *Journal of Gastroenterology*, vol. 46, no. 3, pp. 277–288, 2011.

[4] T. Watanabe, K. Yamashita, S. Fujikawa et al., "Involvement of activation of toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis," *Arthritis and Rheumatism*, vol. 64, no. 3, pp. 914–924, 2012.

[5] R. Akitake, T. Watanabe, C. Zaima et al., "Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease," *Gut*, vol. 59, no. 4, pp. 542–545, 2010.

[6] A. Khosroshahi, D. B. Bloch, V. Deshpande, and J. H. Stone, "Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease," *Arthritis and Rheumatism*, vol. 62, no. 6, pp. 1755–1762, 2010.

[7] A. Khosroshahi, M. N. Carruthers, V. Deshpande, S. Unizony, D. B. Bloch, and J. H. Stone, "Rituximab for the treatment of IgG4-related disease: lessons from 10 consecutive patients," *Medicine*, vol. 91, no. 1, pp. 57–66, 2012.

[8] R. C. Aalberse, R. Van Der Gaag, and J. Van Leeuwen, "Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response," *Journal of Immunology*, vol. 130, no. 2, pp. 722–726, 1983.

[9] R. C. Aalberse, S. O. Stapel, J. Schuurman, and T. Rispens, "Immunoglobulin G4: an odd antibody," *Clinical and Experimental Allergy*, vol. 39, no. 4, pp. 469–477, 2009.

[10] P. Bruhns, B. Iannascoli, P. England et al., "Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses," *Blood*, vol. 113, no. 16, pp. 3716–3725, 2009.

[11] M. H. Tao, R. I. F. Smith, and S. L. Morrison, "Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation," *Journal of Experimental Medicine*, vol. 178, no. 2, pp. 661–667, 1993.

[12] S. M. Canfield and S. L. Morrison, "The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the CH2 domain and is modulated by

the hinge region," *Journal of Experimental Medicine*, vol. 173, no. 6, pp. 1483–1491, 1991.

[13] M. van der Neut Kolfschoten, J. Schuurman, M. Losen et al., "Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange," *Science*, vol. 317, no. 5844, pp. 1554–1557, 2007.

[14] J. S. Van Der Zee, P. Van Swieten, and R. C. Aalberse, "Inhibition of complement activation by IgG4 antibodies," *Clinical and Experimental Immunology*, vol. 64, no. 2, pp. 415–422, 1986.

[15] J. Punnonen, G. Aversa, B. G. Cocks et al., "Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 8, pp. 3730–3734, 1993.

[16] T. Platts-Mills, J. Vaughan, S. Squillace, J. Woodfolk, and R. Sporik, "Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study," *Lancet*, vol. 357, no. 9258, pp. 752–756, 2001.

[17] P. Jeannin, S. Lecoanet, Y. Delneste, J. F. Gauchat, and J. Y. Bonnefoy, "IgE versus IgG4 production can be differentially regulated by IL-10," *Journal of Immunology*, vol. 160, no. 7, pp. 3555–3561, 1998.

[18] Y. Zen, T. Fujii, K. Harada et al., "Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis," *Hepatology*, vol. 45, no. 6, pp. 1538–1546, 2007.

[19] H. Miyoshi, K. Uchida, T. Taniguchi et al., "Circulating naïve and $CD4^+CD25^{high}$ regulatory T cells in patients with autoimmune pancreatitis," *Pancreas*, vol. 36, no. 2, pp. 133–140, 2008.

[20] F. E. Lund and T. D. Randall, "Effector and regulatory B cells: modulators of $CD4^+$ T cell immunity," *Nature Reviews Immunology*, vol. 10, no. 4, pp. 236–247, 2010.

[21] W. Van de Veen, personal communication.

[22] P. G. Calkhoven, M. Aalbers, V. L. Koshte et al., "Relationship between IgG1 and IgG4 antibodies to foods and the development of IgE antibodies to inhalant allergens. II. Increased levels of IgG antibodies to foods in children who subsequently develop IgE antibodies to inhalant allergens," *Clinical and Experimental Allergy*, vol. 21, no. 1, pp. 99–107, 1991.

[23] V. L. Koshte, M. Aalbers, P. G. Calkhoven, and R. C. Aalberse, "The potent IgG4-inducing antigen in banana is a mannose-binding lectin, BanLec-I," *International Archives of Allergy and Immunology*, vol. 97, no. 1, pp. 17–24, 1992.

[24] V. L. Koshte, W. Van Dijk, M. E. Van der Stelt, and R. C. Allbers, "Isolation and characterization of BanLec-I, a mannoside-binding lectin from Musa paradisiac (banana)," *Biochemical Journal*, vol. 272, no. 3, pp. 721–726, 1990.

[25] V. Coelho, S. Krysov, A. M. Ghaemmaghami et al., "Glycosylation of surface Ig creates a functional bridge between human follicular lymphoma and microenvironmental lectins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 43, pp. 18587–18592, 2010.

[26] S. T. Saab, J. L. Hornick, C. D. Fletcher, S. J. Olson, and C. M. Coffin, "IgG4 plasma cells in inflammatory myofibroblastic tumor: inflammatory marker or pathogenic link," *Modern Pathology*, vol. 24, no. 4, pp. 606–612, 2011.

[27] H. Yamamoto, H. Yamaguchi, S. Aishima et al., "Inflammatory myofibroblastic tumor versus igg4-related sclerosing disease and inflammatory pseudotumor: a comparative clinicopathologic study," *American Journal of Surgical Pathology*, vol. 33, no. 9, pp. 1330–1340, 2009.

[28] M. Zaidan, P. Cervera-Pierot, S. De Seigneux et al., "Evidence of follicular T-cell implication in a case of IgG4-related systemic disease with interstitial nephritis," *Nephrology Dialysis Transplantation*, vol. 26, no. 6, pp. 2047–2050, 2011.

[29] A. Khosroshahi and J. H. Stone, "A clinical overview of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 57–66, 2011.

[30] J. R. Stone, "Aortitis, periaortitis, and retroperitoneal fibrosis, as manifestations of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 88–94, 2011.

[31] R. P. Sah and S. T. Chari, "Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 108–113, 2011.

[32] Y. Sato, K. Notohara, M. Kojima, K. Takata, Y. Masaki, and T. Yoshino, "IgG4-related disease: Historical overview and pathology of hematological disorders: review Article," *Pathology International*, vol. 60, no. 4, pp. 247–258, 2010.

[33] T. Kisseleva and D. A. Brenner, "Mechanisms of fibrogenesis," *Experimental Biology and Medicine*, vol. 233, no. 2, pp. 109–122, 2008.

[34] S. B. Lee and R. Kalluri, "Mechanistic connection between inflammation and fibrosis," *Kidney International*, vol. 78, no. 119, pp. S22–S26, 2010.

[35] A. Bellini and S. Mattoli, "The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses," *Laboratory Investigation*, vol. 87, no. 9, pp. 858–870, 2007.

[36] R. A. Reilkoff, R. Bucala, and E. L. Herzog, "Fibrocytes: emerging effector cells in chronic inflammation," *Nature Reviews Immunology*, vol. 11, no. 6, pp. 427–435, 2011.

[37] T. A. Wynn and L. Barron, "Macrophages: master regulators of inflammation and fibrosis," *Seminars in Liver Disease*, vol. 30, no. 3, pp. 245–257, 2010.

[38] M. V. Apte, P. S. Haber, T. L. Applegate et al., "Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture," *Gut*, vol. 43, no. 1, pp. 128–133, 1998.

[39] K. Wake, "Perisinusoidal stellate cells (fat-storing cells, interstitial cell, lipocytes), their related structure in and around the liver sinusoids, and vitamin A storing cells in extrahepatic organs," *International Review of Cytology*, vol. 66, pp. 303–353, 1980.

[40] D. F. Brandão, F. S. Ramalho, A. L. C. Martinelli, S. Zucoloto, and L. N. Z. Ramalho, "Relationship between plasma cells and hepatic stellate cells in autoimmune hepatitis," *Pathology Research and Practice*, vol. 206, no. 12, pp. 800–804, 2010.

[41] M. Apte, R. Pirola, and J. Wilson, "The fibrosis of chronic pancreatitis: new insights into the role of pancreatic stellate cells," *Antioxidants and Redox Signaling*, vol. 15, no. 10, pp. 2711–2722, 2011.

[42] P. Meister, E. Konrad, and N. Hoehne, "Incidence and histological structure of the storiform pattern in benign and malignant fibrous histiocytomas," *Virchows Archiv*, vol. 393, no. 1, pp. 93–101, 1981.

[43] V. Brinkmann, C. H. Heusser, J. Baer, E. Kilchherr, and F. Erard, "Interferon-alpha suppresses the capacity of T cells to help antibody production by human B cells," *Journal of Interferon Research*, vol. 12, no. 4, pp. 267–274, 1992.

[44] I. Turesson, "Distribution of immunoglobulin containing cells in human bone marrow and lymphoid tissues," *Acta Medica Scandinavica*, vol. 199, no. 4, pp. 293–304, 1976.

[45] R. Pabst, M. W. Russell, and P. Brandtzaeg, "Tissue distribution of lymphocytes and plasma cells and the role of the gut," *Trends in Immunology*, vol. 29, no. 5, pp. 206–208, 2008.

Review Article

Are Classification Criteria for IgG4-RD Now Possible? The Concept of IgG4-Related Disease and Proposal of Comprehensive Diagnostic Criteria in Japan

Kazuichi Okazaki[1] and Hisanori Umehara[2]

[1] *Division of Gastroenterology and Hepatology, The Third Department of Internal Medicine, Kansai Medical University, Shinmachi, Hirakata, Osaka 573-1197, Japan*

[2] *Division of Hematology and Immunology, Department of Internal Medicine, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Kanazawa, Japan*

Correspondence should be addressed to Kazuichi Okazaki, okazaki@hirakata.kmu.ac.jp

Recent studies suggest simultaneous or metachronous lesions in multiorgans characterized by elevated serum levels of IgG4 and abundant infiltration of IgG4-positive plasma cells with various degrees of fibrosis. Two Japanese research committees for IgG4-RD, one from fibrosclerosis (Okazaki team) and the other from lymph proliferation (Umehara team) supported by the "Research Program for Intractable Disease" of the Ministry of Health, Labor, and Welfare of Japan, have agreed with the unified nomenclature as "IgG4-RD" and proposed the comprehensive diagnostic criteria (CDC) for IgG4-RD. Validation of the CDC demonstrated satisfactory sensitivity for the practical use of general physicians and nonspecialists but low sensitivity in the organs to be difficult in taking biopsy specimens such as type1 autoimmune pancreatitis (IgG4-related AIP), compared with IgG4-related sialadenitis/dacryoadenitis (Mikulicz's disease) and IgG4-related kidney disease. Although the diagnostic criteria covering all IgG4-RD are hard to be established, combination with the CDC and organ-specific diagnostic criteria should improve sensitivity.

1. Introduction

Recent studies have suggested simultaneous or metachronous lesions in multiorgans characterized by elevated serum levels of IgG4 and abundant infiltration of IgG4-positive plasma cells with various degrees of fibrosis, which lead us to propose the concept of a systemic disease [1, 4, 10, 23, 24]. However, there are many synonyms suggesting a systemic disease, such as IgG4-related autoimmune disease [1], IgG4-related sclerosing disease [4], IgG4-related plasmacytic syndrome (SIPS) [23], IgG4-related multiorgan lymphoproliferative syndrome (IgG4-MOLPS) [10], and systemic IgG4-related disease, all of which may refer to the same conditions [24, 25] (Table 1). To simplify these conditions, members of two Japanese research committees for IgG4-related disease, one from view of fibrosclerosis (Chaired by Prof. Okazaki) [24] and the other from lymph proliferation (Chaired by

Professor. Umehara H) [25], both of which are supported by the "Research for Intractable Disease" Program from the Ministry of Health, Labor, and Welfare of Japan, have agreed with unification of different nomenclatures as "IgG4-related disease (IgG4-RD)" and proposed the comprehensive diagnostic criteria (CDC) for IgG4-RD [15]. As it still remains unclear whether pathogenetic mechanisms in each involved organ-are same or not, the term IgG4-RD was appointed as minimally reflecting these conditions to avoid misdiagnosis of malignancy as much as possible.

2. The Concept of IgG4-Related Disease

The two Japanese research committees independently analyzed the clinical features and conditions of IgG4-RD and finally resulted in the following consensus with close collaboration [15, 24, 25]. (1) Patients with IgG4-RD show

TABLE 1: Nomenclatures of IgG4-related conditions.

Nomenclature	Authors	(year)
IgG4-related autoimmune disease	Kamisawa et al. [1]	(2003)
IgG4-associated multifocal systemic fibrosis	van der Vliet and Perenboom [2]	(2004)
IgG4-related systemic disease	Kamisawa et al. [3]	(2004)
IgG4-related sclerosing disease	Kamisawa et al. [4–7]	(2006)
Hyper-IgG4 disease	Neild et al. [8]	(2006)
IgG4-related disease	Zen et al. [9]	(2007)
Systemic IgG4 plasmacytic syndrome (SIPS)	Masaki et al. [10]	(2009)
IgG4-related multiorgan Lymphoproliferative syndrome (IgG4-MOLPS)	Masaki et al. [10]	(2009)
IgG4-associated disease	Geyer et al. [11]	(2010)

diffuse/focal organ enlargement, with mass-forming or nodular/thickened lesions in various organs, including the central nervous system [26], lachrymal/salivary glands [10, 23], thyroid gland [27, 28], lungs [29], pancreas [30, 31], biliary duct [32], liver [33], gastrointestinal tract [34, 35], kidneys [36], prostate gland [37], retroperitoneum [38], skin [39], lymph nodes [5, 40, 41], and artery [42, 43]. These conditions are quite similar to multifocal idiopathic fibrosclerosis (MIF) [44]. (2) These multiorgan lesions may occur synchronously or metachronously, with the prominent infiltration of lymphocytes and IgG4-positive plasmacytes with fibrosis. (3) IgG4-RD mainly affects middle-aged to elderly men except for IgG4-related dacryoadenitis/sialadenitis. Although clinical symptoms depending on involved organs are relatively mild, some patients develop serious complications such as obstructive jaundice due to hepatic, gallbladder, or pancreatic lesions; hydronephrosis due to retroperitoneal fibrosis; respiratory symptoms due to pulmonary lesions. (4) Steroid treatment is effective in many patient with IgG4-RD. However, prognosis and risk factors of recurrence still remain unclear. (5) Although the infiltration of IgG4-positive cells and increased serum concentrations of IgG4 characteristic of IgG4-RD, the severity of fibrosis is dependent on the individual organs involved. For example, storiform fibrosis and obliterative phlebitis are characteristic of pancreatic, biliary tract, and retroperitoneal lesions but are rarely observed in lachrymal/salivary glands or lymph nodes.

3. IgG4-Related Disease (IgG4-RD) as the Comprehensive Nomenclature [24, 25]

In addition to MIF, there are many synonyms, such as IgG4-related autoimmune disease [1], "IgG4-related sclerosing disease" [4], IgG4-related plasmacytic disease (SIPS) [23], and "IgG4 + sMOLPS" [10], all of which may refer to the same conditions. It has been debated which one is the most appropriate. Storiform fibrosis and obliterative phlebitis are

characteristic of biliopancreatic, retroperitoneal, and renal lesions, but rarely observed in lachrymal/salivary glands and lymphnodes [24, 25]. Then, the nomenclature of "IgG4-related sclerosing disease" is mainly based on the fibrous swollen organs, whereas those of "IgG4-SIPS" and "IgG4-MOLPS" are based on lymphoplasmacytic proliferation and swollen lymph nodes without fibrosis [24, 25]. Although most patients have multiorgan lesions synchronously or metachronously, about 10–20% of the patients show a solitary organ involved without confirming other organ involvement [24, 25]. Therefore, it is unclear whether the pathogenetic mechanism is same among individual organs or not. In addition to IgG4-RD, IgG4-associate conditions such as high serum levels of IgG4 or abundant infiltration of IgG4-positive cells were reported in some patients with malignancy; pancreatic [6, 45], biliary [46] and salivary cancer [47], gastrointestinal sarcoma [48], and ocular adnexal lymphoma [49–51]. Therefore, the term "systemic" may lead us to misdiagnosis of other organ lesions showing IgG4-related conditions in cases of malignancy [51]. Based on these findings, the members of Umehara and Okazaki teams have agreed that the term "IgG4-related disease" is appointed as minimally accepting these conditions at this moment.

4. Comprehensive Diagnostic Criteria for IgG4-RD [15, 24, 25]

The patients with IgG-4-related disease show organ enlargement or nodular/hyperplastic lesions in organs in the entire body, synchronously or metachronously, due to the prominent infiltration and fibrosis of lymphocytes and plasmacytes; however, the causes of the disease are still not clear. The organs known to be affected include the central nervous system, lacrimal/salivary glands, thyroid gland, lungs, pancreas, biliary duct, liver, gastrointestinal tracts, kidneys, prostate gland, retroperitoneum, skin, arteries, and lymph nodes. Although it remains unclear whether this disease is the same as multifocal fibrosclerosis, that is a possibility. Clinical symptoms vary depending on the organ in which the lesions are located, which suggests that it is hard to establish criteria covering all patients with IgG4-RD. Therefore, specific diagnostic criteria are required for each involved organ such IgG4-related Mikulicz's disease (IgG4-related dacryoadenitis/sialadenitis [12] (Table 2), type 1 AIP (IgG4-related pancreatitis) [13] (Table 3), and IgG4-related kidney disease [14, 41] (Table 4). However, these organ-specific criteria do not cover other organs or are not familiar to general clinicians and specialists. Moreover, to avoid misdiagnosis of malignancy, all physicians have to know this emerging disease entity and can make a diagnosis of IgG4-RD. Therefore, the CDC for IgG4-RD, containing three major criteria (clinical, hematological and histopathological examinations), have been proposed for practical use of general physicians and nonspecialist [15] (Table 5). Although sensitivity of the CDC for definitive IgG4-RD is low in the organs to be difficult in taking biopsy specimens, it can detect possible cases of IgG4-RD. In the probable or possible cases, organ specific criteria should be used concurrently.

TABLE 2: Diagnostic criteria for IgG4+ Mikulicz's disease [12] (approved by the Japanese Society for Sjögren's Syndrome, 2008).

(1) Symmetrical swelling of at least 2 pairs of lachrymal, parotid, and submandibular glands continuing for more than 3 months,

(2) elevated serum IgG4 (>135 mg/dL),

or

(3) histopathological features including lymphocyte and IgG4+ plasma cell infiltration (IgG4+ plasma cells/IgG+ plasma cells > 50%) with typical tissue fibrosis or sclerosis.

Differential diagnosis is necessary from other disorders, including sarcoidosis, Castleman's disease, Wegener's granulomatosis, lymphoma, and cancer. Although the diagnostic criteria for Sjögren's syndrome (SS) may also include some patients with IgG4+ Mikulicz's disease, the clinicopathological conditions of patients with typical SS and IgG4+ Mikulicz's disease are different.

(1) *Clinical Examination.* Physical examinations and imaging on US/CT/MRI can show the characteristic diffuse/localized swelling, masses, or thickness in single or multiple organs (Figure 1).

(2) *Immunological Examination*

(a) *Increase of Serum Levels of IgG4.* The cutoff value for serum IgG4 concentration, 135 mg/dL, was based on receiver operating characteristic (ROC) curves, and its validity was confirmed in patients with autoimmune pancreatitis [7] (Table 6). In patients with single-organ involvement and serum IgG4 concentration less than 135 mg/dL, the IgG4/IgG ratio may be helpful in making a diagnosis.

However, elevated IgG4 may be also observed in other diseases (e.g., atopic dermatitis, pemphigus, asthma, and multicentric Castleman's disease), especially in about 10% of malignancy, which suggests that high serum IgG4 is not necessarily specific marker of IgG4-RD [6]. Although a high cutoff value with >270 mg/dL of IgG4 increases specificity but decreased sensitivity of IgG4-RD differing from pancreatic cancer [45]. Therefore, at present, the significance of elevated IgG4 in the pathogenesis/pathophysiology of IgG4-RD still remains unknown.

(b) *Other Immunological Markers.* In addition to increased serum levels of IgG4, high serum levels of polyclonal γ-globulin, IgG, and IgE are often, and hypocomplementemia may occur [52]. As these markers are less sensitive for IgG4-RD, they are not included as a diagnostic criterion.

(3) *Histopathologic Examination.* Although tissue biopsies are difficult to obtain from some organs, including the pancreas, retroperitoneum and ocular cavity, histopathological examination is important.

(a) *Marked Lymphocyte and Plasmacyte Infiltration and Fibrosis.* Storiform or swirling fibrosis or obliterative phlebitis is Characteristic of IgG4-RD and may be important in its diagnosis.

(b) *Infiltration of IgG4-Positive Plasma Cells.* IgG4/IgG-positive cells more than 40% [53] or 50% [12] have been reported in lymphnodes of the patients with IgG4-RD. On the other hand, more than 10 IgG4-positive plasma cells are recommended that in diagnosis of type 1 AIP [13]. Based on these findings, the CDC for IgG4-RD recommend both the ratio of IgG4/IgG-positive cells >40% and infiltration of >10 IgG4-positive plasma cells/HPF for the definitive diagnosis [15]. Eosinophilic infiltration is often observed along with infiltration of IgG4-positive cells. It is noted that reactive infiltration of IgG4-positive cells and fibrosis may be observed in various diseases and clinical conditions, such as rheumatoid synovitis, inflammatory oral and skin lesions, and around cancer. However, it is noted that some additional immune-mediated conditions with increased serum interleukin-6 (IL-6) such as multicentric Castleman's disease may show elevated serum IgG4 and/or IgG4+/IgG+ plasma cell ratios >40%.

(4) *Prohibition of Facile Steroid Treatment in the CDC for IgG4-RD.* Patients with malignant lymphoma or paraneoplastic lesions can sometimes be improved by steroid administration. Therefore, steroid trials should be strictly avoided. Efforts should be made to collect tissue samples for diagnosis. However, patients having disease in organs difficult to biopsy, such as the pancreas, retroperitoneum, and pituitary, and respond to steroids may possibly have IgG4-RD. In accordance with the guidelines for treatment of autoimmune pancreatitis, patients should be started on 0.5-0.6 mg/kg/day/prednisolone. If patients do not respond to the initial steroid therapy, the diagnosis should be reviewed again.

(5) *Diseases to be Excluded or Differentiated*

(a) *Malignancies (e.g., Cancer, Lymphoma).* In cases of malignancy in the involved organs, it is essential to determine whether malignant cells are present histopathologically.

(b) *Similar Diseases.* Other similar benign diseases including Sjögren's syndrome, primary sclerosing cholangitis, multicentric Castleman's disease, idiopathic retroperitoneal fibrosis, Wegener's granulomatosis, sarcoidosis, and Churg-Strauss syndrome should be differentially diagnosed using the diagnostic criteria for each disease. It is noted that multicentric Castleman's disease, one of hyper IL-6 syndromes should be excluded from IgG4-RD, even if the CDC for IgG4-RD are fulfilled.

Table 3: International Consensus Diagnostic Criteria (ICDC) for autoimmune pancreatitis [13].

Diagnosis	Primary basic for diagnosis	Imaging Evidence	Collateral evidence
Definitive type 1 AIP	Histology	Typical/indeterminate	Histologically confirmed LPSP (level 1 H)
	Imaging	Typical	Any non-D level 1/level 2
	Imaging	Indeterminate	Two or more from level 1 (+level 2 D*)
	Response to steroid	Indeterminate	Level 1 S/OOI + Rt or level 1 D + level 2 S/OOI/H + Rt
Probable type 1 AIP		Indeterminate	Level 2 S/OOI/H + Rt

*Level 2 D is counted as level 1 in this setting.

	Criterion	Level 1	Level 2
P	Parenchymal imaging	Typical: diffuse enlargement with delayed enhancement (sometimes associated with rim-like enhancement)	Indeterminate (including atypical‡): segmental/focal enlargement with delayed enhancement
D	Ductal imaging (ERP)	Long (>1/3 length of the main pancreatic duct) or multiple strictures without marked upstream dilatation	Segmental/focal narrowing without marked upstream dilatation (duct size, <5 mm)
S	Serology	IgG4, >2× upper limit of normal value a or b	IgG4, 1-2× upper limit of normal value a or b
OOI	Other organ involvement	(a) Histology of extrapancreatic organs: any three of the following: (1) marked lymphoplasmacytic infiltration with fibrosis and without granulocytic infiltration; (2) storiform fibrosis; (3) obliterative phlebitis; (4) abundant (>10 cells/HPF) IgG4-positive cells. (b) Typical radiological evidence at least one of the following: (1) segmental/multiple proximal (hilar/intrahepatic) or proximal and distal bile duct stricture; (2) retroperitoneal fibrosis;	(a) Histology of extrapancreatic organs including endoscopic biopsies of bile duct‡: both of the following: (1) marked lymphoplasmacytic infiltration without granulocytic infiltration; (2) abundant (>10 cells/HPF) IgG4-positive cells. (b) Physical or radiological evidence: at least one of the following; (1) symmetrically enlarged salivary/lachrymal glands; (2) radiological evidence of renal involvement described in association with AIP.
H	Histology of the pancreas	LPSP (core biopsy/resection): at least 3 of the following: (1) periductal lymphoplasmacytic infiltration without granulocytic infiltration; (2) obliterative phlebitis; (3) storiform fibrosis; (4) abundant (>10 cells HPF) IgG4-positive cells.	LPSP (core biopsy): any 2 of the following: (1) periductal lymphoplasmacytic infiltrate without granulocytic infiltration; (2) obliterative phlebitis; (3) storiform fibrosis; (4) abundant (>10 cells/HPF) IgG4-positive cells.
Response to steroid (Rt)*	Diagnostic steroid trial	Rapid (≤2 wk) radiologically demonstrable resolution or marked improvement in pancreatic/extrapancreatic manifestations	

Table 4: Diagnostic criteria for IgG4-related kidney disease [14].

(1) Presence of some kidney damage, as manifested by abnormal urinalysis or urine marker(s) or decreased kidney function with either elevated serum IgG or IgE or hypocomplementemia

(2) Abnormal renal radiologic findings:

   (a) multiple low-density lesions on enhanced computed tomography;

   (b) diffuse kidney enlargement;

   (c) hypovascular solitary mass in the kidney;

   (d) hypertrophic lesion of the renal pelvic wall without irregularities of the renal pelvic surface.

(3) Elevated serum IgG4 level (>135 mg/dL)

(4) Histological findings in the kidney:

   (a) dense lymphoplasmacytic infiltration by >10 IgG4-positive plasma cells/high power field (HPF) and/or IgG4+/IgG+ positive plasma cells > 40%;

   (b) characteristic (sclero-) fibrosis surrounding nests of lymphocytes and/or plasma cells;

(5) Histological findings in extrarenal organ(s):

   dense lymphoplasmacytic infiltration by >10 IgG4-positive plasma cells/HPF and/or IgG4/IgG-positive plasma cells > 40%

Definite: (1) + (3) + (4) (a), (b)

           (2) + (3) + (4) (a), (b)

           (2) + (3) + (5)

           (1) + (3) + (4) (a) + (5)

Probable: (1) + (4) (a), (b)

           (2) + (4) (a), (b)

           (2) + (5)

           (3) + (4) (a), (b)

Possible: (1) + (3)

           (2) + (3)

           (1) + (4) (a)

           (2) + (4) (a)

Appendix:

   (1) Clinically and histologically, the following diseases should be excluded:

      Wegener's granulomatosis, Churg-Strauss syndrome, and extramedullary plasmacytoma.

   (2) Radiologically, the following diseases should be excluded:

      Malignant lymphoma, urinary tract carcinomas, renal infarction, and pyelonephritis.

(Rarely, Wegener's granulomatosis, sarcoidosisand metastatic carcinoma)

Table 5: Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011 [15].

[Concept]

IgG4-related disease (IgG4-RD) shows organ enlargement or nodular/hyperplastic lesions in various organs concurrently or metachronously, due to marked infiltration of lymphocytes and IgG4-positive plasma cells, as well as fibrosis of unknown etiology. IgG4-RD affects various organs, including the pancreas, bile duct, lacrimal gland, salivary gland, central nervous system, thyroid, lung, liver, gastrointestinal tract, kidney, prostate, retroperitoneum, arteries, lymph nodes, skin, and breast. Although many patients with IgG4-RD have lesions in several organs, either synchronously or metachronously, others show involvement of a single organ. Clinical symptoms vary depending on the affected organ, and some patients may experience serious complications, such as obstruction or compression symptoms due to organomegaly or hypertrophy and organ dysfunction caused by cellular infiltration or fibrosis. Steroid therapy is often effective.

[Comprehensive clinical diagnostic criteria for IgG4-RD, 2011]

(1) Clinical examination shows characteristic diffuse/localized swelling or masses in single or multiple organs.

(2) Hematological examination shows elevated serum IgG4 concentrations (≥135 mg/dL).

(3) Histopathologic examination shows;

   (1) marked lymphocyte and plasmacyte infiltration and fibrosis

   (2) infiltration of IgG4-positive plasma cells: ratio of IgG4/IgG positive cells > 40% and > 10 IgG4-positive plasma cells/HPF.

Definite: (1) + (2) + (3), Probable: (1) + (3), Possible: (1) + (2)

However, it is important to differentiate IgG4-RD from malignant tumors of each organ (e.g. cancer, lymphoma) and similar diseases (e.g. Sjögren's syndrome, primary sclerosing cholangitis, Castleman's disease, secondary retroperitoneal fibrosis, Wegener's granulomatosis, sarcoidosis, and Churg-Strauss syndrome) by additional histopathological examination. Even when patients cannot be diagnosed using the CCD criteria, they may be diagnosed using organ-specific diagnostic criteria for IgG4RD.

TABLE 6: Sensitivity and specificity of serum levels of IgG4 in patients with type 1 AIP.

	Cut-off	Sensitivity		Specificity	
	mg/dL	n	Median/(range)	n	(vs cancer)
Japan	135				
Okazaki et al. [16]		71	80% 410 (3–3670)	101	98%
Okazaki et al. [17]		52	73% 505 (43–1540)		NS
Kawa et al. [18]		64	92% 618 (8–2855)	80	98%
Korea	135				
Choi et al. [19]		30	73% 473 (10–1764)	76	99%
USA	140				
Ghazale et al. [20]		45	76% 550 (16–2890)	135	90%
Raina et al. [21]		26	44% (8–825)	NS	
Italy	135				
	(focal)	55	66% 267		NS
Frulloni et al. [22]	(diffuse)	32	27% 78		

TABLE 7: Validation of a combination of CDC and organ-specific criteria for type 1 AIP.

Compared with pancreas cancer, the sensitivity of comprehensive criteria for definite/probable AIP was 0%, but 78% for possible AIP, and specificity was 100% in any groups. Although it is hard to take an enough size of specimen in diagnosis of AIP malignancy can be usually denied by EUS-FNA. Therefore, the CDC are enough for detecting possible AIP, but not for definite/probable AIP.

AIP (n = 60) PaCa (n = 17) Total (n = 77)	JPS 2006	ICDC for type 1 AIP	CDC for IgG4-RD	
Diagnosis of AIP	Definite AIP	Definite/probable AIP	Definite/probable AIP	Possible
sensitivity	70%	97%	0%	78%
specificity	100%	100%	100%	100%
PPV	100%	100%	0%	100%
NPV	49%	8%	100%	57%
accuracy	77%	95%	22%	83%

PaCa: pancreas cancer, PPV: positive predictive value, NPV: negative predictive value.
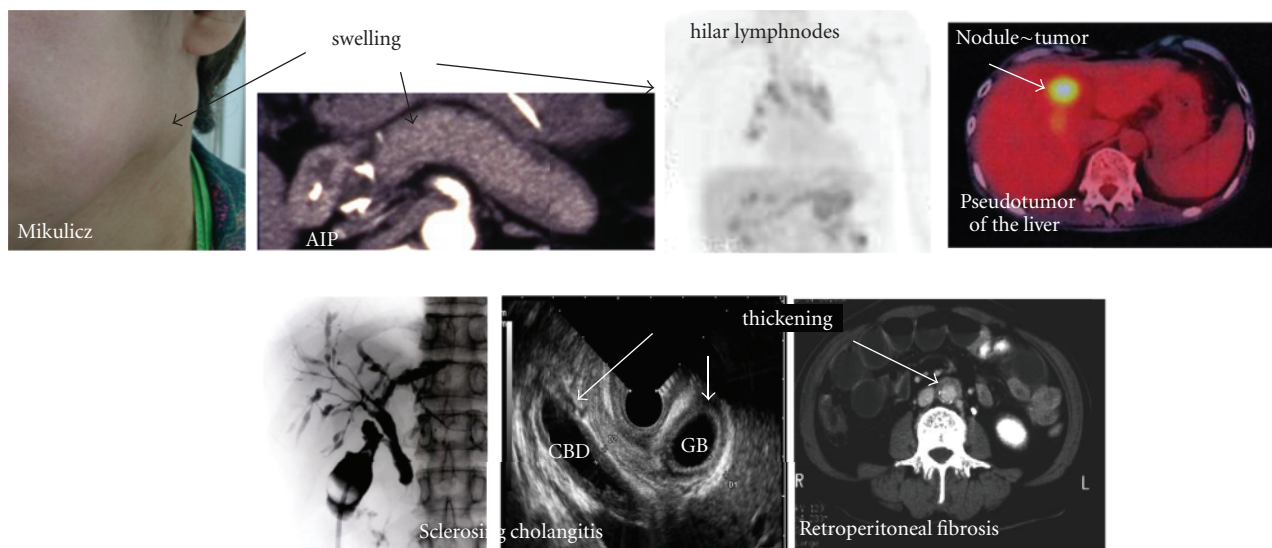

FIGURE 1: Clinical findings of IgG4-related disease. Physical examinations and imaging on US/CT/MRI can show the characteristic diffuse/localized swelling, masses, or thickness in single or multiple organs.
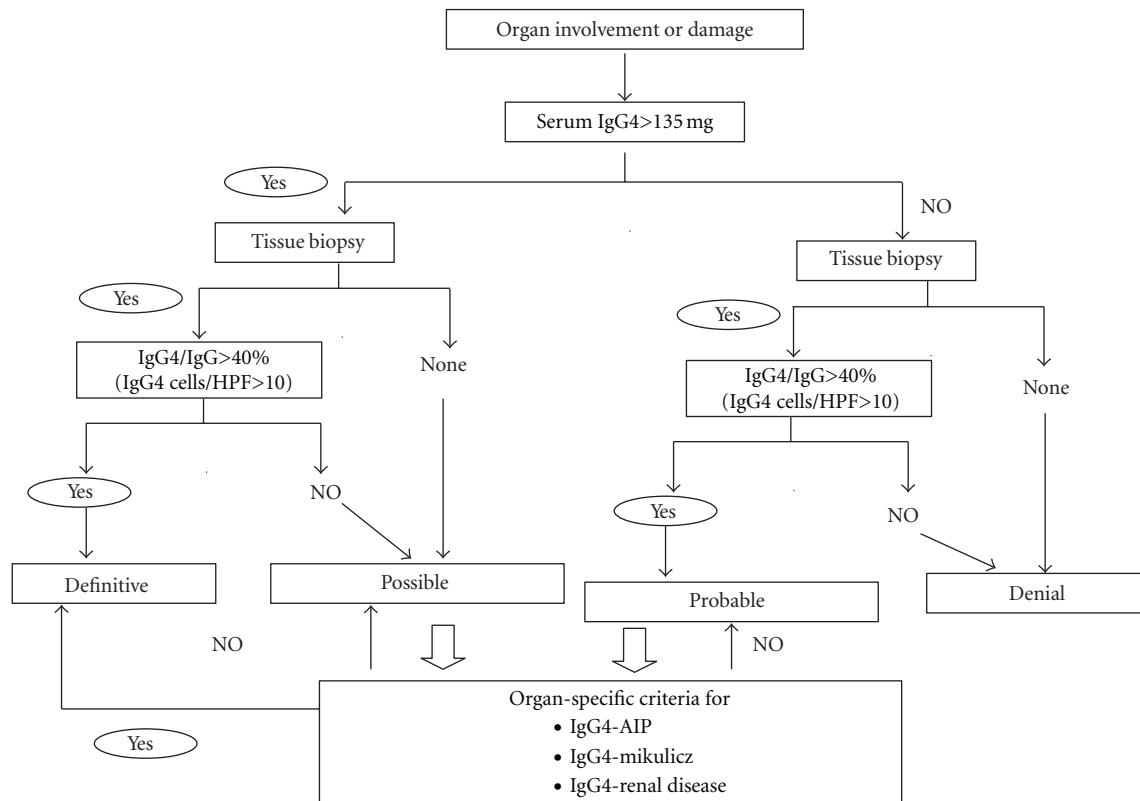
Figure 2: Diagnostic algorithm for IgG4-RD in Japan.

5. Sensitivity and Specificity of the CDC Criteria and Diagnostic Algorithm for IgG4-RD

The sensitivity of CDC for definitive/probable IgG4-RD is satisfactory in IgG4-related MD [12] and IgG4-related KD [14], but not in type 1 AIP [6, 13]. The major reason of low sensitivity in type 1 AIP is that enough biopsy samples of the pancreas are not easily obtained in most of these patients. In addition, endoscopic ultrasonography (EUS), guide fine needle aspiration (FNA), is available in a few of institutes in Japan, for examples only 16 of 226 (7%) board member institutes in Kink district of Japan Gastroenterological Endoscopy Society (JGES). On the other hand, the sensitivity of the CDC for possible IgG4-RD is satisfactory in type 1 AIP (Table 7). In contrast, patients with type 1 AIP could not be diagnosed by the comprehensive diagnostic criteria (0%) for definite, because biopsies could not be obtained from most of these patients. Therefore, combination of the CDC and organ-specific criteria should increase the sensitivity of diagnosis, even in the possible cases of IgG4-RD.

Based on these findings, a diagnostic algorithm for IgG4-RD in combination with the CDC and other organ-specific criteria has been proposed, although they have a limitation to the utility of the criteria proposed [15] (Figure 2). In patients with (a) organ enlargement, mass or nodular lesions, or organ dysfunction, performing of both (b) measurement of serum IgG4 and (c) tissue biopsy is recommended. In the cases with >135 mg/dL of IgG4, diagnostic histopathological findings of >10 IgG4 cells/HPF and an IgG4/IgG cell ratio >40 can diagnose them as definitive AIP. In possible or probable cases fulfilling criterion (a) with (b), or (c), organ-specific criteria for each disease should be applied. It is important to differentiate IgG4-RD from malignant tumors of each organ (e.g., cancer, lymphoma) and similar diseases (e.g., Sjögren's syndrome, primary sclerosing cholangitis, Castleman's disease, secondary retroperitoneal fibrosis, Wegener's granulomatosis, sarcoidosis, and Churg-Strauss syndrome) by additional histopathological examination. Future studies including other organ diseases similar to IgG4-RD are needed to establish the diagnostic efficacy of CDC.

6. Conclusion

"All Japan Research Team for IgG4-RD" unified the nomenclatures as "IgG4-related disease (IgG4-RD)" and proposed the comprehensive diagnostic criteria (CDC) for IgG4-RD. The CDC for IgG4-RD was made for the practical use and for general physicians to differentiate IgG4-RD from malignancy or similar diseases as much as possible. Although sensitivity of the CDC for definitive IgG4-RD is low in the organs to

be difficult in taking biopsy specimens, it can detect possible cases of IgG4-RD. In the probable or possible cases, organ-specific criteria should be used concurrently.

Authors' Contribution

K. Okazaki and H. Umehara declare that they equally contributed to this work.

Disclosure

Acknowledgments

References

[1] T. Kamisawa, N. Funata, Y. Hayashi et al., "A new clinicopathological entity of IgG4-related autoimmune disease," *Journal of Gastroenterology*, vol. 38, no. 10, pp. 982–984, 2003.

[2] H. J. van der Vliet and R. M. Perenboom, "Multiple pseudotumors in IgG4-associated multifocal systemic fibrosis," *Annals of Internal Medicine*, vol. 141, no. 11, pp. 896–897, 2004.

[3] T. Kamisawa, N. Funata, and Y. Hayashi, "Lymphoplasmacytic sclerosing pancreatitis is a pancreatic lesion of IgG4-related systemic disease," *The American Journal of Surgical Pathology*, vol. 27, no. 8, pp. 1119–1127, 2003.

[4] T. Kamisawa and A. Okamoto, "Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 613–625, 2006.

[5] T. Kamisawa, H. Nakajima, N. Egawa, N. Funata, K. Tsuruta, and A. Okamoto, "IgG4-related sclerosing disease incorporating sclerosing pancreatitis, cholangitis, sialadenitis and retroperitoneal fibrosis with lymphadenopathy," *Pancreatology*, vol. 6, no. 1-2, pp. 132–137, 2006.

[6] T. Kamisawa, P. Y. Chen, Y. Tu et al., "Pancreatic cancer with a high serum IgG4 concentration," *World Journal of Gastroenterology*, vol. 12, no. 38, pp. 6225–6228, 2006.

[7] K. Okazaki, S. Kawa, T. Kamisawa et al., "Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 626–631, 2006.

[8] G. H. Neild, M. Rodriguez-Justo, C. Wall, and J. O. Connolly, "Hyper-IgG4 disease: report and characterisation of a new disease," *BMC Medicine*, vol. 4, article 23, 2006.

[9] Y. Zen, T. Fujii, Y. Sato, S. Masuda, and Y. Nakanuma, "Pathological classification of hepatic inflammatory pseudotumor with respect to IgG4-related disease," *Modern Pathology*, vol. 20, no. 8, pp. 884–894, 2007.

[10] Y. Masaki, L. Dong, N. Kurose et al., "Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders," *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1310–1315, 2009.

[11] J. T. Geyer, J. A. Ferry, N. L. Harris et al., "Chronic sclerosing sialadenitis (Küttner tumor) is an IgG4-associated disease," *American Journal of Surgical Pathology*, vol. 34, no. 2, pp. 202–210, 2010.

[12] Y. Masaki, S. Sugai, and H. Umehara, "IgG4-related diseases including Mikulicz's disease and sclerosing pancreatitis: diagnostic insights," *Journal of Rheumatology*, vol. 37, no. 7, pp. 1380–1385, 2010.

[13] T. Shimosegawa, S. T. Chari, L. Frulloni et al., "International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the international association of pancreatology," *Pancreas*, vol. 40, no. 3, pp. 352–358, 2011.

[14] M. Kawano, T. Saeki, H. Nakashima et al., "Proposal for diagnostic criteria for IgG4-related kidney disease," *Experimental Nephrology*, vol. 15, no. 5, pp. 615–626, 2011.

[15] H. Umehara, K. Okazaki, Y. Masaki et al., "Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011," *Modern Rheumatology*, vol. 22, no. 1, pp. 21–30, 2012.

[16] K. Okazaki, S. Kawa, T. Kamisawa et al., "Japanese clinical guidelines for autoimmune pancreatitis," *Pancreas*, vol. 38, no. 8, pp. 849–866, 2009.

[17] K. Okazaki, K. Uchida, M. Matsushita, and M. Takaoka, "How to diagnose autoimmune pancreatitis by the revised Japanese clinical criteria," *Journal of Gastroenterology*, vol. 42, supplement 18, pp. 32–38, 2007.

[18] S. Kawa, K. Okazaki, T. Kamisawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: II. Extrapancreatic lesions, differential diagnosis," *Journal of Gastroenterology*, vol. 45, no. 4, pp. 355–369, 2010.

[19] E. K. Choi, M. H. Kim, T. Y. Lee et al., "The sensitivity and specificity of serum immunoglobulin G and immunoglobulin G4 levels in the diagnosis of autoimmune chronic pancreatitis: Korean experience," *Pancreas*, vol. 35, no. 2, pp. 156–161, 2007.

[20] A. Ghazale, S. T. Chari, T. C. Smyrk et al., "Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1646–1653, 2007.

[21] A. Raina, D. Yadav, A. M. Krasinskas et al., "Evaluation and management of autoimmune pancreatitis: experience at a large us center," *American Journal of Gastroenterology*, vol. 104, no. 9, pp. 2295–2306, 2009.

[22] L. Frulloni, C. Scattolini, M. Falconi et al., "Autoimmune pancreatitis: differences between the focal and diffuse forms in 87 patients," *American Journal of Gastroenterology*, vol. 104, no. 9, pp. 2288–2294, 2009.

[23] M. Yamamoto, M. Ohara, C. Suzuki et al., "Elevated IgG4 concentrations in serum of patients with Mikulicz's disease," *Scandinavian Journal of Rheumatology*, vol. 33, no. 6, pp. 432–433, 2004.

[24] K. Okazaki, K. Uchida, H. Miyoshi, T. Ikeura, M. Takaoka, and A. Nishio, "Recent concept of autoimmune pancreatitis and IgG4-related disease," *Clinical Reviews in Allergy and Immunology*, vol. 41, no. 2, pp. 126–138, 2010.

[25] H. Umehara, K. Okazaki, Y. Masaki et al., "A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details," *Modern Rheumatology*, vol. 22, no. 1, pp. 1–14, 2012.

[26] A. Shimatsu, Y. Oki, I. Fujisawa, and T. Sano, "Pituitary and stalk lesions (Infundibulo-hypophysitis) associated with

immunoglobulin G4-related systemic disease: an emerging clinical entity," *Endocrine Journal*, vol. 56, no. 9, pp. 1033–1041, 2009.

[27] M. Dahlgren, A. Khosroshahi, G. P. Nielsen, V. Deshpande, and J. H. Stone, "Riedel's thyroiditis and multifocal fibrosclerosis are part of the IgG4-related systemic disease spectrum," *Arthritis Care and Research*, vol. 62, no. 9, pp. 1312–1318, 2010.

[28] M. Kojima, M. Hirokawa, H. Kuma et al., "Distribution of IgG4- and/or IgG-positive plasma cells in Hashimoto's thyroiditis: an immunohistochemical study," *Pathobiology*, vol. 77, no. 5, pp. 267–272, 2010.

[29] Y. Zen, D. Inoue, A. Kitao et al., "IgG4-related lung and pleural disease: a clinicopathologic study of 21 cases," *American Journal of Surgical Pathology*, vol. 33, no. 12, pp. 1886–1893, 2009.

[30] K. Yoshida, F. Toki, T. Takeuchi, S. I. Watanabe, K. Shiratori, and N. Hayashi, "Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis," *Digestive Diseases and Sciences*, vol. 40, no. 7, pp. 1561–1568, 1995.

[31] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[32] T. Nakazawa, H. Ohara, H. Sano et al., "Cholangiography can discriminate sclerosing cholangitis with autoimmune pancreatitis from primary sclerosing cholangitis," *Gastrointestinal Endoscopy*, vol. 60, no. 6, pp. 937–944, 2004.

[33] S. Eguchi, M. Takatsuki, M. Hidaka et al., "De novo autoimmune hepatitis after living donor liver transplantation is unlikely to be related to immunoglobulin subtype 4-related immune disease," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 7, part 2, pp. e165–e169, 2008.

[34] T. Fujita, T. Ando, M. Sakakibara, W. Hosoda, and H. Goto, "Refractory gastric ulcer with abundant IgG4-positive plasma cell infiltration: a case report," *World Journal of Gastroenterology*, vol. 16, no. 17, pp. 2183–2186, 2010.

[35] K. Ueno, T. Watanabe, Y. Kawata et al., "IgG4-related autoimmune pancreatitis involving the colonic mucosa," *European Journal of Gastroenterology and Hepatology*, vol. 20, no. 11, pp. 1118–1121, 2008.

[36] Y. Uchiyama-Tanaka, Y. Mori, T. Kimura et al., "Acute tubulointerstitial nephritis associated with autoimmune-related pancreatitis," *American Journal of Kidney Diseases*, vol. 43, no. 3, pp. e18–e25, 2004.

[37] I. Nishimori, T. Kohsaki, S. Onishi et al., "IgG4-related autoimmune prostatitis: two cases with or without autoimmune pancreatitis," *Internal Medicine*, vol. 46, no. 24, pp. 1983–1989, 2007.

[38] H. Hamano, S. Kawa, Y. Ochi et al., "Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis," *The Lancet*, vol. 359, no. 9315, pp. 1403–1404, 2002.

[39] A. Miyagawa-Hayashino, Y. Matsumura, F. Kawakami et al., "High ratio of IgG4-positive plasma cell infiltration in cutaneous plasmacytosis—is this a cutaneous manifestation of IgG4-related disease?" *Human Pathology*, vol. 40, no. 9, pp. 1269–1277, 2009.

[40] W. Cheuk, H. K. L. Yuen, S. Y. Y. Chu, E. K. W. Chiu, L. K. Lam, and J. K. C. Chan, "Lymphadenopathy of IgG4-related sclerosing disease," *American Journal of Surgical Pathology*, vol. 32, no. 5, pp. 671–681, 2008.

[41] Y. Sato, M. Kojima, K. Takata et al., "Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease," *Modern Pathology*, vol. 22, no. 4, pp. 589–599, 2009.

[42] S. Kasashima, Y. Zen, A. Kawashima et al., "Inflammatory abdominal aortic aneurysm: close relationship to IgG4-related periaortitis," *American Journal of Surgical Pathology*, vol. 32, no. 2, pp. 197–204, 2008.

[43] J. H. Stone, A. Khosroshahi, A. Hilgenberg, A. Spooner, E. M. Isselbacher, and J. R. Stone, "IgG4-related systemic disease and lymphoplasmacytic aortitis," *Arthritis and Rheumatism*, vol. 60, no. 10, pp. 3139–3145, 2009.

[44] D. E. Comings, K. B. Skubi, J. van Eyes, and A. G. Motulsky, "Familial multifocal fibrosclerosis. Findings suggesting that retroperitoneal fibrosis, mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, and pseudotumor of the orbit may be different manifestations of a single disease," *Annals of Internal Medicine*, vol. 66, no. 5, pp. 884–892, 1967.

[45] A. Ghazale, S. T. Chari, T. C. Smyrk et al., "Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1646–1653, 2007.

[46] B. K. Straub, I. Esposito, D. Gotthardt et al., "IgG4-associated cholangitis with cholangiocarcinoma," *Virchows Archiv*, vol. 458, no. 6, pp. 761–765, 2011.

[47] J. Gill, N. Angelo, M. L. Yeong, and N. McIvor, "Salivary duct carcinoma arising in IgG4-related autoimmune disease of the parotid gland," *Human Pathology*, vol. 40, no. 6, pp. 881–886, 2009.

[48] M. Joo, S. H. Chang, H. Kim, J. M. Gardner, and J. Y. Ro, "Primary gastrointestinal clear cell sarcoma: report of 2 cases, one case associated with IgG4-related sclerosing disease, and review of literature," *Annals of Diagnostic Pathology*, vol. 13, no. 1, pp. 30–35, 2009.

[49] W. Cheuk, H. K. L. Yuen, A. C. L. Chan et al., "Ocular adnexal lymphoma associated with IgG4+ chronic sclerosing dacryoadenitis: a previously undescribed complication of IgG4-related sclerosing disease," *American Journal of Surgical Pathology*, vol. 32, no. 8, pp. 1159–1167, 2008.

[50] T. Kubota, S. Moritani, T. Yoshino, H. Nagai, and H. Terasaki, "Ocular adnexal marginal zone B cell lymphoma infiltrated by IgG4-positive plasma cells," *Journal of Clinical Pathology*, vol. 63, no. 12, pp. 1059–1065, 2010.

[51] M. Yamamoto, H. Takahashi, T. Tabeya et al., "Risk of malignancies in IgG4-related disease," *Modern Rheumatology*. In press.

[52] T. Muraki, H. Hamano, Y. Ochi et al., "Autoimmune pancreatitis and complement activation system," *Pancreas*, vol. 32, no. 1, pp. 16–21, 2006.

[53] Y. Zen, K. Harada, M. Sasaki et al., "Distribution of IgG4- and/or IgG-positive plasma cells in Hashimoto's thyroiditis: an immunohistochemical study," *Pathobiology*, vol. 77, no. 5, pp. 267–272, 2010.

Clinical Study

Spectrum of Disorders Associated with Elevated Serum IgG4 Levels Encountered in Clinical Practice

Jay H. Ryu,[1] Ryohei Horie,[1] Hiroshi Sekiguchi,[1] Tobias Peikert,[1] and Eunhee S. Yi[2]

[1] *Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Gonda 18 South, 200 First St. SW, Rochester, MN 55905, USA*
[2] *Division of Anatomic Pathology, Mayo Clinic, Rochester, MN 55905, USA*

Correspondence should be addressed to Jay H. Ryu, ryu.jay@mayo.edu

IgG4-related disease (IgG4-RD) is a recently described systemic fibroinflammatory disease associated with elevated circulating levels of IgG4 and manifests a wide spectrum of clinical presentations. Although serum IgG4 level has been described to be the most sensitive and specific laboratory test for the diagnosis of IgG4-RD, it is recognized that an elevated serum IgG4 level can be encountered in other diseases. In this study, we sought to identify the frequency of IgG4-RD and other disease associations in patients with elevated serum IgG4 levels seen in clinical practice. Among 3,300 patients who underwent IgG subclass testing over a 2-year period from January 2009 to December 2010, 158 (4.8%) had an elevated serum IgG4 level (>140 mg/dL). IgG4 subclass testing was performed for evaluation of suspected IgG4-RD or immunodeficiency. Twenty-nine patients (18.4%) had definite or possible IgG4-RD. Among those patients without IgG4-RD, a broad spectrum of biliary tract, pancreatic, liver, and lung diseases, as well as systemic vasculitis, was diagnosed. We conclude that patients with elevated serum IgG4 levels encountered in clinical practice manifest a wide array of disorders, and only a small minority of them has IgG4-RD.

1. Introduction

IgG4-related disease (IgG4-RD) is a recently described systemic fibroinflammatory disease associated with elevated circulating levels of IgG4 [1–4]. The pathologic lesion of IgG4-RD is characterized by lymphoplasmacytic inflammation with increased numbers of IgG4-positive plasma cells, fibrosis, and phlebitis. Although initial descriptions of this disorder focused on its pancreatic presentation (autoimmune pancreatitis), it has become apparent that IgG4-RD is a systemic disease associated with a wide spectrum of clinical manifestations involving virtually any organ in the body.

As initially observed in patients with autoimmune pancreatitis, the majority of patients with IgG4-RD have an elevated serum IgG4 level. Although serum IgG4 level has been described to be the most sensitive and specific laboratory test for the diagnosis of IgG4-RD, it is recognized that an elevated serum IgG4 level can be encountered in other diseases such as pancreatic cancer [5], atopic diseases [6], and infections [7, 8]. Furthermore, serum IgG4 level is elevated in up to 5% of the normal population [9, 10].

In this study, we sought to identify the spectrum of diseases associated with elevated serum IgG4 levels in patients encountered in clinical practice and the frequency of IgG4-RD in this population.

2. Methods

Using a computer-assisted search, we identified all patients who had serum IgG subclass levels determined on one or more occasions at Mayo Rochester, MN, USA, during the 2-year period from January 1, 2009 to December 31, 2010 and selected those with an elevated serum IgG4 concentration (>140 mg/dL) for analysis [10]. The concentrations of IgG subclass proteins in serum were measured in the Mayo Clinic Clinical Laboratory by automated nephelometry in which the concentrations of each protein were determined from standard curves [11]. Human IgG4 latex reagent (Binding Site Group Ltd, Birmingham, UK) was used in quantifying the serum IgG4 concentration. Medical records of those patients with elevated serum IgG4 levels were reviewed to extract data regarding age, sex, clinical presentation, serum

IgG4 level, indication for serum IgG subclass determination, imaging and biopsy results, and diagnoses. The main diagnosis that resulted from evaluation of the presenting clinical issue at the time of the serum IgG subclass testing was identified.

For this study, the diagnostic criteria similar to those proposed by Umehara and colleagues were employed [12]. The "definite" diagnosis of IgG4-RD required the following criteria in addition to the known serum IgG4 elevation: (1) clinical and/or radiologic evidence of lesions consistent with IgG4-RD in one or more organs as previously described in the literature and (2) IgG4 staining showing greater than 10 IgG4+ cells/high-power field and IgG4+/IgG+ ratio greater than 40% in the presence of lymphoplasmacytic infiltration and fibrosis. Patients who fulfill the organ-specific criteria for IgG4-related autoimmune pancreatitis, IgG4-related Mikulicz's disease, and IgG4-related kidney disease were also designated as having "definite" IgG4-RD [12]. Those patients clinically diagnosed based on clinical and imaging features along with an elevated serum IgG4 level but not fulfilling histopathologic criteria outlined above or in the absence of tissue biopsy and exhibiting improvement with corticosteroid therapy were designated as "possible" IgG4-RD in the absence of any other more likely diagnoses. None of our patients met the criteria for "probable" IgG4-RD outlined by Umehara and colleagues [12]. Non-IgG4-RD diagnoses were determined based on the results of the diagnostic evaluation, the clinicians' diagnostic impression, and the subsequent clinical course. Approval was obtained from the Mayo Foundation Institutional Review Board prior to beginning the study.

2.1. Statistical Analysis. Data are presented as mean ± SD and percentages for categorical variables unless stated otherwise. Demographic data were compared using the Fisher's exact test. Means of continuous variables were compared between groups with a two-sample t-test. Serum IgG4 levels between groups were compared using the Wilcoxon rank-sum test. In all cases P-values <0.05 were considered statistically significant.

3. Results

We identified 3,300 consecutive patients who had their serum IgG subclass testing performed on one or more occasions during the 2-year interval from January 1, 2009 to December 31, 2010; 158 patients (4.8%) had at least one high serum IgG4 level (>140 mg/dL). The demographic features and serum IgG4 level of these 158 patients are outlined in Table 1. Indications for IgG subclass testing were evaluation for possible IgG4-RD in 104 patients (65.8%) and to assess for immunodeficiency (e.g., patients with recurrent or chronic infections) in 54 patients (34.2%).

Twenty-nine patients (18.4%) met the criteria for definite or possible IgG4-RD (Table 2). The mean age of those with IgG4-RD was older compared to those without IgG4-RD (58.3 ± 16.9 versus 49.9 ± 20.8, $P < 0.05$) but the gender distribution was not different ($P = 0.29$). The serum IgG4 level was significantly higher in those with IgG4-RD

TABLE 1: Demographics and serum IgG4 levels ($n = 158$).

Characteristic	All patients	IgG4-RD ($n = 29$)	No IgG4-RD ($n = 129$)
Age, yr	51.4 ± 20.3	58.3 ± 16.9	49.9 ± 20.8
Male sex, no. (%)	98 (62)	21 (72)	77 (60)
Serum IgG4 level, mg/dL, median (range)	202 (141–3150)	223 (149–3150)	187 (141–1030)

Data are presented as mean ± SD, unless otherwise indicated.
IgG4-RD: IgG4-related disease.
IgG4-RD includes definite and possible cases.

FIGURE 1: Serum IgG4 levels for definite IgG4-RD, possible IgG4-RD, and non-IgG4-RD subgroups. Median and interquartile range (25% to 75%) are depicted.

compared to those with non-IgG4-RD diagnoses ($P < 0.001$) (Table 1). Furthermore, mean serum IgG4 level was higher for those with definite IgG4-RD (940 ± 990) compared to possible IgG4-RD (329 ± 318) which, in turn, was higher compared to non-IgG4-RD subgroup (226 ± 127) ($P < 0.05$) (Figure 1). The mean serum IgG4/IgG ratio was significantly higher in patients with IgG4-RD (definite and possible) compared to non-IgG4-RD patients (0.263 ± 0.239 versus 0.148 ± 0.061, $P < 0.01$), but there was substantial overlap in individual values between the two groups as shown in Figure 2. Among 29 patients with IgG4-RD, 10 patients met the criteria for definite IgG4-RD which included supportive histopathologic findings on tissue biopsy. Of 19 patients with possible IgG4-RD, 12 had undergone biopsy procedures but the biopsy findings did not meet the criteria for definite or probable IgG4-RD diagnosis.

In the remaining 129 patients who did not have IgG4-RD, the most common diagnoses were primary sclerosing cholangitis, bronchiectasis, non-IgG4-related pancreatitis, vasculitis, chronic rhinosinusitis, and pancreatic or bile duct cancer. No specific diagnosis was achieved in 29 patients;

TABLE 2: Spectrum of diagnoses associated with high serum IgG4 Levels ($n = 158$).

Diagnosis	No. of patients (%)
IgG4-related disease	29 (18.4)
Definite	10
Probable	0
Possible	19
Respiratory diseases	32 (20.3)
Bronchiectasis	11
Chronic rhinosinusitis	7
Asthma	4
Idiopathic pulmonary fibrosis	3
Sarcoidosis	2
Other respiratory diseases*	5
Biliary tract diseases	26 (16.5)
Primary sclerosing cholangitis	17
Cholangiocarcinoma	6
Biliary stricture or stone	3
Pancreatic diseases	19 (12.0)
Pancreatitis, not IgG4 related	10
Pancreatic cancer	6
Other pancreatic diseases†	3
Cirrhosis and other liver diseases	9 (5.7)
Vasculitis	9 (5.7)
Granulomatosis with polyangiitis (Wegener's)	5
The Churg-Strauss syndrome	3
Polyarteritis nodosa	1
Atopic dermatitis	1 (0.6)
Miscellaneous diseases‡	4 (2.5)
No specific diagnosis	29 (18.4)

* Other respiratory diseases (5 patients) included chronic pleuritis, emphysema, fibrosing mediastinitis, hypersensitivity pneumonitis, and recurrent pneumonias, respectively.
† Other pancreatic diseases (3 patients) included intraductal papillary mucinous neoplasm, pancreatic cyst, and pancreatic insufficiency, respectively.
‡ Miscellaneous diseases (4 patients) included lactose intolerance, neurofibromatosis, polymyositis, and psoriasis, respectively.
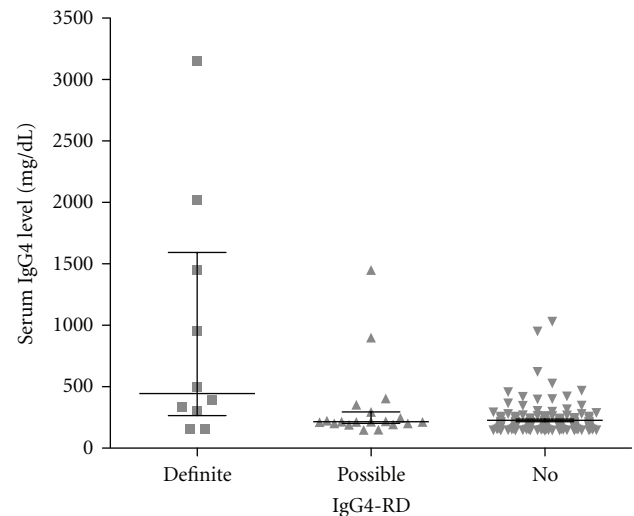

FIGURE 2: Serum IgG4/IgG ratios for IgG4-RD (definite and possible IgG4-RD) and non-IgG4-RD subgroups. Median and interquartile range (25% to 75%) are depicted.
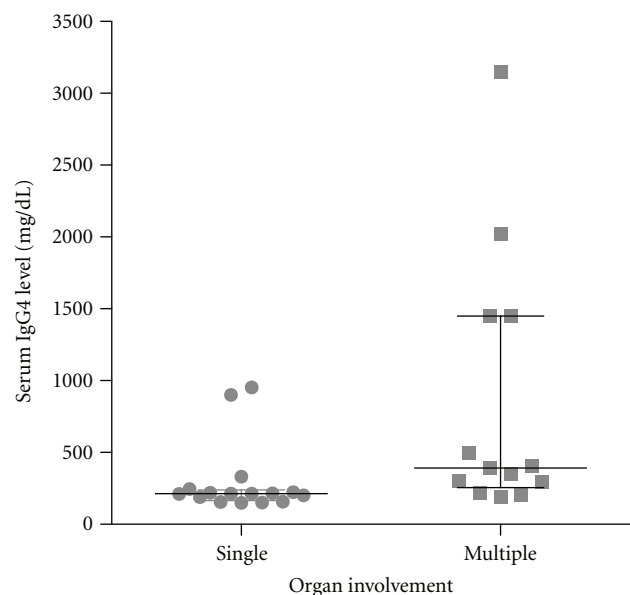
FIGURE 3: Serum IgG4 levels for patients with single-organ and multiple-(two or more) organ involvement. Median and interquartile range (25% to 75%) are depicted.

most common presenting complaints in these patients included abdominal pain, fevers, and lymphadenopathy.

Pattern of organ involvement for these patients with IgG4-RD is outlined in Table 3. Pancreas, bile ducts, and orbital structures were most commonly involved. However, these patients exhibited involvement in a number of other organs including the salivary glands, retroperitoneal region, lymph nodes, kidney, lung, pleura, sinuses, gastrointestinal tract, and testis. Patients with two or more organ involvements ($n = 13$) had a higher mean serum IgG4 level (840 ± 914) compared to those with single organ involvement (296 ± 250) ($P < 0.01$) (Figure 3).

All except one patient (surgical resection of a biliary lesion) with IgG4-RD (definite or possible) were treated with prednisone and improved. Twelve of 28 patients initially treated with prednisone experienced subsequent relapses requiring reinstitution of prednisone alone or in combination with another immunosuppressive agent (including azathioprine, methotrexate, and rituximab). Median duration of followup was 23 months (range, 1 to 126 months).

4. Discussion

In this study we found a broad spectrum of diagnoses to be associated with elevated serum IgG4 levels encountered in the clinical practice setting; less than one-fifth of these

Table 3: Spectrum of organ involvement in patients with IgG4-RD ($n = 29$).

Organ	Definite cases, n ($n = 10$)	Possible cases, n ($n = 19$)	All cases, n (%)
Pancreas	6	12	18 (62)
Bile ducts	2	9	11 (38)
Orbit	2	2	4 (14)
Retroperitoneum	1	2	3 (10)
Salivary glands	2	1	3 (10)
Lymph nodes	1	1	2 (7)
Lung	1	1	2 (7)
Gastrointestinal	1	1	2 (7)
Kidney	1	0	1 (3)
Pleura	1	0	1 (3)
Sinus	1	0	1 (3)
Mesentery	0	1	1 (3)
Testis	1	0	1 (3)

patients manifested evidence for IgG4-RD. IgG4 subclass testing was ordered by clinicians for evaluation of possible IgG4-RD or immunodeficiency, and thus it is not surprising that various types of non-IgG4-related pancreatic and biliary tract disorders were included along with chronic infections, for example, bronchiectasis and sinusitis.

At the present time, there is no published international consensus on the diagnostic criteria for IgG4-RD. Most authors agree that definitive diagnosis of IgG4-RD requires histologic confirmation that includes the presence of characteristic histopathologic features (lymphoplasmacytic infiltration, fibrosis, and obliterative phlebitis or arteritis) along with immunostaining that demonstrates increased numbers of IgG4+ cells. Various authors have used different cutoffs for IgG4 staining criteria. For the purposes of this study, we used the diagnostic criteria recently published by Umehara and colleagues [12] for "definite," "probable," and "possible" IgG4-RD cases. In clinical practice, there are patients in whom the diagnosis of IgG4-RD is likely and are empirically treated without biopsy confirmation. This may occur when patients decline invasive procedures or the initial biopsy specimen is nondiagnostic and additional biopsies are not pursued for based on patient preference, perceived risks, or lack of any other likely diagnosis.

All patients included in this analysis exhibited elevated serum IgG4 levels and the degree of this elevation was not used in determining the presence or absence of IgG4-RD. Our patients with IgG4-RD exhibited a significantly higher serum IgG4 levels compared to those without IgG4-RD although there was an overlap in their serum IgG4 values. Some authors have suggested that a serum IgG4 concentration that is more than twice the upper limit of normal (>280 mg/dL) is highly specific for IgG-RD [13]. In this study 13 of 29 patients (45%) with IgG4-RD and 18 of 129 patient (14%) without IgG4-RD had a serum IgG4 concentration that was >280 mg/dL.

The sensitivity of elevated serum IgG4 levels in the diagnosis of IgG4-RD has been reported to be in the range of 67% to 95% and specificity to be 90% to 97% [1, 2, 10, 13–17]. Serum IgG4 elevation is present in 5% of the normal population [9, 10] and has been observed in patients with other disorders. For example, serum IgG4 levels have been reported to be elevated in 7% to 10% of patients with pancreatic cancer [5, 13]. Similarly, serum IgG4 elevation has been seen in 5% to 9% of patients with other forms of pancreatitis and benign pancreatic tumors [13]. Similar spectrum of pancreatic diseases was also seen in our study cohort.

Nonpancreatic disorders have also been associated with elevated serum IgG4 levels. These include skin diseases including atopic dermatitis [6] and pemphigus vulgaris [18, 19], as well as parasitic diseases [7, 8]. Our study cohort included one patient with atopic dermatitis, but none had pemphigus or parasitic diseases.

One-fifth of our cohort had various respiratory diseases. Van Nieuwkoop and colleagues [20] had previously described an association between a polyclonal increase in serum IgG4 subclass with acquired respiratory diseases. Bronchiectasis and chronic rhinosinusitis were most respiratory disorders in our study cohort likely reflecting the fact that suspected immunodeficiency was one of the main indications for IgG subclass testing in this population.

Primary sclerosing cholangitis (PSC) was the single most common diagnosis in the non-IgG4-RD group. Nine of 17 patients with PSC had underlying inflammatory bowel disease. Elevated serum IgG4 levels have been described in 9% to 12% of patients with PSC [21, 22]. Since differentiation of autoimmune pancreatitis from pancreatic cancer and biliary tract disease is a common clinical indication for IgG subclass testing, it seems reasonable to expect that patients with various types of biliary tract diseases including PSC and cholangiocarcinoma will be encountered in those patients with elevated serum IgG4 levels.

Vascular involvement has been seen in IgG4-RD mainly in the form of aortitis, periaortitis, and inflammatory abdominal aortic aneurysm [2, 23, 24]. Recently, elevated serum IgG4 levels have been reported in patients with Churg-Strauss syndrome [25, 26] and hypocomplementemic urticarial vasculitis [27]. Additionally, IgG4 antiproteinase 3 autoantibodies have been demonstrated to stimulate neutrophils to undergo a proinflammatory response suggesting potential relevance in the pathogenesis of granulomatosis with polyangiitis (Wegener's) [28]. In this regard, it is interesting to note that nine patients in our study cohort had systemic vasculitis including granulomatosis with polyangiitis and the Churg-Strauss syndrome. Prevalence of high serum IgG4 level in patients with ANCA-associated vasculitis and the relevance of IgG4 in the pathogenesis of these disorders need to be explored further.

Although IgG4-related hepatopathy and hepatic inflammatory pseudotumors have been described [29–31], none of our patients with liver disease and elevated serum IgG4 levels fulfilled the criteria for IgG4-RD. They had other identifiable causes including hepatitis C, drugs, sarcoidosis, and primary biliary cirrhosis.

As previously noted, serum IgG4 level is elevated in 5% of the normal population [9, 10]. Similarly, 4.8% of 3,300 consecutive patients undergoing serum IgG subclass testing at our institution over a 2-year period exhibited an elevated serum IgG4 level. It seems reasonable to assume that elevated serum IgG4 level will be found coincidentally in a small portion of various disease populations that are subjected to IgG subclass testing, for example, patients with bronchiectasis. None of our 54 patients with high serum IgG4 level who had undergone IgG subclass testing for the indication of suspected immunodeficiency had evidence of IgG4-RD. It appears unlikely that there is a causal relationship between the elevated serum IgG4 level and many of the diseases listed in Table 2. It remains to be determined whether elevated serum IgG4 level is a relevant finding in other disorders other than those recognized currently as IgG4-RD.

There are several limitations to this study. This study was a retrospective survey with analysis limited to the clinical data available in medical records and imaging studies. The diagnostic evaluation for these patients was performed by various clinicians at our institution according to their own clinical judgment and patient context. It is possible that some cases of IgG4-RD may have been missed particularly in those patients without a specific diagnosis. In addition, the extent of organ involvement may have been underestimated in patients with IgG4-RD due to lack of relevant imaging studies or biopsy specimens in the absence of standard methodical evaluation.

We conclude that only a minority of patients with elevated serum IgG4 levels encountered in clinical practice have IgG4-RD. Furthermore, elevated serum IgG4 levels can be seen in patients with many different diseases, most of which likely represent coincidental occurrence. Our findings reinforce the principle that an elevated serum IgG4 level in isolation is of limited diagnostic utility.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

[1] T. Kamisawa and A. Okamoto, "IgG4-related sclerosing disease," *World Journal of Gastroenterology*, vol. 14, no. 25, pp. 3948–3955, 2008.

[2] Y. Zen and Y. Nakanuma, "IgG4-related disease: a cross-sectional study of 114 cases," *American Journal of Surgical Pathology*, vol. 34, no. 12, pp. 1812–1819, 2010.

[3] W. Cheuk and J. K. C. Chan, "IgG4-related sclerosing disease a critical appraisal of an evolving clinicopathologic entity," *Advances in Anatomic Pathology*, vol. 17, no. 5, pp. 303–332, 2010.

[4] A. Khosroshahi and J. H. Stone, "A clinical overview of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 57–66, 2011.

[5] A. Raina, A. M. Krasinskas, J. B. Greer et al., "Serum immunoglobulin G fraction 4 levels in pancreatic cancer: elevations not associated with autoimmune pancreatitis," *Archives of Pathology and Laboratory Medicine*, vol. 132, no. 1, pp. 48–53, 2008.

[6] R. C. Aalberse, F. Van Milligen, K. Y. Tan, and S. O. Stapel, "Allergen-specific IgG4 in atopic disease," *Allergy*, vol. 48, no. 8, pp. 559–569, 1993.

[7] A. Kurniawan, M. Yazdanbakhsh, R. Van Ree et al., "Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis," *Journal of Immunology*, vol. 150, no. 9, pp. 3941–3950, 1993.

[8] M. Satoh, H. Toma, Y. Sato et al., "Production of a high level of specific IgG4 antibody associated with resistance to albendazole treatment in HLA-DRB1*0901-positive patients with strongyloidiasis," *American Journal of Tropical Medicine and Hygiene*, vol. 61, no. 4, pp. 668–671, 1999.

[9] A. Nirula, S. M. Glaser, S. L. Kalled, and F. R. Taylora, "What is IgG4? A review of the biology of a unique immunoglobulin subtype," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 119–124, 2011.

[10] R. P. Sah and S. T. Chari, "Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 108–113, 2011.

[11] U. Schauer, F. Stemberg, C. H. L. Rieger et al., "IgG subclass concentrations in certified reference material 470 and reference values for children and adults determined with the binding site reagents," *Clinical Chemistry*, vol. 49, no. 11, pp. 1924–1929, 2003.

[12] H. Umehara, K. Okazaki, Y. Masaki et al., "Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD)," *Modern Rheumatology*, vol. 22, no. 1, pp. 21–30, 2011.

[13] A. Ghazale, S. T. Chari, T. C. Smyrk et al., "Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1646–1653, 2007.

[14] I. Naitoh, T. Nakazawa, H. Ohara et al., "Comparative evaluation of the Japanese diagnostic criteria for autoimmune pancreatitis," *Pancreas*, vol. 39, no. 8, pp. 1173–1179, 2010.

[15] R. Sadler, R. W. Chapman, D. Simpson et al., "The diagnostic significance of serum IgG4 levels in patients with autoimmune pancreatitis: a UK study," *European Journal of Gastroenterology and Hepatology*, vol. 23, no. 2, pp. 139–145, 2011.

[16] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[17] T. Tabata, T. Kamisawa, K. Takuma et al., "Serum IgG4 concentrations and IgG4-related sclerosing disease," *Clinica Chimica Acta*, vol. 408, no. 1-2, pp. 25–28, 2009.

[18] R. Hussain, R. W. Poindexter, and E. A. Ottesen, "Control of allergic reactivity in human filariasis: predominant localization of blocking antibody to the IgG4 subclass," *Journal of Immunology*, vol. 148, no. 9, pp. 2731–2737, 1992.

[19] Y. Shirakata, S. Shiraishi, K. Sayama, and Y. Miki, "Subclass characteristics of IgG autoantibodies in bullous pemphigoid and pemphigus," *Journal of Dermatology*, vol. 17, no. 11, pp. 661–666, 1990.

[20] J. A. Van Nieuwkoop, A. Brand, J. Radl, and F. Skvaril, "Increased levels of IgG4 subclass in 5 patients with acquired respiratory disease," *International Archives of Allergy and Applied Immunology*, vol. 67, no. 1, pp. 61–65, 1982.

[21] F. D. Mendes, R. Jorgensen, J. Keach et al., "Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis," *American Journal of Gastroenterology*, vol. 101, no. 9, pp. 2070–2075, 2006.

[22] E. Björnsson, S. Chari, M. Silveira et al., "Primary sclerosing cholangitis associated with elevated immunoglobulinG4:

clinical characteristics and response to therapy," *American Journal of Therapeutics*, vol. 18, no. 3, pp. 198–205, 2011.

[23] J. R. Stone, "Aortitis, periaortitis, and retroperitoneal fibrosis, as manifestations of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 88–94, 2011.

[24] S. Kasashima and Y. Zen, "IgG4-related inflammatory abdominal aortic aneurysm," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 18–23, 2011.

[25] M. Yamamoto, H. Takahashi, C. Suzuki et al., "Analysis of serum IgG subclasses in churg-strauss syndrome-The meaning of elevated serum levels of IgG4," *Internal Medicine*, vol. 49, no. 14, pp. 1365–1370, 2010.

[26] A. Vaglio, J. D. Strehl, B. Manger et al., "IgG4 immune response in Churg-Strauss syndrome," *Annals of Rheumatic Diseases*, vol. 71, no. 3, pp. 390–393, 2011.

[27] R. Wakamatsu, H. Watanabe, K. Suzuki et al., "Hypocomplementemic urticarial vasculitis syndrome is associated with high levels of serum IgG4: a clinical Manifestation that Mimics IgG4-related disease," *Internal Medicine*, vol. 50, no. 10, pp. 1109–1112, 2011.

[28] A. Hussain, T. Pankhurst, M. Goodall et al., "Chimeric IgG4 PR3-ANCA induces selective inflammatory responses from neutrophils through engagement of Fcγ receptors," *Immunology*, vol. 128, no. 2, pp. 236–244, 2009.

[29] Y. Zen, T. Fujii, Y. Sato, S. Masuda, and Y. Nakanuma, "Pathological classification of hepatic inflammatory pseudotumor with respect to IgG4-related disease," *Modern Pathology*, vol. 20, no. 8, pp. 884–894, 2007.

[30] T. Umemura, Y. Zen, H. Hamano, S. Kawa, Y. Nakanuma, and K. Kiyosawa, "Immunoglobin G4-hepatopathy: association of immunoglobin G4-bearing plasma cells in liver with autoimmune pancreatitis," *Hepatology*, vol. 46, no. 2, pp. 463–471, 2007.

[31] S. L. Taylor and S. Haque, "Hepatobiliary pathology," *Current Opinion in Gastroenterology*, vol. 27, no. 3, pp. 248–255, 2011.

Clinical Study

IgG4-Related Disease Is Not Associated with Antibody to the Phospholipase A2 Receptor

Arezou Khosroshahi,[1] Rivka Ayalon,[2] Laurence H. Beck Jr.,[2] David J. Salant,[2] Donald B. Bloch,[3] and John H. Stone[1]

[1] *Department of Medicine, Harvard Medical School and Rheumatology Unit, Massachusetts General Hospital, Boston, MA 02114, USA*
[2] *Section of Nephrology, Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA*
[3] *Department of Medicine, Harvard Medical School and The Division of Rheumatology, Allergy and Immunology and The Center for Immunology and Inflammatory Diseases of the General Medical Services, Massachusetts General Hospital, Boston, MA 02114, USA*

Correspondence should be addressed to John H. Stone, jhstone@partners.org

Patients with IgG4-related disease (IgG4-RD) share histopathological characteristics that are similar across affected organs. The finding of infiltration with IgG4+ plasma cells in the proper clinical and histopathological contexts connects a large number of clinical entities that were viewed previously as separate conditions. The renal involvement in IgG4-RD is usually characterized by tubulointerstitial nephritis, but membranous nephropathy has also been reported to be one of the renal complications of IgG4-RD. The recent discovery that a high proportion of patients with idiopathic membranous nephropathy (IMN) have IgG4 autoantibodies to the M-type phospholipase A2 receptor (PLA2R) in the circulation and glomerular immune deposits, together with the profound IgG4 hypergammaglobulinemia and occasional reports of membranous nephropathy in IgG4-RD, raised the question of a common antigen. To assess the presence of anti-PLA2R antibody in patients with IgG4-RD, we screened sera from 28 IgG4-RD patients by immunoblot. None of the patients in this cohort had detectable circulating anti-PLA2R antibodies. This study suggests that despite some clinical and serological overlaps between IgG4-RD and IMN, anti-PLA2R antibodies do not play a role in the pathogenesis of IgG4-RD. Additional studies of IgG4-RD with evidence of membranous nephropathy are important to exclude any definite relationship.

1. Introduction

IgG4-related disease (IgG4-RD) is a multiorgan system fibroinflammatory condition defined by a tendency to form tumorous lesions in various organs including the pancreas, salivary and lacrimal glands, biliary tract, liver, lung, and kidney, aorta [1]. The histopathologic findings are remarkably similar across all organs in this disease. The distinctive pathologic features include a dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells, storiform fibrosis, obliterative phlebitis, and eosinophilia [2]. Frequent elevations of serum IgG4 in patients with IgG4-RD and significant clinical responses to glucocorticoids are other hallmarks of this condition [3]. The relationship between elevated serum IgG4 and distinctive patterns of organ involvement was first

recognized in autoimmune pancreatitis [4], but subsequent observations led to the identification of this disease in nearly all organ systems [1, 2, 5].

Idiopathic membranous nephropathy (IMN) is an organ-specific autoimmune disorder and a leading cause of nephrotic syndrome in adults. Until recently, the etiology of this condition was unknown, but studies in experimental MN had established that circulating antibodies bind to a target antigen on glomerular podocytes and form antigen-antibody complexes that cause podocyte injury and proteinuria [6]. In 2009, Beck et al. discovered that a high proportion of patients with IMN have circulating IgG4 autoantibodies that bind to the M-type phospholipase A2 receptor (PLA2R), a transmembrane glycoprotein, and member of the mannose receptor family expressed on human glomerular

podocytes [7]. This finding is congruent with previous reports that IgG4 predominates in the immune deposits of renal biopsy specimens of IMN. This predominance of IgG4 is not observed in secondary—or lupus-associated—membranous nephropathy [8]. Studies of patients in several different cohorts have indicated that 70–80% of patients with IMN have anti-PLA2R antibodies that are of the IgG4 subclass [7, 9–11]. Of note, however, hypergammaglobulinemia and elevated serumIgG4 concentrations are not reported in IMN patients.

IgG4-RD and IMN both appear to respond well to B cell depletion treatment with rituximab [9, 12, 13]. The early experience with B cell depletion in IgG4-RD suggests that rituximab (RTX) has a targeted effect on serum IgG4 : IgG4 decreases rapidly following B cell depletion while the concentrations of other IgG subclasses remain stable [12, 13]. RTX has also been reported in case series to be effective in IMN [14, 15]. A decline in anti-PLA2R antibodies has been shown to precede the clinical improvement of patients with membranous nephropathy [9]. A randomized clinical trial of RTX in IMN is now under way (Clinicaltrials.gov identifier NCT01180036).

Membranous nephropathy has been reported in some patients with IgG4-RD [16–18], but the principal renal manifestation of IgG4-RD is tubulointerstitial nephritis [19, 20], characterized by interstitial fibrosis and infiltration of lymphocytes and IgG4-positive plasma cells. Immune complex deposition and membranous glomerulonephritis have been shown to coexist with tubulointerstitial nephritis in a minority of patients with IgG4-RD [21, 22]. Cravedi et al. [23] recently described a patient with IgG4-RD who had pancreatic and salivary gland involvement and subsequently developed proteinuria. A renal biopsy showed features of membranous nephropathy. A search for anti-PLA2R antibodies in that patient's serum was negative. Likewise, anti-PLA2R antibodies were not detected in the case of IgG4-RD and membranous nephropathy reported by Fervenza et al. [18].

Because of certainclinical and pathological features of IgG4-RD and IMN overlap, the shared association with antibodies of the IgG4 subclass, and the ostensible improvement that both diseases demonstrate in response to B cell depletion, we assayed sera from patients in our longitudinal IgG4-RD registry for antibodies directed against PLA2R.

2. Material and Methods

2.1. Patients. Between July 2009 and September 2011, we obtained serum samples from 28 patients with IgG4-RD. All patients were enrolled in the Massachusetts General Hospital IgG4-RD Registry. The screening of human sera for anti-PLA2R antibodies was approved by the Institutional Review Boards at both the Massachusetts General Hospital and Boston University Medical Center.

2.2. Inclusion Criteria for the IgG4-RD Registry. Patients were eligible to participate in the study if they had a biopsy-confirmed diagnosis of IgG4-RD. Histopathologic features

considered to be highly suggestive of IgG4-RD diagnosis included lymphoplasmacytic infiltrates and storiform fibrosis within involved organs. Obliterative phlebitis and mild-to-moderate tissue eosinophilia were observed frequently but were not required for the diagnosis [24]. In addition, all patients had either an IgG4/IgG plasma cell ratio of >50% within the affected organs or more than 30 IgG4-bearing plasma cells/high-power field (hpf). Elevated serum IgG4 was not required for the diagnosis of IgG4-RD.

2.3. IgG4 Plasma Cell Quantitation. Immunohistochemical staining was performed as previously described [12]. Formalin-fixed, paraffin-embedded tissue sections were stained with antibodies to IgG4 (Zymed, 1 : 200 dilution) or IgG (Dako, 1 : 3000 dilution). For each specimen, the number of IgG4+ plasma cells and the total number of IgG+ plasma cells were assessed in three nonoverlapping high-power fields (400x). The three fields with the highest number of IgG4+ plasma cells were selected for quantitation, and the ratio of IgG4+/IgG+ plasma cells was determined.

2.4. Serum IgG4 Assay. Serum IgG4 concentrations were measured by nephelometry (Mayo Medical Laboratories New England, Andover, Massachusetts).

2.5. Immunoblot Assay for the Detection of Anti-PLA2R Antibodies. Patients' sera were tested for the presence of anti-PLA2R antibodies by immunoblot, under nonreducing conditions, as previously described [7]. Recombinant human PLA2R was fractionated by polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Individual lanes were cut and incubated overnight at 4°C with serum samples diluted 1 : 25 in Tris-buffered saline containing 0.2% Tween-20 and 10% skim milk. Serum from a patient with IMN, previously shown to have anti-PLA2R antibodies, was used as a positive control. IgG subclass-specific sheep anti-human IgG4 (The Binding Site, San Diego, CA) was used at 1 : 3000. Sheep IgG was subsequently detected with species-specific, horseradish peroxidase-conjugated donkey anti-sheep IgG (Jackson ImmunoResearch, West Grove, PA), followed by reaction in a chemiluminescent substrate and exposure to radiographic film for two minutes. A band corresponding to the size of PLA2R was judged to represent the presence of anti-PLA2R antibodies.

3. Results

Demographic Features. Sera from 28 patients were tested for the presence of anti-PLA2R antibodies. The patients' baseline characteristics are shown in Table 1.The IgG4-RD patients included 15 men and 13 women, with an average age of 57 years (range: 24–82).

Clinical manifestations of IgG4-RD. The patients' manifestations of IgG4-RD covered the full range of disease expression [25], including clinically evident renal disease in two patients (see below). Multiorgan system IgG4-RD was observed in 11 patients (39%). The most commonly involved organs

TABLE 1: IgG4-RD patients' characteristics.

Patient	Age	Sex	Serum IgG4 mg/dL	Serum IgG mg/dL	ANA	Prednisonetreatment	Organ involved
1	67	M	1340	2450	+1 : 1280 S	Yes	aorta, lymph node
2	76	M	2050	2310	+1 : 40 S	Yes	pancreas, biliary tract, aorta, lymph node, salivary gland
3	55	M	1500	1640	+1 : 160 S	Yes	lacrimal and salivary glands
4	51	F	401	726	+1 : 5120 C	Yes	lacrimal and salivary glands
5	60	M	365	2570	+1 : 40 S	Yes	aorta
6	58	M	894	1210	+1 : 40 S	No	lymph node
7	41	M	200	766	+1 : 40 H	No	lymph node
8	24	M	47	100	—	Yes	orbital tissue
9	72	F	429	1170	+1 : 320 S	Yes	lacrimal & salivary glands Skin
10	52	F	62	1280	—	Yes	biliary tract
11	40	F	98	1090	+1 : 160 S	Yes	pancreas, lung, thyroid, lacrimal gland
12	59	M	27	1280	+1 : 40 S	Yes	retroperitoneum
13	48	F	9	1240	+1 : 40 S	No	pericardium
14	63	M	386	1500	+1 : 40 S	Yes	retroperitoneum, kidney
15	56	M	670	1980	+1 : 640 H	Yes	orbital tissue
16	74	F	28	1830	+1 : 40 S	Yes	kidney, lacrimal gland, pancreas, and biliary tract
17	39	M	53	1460	+1 : 40 S	Yes	retroperitoneum
18	51	F	68	1190	+1 : 40 S	No	lymph node
19	33	F	139	1570	+1 : 160 S	No	lymph node
20	58	F	194	1160	+1 : 40 H	Yes	tonsils
21	41	F	240	1710	+1 : 160 H	No	salivary gland
22	63	F	30	923	+1 : 320 S	Yes	orbital tissue
23	51	F	80	1300	—	Yes	pachymeninges
24	82	M	59	1180	—	No	lymph node
25	66	F	154	769	+1:160 S	Yes	retroperitoneum, lymph node, salivary gland
26	72	M	12	1000	+1 : 40 S	Yes	pancreas, salivary gland
27	70	M	164	1230	+1 : 160 S	Yes	pancreas, biliary tract, salivary gland
28	61	M	50	1650	+1 : 160 S	Yes	pancreas, retroperitoneum, hard palate, lymph node

S: Speckled H: Homogeneous C: Centromere.

and tissues were the lymph nodes ($n = 9$), salivary glands ($n = 8$), and orbital regions ($n = 7$). Five patients had IgG4-related pancreatitis (type 1 AIP), and four had IgG4-related sclerosing cholangitis. The other sites involved were the retroperitoneum ($n = 4$), aorta ($n = 3$), and the skin, pericardium, lung, thyroid gland, and tonsils (1 patient each).

Renal Disease. Two patients had evidence of kidney dysfunction, characterized by renal masses and proteinuria. Patient 16 [26] was found to have bilateral kidney masses on a magnetic resonance imaging study performed for evaluation of IgG4-related sclerosing cholangitis. Renal biopsy showed a destructive tubulointerstitial infiltrate with prominent fibrosis. The inflammatory infiltrate was composed predominantly of IgG4+ plasma cells. There were significant quantities of electron dense deposits within the thickened tubular basement membrane, and small electron-dense

deposits were seen scattered within the fibrotic interstitium. The glomeruli were free of deposits.

Patient 14 was diagnosed with IgG4-related retroperitoneal fibrosis. Acute renal failure and proteinuria emerged a year after the diagnosis of IgG4-RD and resolved with glucocorticoid treatment. Kidney biopsy showed tubular injury, periglomerular fibrosis, interstitial inflammation, and the presence of IgG4+ plasma cells. Immunofluorescence microscopy showed no tubular basement membrane or glomerular basement membrane deposits. There were prominent mesangial granular deposits of IgG, IgA, IgM, and C3.

Treatment before Serum Sampling. Most of the patients were entered into the IgG4-RD Registry after the initiation of prednisone treatment. Twenty-one (75%) of the 28 patients were receiving glucocorticoids at the time their serum IgG4 was measured for the first time. Fifteen (54%) of the 28
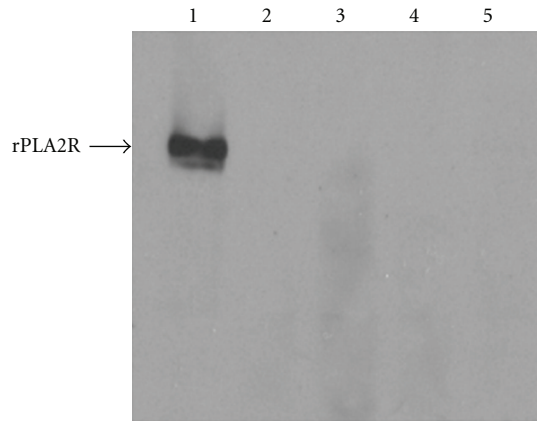
FIGURE 1: Representative immunoblot demonstrating that sera from patients with IgG4-RD (lanes 2–5) lack detectable reactivity with rPLA2R. Serum from a patient with idiopathic membranous nephropathy (lane 1) was used as a positive control.

patients had elevated serum IgG4. Among those patients with elevated serum IgG4 concentrations at baseline, the mean IgG4 was 640 mg/dL (normal <135 mg/dL). The mean serum IgG4 concentration for all patients in the cohort was 342 mg/dL. Among the seven patients who were not treated with glucocorticoids before the diagnosis of IgG4-RD and serum sampling, the mean IgG4 level was 230 mg/dL.

Other Autoantibody Testing. Patients were tested for rheumatoid factor by nephelometry, which was positive in 2 patients (7%). Antinuclear antibody (ANA) testing by immunofluorescence using the HEp-2 substrate was positive in 24 patients (85%). Patient 4 had a strongly positive ANA in an anticentromere pattern but had no clinical features of limited systemic sclerosis or Sjögren's syndrome, and salivary gland histopathology disclosed findings diagnostic of IgG4-RD, not Sjögren's syndrome. Enzyme-linked immunoabsorbent assays for antibodies against Ro, La, Sm, and RNP antigens were negative in all 28 patients.

Anti-PLA2R antibody assays. The sera from these 28 patients with IgG4-RD were tested for the presence of autoantibodies reactive with PLA2R. None of the serum samples contained detectable anti-PLA2R antibodies when screened at a dilution of 1 : 25 (Figure 1).

4. Discussion

IgG4-RD and IMN have significant overlap in their renal manifestations. Although membranous nephropathy is most often a renal limited autoimmune disease (so-called idiopathic or primary MN), membranous lesions and nephrotic syndrome have also been reported in IgG4-RD [23] and may accompany the more usual interstitial nephritis typical of IgG4-RD [16, 27]. Both IgG-RD and IMN are associated with perturbations in the IgG4 antibody subclass. The autoantibody recently linked to IMN is generally of the IgG4 subclass, and IgG4 hypergammaglobulinemia—occasionally

present up to 25 times the upper limit of normal—occurs in 70% of patients with IgG4-RD [28]. In addition, many cases of both IMN and IgG4-RD are exquisitely sensitive to treatment with rituximab. B cell depletion is associated with declines in the titers of anti-PLA2R in IMN and the level of IgG4 hypergammaglobulinemia in IgG-RD. Despite the similarities between IMN and IgG4-RD, the principal finding of this study is that the likelihood of a relationship between IgG4-RD and autoantibodies to the PLA2R is low. These antibodies were not identified in any of the 28 patients evaluated in this study. The absence of anti-PLA2R in two previous cases of IgG4-RD accompanied by membranous nephropathy is noteworthy [18, 23].

There are a variety of potential explanations for this finding. The first is that anti-PLA2R antibodies do not play a role in IgG4-RD and that a different antigen-antibody system is at play in those few cases that develop membranous nephropathy [23]. This is consistent with the fact that although important similarities exist between these two conditions, fundamental differences also exist. Whereas IMN is a renal-limited lesion, IgG4-RD is a multiorgan disease [25]. IMN patients do not demonstrate elevated serum IgG4 and decreased complement levels which are seen in some patients with IgG4-RD. Furthermore, although membranous GN has been described in IgG4-RD, the renal lesion most characteristic of IgG4-RD is tubulointerstitial nephritis. Finally, the other pathological features that are central to IgG4-RD, namely, storiform fibrosis, obliterative phlebitis, and the infiltration of large numbers of IgG4+ plasma cells are absent in IMN. The glomerular lesions of IgG4-RD described to date have included mesangial proliferative glomerulonephritis, membranous nephropathy, membranoproliferative glomerulonephritis, and endocapillary proliferative glomerulonephritis [19, 29].

A second possible explanation for our inability to detect the anti-PLA2R antibodies in this IgG4-RD patient cohort is that 75% of the patients received glucocorticoids prior to their entry to the study. Although glucocorticoids may have reduced the level of anti-PLA2R antibodies below the level of detection by the immunoblot assay used in this study, they are generally ineffective when used alone for treatment of IMN. Moreover, we did not detect these antibodies even in patients who had not been treated with glucocorticoids prior to serum sampling ($n = 7$).

A third potential explanation for our failure to find any relationship between the presence of anti-PLA2R antibodies and IgG4-RD is that IgG4-RD is not a single disease but rather a pathologic syndrome in which certain mechanisms operate across organ systems. If this explanation were true, then anti-PLA2R antibodies might play a role in some disease subsets of IgG4-RD, particularly the renal disease subset. The renal disease subset of IgG4-RD is underrepresented in our Registry as only two patients had overt renal disease, and none had biopsy-proven membranous glomerulonephropathy or nephrotic range proteinuria. An expanded study that includes a larger number of IgG4-RD patients with renal involvement of this nature is important to exclude definitively any relationship between IgG4-RD and antibodies to the PLA2R.

Given the consistency of pathological features across involved organs in IgG4-RD, we believe that our findings likely represent the true nature of the relationship between IgG4-RD and antibodies to the PLA2-R, namely, that there is none. The significance of IgG4 hypergammaglobulinemia and IgG4+ plasma cell infiltration into involved organs in patients with IgG4-RD—whether they are pathogenic or just "innocent bystanders"—remains to be clarified.

Authors' Contributions

A. Khosroshahi and R. Ayalon contributed equally to this paper.

Acknowledgments

References

[1] J. H. Stone, A. Khosroshahi, V. Deshpande, J. K. Chan, J. Heathcote, and S. Chari, "IgG4-related disease: recommendations for the nomenclature of this condition and its individual organ system manifestations," *Arthritis and Rheumatism*. In press.

[2] V. Deshpande, Y. Zen, J. K. C. Chan et al., "Consensus statement on the pathology of IgG4-related disease," *Modern Pathology*. In press.

[3] W. Cheuk and J. K. C. Chan, "IgG4-related sclerosing disease a critical appraisal of an evolving clinicopathologic entity," *Advances in Anatomic Pathology*, vol. 17, no. 5, pp. 303–332, 2010.

[4] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[5] T. Kamisawa, N. Funata, Y. Hayashi et al., "A new clinicopathological entity of IgG4-related autoimmune disease," *Journal of Gastroenterology*, vol. 38, no. 10, pp. 982–984, 2003.

[6] L. H. Beck Jr. and D. J. Salant, "Membranous nephropathy: recent travels and new roads ahead," *Kidney International*, vol. 77, no. 9, pp. 765–770, 2010.

[7] L. H. Beck Jr., R. G. B. Bonegio, G. Lambeau et al., "M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy," *The New England Journal of Medicine*, vol. 361, no. 1, pp. 11–21, 2009.

[8] A. Kuroki, M. Iyoda, T. Shibata, and T. Sugisaki, "Th2 cytokines increase and stimulate B cells to produce IgG4 in idiopathic membranous nephropathy," *Kidney International*, vol. 68, no. 1, pp. 302–310, 2005.

[9] L. H. Beck Jr., F. C. Fervenza, D. M. Beck et al., "Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy," *Journal of the American Society of Nephrology*, vol. 22, no. 8, pp. 1543–1550, 2011.

[10] J. M. Hofstra, L. H. Beck Jr., D. M. Beck, J. F. Wetzels, and D. J. Salant, "Anti-phospholipase a2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy," *Clinical Journal of the American Society of Nephrology*, vol. 6, no. 6, pp. 1286–1291, 2011.

[11] W. Qin, L. H. Beck Jr., C. Zeng et al., "Anti-phospholipase A2 receptor antibody in membranous nephropathy," *Journal of the American Society of Nephrology*, vol. 22, no. 6, pp. 1137–1143, 2011.

[12] A. Khosroshahi, D. B. Bloch, V. Deshpande, and J. H. Stone, "Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease," *Arthritis and Rheumatism*, vol. 62, no. 6, pp. 1755–1762, 2010.

[13] A. Khosroshahi, M. N. Carruthers, V. Deshpande, S. Unizony, D. B. Bloch, and J. H. Stone, "Rituximab for the treatment of IgG4-related disease: lessons from 10 consecutive patients," *Medicine*, vol. 91, no. 1, pp. 57–66, 2012.

[14] P. Cravedi, M. C. Sghirlanzoni, M. Marasà, A. Salerno, G. Remuzzi, and P. Ruggenenti, "Efficacy and safety of rituximab second-line therapy for membranous nephropathy: a prospective, matched-cohort study," *American Journal of Nephrology*, vol. 33, no. 5, pp. 461–468, 2011.

[15] F. C. Fervenza, R. S. Abraham, S. B. Erickson et al., "Rituximab therapy in idiopathic membranous nephropathy: a 2-year study," *Clinical Journal of the American Society of Nephrology*, vol. 5, no. 12, pp. 2188–2198, 2010.

[16] S. J. W. Watson, D. A. S. Jenkins, and C. O. S. Bellamy, "Nephropathy in IgG4-related systemic disease," *American Journal of Surgical Pathology*, vol. 30, no. 11, pp. 1472–1477, 2006.

[17] T. Saeki, S. Nishi, N. Imai et al., "Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis," *Kidney International*, vol. 78, no. 10, pp. 1016–1023, 2010.

[18] F. C. Fervenza, G. Downer, L. H. Beck Jr., and S. Sethi, "IgG4-related tubulointerstitial nephritis with membranous nephropathy," *American Journal of Kidney Diseases*, vol. 58, no. 2, pp. 320–324, 2011.

[19] L. D. Cornell, "IgG4-related tubulointerstitial nephritis," *Kidney International*, vol. 78, no. 10, pp. 951–953, 2010.

[20] M. Kawano, T. Saeki, H. Nakashima et al., "Proposal for diagnostic criteria for IgG4-related kidney disease," *Clinical and Experimental Nephrology*, vol. 15, no. 5, pp. 615–626, 2011.

[21] T. Saeki, A. Saito, H. Yamazaki et al., "Tubulointerstitial nephritis associated with IgG4-related systemic disease," *Clinical and Experimental Nephrology*, vol. 11, no. 2, pp. 168–173, 2007.

[22] J. Morimoto, Y. Hasegawa, H. Fukushima et al., "Membranoproliferative glomerulonephritis-like glomerular disease and concurrent tubulointerstitial nephritis complicating IgG4-related autoimmune pancreatitis," *Internal Medicine*, vol. 48, no. 3, pp. 157–162, 2009.

[23] P. Cravedi, M. Abbate, E. Gagliardini et al., "Membranous nephropathy associated with IgG4-related disease," *American Journal of Kidney Diseases*, vol. 58, no. 2, pp. 272–275, 2011.

[24] Y. Zen and Y. Nakanuma, "IgG4-related disease: a cross-sectional study of 114 cases," *American Journal of Surgical Pathology*, vol. 34, no. 12, pp. 1812–1819, 2010.

[25] A. Khosroshahi and J. H. Stone, "A clinical overview of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 57–66, 2011.

[26] L. D. Cornell, S. L. Chicano, V. Deshpande et al., "Pseudotumors due to IgG4 immune-complex tubulointerstitial nephritis associated with autoimmune pancreatocentric disease," *American Journal of Surgical Pathology*, vol. 31, no. 10, pp. 1586–1597, 2007.

[27] T. Saeki, N. Imai, T. Ito, H. Yamazaki, and S. Nishi, "Membranous nephropathy associated with IgG4-related systemic disease and without autoimmune pancreatitis," *Clinical Nephrology*, vol. 71, no. 2, pp. 173–178, 2009.

[28] R. Sadler, R. W. Chapman, D. Simpson et al., "The diagnostic significance of serum IgG4 levels in patients with autoimmune pancreatitis: a UK study," *European Journal of Gastroenterology and Hepatology*, vol. 23, no. 2, pp. 139–145, 2011.

[29] S. Nishi, N. Imai, K. Yoshida, Y. Ito, and T. Saeki, "Clinicopathological findings of immunoglobulin G4-related kidney disease," *Clinical and Experimental Nephrology*, vol. 15, no. 6, pp. 810–819, 2011.

Clinical Study

Cutoff Values of Serum IgG4 and Histopathological IgG4+ Plasma Cells for Diagnosis of Patients with IgG4-Related Disease

**Yasufumi Masaki,[1] Nozomu Kurose,[2] Motohisa Yamamoto,[3]
Hiroki Takahashi,[3] Takako Saeki,[4] Atsushi Azumi,[5] Shinji Nakada,[6] Shoko Matsui,[7]
Tomoki Origuchi,[8] Susumu Nishiyama,[9] Kazunori Yamada,[10] Mitsuhiro Kawano,[10]
Akira Hirabayashi,[11] Keita Fujikawa,[12] Tomoko Sugiura,[13] Masanobu Horikoshi,[14]
Naoto Umeda,[14] Hiroshi Minato,[2] Takuji Nakamura,[1] Haruka Iwao,[1] Akio Nakajima,[1]
Miyuki Miki,[1] Tomoyuki Sakai,[1] Toshioki Sawaki,[1] Takafumi Kawanami,[1]
Yoshimasa Fujita,[1] Masao Tanaka,[1] Toshihiro Fukushima,[1] Katumi Eguchi,[8]
Susumu Sugai,[1] and Hisanori Umehara[1]**

[1] Hematology and Immunology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Kahoku-gun, Ishikawa 920-0293, Japan
[2] Department of Pathology and Laboratory Medicine, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Kahoku-gun, Ishikawa 920-0293, Japan
[3] First Department of Internal Medicine, School of Medicine, Sapporo Medical University, S1 W17 Chuo-ku, Sapporo, Hokkaido 060-8556, Japan
[4] Department of Internal Medicine, Nagaoka Red Cross Hospital, Sensyu-2 297-1, Nagaoka-shi, Niigata 940-2085, Japan
[5] Ophthalmology Department, Kobe Kaisei Hospital, Shinohara Kitamachi 3-11-15, Nada Ku, Kobe City, Hyogo 657-0068, Japan
[6] Department of Japanese Oriental Medicine, University of Toyama, 2630 Sugitani, Toyama city, Toyama 930-0194, Japan
[7] First Department of Internal Medicine, University of Toyama, 2630 Sugitani, Toyama city, Toyama 930-0194, Japan
[8] Nagasaki Graduate School of Health Sciences, 1-7-1 Sakamoto, Nagasaki city, Nagasaki 852-8501, Japan
[9] Rheumatic Disease Center, Kurashiki Medical Center, 250 Bakuro-chou, Kurashiki, Okayama 710-8522, Japan
[10]Division of Rheumatology, Department of Internal Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8641, Japan
[11]Hiroshima Bay Clinic, 2-2-19 Yanoshin-machi, Aki-ku, Hiroshima city, Hiroshima 736-0084, Japan
[12]Department of Internal Medicine, Isahaya General Hospital, 24-1 Eisohohigasi-macki, Isahaya, Nagasaki 854-8501, Japan
[13]Sugiura Clinic, 2-8-3 Imaichi-chou, Kitashin-machi, Izumo, Shimane 693-0002, Japan
[14]Department of Rheumatology, University of Tsukuba, 2-1-1 Amakubo, Tsukuba, Ibaraki 305-8576, Japan

Correspondence should be addressed to Yasufumi Masaki, yasum@kanazawa-med.ac.jp

IgG4-related disease is a new disease classification established in Japan in the 21st century. Patients with IgG4-related disease display hyper-IgG4-gammaglobulinemia, massive infiltration of IgG4+ plasma cells into tissue, and good response to glucocorticoids. Since IgG4 overexpression is also observed in other disorders, it is necessary to diagnose IgG4-related disease carefully and correctly. We therefore sought to determine cutoff values for serum IgG4 and IgG4/IgG and for IgG4+/IgG+ plasma cells in tissue diagnostic of IgG4-related disease. *Patients and Methods*. We retrospectively analyzed serum IgG4 concentrations and IgG4/IgG ratio and IgG4+/IgG+ plasma cell ratio in tissues of 132 patients with IgG4-related disease and 48 patients with other disorders. *Result*. Serum IgG4 >135 mg/dl demonstrated a sensitivity of 97.0% and a specificity of 79.6% in diagnosing IgG4-related disease, and serum IgG4/IgG ratios >8% had a sensitivity and specificity of 95.5% and 87.5%, respectively. IgG4+cell/IgG+ cell ratio in tissues >40% had a sensitivity and specificity of 94.4% and 85.7%, respectively. However, the number of IgG4+ cells was reduced in severely fibrotic parts of tissues. *Conclusion*. Although a recent unanimous consensus of all relevant researchers in Japan recently established the diagnostic criteria for IgG4-related disease, findings such as ours indicate that further discussion is needed.

1. Introduction

IgG4-related disease (IgG4-RD), a new disease classification first established in Japan in the 21st century, is characterized by hyper-IgG4-gammaglobulinemia and massive infiltration of IgG4-positive plasma cells into various swollen organs [1–10]. In general, a serum IgG4 concentration >135 mg/dL has been established as the cutoff value for the diagnosis of patients with IgG4-RD and is used in the joint consensus criteria of the Okazaki and Umehara groups investigating IgG4-RD for the Ministry of Health, Labor, and Welfare of Japan [11].

Some patients with early or limited stage IgG4-RD, however, may show the sufficient pathological characteristics and clinical features of this disease, such as good response to glucocorticoids, despite having serum IgG4 concentrations <135 mg/dL. In addition, an IgG4+/IgG+ ratio >40% in tissue and >10 cells/high-power field (HPF) have been used in the histopathologic diagnosis of IgG4-RD in Japan. Thus, a proper diagnosis of these patients may require the use of other criteria, including IgG4+/IgG plasma cell ratio. Since lower cutoff values may increase sensitivity while decreasing specificity, it is necessary to establish accurate cut off values for this ratio.

To better establish the diagnostic criteria for IgG4-RD, we, the members of the IgG4+MOLPS/Mikulicz's disease research group in Japan, sought to determine the cutoff values for serum IgG4 and IgG4/IgG and for IgG4+/IgG+ plasma cells in tissue diagnostic of IgG4-RD using retrospectively collected data.

2. Materials and Methods

2.1. Measurement of Serum IgG4 Concentration. Serum IgG4 concentrations and IgG4/IgG ratio and the ratio of IgG4+/IgG+ plasma cells in tissue were determined in 132 patients with IgG4-RD and 48 patients with other disorders registered retrospectively in the IgG4+MOLPS/Mikulicz's disease research group (Table 1). The 48 patients with other disorders included 33 with Sjögren's syndrome, 3 with multicentric Castleman's disease (MCD), 3 with B-cell lymphoma, 2 with sarcoidosis, and 1 each with Kimura's disease, ulcerative colitis, autoimmune hepatitis, IgG-type monoclonal gammopathy of undetermined significance, progressive transformation of the germinal center, scleritis, and severe keratoconjunctivitis sicca. The study was approved by the review boards of Kanazawa Medical University and all other collaborating institutions, and all patients provided written informed consent for the use of their data and samples. The sensitivities, specificities, and ROC curves of serum IgG4 >135 mg/dL and various serum IgG4/IgG ratios were statistically analyzed using SPSS v.11 (SPSS Inc., Chicago, IL, USA).

Patients with borderline IgG4-RD were defined carefully as those with (1) >40% IgG4+/IgG+ plasma cells in tissue, (2) strict pathological differential, and (3) a typical clinical

Table 1: Involved organs in 132 patients with IgG4-RD. More than half (64 cases) the patients in our series had so-called Mikulicz's disease, with symmetrical swelling of at least two sets of lacrimal, parotid, or submandibular glands.

Involved organ	Number of patients
Total	132
Lacrimal gland	75
Parotid gland	32
Submandibular gland	74
Pancreas	20
Kidney	21
Lung	6
Lymph node	4
Thyroid	4
Liver	3
Retroperitoneum	3
Dura	1
Hypophysis	1
Bile duct	1

course (spontaneous regression or no change without treatment, or good response to an initial daily dose of <0.6 mg/kg prednisolone).

2.2. Analysis of IgG4+ Cells in Tissue. The numbers of IgG4+ and IgG+ cells in tissue samples from 36 patients with IgG4-RD and from 21 with other disorders were determined by counting cells counts in 5 high-power fields (HPF) under light microscopy. We also recounted cell areas of 17 samples from patients with IgG4-RD that contained both fibrotic and nonfibrotic parts.

We also assessed the sensitivity and specificity of IgG4+/IgG+ cell ratios >10%, >20%, >30%, >40%, and >50%, and of >10, >20, >30, >40 and >50 IgG4+cells/HPF, as well as the presence of obliterative phlebitis, storiform fibrosis, eosinophilia, fibrosis, and lymphocyte infiltration as determined by hematoxylin and eosin staining in the diagnosis of IgG4-RD.

Tissue samples with borderline IgG4-RD were defined as those with (1) serum IgG4 >135 mg/dL, (2) strict pathological differential, and (3) typical clinical course. Clinical course including response to steroid is not included in the comprehensive diagnostic criteria for IgG4-RD, to avoid the needless treatment with steroid of patients suspected of having IgG4-RD. As this was a retrospective analysis, however, we analyzed the clinical course of these borderline patients, including their response to steroid treatment.

3. Results

3.1. Serum IgG4 Concentration. A serum IgG4 cutoff value >135 mg/dL had a sensitivity of 97.0% and a specificity of 79.6% for the diagnosis of IgG4-RD. In 4 patients with relatively small and restricted lesions, however, this criterion was not adequate to diagnose IgG4-RD, although all had a
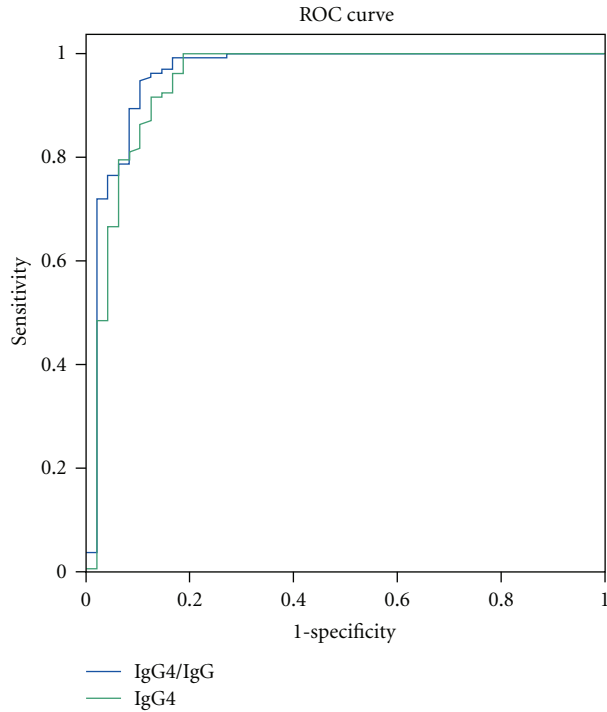
TABLE 2: Serum IgG and IgG4 concentrations and IgG4/IgG4 ratio of patients with false-positive and false-negative diagnoses of IgG4-RD.

False positives			
Diagnosis	IgG (mg/dL)	IgG4 (mg/dL)	IgG4/IgG
(1) MCD	7,080	4,560	64.4%
(2) MCD	4,420	690	15.6%
(3) B-cell lymphoma	2,510	456	18.2%
(4) MCD	2,960	295	10.0%
(5) MCD	2,476	295	11.9%
(6) B-cell lymphoma	4,010	271	6.8%
(7) Scleritis	2,900	232	8.0%
(8) Sarcoidosis	1,380	189	13.7%
(9) Sjögren's syndrome	3,920	171	4.4%
(10) Malignant lymphoma	1,930	141	7.3%
False negatives			
Diagnosis	IgG(mg/dL)	IgG4(mg/dL)	IgG4/IgG
(1) IgG4-RD	1,149	125	10.9%
(2) IgG4-RD	1,210	123	10.2%
(3) IgG4-RD	1,228	111	9.0%
(4) IgG4-RD	1,260	106	8.4%

IgG4-RD: IgG4-related disease; MCD: multicentric Castleman's disease.

TABLE 3: Sensitivity and specificity of serum cutoff values in the diagnosis of IgG4-RD.

	Sensitivity	Specificity
IgG4 > 135 mg/dL	97.0%	79.6%
IgG4/IgG > 5%	99.2%	83.3%
IgG4/IgG > 6%	97.0%	83.3%
IgG4/IgG > 7%	97.0%	85.4%
IgG4/IgG > 8%	95.5%	87.5%
IgG4/IgG > 9%	92.4%	89.6%
IgG4/IgG > 10%	89.4%	91.7%

histopathology and clinical course compatible with IgG4-RD (Table 2). All 4 patients were diagnosed with IgG4-RD based on a serum IgG4/IgG ratio >8%.

In contrast, neither a serum IgG4 cutoff of >135 mg/dL nor a serum IgG4/IgG ratio >8% was adequate for the diagnosis of four patients with MCD and one each with B-cell lymphoma, scleritis, and Sjögren's syndrome, because all of these patients had hyper-IgG4-globulinemia associated with polyclonal gammopathy. We therefore estimated the sensitivity and specificity of various IgG4/IgG ratios (Table 3). We found that the ROC curves for absolute serum IgG4 concentration and serum IgG4/IgG ratio were almost identical (Figure 1).

3.2. Analysis of IgG4+ Cells in Tissue Samples. We also assessed the ability of the ratio of IgG4+/IgG+ plasma cell ratios in 5 HPFs of tissue samples to diagnose IgG4-RD. We found that a ratio >40% had a sensitivity of 94.4% and a specificity of 85.7% (Table 4). Although >10 IgG4+ cells per HPF had a sensitivity of 100%, They had specificities of only 38.1%.

In tissues containing both fibrotic and nonfibrotic areas, we counting the number cells in each part showed that fibrotic areas contained fewer IgG4+ cells (Table 5).

We also assessed the ability of obliterative phlebitis and storiform fibrosis to diagnose IgG4-RD. Although both had specificities of 100%, their sensitivities were not very high (Table 4).

4. Discussion

Serum IgG4 >135 mg/dL has been widely accepted as a cutoff value for diagnosis of IgG4-RD. Although this concentration was determined by comparing patients with IgG4-related sclerosing pancreatitis and those with pancreatic cancer [1], it has also been used to diagnose IgG4-RD involving other organs. For example, we have utilized this cutoff value as a diagnostic criterion for IgG4-related Mikulicz's disease [5] and IgG4-related kidney disease [12], and, in 2011, it was adopted in the comprehensive clinical diagnostic criteria of IgG4-related diseases [11]. Most patients with IgG4-RD show multiple organ involvement at diagnosis, with both high absolute serum IgG4 concentrations and serum IgG4/IgG ratios. However, some patients with early and/or limited stage IgG4-RD do not present with high IgG4-globulinemia (Figure 2), with some not having IgG4 concentrations >135 mg/dL. We have therefore tested the

TABLE 4: Sensitivity and specificity of pathological findings for the diagnosis of IgG4-RD.

	Sensitivity	Specificity
IgG4+/IgG+ > 10%	100.0%	33.3%
IgG4+/IgG+ > 20%	100.0%	47.6%
IgG4+/IgG+ > 30%	100.0%	71.4%
IgG4+/IgG+ > 40%	94.4%	85.7%
IgG4+/IgG+ > 50%	94.4%	95.2%
IgG4+cells/HPF > 10	100.0%	38.1%
IgG4+cells/HPF > 20	97.2%	42.9%
IgG4+cells/HPF > 30	97.2%	61.9%
IgG4+cells/HPF > 40	91.7%	66.7%
IgG4+cells/HPF > 50	86.1%	71.4%
Obliterative phlebitis	54.5%	100.0%
Storiform fibrosis	31.4%	100.0%
Eosinophilia	42.9%	100.0%
Fibrosis	91.4%	82.4%
Lymphocytic infiltration	100.0%	16.7%

TABLE 5: Recounting in each areas of 17 samples containing both fibrotic and nonfibrotic parts. All patients were diagnosed with IgG4-RD but had biopsy specimens that were too small (samples 1–14) or with relatively large fibrotic areas inadequate to diagnose IgG4-RD (samples 15–17). All samples had >10 IgG4+ cells per HPF.

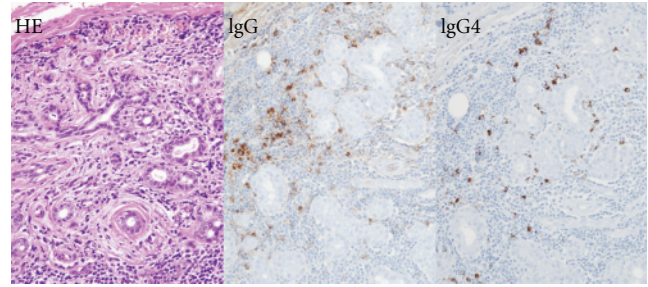| Tissue	Fibrosis+	Fibrosis−
(1) Pancreas	76.6%	94.0%
(2) Submandibular gland	75.4%	89.2%
(3) Submandibular gland	72.1%	73.0%
(4) Submandibular gland	72.1%	97.3%
(5) Submandibular gland	71.8%	99.1%
(6) Pancreas	67.7%	95.0%
(7) Labial salivary glands	65.0%	70.8%
(8) Lung	58.9%	94.4%
(9) Submandibular gland	49.2%	68.9%
(10) Gall bladder	48.6%	94.0%
(11) Bile duct	46.8%	95.0%
(12) Submandibular gland	46.2%	74.1%
(13) Orbit	44.2%	94.4%
(14) Submandibular gland	43.6%	95.0%
(15) Submandibular gland	33.3%	95.0%
(16) Submandibular gland	25.9%	51.5%
(17) Labial salivary glands	8.0%	76.2%

FIGURE 2: Histopathology of a patient with limited stage IgG4-related dacryoadenitis. A 35-year-old woman presented with swelling of her bilateral lacrimal glands. Her serum IgG and IgG4 concentrations were 1,149 mg/dL and 125 mg/dL, respectively, and her serum IgG4/IgG ratio was 10.88%. Histopathological examination of a lacrimal gland biopsy revealed IgG4+ plasma cell infiltration, with an IgG4+/IgG+ cell ratio of 74.7%, and 53 IgG4+ cells per HPF, although sclerotic changes were not severe. Treatment with prednisolone 20 mg/day resulted in a rapid and dramatic improvement in symptoms. Although using an absolute serum IgG4 cutoff concentration of >135 mg did not result in diagnosis of IgG4-RD, her clinical course and histopathology were typical of IgG4-RD. However, using a serum IgG4/IgG ratio >8% as a cutoff value resulted in a diagnosis of IgG4-RD.

ability of alternative criteria to diagnose for IgG4-RD. Although we found that a serum IgG4/IgG ratio >5% had the highest sensitivity, the normal ratio is about 5-6%, making this cut off value misleading. An IgG4/IgG ratio >8% had a sensitivity similar to that of absolute IgG4 >135 mg/dL, but a greater specificity, enabling us to diagnose 4 patients with lower absolute IgG4 concentrations as having IgG4-RD (Table 2). Since the standard cut off of absolute IgG4 >135 mg/dL demonstrated excellent sensitivity and specificity, it should be utilized, except for patients with early and/or limited IgG4-RD, for whom we propose using an IgG4/IgG ratio >8%.

Careful diagnosis is required in patients with lower IgG4 concentrations, since those patients may have other distinct disorders with different clinical features than IgG4-RD. Patients with untypical clinical courses, including glucocorticoid refractoriness, should be reassessed.

IgG4+/IgG+ plasma cell ratios in tissue >40% and >50%, and >10 IgG4+ cells per HPF have been used for the diagnosis of IgG4-RD. We found that an IgG4+/IgG+ cell ratio >40% in tissue had a sensitivity of 94.4% and a specificity of 85.7% in the diagnosis of IgG4-RD. We also found that IgG4+ plasma cell concentrations in tissue were diminished in fibrotic tissue areas, suggesting that a ratio >40% is a better histopathologic cutoff value. The presence of obliterative phlebitis and storiform fibrosis demonstrated specificities of 100%, but their sensitivities were much lower, indicating that these findings would be useful when added to, but not in place of, other results.

Since patients with disorders such as MCD and lymphoma may demonstrate hyper-IgG4-gammaglobulinemia and massive IgG4+ plasma cell infiltration in tissue, serum IgG4 concentration and IgG4+ cells in tissue are not specific indicators of IgG4-RD. Rather, a diagnosis of IgG4-RD should be based on the overall balance of clinical features, such as disease distribution throughout the body, clinical course, serum concentrations, and histopathology.

The pathologic consensus statement of the first international Symposium on IgG4-RD in Boston did not adopt IgG4+/IgG+ cell ratio in tissue as diagnostic, although it

did suggest cutoffs for numbers of IgG4+ cells in HPFs of various organs. This, however, may be confusing for many pathologists and physicians. Although pathologic findings are very important in the diagnosis for IgG4-RD, clinical features and serological findings should be included.

Recently, some patients with IgG4-RD were found to have lymphoma [13, 14] and other types of cancer [15, 16]. Thus IgG4-RD may not always be a benign disease with good prognosis. Many patients referred to our centers with glucocorticoid refractory IgG4-RD were diagnosed incorrectly, suggesting the need for more accurate diagnostic criteria for these diseases.

Acknowledgments

References

[1] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[2] T. Kamisawa and A. Okamoto, "Autoimmune pancreatitis: Proposal of IgG4-related sclerosing disease," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 613–625, 2006.

[3] Y. Masaki, L. Dong, N. Kurose et al., "Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders," *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1310–1315, 2009.

[4] M. Yamamoto, H. Takahashi, K. Hasebe et al., "The analysis of interleukin-6 in patients with systemic IgG4-related plasmacytic syndrome—expansion of SIPS to the territory of Castleman's disease," *Rheumatology*, vol. 48, no. 7, pp. 860–862, 2009.

[5] Y. Masaki, S. Sugai, and H. Umehara, "IgG4-related diseases including Mikulicz's disease and sclerosing pancreatitis: diagnostic insights," *Journal of Rheumatology*, vol. 37, no. 7, pp. 1380–1385, 2010.

[6] Y. Masaki, H. Iwao, A. Nakajima, M. Miki, S. Sugai, and H. Umehara, "IgG4-related disease (IgG4+MOLPS)—diagnostic criteria and diagnostic problems," *Current Immunology Reviews*, vol. 7, no. 2, pp. 172–177, 2011.

[7] Y. Masaki, N. Kurose, and H. Umehara, "IgG4-related disease: a novel lymphoproliferative disorder discovered and established in Japan in the 21st century," *Journal of Clinical and Experimental Hematopathology*, vol. 51, pp. 13–20, 2010.

[8] T. Saeki, S. Nishi, N. Imai et al., "Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis," *Kidney International*, vol. 78, no. 10, pp. 1016–1023, 2010.

[9] Y. Sato, K. Notohara, M. Kojima, K. Takata, Y. Masaki, and T. Yoshino, "IgG4-related disease: Historical overview and pathology of hematological disorders," *Pathology International*, vol. 60, no. 4, pp. 247–258, 2010.

[10] H. Umehara, K. Okazaki, Y. Masaki et al., " The Research Program for Intractable Disease by Ministry of Health, Labor and Welfare (MHLW) Japan G4 team. A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details," *Modern Rheumatology*, vol. 22, pp. 1–14, 2012.

[11] H. Umehara, K. Okazaki, Y. Masaki et al., "Comprehensive diagnostic criteria (CDC) for IgG4-related disease (IgG4RD) 2011," *Modern Rheumatology*, vol. 22, pp. 21–30, 2012.

[12] M. Kawano, T. Saeki, H. Nakashima et al., "Proposal for diagnostic criteria for IgG4-related kidney disease," *Clinical and Experimental Nephrology*, vol. 15, pp. 615–626, 2011.

[13] W. Cheuk, H. K. L. Yuen, A. C. L. Chan et al., "Ocular adnexal lymphoma associated with IgG4+ chronic sclerosing dacryoadenitis: a previously undescribed complication of IgG4-related sclerosing disease," *American Journal of Surgical Pathology*, vol. 32, no. 8, pp. 1159–1167, 2008.

[14] Y. Sato, K. I. Ohshima, K. Ichimura et al., "Ocular adnexal IgG4-related disease has uniform clinicopathology," *Pathology International*, vol. 58, no. 8, pp. 465–470, 2008.

[15] O. Hyoung-Chul, K. J. Gyu, K. J. Wook et al., "Early bile duct cancer in a background of sclerosing cholangitis and autoimmune pancreatitis," *Internal Medicine*, vol. 47, no. 23, pp. 2025–2028, 2008.

[16] T. Kamisawa, P. Y. Chen, Y. Tu et al., "Pancreatic cancer with a high serum IgG4 concentration," *World Journal of Gastroenterology*, vol. 12, no. 38, pp. 6225–6228, 2006.

Research Article

Histopathologic Overlap between Fibrosing Mediastinitis and IgG4-Related Disease

Tobias Peikert,[1] Bijayee Shrestha,[2] Marie Christine Aubry,[2] Thomas V. Colby,[3] Jay H. Ryu,[1] Hiroshi Sekiguchi,[1] Thomas C. Smyrk,[2] Ulrich Specks,[1] and Eunhee S. Yi[2]

[1] *Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN 55905, USA*
[2] *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA*
[3] *Department of Laboratory Medicine and Pathology, Mayo Clinic, Scottsdale, AZ 85259, USA*

Correspondence should be addressed to Tobias Peikert, peikert.tobias@mayo.edu

Fibrosing mediastinitis (FM) and IgG4-related disease (IgG4-RD) are two fibroinflammatory disorders with potentially overlapping clinical and radiological features. In this paper, we looked for histopathologic features of IgG4-RD and enumerated infiltrating IgG4-positive plasma cells within mediastinal tissue biopsies from FM patients. We identified 15 consecutive FM surgical mediastinal tissue biopsies between 1985 and 2006. All patients satisfied the clinical and radiological diagnostic criteria for FM. All patients had either serological or radiological evidence of prior histoplasmosis or granulomatous disease, respectively. Formalin-fixed paraffin-embedded tissue sections of all patients were stained for H&E, IgG, and IgG4. Three samples met the predefined diagnostic criteria for IgG4-RD. In addition, characteristic histopathologic changes of IgG4-RD in the absence of diagnostic numbers of tissue infiltrating IgG4-positive plasma cells were seen in a number of additional cases (storiform cell-rich fibrosis in 11 cases, lymphoplasmacytic infiltrate in 7 cases, and obliterative phlebitis/arteritis in 2 cases). We conclude that up to one-third of histoplasmosis or granulomatous-disease-associated FM cases demonstrate histopathological features of IgG4-RD spectrum. Whether these changes occur as the host immune response against Histoplasma or represent a manifestation of IgG4-RD remains to be determined. Studies to prospectively identify these cases and evaluate their therapeutic responses to glucocorticoids and/or other immunosuppressive agents such as rituximab are warranted.

1. Background

IgG4-related disease (IgG4-RD) is recognized to include a growing number of fibroinflammatory disorders [1–5]. Histopathologic evaluation typically demonstrates distinctive cellular fibrosis organized in an irregular whorled pattern (often referred to as "storiform fibrosis"), obliterative phlebitis/arteritis, and prominent lymphoplasmacytic tissue infiltration [6]. Tissue immunostaining and serum IgG-subclass assessment characteristically reveal large numbers of IgG4 producing plasma cells and elevated serum IgG4 levels, respectively [6].

IgG4-RD was first described in the context of autoimmune pancreatitis presenting with obstructive jaundice due to a space-occupying lesion within the pancreas [7, 8].

Since these initial reports, IgG4-RD has been demonstrated to involve various other organs including the biliary tree (sclerosing cholangitis), salivary (sclerosing sialadenitis), and lacrimal glands (sclerosing dacroadenitis) in isolation or in combination (multisystem involvement) [1–5, 9]. IgG4-RD is typically characterized by clinical and radiographic evidence of an idiopathic metabolically active (e.g., fluorodeoxyglucose-avid) space-occupying lesions within different organs [1–5, 9, 10]. Therapeutically, patients with IgG4-RD typically respond to immunosuppressive therapy with glucocorticoids [11].

Fibrosing mediastinitis (FM), also called sclerosing mediastinitis, is a rare syndrome characterized by an aggressive fibroinflammatory process within the mediastinum [12–15]. Progressive fibrosis caused by the proliferation of

invasive fibrous tissue within the mediastinum frequently results in compression and functional compromise of vital mediastinal structures [13–15]. Consequently, FM can lead to substantial disease-related morbidity and perhaps even increased mortality [13, 15].

Although the pathogenesis of FM remains unknown, radiographic, serologic, or histopathologic evidence of prior *Histoplasma capsulatum* infection can often be documented. In endemic areas of North America, the majority of FM cases are thought to represent a rare hypersensitivity reaction to this infection [13–18]. Additional infectious triggers implicated in the pathogenesis of FM include other fungal and mycobacterial organisms associated with granulomatous mediastinitis [13–18]. Finally, there are rare immune-mediated (idiopathic) and drug-induced (e.g., methysergide) cases of FM [13–18]. Interestingly, patients with idiopathic immune-mediated FM frequently have other disease manifestations such as retroperitoneal fibrosis or Riedels thyroiditis, all of which have been associated with the IgG4-RD spectrum [6, 13–18]. Except for selected patients with the idiopathic immune-mediated variant of FM, therapeutic successes using systemic glucocorticoids and other immunosuppressive agents are exceptionally rare [14].

Chest computed tomography in "granulomatous-infection-associated" FM characteristically demonstrates focal, commonly calcified, and most commonly right-sided mediastinal mass lesions. This contrasts the diffuse noncalcified mediastinal infiltration classically seen in idiopathic immune-mediated or drug-induced cases [19, 20]. Given the high diagnostic yield of chest radiological evidence of a focal, calcified mediastinal mass compromising other mediastinal structures, diagnostic tissue biopsies are currently largely reserved to exclude alternative diagnoses such as mediastinal malignancies [14, 20].

Mediastinal lymphadenopathy is one of the most frequent extrapancreatic disease manifestations in patients with IgG4-RD [21, 22]. However, up to date only a single case of FM attributed to IgG4-RD disease has been reported in the medical literature. This Japanese patient had a clinical and radiographic presentation consistent with idiopathic immune-mediated FM but demonstrated histopathological changes typical of IgG4-RD, had an elevated serum IgG4 level, and responded favorably to glucocorticoid therapy [23]. Fibrosis within the mediastinum without compression of mediastinal structures has been reported in the context of patients with other disease manifestations of IgG4-RD [24–26].

We and others have recently demonstrated that mediastinal biopsies from consecutive patients with FM frequently contain large numbers of inflammatory cells including a high number of CD138- (syndecan-1-) positive plasma cells [12, 14]. Consequently, FM is now considered to represent a fibroinflammatory rather than a purely fibrotic disease process. Based on these fibroinflammatory changes associated with the local accumulation of plasma cells, we hypothesized that a subset of FM cases may belong in the IgG4-RD spectrum and demonstrate histopathological and immunological changes consistent with IgG4-RD.

2. Patients and Methods

A search of the Mayo Clinic pathology database for a histopathological diagnosis of FM (we used the search terms of *fibrosing mediastinitis, sclerosing mediastinitis,* and *mediastinal fibrosis*) between 1985 and 2006 identified 21 biopsy specimens in the Mayo Clinic tissue registry. The medical records of these cases were reviewed and FM cases were defined clinically by the presence of chest radiological evidence of an infiltrative (crossing tissue planes) mediastinal process associated with the invasion or obstruction of mediastinal structures. Based upon evidence of coexisting malignancies, two patients (one with malignant thymoma and one with desmoplastic mesothelioma) were excluded. The histopathology of the remaining cases was independently reviewed by two of the investigators (TVC and ESY) and a diagnosis of FM was confirmed in 15 patients. Four cases were excluded due to the absence of invasive fibrosis. The clinical and radiographic features and the characterization of adaptive immune response (immunostaining for CD3, CD8, CD20, CD138, and S100) of these cases have been described in detail elsewhere [14].

Immunostaining was performed using a DAKO auto-staining system (DAKO, Carpinteria, CA, USA). The monoclonal mouse anti-human IgG4 antibody (clone HP6025, dilution 1 : 100, Zymed, San Francisco, CA, USA) was used to stain formalin-fixed, paraffin-embedded tissue sections. In a similar fashion, IgG staining was performed on consecutive tissue sections using the polyclonal rabbit anti-human IgG antibody (IS512, dilution 1 : 10,000, DAKO, Carpinteria, CA, USA).

The presence and number of IgG- and IgG4-positive cells was evaluated in all 15 FM cases. To enumerate IgG and IgG4-positive plasma cells, the entire slide was scanned at low power for the areas with the highest IgG4-positive plasma cell density. For each case, the three high power fields (hpf) with the highest density were photographed using a magnification of 40x (Nikon Eclipse E400 microscope, field diameter 0.55 mm and Olympus DP70 camera, $0.0645\,mm^2$). The corresponding areas were also photographed on the IgG-stained slides. The number of IgG4- and IgG-positive plasma cells was determined in 3 hpf by manually counting positive cells on the photomicrographs and cell numbers were averaged. The number of IgG4-positive cells/hpf was determined, and the fraction of IgG4-positive cells of all IgG-positive plasma cells [%] was calculated for each case.

3. Definitions

3.1. Clinical Definition of FM.
Presence of radiological evidence of an infiltrative, space-occupying mediastinal process with associated pulmonary vascular, airway, superior vena cava (SVC), or esophageal compression. Patients with mediastinal malignancies and/or prior mediastinal radiation therapy were excluded [14, 20].

3.2. Histological Case Definition of FM.
A histopathological diagnosis of FM required the presence of extensive tissue fibrosis. This fibrous tissue typically infiltrates and obliterates

TABLE 1: Radiological, microbiological and histological characteristics of the 15 FM patients.

Age/gender	Histological features of IgG4-RD	Chest radiology	Histoplasmosis/granulomatous disease	Histological granulomatous inflammation
25/M	Definite	Right mediastinal mass	Suggestive	Absent
32/M	Definite	Calcified left hilar mass	Conclusive	Present
65/F	Definite	Right mediastinal mass	Conclusive	Present
51/F	Absent	Calcified left cervical and right hilar mass	Conclusive	Present
31/F	Absent	Calcified right mediastinal mass	Suggestive	Absent
27/F	Absent	Calcified right mediastinal mass	Conclusive	Present
27/F	Absent	Diffuse mediastinal infiltration	Suggestive	Present
48/F	Absent	Left mediastinal mass	Suggestive	Present
35/M	Absent	Calcified bilateral hilar masses	Conclusive	Present
43/F	Absent	Right mediastinal mass	Not available	Present
44/M	Absent	Calcified anterior mediastinal mass	Not available	Absent
27/F	Absent	Calcified right mediastinal mass	Conclusive	Present
59/F	Absent	Calcified right mediastinal mass	Conclusive	Present
36/F	Absent	Calcified right mediastinal mass	Conclusive	Absent
58/F	Absent	Calcified right mediastinal mass	Not available	Absent

(a)

(b)

(c)
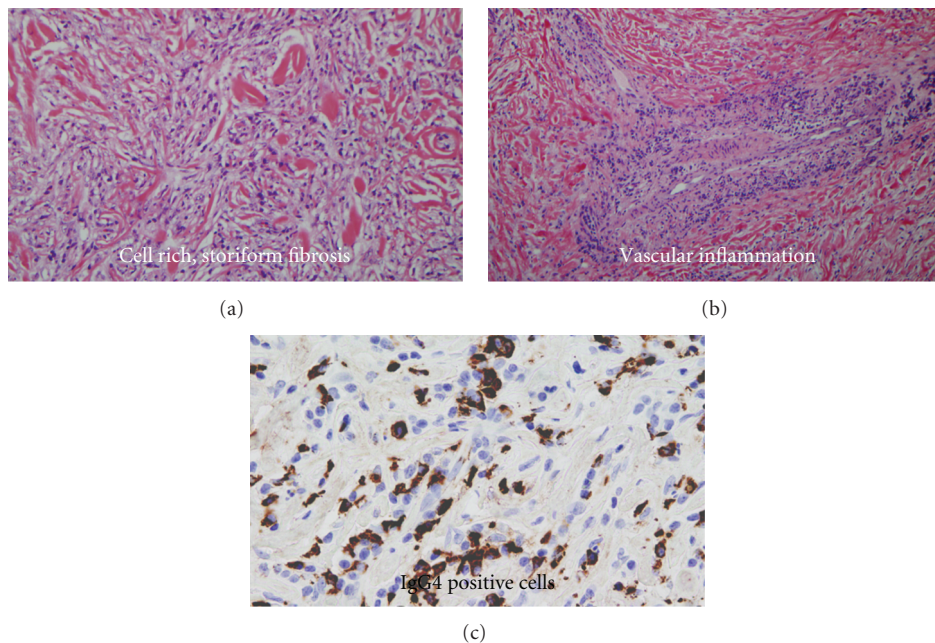
FIGURE 1: Histopathological changes and IgG4 immunostaining demonstrate an overlap between FM and IgG4-RD in a subset of patients ($n = 3$), (a). cell rich, storiform fibrosis, (b). vascular inflammation and (c). IgG4 positive plasma cells. (representative images selected from 3 patients).

adipose tissue with or without patchy mononuclear immune cell infiltration in the absence of malignancy.

3.3. Histoplasma Capsulatum Infection.

A *conclusive* diagnosis of infection was assumed in the presence of a positive fungal stain (Grocott methenamine silver (GMS)) or culture of the biopsy tissue specimens and/or serologic titer $\geq 1:32$ and/or presence of an M or H band by complement fixation/immunodiffusion. A *suggestive* diagnosis was defined as a serologic titer $>1:8$ and/or radiological features (pulmonary, splenic, and/or hepatic granulomas) suggestive of previous granulomatous infection.

3.4. Granulomatous Disease.

Patients with radiological features of prior granulomatous disease, histological evidence of granulomatous inflammation, or a localized calcified mass lesion within the mediastinum were classified as previous granulomatous disease [20].

3.5. IgG4-RD. IgG4-RD was defined using the following definition: *definite case of IgG4-RD*: at least two of the following three histological features: (1) lymphoplasmacytic infiltrate, (2) storiform-type fibrosis, or (3) obliterative phlebitis/arteritis plus $\geq$50 IgG4-positive cells/hpf with an IgG4$^+$/IgG$^+$ ratio $\geq$40%. *Probable case of IgG4-RD*: one histological feature plus $\geq$50 IgG4-positive cells/hpf with an IgG4$^+$/IgG$^+$ ratio $\geq$40%. *Unlikely case of IgG4-RD*: remaining cases. This definition is based on the consensus recommendations provided by a panel of experts during the International Symposium on IgG4-Related Disease, Boston, MA, October 2011 (http://www2.massgeneral.org/pathology/symposium/IgG4_related_systemic_dis.asp).

The Mayo Clinic Institutional Review Board (IRB) approved the study.

4. Results

All 15 patients underwent surgical resection/debulking (7 patients) or surgical biopsy (8 patients) at Mayo Clinic, Rochester, MN, between 1985 and 2006 [14].

Histopathological examination universally demonstrated the proliferation of fibrous tissue with associated infiltration of the surrounding mediastinal fat and soft tissues. Surgical sampling of the adjacent lymph nodes was performed in 8 of 15 cases. In 6 cases, the lymph node samples revealed extensive perinodal fibrosis, extending more than 2 mm beyond the capsule. One case exhibited only mild perinodal sclerosis with fibrous reaction less than 2 mm. In another case, perinodal scarring was absent. In 10 of 15 cases histopathologic evidence of prior granulomatous disease with associated necrosis (with or without calcification) and a surrounding dense fibrotic rim was identified. GMS staining detected characteristic yeast forms for *Histoplasma capsulatum* in 6 of 13 cases. The organisms were typically detected in the necrotic areas of granulomas (Table 1).

Three cases met the predefined diagnostic histological criteria for definite IgG4-RD (FM IgG4-RD) (Figure 1). The absolute numbers of IgG4-positive plasma cells and the IgG4$^+$/IgG$^+$ ratios are summarized in Figure 2. No cases of probable IgG4-RD were identified. Interestingly, despite the absence of the required number and ratio of IgG4-positive plasma cells, characteristic histopathological findings of IgG4-RD such as cell-rich storiform fibrosis and lymphoplasmacytic infiltration were frequently present in the remaining non-IgG4-RD cases (Table 2).

Unfortunately, serum IgG4 levels were not available for any of these patients and none of them was treated with glucocorticoids or other immunosuppressants. The demographic, clinical, and radiological characteristics are summarized in Table 3. Based upon the geographic location of our institution in the Midwestern United States, it is not surprising that all of our cases had evidence of prior granulomatous infections, predominantly histoplasmosis (Table 1). There were no idiopathic immune-mediated cases among our 15 FM patients. Overall there were no significant differences between FM IgG4-RD and FM-non-IgG4-RD cases
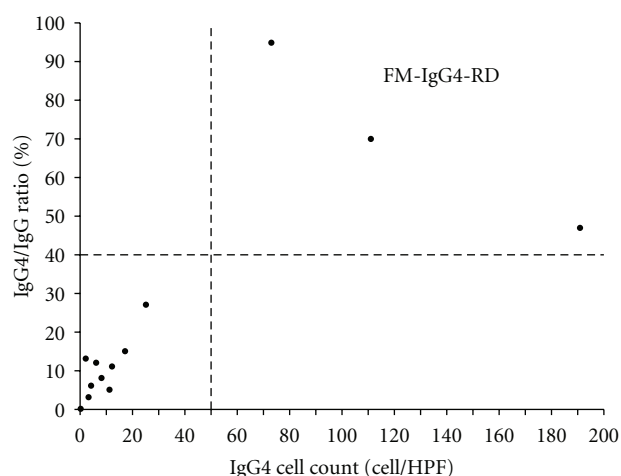
FIGURE 2: Number of IgG4-positive plasma cells and the corresponding IgG4$^+$/IgG$^+$ ratios in FM patients (n = 15, 3 patients had no IgG4 positive plasma cells).

TABLE 2: Distribution of the diagnostic criteria of IgG4-RD.

	FM IgG4-RD (N = 3)	FM-non-IgG4-RD (N = 12)
$\geq$50 IgG4-positive plasma cells/HPF and $\geq$40% IgG4/IgG ratio, case (%)	3 (100)	0 (0)
Lymphoplasmacytic infiltrate, cases (%)	3 (100)	7 (58)
Storiform, cell-rich fibrosis, cases (%)	3 (100)	11 (91)
Obliterative phlebitis/ arteritis, cases (%)	2 (66)	2 (16)

(Table 3). None of our patients had disease manifestations of IgG4-RD outside the mediastinum.

5. Discussion

The precise etiology of FM remains indeterminate. It likely represents a clinical-pathological syndrome attributable to various triggers including infectious pathogens causing granulomatous mediastinitis, drug toxicity and idiopathic immune-mediated cases [13–15]. The majority of FM cases respond suboptimally to antimicrobial, immunosuppressive, and antifibrotic treatments [13–15]. Therapeutic responses of patients with histoplasmosis/granulomatous-disease-associated FM are exceedingly rare. Better understanding of the pathogenesis in specific cases of FM may ultimately result in improved individualized therapies for subgroups of patients.

By definition, IgG4-RD is currently considered an idiopathic fibroinflammatory disorder [1, 2, 4, 6, 9]. It is characterized by the expansion of IgG4-producing plasma cells. The triggers and pathogenesis of IgG4-RD remain undefined [1, 2, 4, 6, 9]. Yet, the disease is typically responsive to glucocorticoid therapy [1, 2, 4, 6, 9]. Furthermore,

TABLE 3: Demographic, clinical, and radiological features of FM IgG4-RD and FM-non-IgG4-RD cases.

	FM IgG4-RD (N = 3)	FM-non-IgG4-RD (N = 12)	P-value
Age (Median, range (years))	32 (25–65)	39.5 (27–59)	NS
Gender (Women, number of cases (%))	1 (33)	10 (83)	NS
Radiological disease localization			
Right	2	7	
Left	0	1	NS
Bilateral	1	4	
Organ compression			
Superior vena cava	1	3	
Pulmonary vascular	1	3	NS
Airway	1	5	
Esophagus	0	1	
Histoplasmosis (N = 13)			
Conclusive	2	7	
Suggestive	1	2	NS
Absent	0	1	

refractory IgG4-RD cases frequently improve following the depletion of B-lymphocytes with rituximab [11, 27]. Unfortunately, current clinical, serologic, and pathological diagnostic criteria for IgG4-RD, especially outside the pancreas, lack high diagnostic accuracy. The diagnostic criteria for most other organ systems including the mediastinum are exclusively based on expert consensus or extrapolated from observations in the pancreas, salivary, and lacrimal glands. Consequently, a diagnosis of IgG4-RD requires the exclusion of all diseases that can mimic the disorder. Important mimics include malignancies, infections, and vasculitides [28–30].

The clinical, radiological, and pathological presentation of FM as a metabolically active space-occupying fibro-inflammatory disease process within the mediastinum is highly compatible with the disease manifestations of IgG4-RD in other organs such as the pancreas and the salivary glands. Herein we demonstrate that a subset of histoplasmosis/granulomatous-disease-associated FM cases exhibits the histopathological and immunological characteristics consistent with a definite diagnosis of IgG4-RD: lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis/arteritis, and an accumulation of IgG4-positive plasma cells (≥50 cells/hpf and ≥40% of IgG4/IgG-positive plasma cells). Therefore, this subgroup of FM cases may be part of the ever-expanding disease spectrum of IgG4-RD, and perhaps respond favorably to immunosuppressive therapy with glucocorticoids or rituximab. Interestingly, the tissue samples of these patients also contained a large number of CD20-positive B-lymphocytes [14]. Alternatively, these histopathological findings and the expansion of IgG4-positive plasma cells within the tissue biopsies of these patients may represent a subset of an IgG4 dominant fibroinflammatory response triggered by histoplasmosis or other granulomatous diseases. It is conceivable, that this response pattern is indicative of the presence of chronic infection or persistence of foreign antigens within the tissue. Our study has several limitations.

Due to the retrospective design our analysis of the clinical, radiological and serologic data is constrained by the data documented in the medical records by a diverse team of care providers. Furthermore, the infrequent utilization of diagnostic tissue biopsies and surgical debulking procedures in FM patients restricted our analysis of the histopathologic pattern and number of IgG4-positive plasma cells to a relatively small subset of FM patients (small study size). However, we have previously demonstrated that these 15 patients were representative of a large FM cohort treated at a Midwestern tertiary referral center [14]. Our study does not include any tissue specimens from patients with idiopathic immune-mediated FM, which based on the common association with other IgG4-RD and response to immunosuppressive therapy would perhaps be even more likely to represent a manifestation of IgG4-RD. Moreover, serum IgG4 measurements were not performed in these patients, and therapeutic glucocorticoid use was not reported for any of these patients.

In summary, we conclude that there is an overlap of the histopathologic features between histoplasmosis/granulomatous-disease-associated FM and IgG4-RD. A subset, approximately 20%, of these FM cases may indeed be part of the IgG4-RD spectrum. Although the exact pathogenesis and natural history of these cases remains unknown and may differ from classic IgG4-RD, the prospective identification of this subgroup of patients with characteristic IgG4-RD histopathology and a hyper-IgG4 immune response would be extremely valuable. This strategy can perhaps facilitate the identification of a subset of FM patients more likely to respond to immunosuppressive therapy. Consequently, we propose the prospective identification of this subgroup of FM patients based upon the presence of an elevated serum IgG4 level >140 mg/dL and/or characteristic histopathological

findings and accumulation of IgG4-positive plasma cells within the mediastinal tissue. As the use of chest computed tomography has largely reduced the need for routine surgical tissue biopsies to establish a diagnosis of FM, based on the vast experience of the successful use of endoscopic needle biopsies for the diagnosis of IgG4-RD in the pancreas, we recommend to obtain transbronchoscopic ultrasound-guided needle biopsies if surgical biopsies are not needed for diagnosis [20, 31, 32]. This approach would allow the identification of a patient population to prospectively evaluate the treatment effects of glucocorticoids and/or other immunosuppressive agents such as rituximab in FM patients with diagnostic features of IgG4-RD (FM IgG4-RD).

Acknowledgment

References

[1] W. Cheuk and J. K. C. Chan, "IgG4-related sclerosing disease a critical appraisal of an evolving clinicopathologic entity," *Advances in Anatomic Pathology*, vol. 17, no. 5, pp. 303–332, 2010.

[2] T. Kamisawa and A. Okamoto, "IgG4-related sclerosing disease," *World Journal of Gastroenterology*, vol. 14, no. 25, pp. 3948–3955, 2008.

[3] A. Khosroshahi and J. H. Stone, "A clinical overview of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 57–66, 2011.

[4] Y. Zen and Y. Nakanuma, "IgG4-related disease: a cross-sectional study of 114 cases," *American Journal of Surgical Pathology*, vol. 34, no. 12, pp. 1812–1819, 2010.

[5] M. Ebbo, L. Daniel, M. Pavic et al., "IgG4-related systemic disease: features and treatment response in a French cohort: results of a multicenter registry," *Medicine*, vol. 91, no. 1, pp. 49–56, 2012.

[6] M. N. Carruthers, J. H. Stone, and A. Khosroshahi, "The latest on IgG4-RD: a rapidly emerging disease," *Current Opinion in Rheumatology*, vol. 24, no. 1, pp. 60–69, 2012.

[7] T. Kamisawa and A. Okamoto, "Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 613–625, 2006.

[8] K. Yoshida, F. Toki, T. Takeuchi, S. I. Watanabe, K. Shiratori, and N. Hayashi, "Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis," *Digestive Diseases and Sciences*, vol. 40, no. 7, pp. 1561–1568, 1995.

[9] A. Khosroshahi, V. Deshpande, and J. H. Stone, "The clinical and pathological features of IgG(4)-related disease," *Current Rheumatology Reports*, vol. 13, no. 6, pp. 473–481, 2011.

[10] K. Nakatani, Y. Nakamoto, and K. Togashi, "Utility of FDG PET/CT in IgG4-related systemic disease," *Clinical Radiology*, vol. 67, no. 4, pp. 297–305, 2012.

[11] A. Khosroshahi and J. H. Stone, "Treatment approaches to IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 67–71, 2011.

[12] D. B. Flieder, S. Suster, and C. A. Moran, "Idiopathic fibroinflammatory (Fibrosing/Sclerosing) lesions of the mediastinum: a study of 30 cases with emphasis on morphologic heterogeneity," *Modern Pathology*, vol. 12, no. 3, pp. 257–264, 1999.

[13] J. E. Loyd, B. F. Tillman, J. B. Atkinson, and R. M. Des Prez, "Mediastinal fibrosis complicating histoplasmosis," *Medicine*, vol. 67, no. 5, pp. 295–310, 1988.

[14] T. Peikert, T. V. Colby, D. E. Midthun et al., "Fibrosing mediastinitis: clinical presentation, therapeutic outcomes, and adaptive immune response," *Medicine*, vol. 90, no. 6, pp. 412–423, 2011.

[15] S. E. Rossi, H. P. McAdams, M. L. Rosado-de-Christenson, T. J. Franks, and J. R. Galvin, "From the archives of the AFIP: fibrosing mediastinitis," *Radiographics*, vol. 21, no. 3, pp. 737–757, 2001.

[16] R. A. Goodwin, J. A. Nickell, and R. M. Des Prez, "Mediastinal fibrosis complicating healed primary histoplasmosis and tuberculosis," *Medicine*, vol. 51, no. 3, pp. 227–246, 1972.

[17] C. G. Schowengerdt, R. Suyemoto, and F. B. Main, "Granulomatous and fibrous mediastinitis. A review and analysis of 180 cases," *Journal of Thoracic and Cardiovascular Surgery*, vol. 57, no. 3, pp. 365–379, 1969.

[18] S. E. Straus and E. S. Jacobson, "The spectrum of histoplasmosis in a general hospital: a review of 55 cases diagnosed at Barnes Hospital between 1966 and 1977," *American Journal of the Medical Sciences*, vol. 279, no. 3, pp. 147–158, 1980.

[19] A. Devaraj, N. Griffin, A. G. Nicholson, and S. P. G. Padley, "Computed tomography findings in fibrosing mediastinitis," *Clinical Radiology*, vol. 62, no. 8, pp. 781–786, 2007.

[20] A. D. Sherrick, L. R. Brown, G. F. Harms, and J. L. Myers, "The radiographic findings of fibrosing mediastinitis," *Chest*, vol. 106, no. 2, pp. 484–489, 1994.

[21] I. Naitoh, T. Nakazawa, H. Ohara et al., "Clinical significance of extrapancreatic lesions in autoimmune pancreatitis," *Pancreas*, vol. 39, no. 1, pp. e1–e5, 2010.

[22] K. Tsushima, T. Tanabe, H. Yamamoto et al., "Pulmonary involvement of autoimmune pancreatitis," *European Journal of Clinical Investigation*, vol. 39, no. 8, pp. 714–722, 2009.

[23] M. Inoue, N. Nose, H. Nishikawa, M. Takahashi, Y. Zen, and M. Kawaguchi, "Successful treatment of sclerosing mediastinitis with a high serum IgG4 level," *General Thoracic and Cardiovascular Surgery*, vol. 55, no. 10, pp. 431–433, 2007.

[24] A. Sakamoto, R. Nagai, K. Saito et al., "Idiopathic retroperitoneal fibrosis, inflammatory aortic aneurysm, and inflammatory pericarditis-Retrospective analysis of 11 case histories," *Journal of Cardiology*, vol. 59, no. 2, pp. 139–146, 2012.

[25] T. Taniguchi, H. Kobayashi, S. Fukui, K. Ogura, T. Saiga, and M. Okamoto, "A case of multifocal fibrosclerosis involving posterior mediastinal fibrosis, retroperitoneal fibrosis, and a left seminal vesicle with elevated serum IgG4," *Human Pathology*, vol. 37, no. 9, pp. 1237–1239, 2006.

[26] Y. Zen, A. Sawazaki, S. Miyayama, K. Notsumata, N. Tanaka, and Y. Nakanuma, "A case of retroperitoneal and mediastinal fibrosis exhibiting elevated levels of IgG4 in the absence of sclerosing pancreatitis (autoimmune pancreatitis)," *Human Pathology*, vol. 37, no. 2, pp. 239–243, 2006.

[27] A. Khosroshahi, D. B. Bloch, V. Deshpande, and J. H. Stone, "Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease," *Arthritis and Rheumatism*, vol. 62, no. 6, pp. 1755–1762, 2010.

[28] J. D. Strehl, A. Hartmann, and A. Agaimy, "Numerous IgG4-positive plasma cells are ubiquitous in diverse localised non-specific chronic inflammatory conditions and need to be distinguished from IgG4-related systemic disorders," *Journal of Clinical Pathology*, vol. 64, no. 3, pp. 237–243, 2011.

[29] A. Vaglio, J. D. Strehl, B. Manger et al., "IgG4 immune response in Churg-Strauss syndrome," *Annals of the Rheumatic Diseases*, vol. 71, no. 3, pp. 390–393, 2012.

[30] M. Yamamoto, H. Takahashi, C. Suzuki et al., "Analysis of serum IgG subclasses in churg-strauss syndrome-The meaning of elevated serum levels of IgG4," *Internal Medicine*, vol. 49, no. 14, pp. 1365–1370, 2010.

[31] R. P. Sah and S. T. Chari, "Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 108–113, 2011.

[32] M. J. Levy, M. J. Wiersema, and S. T. Chari, "Chronic pancreatitis: focal pancreatitis or cancer? Is there a role for FNA/biopsy? Autoimmune pancreatitis," *Endoscopy*, vol. 38, supplement 1, pp. S30–S35, 2006.

Clinical Study

Evaluation and Clinical Validity of a New Questionnaire for Mikulicz's Disease

Motohisa Yamamoto,[1] Hiroki Takahashi,[1] Keisuke Ishigami,[1] Hidetaka Yajima,[1] Yui Shimizu,[1] Tetsuya Tabeya,[1] Mikiko Matsui,[1] Chisako Suzuki,[1] Yasuyoshi Naishiro,[1] Hiroyuki Yamamoto,[1] Kohzoh Imai,[2] and Yasuhisa Shinomura[1]

[1] *First Department of Internal Medicine, School of Medicine, Sapporo Medical University, Hokkaido 0608543, Japan*
[2] *Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo 1088639, Japan*

Correspondence should be addressed to Motohisa Yamamoto, mocha@cocoa.plala.or.jp

Objectives. The characteristic features of Mikulicz's disease (MD) are diffuse enlargement of the lacrimal and submandibular glands, elevated levels of serum immunoglobulin (Ig)G4, and abundant infiltration of IgG4-positive plasmacytes into both glands. No disease index is available to properly evaluate MD, so we developed a functional assessment of MD, the Mikulicz's disease activity questionnaire (MAQ), and evaluated its clinical efficacy. *Methods.* We selected 18 patients who were either being treated for MD or who had presented with recurrence. The patients completed a self-assessment and were scored according to the MAQ sheet during each visit between December 2009 and August 2011. Assessment items were in regard to increases or decreases in lacrimal and salivary gland enlargement and severity of sicca symptoms. *Results.* On the first visits, MAQ scores were high, but scores decreased rapidly as treatment progressed. When doses of glucocorticoid were reduced, some patients showed increased scores. Dry-symptom scores increased initially. MAQ scores for patients with recurrent MD gradually increased over several months before relapse. However, some patients displayed no elevation in MAQ scores due to relapses at other sites. *Conclusion.* MAQ score can be used to quantify flares and treatment response and is useful for functional assessment of MD.

1. Background and Purpose

Mikulicz's disease (MD) is a chronic inflammatory disease characterized by diffuse enlargement of the lacrimal and submandibular glands, elevated levels of serum immunoglobulin (Ig)G4, and abundant infiltration of IgG4-positive plasmacytes and fibrosis in both glands. MD is considered as an IgG4-related disease (IgG4-RD) with aspects of systemic disorders [1]. Several epidemiological studies have shown that thousands of patients in Japan have MD [2]. The pathogenesis of IgG4-RD involves a gradual shift from an inflammatory stage to a fibrotic stage. Early intervention with appropriate therapy is necessary to avoid irreversible organ dysfunction. One of the features of MD is a high relapse rate following reductions in glucocorticoid treatment, as this steroid is known to be useful in achieving clinical remission [3].

As a representative chronic disorder, treatment of rheumatoid arthritis (RA) requires precise clinical evaluation and review of the course of treatment [4]. Similar principles would apply to MD, but no clinical index has been available to properly evaluate this pathology. The health assessment questionnaire (HAQ) was developed as a comprehensive measure of outcomes in patients with a wide variety of rheumatic diseases [5], but does not reflect the condition of MD particularly well because MD is a local condition affecting only the lacrimal and salivary glands. A system for assessing disease activity and functional impairment in patients with MD is thus needed, and we therefore developed a functional evaluation system, the "Mikulicz's disease activity questionnaire" (MAQ), and have applied this in daily practice since December 2009. The present study analyzed the clinical efficacy of MAQ in patients with MD.

2. Methods

We conducted a followup study of 18 MD patients from December 2009 to August 2011. Diagnoses were made according to the diagnostic criteria for IgG4-related MD proposed by the Japanese Society for Sjögren's syndrome in 2008 (Figure 4) [6] and by the pathological evaluation of enlarged submandibular glands. Study subjects included patients who had started treatment and patients who presented with relapses. Patients under 20 years old were excluded. At the hospital and related institutes, patients assessed the severity of their own lacrimal and salivary gland swelling and recorded the scores on the MAQ. Levels of serum IgG4 were also measured continuously. We analyzed the serial changes in MAQ score with treatment.

The MAQ score sheet (Figure 1) comprises four questions to assess the degrees to which the lacrimal and salivary glands were enlarged and the occurrence and severity of sicca symptoms. Patients checked the boxes that best corresponded to current symptoms: disappearance of symptoms (0 points); slight improvement of symptoms (1 point); unchanged symptoms (2 points); worsening of symptoms (3 points). MAQ scores were determined as the total of the swelling-evaluation points and dryness-evaluation points, allowing us to compare the conditions of patients compared with the first visit. We also recorded the amount of prescribed glucocorticoid at each visit. No patients were given any guidance including leading questions in this assessment. If marked differences were seen in assessment results between patients and doctors, we asked the doctors to record comments in the MAQ sheets. In MD, relapse was defined as reenlargement of the lacrimal and salivary glands on physical and image findings. In other organs, MD relapse was defined as swelling of organs on systemic enhanced CT examined periodically. However, for renal lesions, we considered the appearance of contrast defects in the renal parenchyma as indicative of flare-up.

Treatment was performed as follows. For patients with failure of organs other than the lacrimal and salivary glands, we initially prescribed 0.8 mg/kg/day of prednisolone (PSL) for 1 month and then reduced the amount by 10% every 2 weeks. For those patients without organ failure, we initially prescribed 0.6 mg/kg/day of PSL [6]. After the dose was decreased to <10 mg/day, we continued to administer a maintenance dose for 6 months. After 6 months, the doctors reduced the amount of steroid if the patient was in clinical remission. If a relapse occurred, the dose of glucocorticoid was increased. In our analysis, clinical remission was defined as no observation of lacrimal or salivary gland enlargement over a 3-month period as determined by physical and imaging findings. Recurrence was defined as the necessity for treatment intervention due to swelling of the glands or detection of other organ dysfunction.

3. Results

3.1. Patient Profiles.
The 41 participants in the followup study comprised 17 men and 24 women. Mean (±standard deviation) age at MD onset was 56.59 ± 11.90 years, and mean age at diagnosis was 58.44 ± 11.52 years. Mean duration of followup from the first visit was 4.00 ± 2.36 years. Seven patients (17.1%) started treatment with glucocorticoid, and 11 patients (26.8%) presented with recurrence during the observation period. As of December 2009, 23 cases had been prescribed with steroid and showed no flare-up during the study period (Table 1).

3.2. Analysis of Patients Starting Treatment.
The 7 patients who began treatment during the observation period showed high MAQ scores of 8.3 ± 1.7 at the first visit. Starting steroid doses were as follows: 40 mg/day in 1 patient; 30 mg/day in 5 patients; 25 mg/day in 1 patient. Doctors continued to prescribe PSL at 4 to 7 mg/day at last visit. As all patients progressed with treatment, MAQ scores including dryness scores rapidly decreased; scores were <1 in all cases when the dose of PSL was reduced to 20 mg/day. Some cases initially presented with sicca symptoms and subsequently showed an increase in questionnaire scores at 8 to 10 mg/day (Figure 2(a)). In untreated patients, however, mean serum level of IgG4 was 368.71 ± 162.28 mg/dL. Patient levels of IgG4 also decreased after the initiation of treatment, but at <15 mg/day of PSL, IgG4 levels in some patients reelevated in advance of reelevations in MAQ scores. Almost all patients showed an elevation of serum IgG4 at the dose point of 5 mg/day (Figure 2(b)). In this study, no cases were seen in which assessments differed markedly between patients and doctors.

3.3. Analysis of Patients Presenting with Relapse.
Among the 11 patients who presented with relapses during observation, 2 experienced more than one relapse. The steroid dose for these patients at the time of relapse was 0 to 16 mg/day, and the mean dose was 6.86 ± 4.54 mg/day. Most of these patients received an additional amount of steroid, but patients with repeated past recurrences received immunosuppressants such as mizoribine or rituximab. Mean MAQ scores at first relapse were 2.7 ± 2.1, with scores of 0 in two cases. Many cases showed a gradual elevation in MAQ scores at 1 to 6 months before relapse (Figure 3(a)). This tendency toward elevation was marked in dryness scores. In two patients who presented with more than one relapse, MAQ scores showed an unstable transition after the PSL dose was increased. We also found that these patients showed elevated levels of serum IgG4 several months before relapse (Figure 3(b)). Levels of serum IgG4 decreased immediately after an increase in steroid dose, but reelevation of serum IgG4 was observed with a reduction of glucocorticoid (data not shown). As with patients starting treatment, no cases showed marked differences in assessments between patients and doctors.

4. Discussions

MD is a chronic inflammatory disorder and can also be termed IgG4-related dacryoadenitis and sialadenitis. Disease activity indices are needed in the assessment of chronic diseases. Glucocorticoid treatment is now uniformly performed for MD, but the disease is known to be likely to relapse with reductions in steroid dose. In our data (SMART:

Mikulicz's disease activity questionnaire

First Department of Internal Medicine, Sapporo Medical University Hospital

Date of visit   *25/ 7 / 2010*   Pt's name

Please tell us your conditions at today.

Please check ☑ in the applicable box.

(A) How do you like the swelling of upper eyelids in comparison with first visit?

Not swell at al	Still swell a little	Swell as the same as first visit	Swell more since first visit
		✓	

(B) How do you like dry eyes in comparison with first visit?

Not dry at al	Still dry a little	Dry as the same as first visit	Dry more since first visit
✓			

(C) How do you like the swelling of parotid and submandibular portion in comparison with first visit?

Not swell at al	Still swell a little	Swell as the same as first visit	Swell more since first visit
	✓		

(D) How do you like dry mouth in comparison with first visit?

Not dry at al	Still dry a little	Dry as the same as first visit	Dry more since first visit
	✓		

Scores of A + C/B + D
Glucocorticoid dose at the last visit

→ *3/1 points*

→ *15 mg/day*

Figure 1: The English version of the Mikulicz's disease activity questionnaire (MAQ). An example of a completed MAQ as used in the clinic during routine visits by patients.

Table 1: Characteristics of analyzed patients with Mikulicz's disease.

(1) Persistent (>3 months), symmetrical swelling of the lacrimal, parotid, and submandibular glands, involving at least two pairs.

(2) Serologically high levels of immunoglobulin (lg)G4 (≥ 13.5 mg/L).

(3) Marked lgG4-positive plasmacyte infiltration ($\geq 50\%$ IgG4-positive/lgG-positive cells in five high power fields) into lacrimal and salivary gland tissues.

In terms of diagnosis, IgG4-related Mikulicz's disease is defined as satisfying item 1 and either item 2 and/or item 3. This form of systemic lgG4-related disease often accompanies multiple organ lesions. Sarcoidosis, Castleman's disease, Wegener's granulomatosis, and malignant lymphoma need to be considered as differential diagnoses.
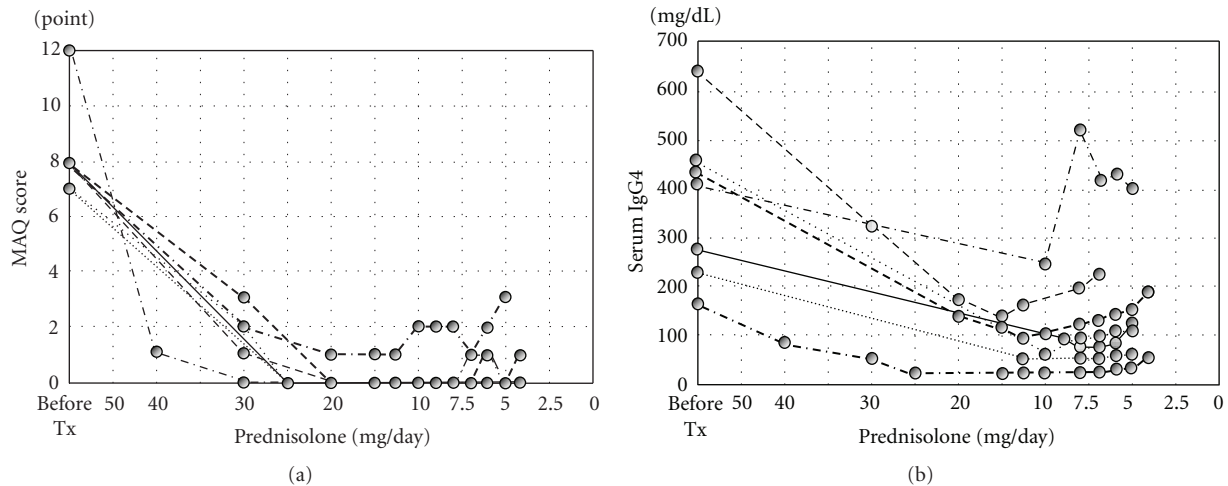
Figure 2: Serial changes in MAQ scores and serum IgG4 levels in patients who started treatment during the observation period. (a) MAQ scores decreased rapidly after starting treatment. Some patients showed initial sicca symptoms, and scores rose when the steroid dose was reduced to 8–10 mg/day. (b) Serum IgG4 levels decreased after initiation of treatment. Some patients showed reelevation of IgG4 levels prior to MAQ scores at <15 mg/day of PSL. Virtually no cases showed any elevation of serum IgG4 at the dosage of 5 mg/day.

Sapporo Medical University and related institutes database for investigation and best treatments of IgG4-related disease), patients withdrawn from steroid presented with mild symptoms (data not shown). We may thus be able to select the dose of glucocorticoid or nonsteroidal treatment, including biologic agents, depending on disease activity during therapy for MD. Recurrence of MD is currently often diagnosed based on physical and imaging findings. On the other hand, risk factors for relapse remain unclear, and no studies have reported analyses of flare-up signs in MD. We therefore developed the MAQ as a clinical evaluation scoring system for MD and analyzed the clinical application of this questionnaire.

The lacrimal and salivary glands are involved in MD. Our database revealed that the chief complaints in most patients are glandular enlargement and impaired secretion. Other complaints, including pain, are rarely observed. We therefore asked patients to assess swelling and dryness in the lacrimal and salivary glands. The degrees of subjective symptoms were set at four levels compared to the first visit: disappearance of symptoms, slight improvement, no change, or progression. This was in consideration of patients, to avoid them wondering which option they should check. This approach to assessment was well received by patients and did not interfere with practice.

As expected, results showed high MAQ scores before treatment and decreased scores after glucocorticoid administration. Many patients showed improvements of more than 5 points, with improvements in both swelling and dryness. We have previously described the efficacy of glucocorticoids in short-term treatment [3]. This analysis demonstrated that the MAQ accurately reflects such results. However, some patients still presented with sicca symptoms after undergoing steroid treatment. MD is not considered prone to destruction of the glands [7], but progressive fibrosis can irreversibly reduce glandular function. Different factors

can also affect sicca symptoms, including mental condition, diabetes mellitus, and concomitant medications. Ideally, each individual factor should be assessed in terms of impacts on dryness, but this is obviously impractical to achieve. The MAQ is expected to adequately evaluate the changing condition of the same patients if no other factors change.

With respect to relapse, the elevation of dryness scores preceded that of swelling scores in most cases. The origin of glandular enlargement in MD remains unclear, but glandular impairment may occur before swelling in MD. The present analysis did not include any missed cases with recurrence. MAQ scores appeared to accurately reflect relapses in MD. However, two patients presented with relapse in other organs despite MAQ scores of 0. One case exhibited relapse from MD alone to bronchitis, and another showed relapse from MD with IgG4-related tubulointerstitial nephritis to retroperitoneal fibrosis alone. In the other cases of flare-up, five patients presented with the involvement of other organs. In all, new lesions in other organs at the time of relapse were revealed in 7 of the 11 cases. This result offers a good reminder that MD is a systemic disease. To address this issue, developing indices for each organ and then combining them is one option, but development of an integrated assessment may be a better approach. The activity index for IgG4-RD requires one such integrated assessment.

The present analysis also examined the association between MAQ scores and serum level of IgG4. Levels of serum IgG4 elevated 3 to 6 months before clinical identification of recurrence. MAQ scores tended to increase later than levels of serum IgG4. Cases presenting with other organ involvements despite an MAQ score of 0 also showed elevated serum IgG4 levels. Tabata pointed out the possibility that concentrations of serum IgG4 reflect IgG4-RD disease activity [8]. Fluctuations in serum IgG4 levels would thus also be important in assessing the disease activity of IgG4-RD. Comparing degrees of elevation of serum IgG4 in flare-ups
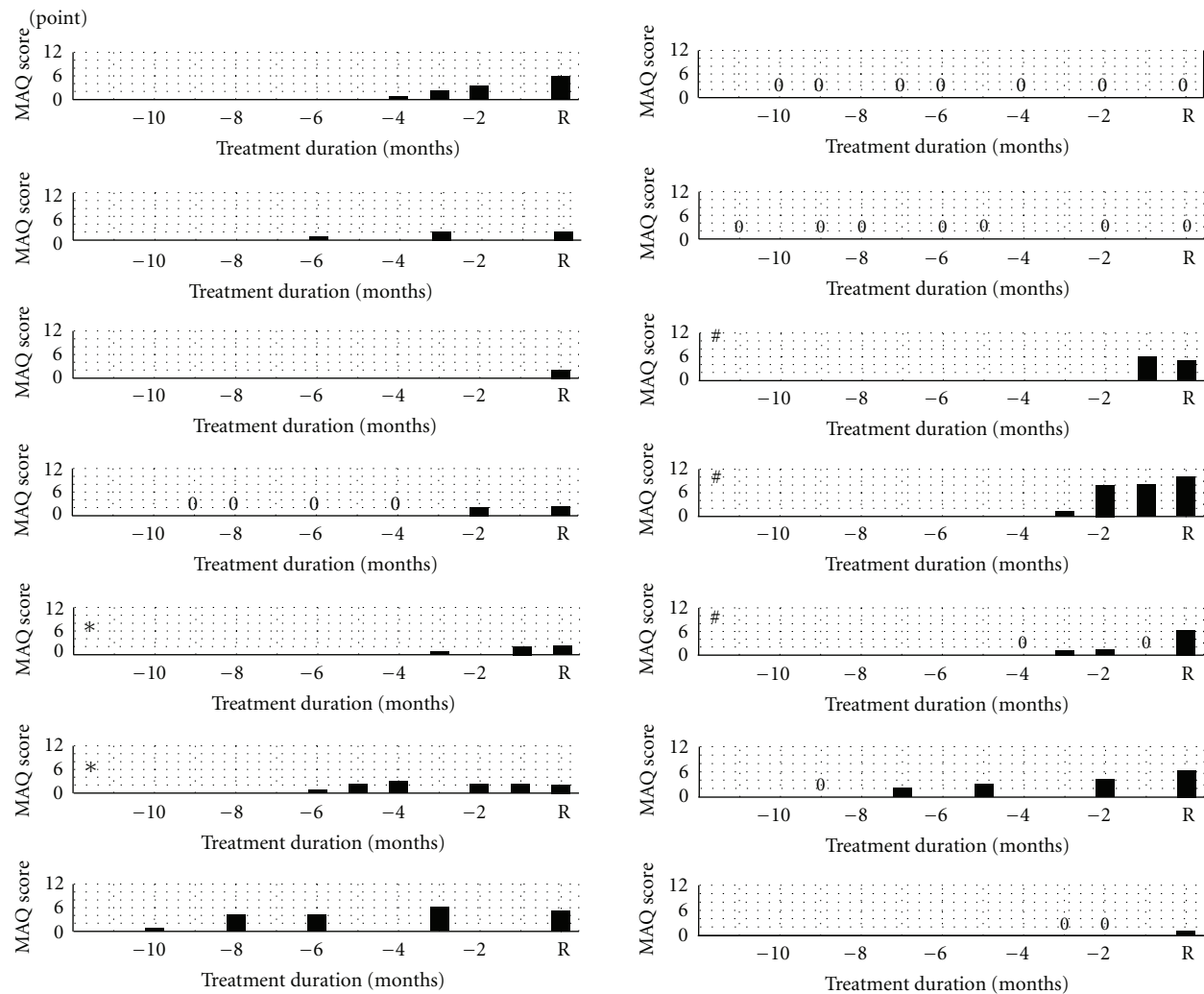
(a)
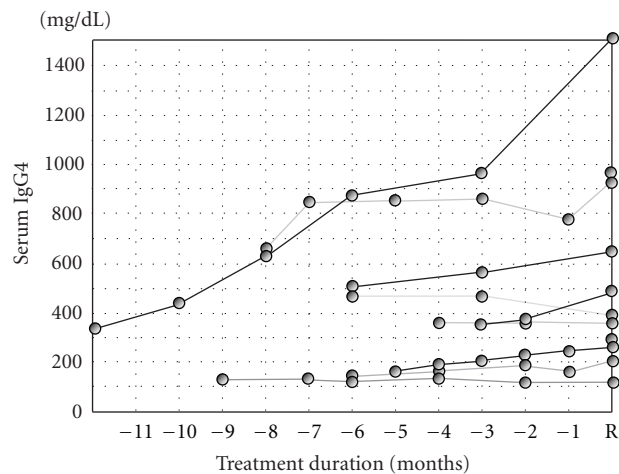
(b)

FIGURE 3: Serial changes in MAQ scores and serum IgG4 levels in patients with relapse (R). (a) Serial changes in MAQ scores. In many cases, MAQ scores had gradually elevated 1 to 6 months before relapse. * and # in the bar graphs denote the same cases. Case * showed two flares, while case # experienced relapse three times. (b) Serial changes in serum IgG4 levels which had elevated several months before relapse.

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                          ┌─────────────────────┐
                          │  Registered cases   │
                          │         72          │
                          └─────────────────────┘
                    ┌──────────────┴──────────────┐
        ┌───────────────────┐        ┌─────────────────────────┐
        │ Transfered or     │        │ Ongoing followup cases  │
        │ dropped out cases │        │           41            │
        │        31         │        └─────────────────────────┘
        └───────────────────┘         ┌──────────┴──────────┐
                        ┌───────────────────┐   ┌─────────────────────────┐
                        │ Already treated or│   │    Analyzed cases       │
                        │ no flare-up cases │   │  (DEC2009-AUG2011)      │
                        │        23         │   │           18            │
                        └───────────────────┘   └─────────────────────────┘
                                          ┌────────────┴────────────┐
                            ┌─────────────────────┐    ┌─────────────────────┐
                            │  Newly treated cases│    │   Relapsed cases    │
                            │          7          │    │        11           │
                            └─────────────────────┘    └─────────────────────┘
```

Male 3 : Female 4   Male 7 : Female 4
Complications: Recurrence pattern:
AIP 1, RF 2, prostatitis 1   Only MD⟶MD + TIN + RF 1, MD + RF 1,
                                                   MD + Lung 2, only BR 1
                                           MD + AIP ⟶MD 1, MD + AIP 1
                                           MD + TIN ⟶ only RF 1
AIP: autoimmune pancreatitis, RF: retroperitoneal fibrosis, MD: Mikulicz's disease,   MD + RF ⟶MD + RF 2
TIN: tubulointerstitial nephritis, BR: bronchitis   MD + AIP + RF ⟶MD + TIN + RF 1
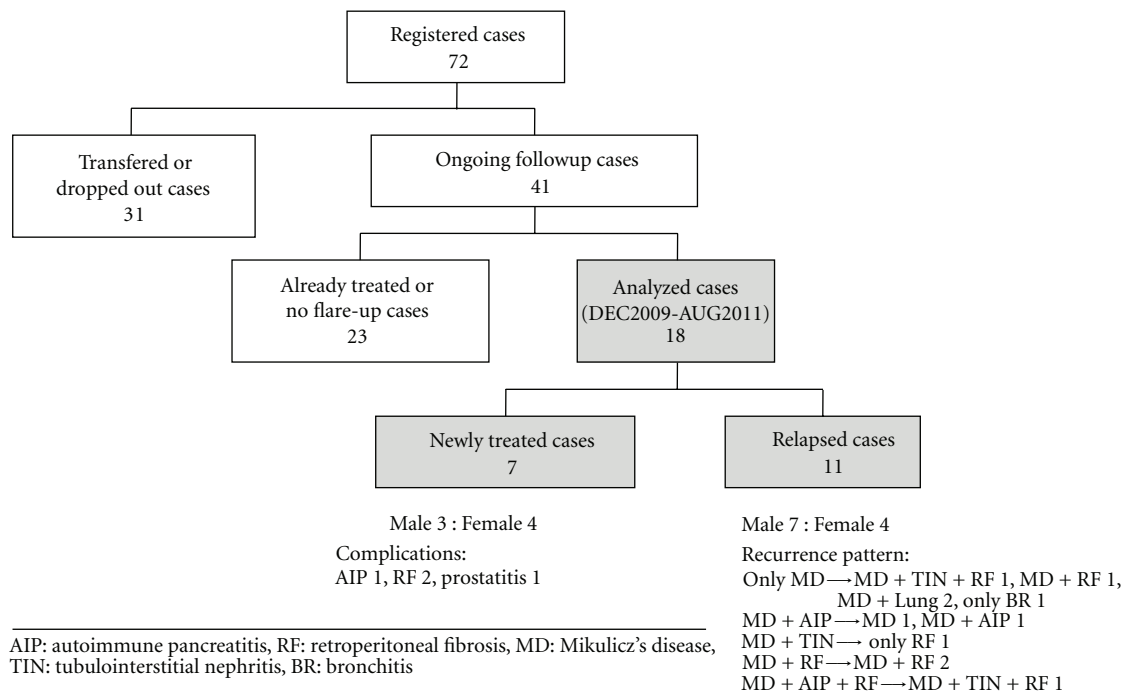
FIGURE 4: Diagnostic criteria for IgG4-related MD (as proposed by the Japanese Society for Sjögren's syndrome in 2008).

among patients is difficult, given the wide variations between individual cases. We therefore did not examine correlations between the degree of IgG4 elevation and changes in MAQ score in the present study.

Clinical research in the present study was designed to maximize the number of cases within a limited period, but this study remains ongoing. As a result, we are hopeful that we will encounter novel findings using the integrated data over the longer term. We would like to perform a multicenter study and ascertain the utility of MAQ scores on a large scale in the future. The MAQ appears to offer a useful assessment of disease activity in MD and may help to catalyze the development of a disease activity index for IgG4-RD.

Conflict of Interests

The authors have no conflict of interests or sources of funding to declare.

Acknowledgment

References

[1] M. Yamamoto, H. Takahashi, M. Ohara et al., "A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease," *Modern Rheumatology*, vol. 16, no. 6, pp. 335–340, 2006.

[2] I. Nishimori, A. Tamakoshi, and M. Otsuki, "Research committee on intractable diseases of the pancreas, Ministry of Health, Labour, and Welfare of Japan. Prevalence of autoimmune pancreatitis in Japan from a nationwide survey in 2002," *Journal of Gastroenterology*, vol. 42, supplement 18, pp. 6–8, 2007.

[3] M. Yamamoto, S. Harada, M. Ohara et al., "Beneficial effects of steroid therapy for Mikulicz's disease," *Rheumatology*, vol. 44, no. 10, pp. 1322–1323, 2005.

[4] J. S. Smolen, D. Aletaha, J. W. J. Bijlsma et al., "Treating rheumatoid arthritis to target: recommendations of an international task force," *Annals of the Rheumatic Diseases*, vol. 69, no. 4, pp. 631–637, 2010.

[5] T. Pincus, J. A. Summey, and S. A. Soraci Jr, "Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment questionnaire," *Arthritis and Rheumatism*, vol. 26, no. 11, pp. 1346–1353, 1983.

[6] M. Yamamoto, H. Takahashi, and Y. Shinomura, "Mikulicz's disease and its extraglandular lesions," *Current Immunology Reviews*, vol. 7, no. 2, pp. 162–171, 2011.

[7] K. Tsubota, H. Fujita, K. Tsuzaka, and T. Takeuchi, "Mikulicz's disease and Sjogren's syndrome," *Investigative Ophthalmology and Visual Science*, vol. 41, no. 7, pp. 1666–1673, 2000.

[8] T. Tabata, T. Kamisawa, K. Takuma et al., "Serial changes of elevated serum IgG4 levels in IgG4-related systemic disease," *Internal Medicine*, vol. 50, no. 2, pp. 69–75, 2011.

Clinical Study

Development of an IgG4-RD Responder Index

Mollie N. Carruthers,[1] John H. Stone,[1] Vikram Deshpande,[2] and Arezou Khosroshahi[1]

[1] *Rheumatology Unit, Division of Rheumatology, Allergy, and Immunology, Department of Medicine, Massachusetts General Hospital, Boston, MA 02114, USA*
[2] *Department of Pathology, Massachustts General Hospital, Harvard Medical School, Boston, MA 02114, USA*

Correspondence should be addressed to John H. Stone, jhstone@partners.org

IgG4-related disease (IgG4-RD) is a multiorgan inflammatory disease in which diverse organ manifestations are linked by common histopathological and immunohistochemical features. Prospective studies of IgG4-RD patients are required to clarify the natural history, long-term prognosis, and treatment approaches in this recently recognized condition. Patients with IgG4-RD have different organ manifestations and are followed by multiple specialties. Divergent approaches to the assessment of patients can complicate the interpretation of studies, emphasizing the critical need for validated outcome measures, particularly assessments of disease activity and response to treatment. We developed a prototype IgG4-RD Responder Index (IgG4-RD RI) based on the approach used in the development of the Birmingham Vasculitis Activity Score for Wegener's granulomatosis (BVAS/WG). The IgG4-RD RI was refined by members of the International IgG4-RD Symposium Organizing Committee in a paper case exercise. The revised instrument was applied retrospectively to fifteen IgG4-RD patients at our institution. Those scores were compared to physician's global assessment scale for the same visits. This paper describes the philosophy and goals of the IgG4-RD RI, the steps in the development of this instrument to date, and future plans for validation of this instrument as an outcome measure.

1. Introduction

Measurement of disease activity is critical for longitudinal assessments in both observational studies and clinical trials. In the field of rheumatology, more than 250 assessment tools have been developed and validated to evaluate pathology, symptoms, function, and health status of patients with rheumatic diseases [1]. Such instruments should be compatible with regulatory requirements of the Food and Drug Administration (FDA) and generally require prospective studies for completion of the validation process [2].

IgG4-related disease (IgG4-RD) is an increasingly recognized immune-mediated disease that is characterized by a lymphoplasmacytic infiltrate enriched with IgG4-positive plasma cells and a distinctive storiform fibrosis of affected organs [3]. Commonly involved organs include the pancreas, biliary tree, orbits, salivary glands, and retroperitoneum, among many others. Organ involvement usually occurs in a metachronous but overlapping fashion. The serum IgG4 level is often but not always elevated [4]. Because of the novelty

of IgG4-RD, little effort to date has been devoted to the development of outcome measures for this newly recognized condition.

A disease responder index is a tool designed to detect any changes in disease activity and identify improvement and worsening in the same and/or different organ systems. A responder index permits objective quantification of the treatment response by providing standardized outcome measures. Assessing clinical response and not simply serologic response is increasingly important to establish endpoints in randomized control trials.

No randomized control trials have been conducted for IgG4-RD treatment to date [5]. Management is based currently on small case series and observational studies. Glucocorticoids are the standard first-line treatment for IgG4-RD and patients whose disease has not reached an advanced stage of fibrosis generally respond well to this treatment, at least initially [5]. Recent data has shown that rituximab can be used successfully to treat IgG4-RD [6].

Two major features of IgG4-RD pose significant challenges for the development of outcome measures. The first is the complex, multiorgan system nature of this disease, which makes it difficult to summarize the state of disease activity across all organs. The second is the fact that the stage of disease activity can differ across organs, such that a patient can have active inflammation likely to respond to immunosuppression in one organ and advanced fibrosis (less likely to respond to treatment) in another.

We have developed an IgG4-RD responder index (IgG4-RD RI) for use as an outcome measure in an ongoing pilot trial of rituximab in this condition. We intend that this instrument will measure not only disease activity but will also incorporate features that capture the need for urgent treatment and catalogue disease-related damage. This paper is designed to provide information on the development and implementation of the IgG4-RD RI. We report the philosophy behind the development of the IgG4-RD RI to date, the steps taken to create the instrument through the enlistment of assistance of international experts in this condition, and the plans for completion of the IgG4-RD RI validation process.

2. Methods

2.1. Overview of the Instrument Development Approach. The IgG4-RD RI was designed to assess disease activity from visit to visit using clinician-generated assessments of both objective and subjective measures. The IgG4-RD RI uses a scoring system from 0–4 for each organ system or site and asks the clinician to rate the extent of disease activity and damage at the time of the clinical encounter. The IgG4-RD RI was revised by the Organizing Committee of the International IgG4-related Disease Symposium, held in Boston in October, 2011 [http://www2.massgeneral.org/pathology/symposium/IgG4_related_systemic_dis.asp]. This group was comprised of 39 experts from 9 countries, with subspecialty expertise in rheumatology, gastroenterology, allergy/immunology, nephrology, surgery, pathology, and radiology. Further revisions were made following a simulation exercise involving six paper case descriptions of real patients, completed by IgG4-RD symposium participants. Finally, both the IgG4-RD RI that emerged from these development steps and a physician global assessment (PGA) were used to assess the disease retrospectively in terms of disease activity and damage. The Pearson's correlation coefficient was then calculated to compare the IgG4-RD RI and PGA responses.

2.2. Model Disease Activity Tool. The IgG4-RD RI was modeled on the Birmingham Vasculitis Activity Score for Wegener's granulomatosis (BVAS/WG) [7]. The BVAS/WG is a formally validated and widely used instrument for the measurement of disease activity in granulomatosis with polyangiitis (formerly Wegener's granulomatosis) and microscopic polyangiitis, a pair of distinct but overlapping conditions often termed antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) [8]. The BVAS/WG is a clinician-scored instrument in which each disease

activity in each organ system is graded "persistent," "worse," or "none" at each clinic visit. The number of items of "persistent" or "worse" for each organ system is totaled and used to quantify the states of disease flare, persistent disease, or remission. The BVAS/WG was selected because of the experience of one of the authors (J. H. Stone) as a lead developer of this instrument; similarities between ANCA-associated vasculitis and IgG4-RD, including the propensities for multi-organ system involvement; the broad range of disease activity between flare and remission; the high frequency of disease-related damage (which must be distinguished from active disease); the absence of reliable biomarkers that necessitates reliance upon clinical indices for longitudinal assessments.

2.3. Scoring Sheet. The IgG4-RD RI, designed to emphasize ease of use, includes specific reminders to consider activity within all organs involved commonly in IgG4-RD (Figure 1). The scoring rules appear in the first box. At each visit, physicians enter a score from 0–4 for each organ/site affected, indicating whether the organ/site is normal, improved, new or recurrent, or worse on treatment. The physician also provides yes/no answers for each organ site to the questions of whether the disease is symptomatic; whether the disease activity requires treatment urgently; whether the organ dysfunction observed is related to damage rather than (or in addition to) active disease. At the end of this table, the serum IgG4 concentration in milligrams per deciliter is entered along with a score of 0–4, indicating whether the IgG4 concentration has improved, become newly or recurrently elevated, or increased despite treatment since the last visit. The scoring scheme for serum IgG4 concentration, therefore, parallels the schemes for individual organ system activity assessment. The cumulative glucocorticoid dose (in prednisone equivalents) since the last visit and total IgG4-RD RI score are then calculated.

2.4. Specific Interpretations of Individual Organ System Scores. The numbers for each organ score refer to disease activity, distinguished from organ dysfunction related to damage:

  (i) "0" signifies the absence of active disease in that organ. A score of 0 is appropriate when the organ system has never been affected by active IgG4-RD, or when previously evident disease within that organ has resolved;

 (ii) "1" indicates that disease activity within an organ has improved but still persists to some degree;

(iii) "2" indicates that the disease within that organ has remained persistent and unchanged since the last visit;

 (iv) "3" indicates the presence of new or recurrent disease activity;

  (v) "4" refers to disease that has worsened despite treatment.

2.5. Organ Site. The organ sites were selected for inclusion in the IgG4-RD RI based on a review of the existing literature

IgG4-RD responder index

Date form completed: (e.g., 7 / July / 2050)

Case number: _____

Scoring rules
Scoring refers to manifestations of disease activity present in the last 28 days

Scoring: 0   Normal or resolved
         1   Improved
         2   Persistent (unchanged from previous visit; still active)
         3   New / recurrence
         4   Worsened despite treatment
Definitions
Organ/site score: the overall level of IgG4-RD activity within a specific organ system
Symptomatic: is the disease manifestation in a particular organ system symptomatic? (Y = yes; N = no)
Urgent disease: disease that requires treatment immediately to prevent serious organ dysfunction (Y = yes; N = no)
            (presence of **urgent disease** within an organ leads to **doubling** of that organ system score)
Damage: organ dysfunction that has occurred as a result of IgG4-RD and is considered permanent (Y = yes; N = no)

Organ/site	Activity			Damage
	Organ/site score (0-4)	Symptomatic (Yes/No)	Urgent (Yes/No)	Present (Yes/No)
Pachymeninges				
Pituitary gland				
Orbits and lacrimal glands				
Salivary glands				
Thyroid				
Lymph nodes				
Lungs				
Aorta and large blood vessels				
Retroperitoneum, mediastinum, and mesentery				
Pancreas				
Bile duct and liver				
Kidney				
Skin				
Other sclerosis/mass formation				

Descriptor	Level mg/dl	Score
Serum IgG4 concentration		

Steroid dose at the time of assessment (prednisone equivalent):
_____ mg/day
Cumulative steroid dose in the past 28 days:
_____ mg prednisone equivalent

Total activity score

Organ/sites ($\times 2$ if urgent) + serum IgG4 score: _____

Total number of urgent organs: _____

Total number of damaged organs: _____

FIGURE 1: IgG4-related Disease Responder Index (IgG4-RD RI) Scoring Sheet this is a sample sheet of the IgG4-RD RI on which physicians score patient's disease activity at a given clinic visit.

(Table 1) [3]. For ease of conceptualization, the sites of potential organ involvement are listed from head to toe. This structure is similar to that of the BVAS/WG scoring sheet, on which disease activity is scored by organ system, and each disease site is assigned a designation of normal, persistent disease activity, and new/worse disease activity, with numerical scores corresponding to each state [7]. Each organ site has specific disease manifestations common to IgG4-RD (the appendix). The IgG4-RD RI category of "other" organ/site involvement is important because the

Table 1: Diseases commonly associated with IgG4-related disease.

IgG4-related spectrum diseases
Idiopathic hypertrophic pachymeningitis
Orbital pseudotumor
Mikulicz's disease
Kuttner's tumor
Eosinophilic angiocentric fibrosis
Riedel's thyroiditis
Idiopathic cervical fibrosis
Pulmonary inflammatory pseudotumor
Chronic sclerosing aortitis
Inflammatory abdominal aortitis
Autoimmune pancreatitis
Sclerosing cholangitis
Retroperitoneal fibrosis
Inflammatory pseudotumor of the kidney

protean nature of this disease makes it impossible to capture all potential sites of disease. In addition, we anticipate that new clinical manifestations of this disease and possible even new sites of organ involvement will be described as the clinical phenotype of this disease is understood more fully.

2.6. Symptoms and Signs of IgG4-RD. Lists of the most common symptoms and signs within a given organ system are included in the IgG4-RD RI instructions (see the appendix), principally as a reminder to the clinician of the possible disease manifestations to consider when scoring disease activity and damage. The physician simply denotes on the form the presence or absence of symptoms for a given IgG4-RD site. This feature of the instrument ensures that a subjective measure is included. Good clinical judgment and a thorough knowledge of the disease manifestations of this condition are essential, as with any clinical responder index.

2.7. Urgency. Capturing the need for urgent treatment is an important aspect of the IgG4-RD RI. Some disease manifestations of IgG4-RD require the immediate institution of treatment to prevent permanent organ damage. For example, IgG4-related sclerosing cholangitis can lead to cirrhosis within several months of diagnosis and requires the prompt initiation of therapy. In contrast, the lymphadenopathy of IgG4-RD remains unchanged for prolonged periods in many patients and may never require treatment. The "urgent" column in the IgG4-RD RI is designed to capture aspects of the disease that require the immediate start of immunosuppression in order to preserve organ function.

The score for an organ site is doubled when the need to initiate treatment for active IgG4-RD at a particular or organ/site is considered urgent. For example, if a patient has new IgG4-related sclerosing cholangitis, the total score for that organ/site would be 6 instead of 3. Similarly, if the patient's biliary status has worsened despite therapy since the time of the last visit and an urgent escalation of therapy is required to treat the IgG4-related sclerosing cholangitis, then that organ score would be 8 rather than 4. Only the score of the individual organ site is doubled in this setting, not the total IgG4-RD RI score.

2.8. Damage. The concept of damage is related directly to that of disease activity. Consequently, the two concepts must be considered in tandem. Organ damage results from active disease and in some cases both active disease and damage can be present in the same organ system simultaneously. In other cases, organ dysfunction is related more to damage than to active IgG4-RD. Immunosuppression must be targeted to active IgG4-RD, not to damage resulting from previously active therapy. The most appropriate use of immunosuppression is to control active disease and prevent disease-related damage. It is particularly ideal to employ immunosuppression at a stage of disease when the histopathology is characterized by a lymphoplasmacytic infiltrate rather than a predominance of acellular fibrosis [9].

The clinical assessment of damage can be challenging. Radiographic studies such as computed tomography (CT) and positron emission tomography with CT (PET-CT) can aid the clinician in determining which organs have been damaged. For example, even conceding that active disease might be present simultaneously with damage within the pancreas, the finding of atrophic changes by CT scan within that organ would be considered to be the result of damage. In such a case, both active disease and disease-related damage should be scored.

2.9. Serum IgG4 Concentration. Scoring also includes a consideration of the serum concentration of IgG4. The serum IgG4 level may become elevated in a patient experiencing an active flare [6, 10]. However, not every patient with IgG4-RD has an elevated serum IgG4 level at baseline, even before treatment. It is well established that classic IgG4-RD can be active in the absence of elevated serum IgG4 concentrations. The IgG4-RD serum level in the IgG4-RD RI is scored according to normal, improved, persistent, new, recurrent, or worsened despite treatment. Patients with rising serum IgG4 levels would have higher scores indicating worsening disease activity.

2.10. Total Scoring. The sum of the disease activity in all of the organ sites plus the serum IgG4 concentration score (also graded on a 0–4 scale) yields the total activity score. An individual active organ site is doubled for urgency and added to the other organ sites. This number can be compared between visits to assess the disease activity over time as well as being used for a clinical trial endpoint. The longitudinal recording of damage, though not included in the overall disease activity score, is essential to the formulation of the patient's overall outcome.

2.11. Glucocorticoid Use. Glucocorticoids are the cornerstone of IgG4-RD treatment, and most patients respond promptly to this treatment, at least initially. Thus, careful recording of the dose of prednisone (or prednisone equivalent) in the
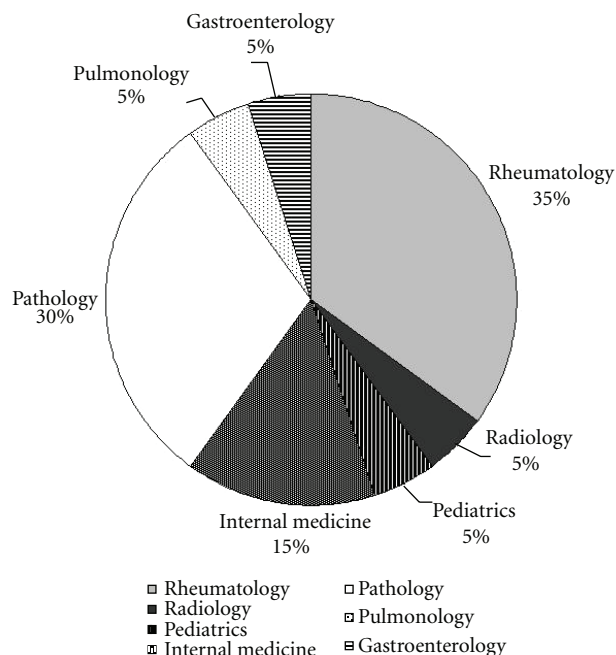
Figure 2: Subspecialties of IgG4-RD RI Simulation Exercise Participants ($n = 21$) there were 21 participants completing the simulation exercises and the pie chart represents the percentage breakdown for each medical subspecialty.

interval between the current visit and the preceding one is essential to a full understanding of the degree of disease activity.

3. Results

3.1. Simulation Exercises. The IgG4-RD RI went through several development stages and iterations before arriving at its current format. A simulation exercise using paper case descriptions of six real patients was sent to attendees of the *International IgG4-RD Symposium* (held in Boston, MA, USA October 2011). Participants were asked to score the simulation exercises using the IgG4-RD RI. The participants received written instructions on how to apply the IgG4-RD RI but did not attend a training session (the appendix). Twenty-one individuals participated in this exercise, providing valuable feedback from a cross-section of investigators interested in IgG4-RD. The physicians who completed the exercises included a variety of subspecialists, particularly rheumatologists and pathologists (Figure 2).

The simulation exercises were presented as clinical vignettes, including data from histories, physical examinations, laboratory results, and radiologic findings for each case. Accompanying each clinical vignette was at least one clinical photograph, radiology study, or histologic image to illustrate the case effectively. The physicians then used all of the information presented in the simulation exercise to complete a separate IgG4-RD RI scoring sheet for each case. The results were scored for each participant against standardized answers prepared by consensus of the four authors.
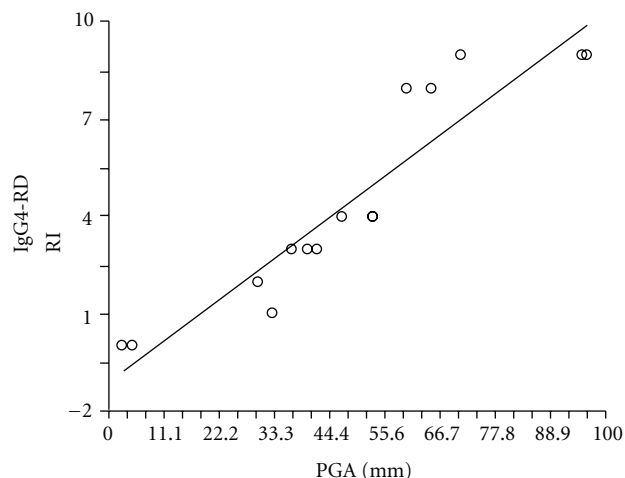

Figure 3: Correlation between physician global assessment (PGA) and IgG4-RD RI the PGA and IgG4-RD RI were compared using linear regression.

The purpose of this exercise was to solicit feedback on the IgG4-RD RI from physicians who were experts in the evaluation of patients with this disorder from different perspectives. Several common scoring errors were observed in this exercise. These included scoring organ involvement in which clinical symptoms and signs had resolved entirely as improved but persistent (i.e., "1") rather than resolved (i.e., "0"). Another scoring discrepancy resulted from incorrectly scoring patients off treatment who were recurrent ("3") as if they were receiving treatment ("4"). A third error was failing to double the organ/site score, when disease requiring treatment urgently was present. Comments from the participants in this exercise contributed substantially to important revisions of the draft instrument. We reformatted the scoring sheet in order to address the common scoring differences from the simulation case exercises.

3.2. Retrospective Application of the IgG4-RD RI. The next step in the development of the IgG4-RD was the retrospective use of the instrument for fifteen individual clinic and in-patient evaluations among patients in the Massachusetts General Hospital IgG4-RD Registry. Two blinded rheumatology experts scored an IgG4-RD patient visit using either the IgG4-RD RI or the physician's global assessment scale (PGA). These results were then compared (Figure 3). The Pearson's correlation coefficient was 0.93 ($P < 0.0001$).

4. Discussion

As the field of IgG4-RD is poised to move beyond the descriptive phase of the disease, validated outcome measures are required to advance the understanding of this condition and the assessment of new treatment approaches. The current iteration of the IgG4-RD RI marks an important step toward the availability of useful outcome measures in this disease. We anticipate that additional validation steps for this instrument will be required, but this paper describes

accurately the philosophy and goals behind the IgG4-RD RI. Its methods and appendices will serve as important guidance documents in the future.

The development efforts to date have created a one-page instrument supported by the instruction manual shown in the appendix. Data included on this single page include indications of disease activity across a full spectrum of potential organ involvement; the serum IgG4 concentration; assessments of the need for treatment on an urgent basis; the recording of damage in organ systems; the sum of recent glucocorticoid use. Expertise with the use of the IgG4-RD RI may, therefore, become a concise and important tool for clinical trials and other investigations related to this disorder.

Although the developers of the IgG4-RD RI have relied significantly upon the BVAS/WG in creating this instrument, the IgG4-RD RI differs in important ways from the BVAS/WG. The "urgent" column in the IgG4-RD RI highlights features of the disease that require the prompt institution of treatment and is, therefore, analogous to the "major" designations given to some organ system manifestations in the BVAS/WG [7]. However, the BVAS/WG does not record disease damage on the same page. Rather, clinical trials in AAV have generally used a separate instrument, the Vasculitis Damage Index [11], for this purpose. Although it is critical that the concepts of disease activity and damage be kept separate and recorded appropriately during clinical assessments, it may be useful to have an indication of damage on the same one-page case report form even if damage does not contribute to the overall disease activity score. This model matches more closely the decision-making process that clinicians undertake on a daily basis in encounters with patients: are the signs of organ dysfunction due to active disease, or are they more accurately a reflection of damage rather than a process that requires more intensive immunosuppression?

The IgG4-RD RI will find its greatest use in the research setting, either in the context of clinical trials or in other types of investigations that require the careful longitudinal assessments of patients' clinical status. Because consistency of its application from visit to visit is critical, it will be most useful to ensure whenever possible that the same investigators complete the IgG4-RD RI for the same patient across all visits.

Significant debate now exists within the community of IgG4-RD investigators about the utility of serum IgG4 concentration measurements in the diagnosis and management of this disorder [12]. Inclusion of the serum IgG4 concentration in the IgG4-RD RI at this point permits an analysis of the value of this measurement in the context of other organ disease assessments. We hypothesize that further analysis of these data will confirm the utility of serial measurements, at least in a subset of patients. This hypothesis, however, requires confirmation through studies of larger numbers of patients in a variety of states of disease activity.

The simulation case exercises illustrated some shortcomings in early iterations of the IgG4-RD RI that led to appropriate revisions of the original index. The experience with the simulation exercise highlighted the importance of adequate training with the instrument prior to its use in the research setting. The IgG4-RD RI is simpler than many clinical assessment tools for multiorgan diseases, but both a thorough understanding of the clinical breadth of IgG4-RD itself and a high degree of familiarity with the index are required in order to employ it effectively. We anticipate that a focused period of instruction for investigators in the context of a formal training course will be required before this tool can be used in the context of a clinical trial.

In conclusion, progress in IgG4-RD will be contingent upon the ability to assess patients rigorously in a longitudinal manner, using validated outcome measures. The IgG4-RD RI described in this paper represents a broad effort at the development of a disease activity and responder index that can be employed in clinical trials and other investigations of patients with this emerging immune-mediated condition. The next steps in validation will include a multicenter study of patients recruited from a core group of sites with extensive experience in the diagnosis and management of this disease.

Appendix

For more information please refer to IgG4-RD RI Instructions Manual. (See Supplementary Material available online at doi:10.1155/2012/259408.)

Acknowledgments

References

[1] P. P. Katz, "Introduction to special issue: Patient outcomes in rheumatology, 2011," *Arthritis Care & Research*, vol. 63, supplment 11, pp. S1–S3, 2011.

[2] R. A. Furie, M. A. Petri, D. J. Wallace et al., "Novel evidence-based systemic lupus erythematosus responder index," *Arthritis Care and Research*, vol. 61, no. 9, pp. 1143–1151, 2009.

[3] A. Khosroshahi and J. H. Stone, "A clinical overview of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 57–66, 2011.

[4] T. Tabata, T. Kamisawa, K. Takuma et al., "Serial changes of elevated serum IgG4 levels in IgG4-related systemic disease," *Internal Medicine*, vol. 50, no. 2, pp. 69–75, 2011.

[5] A. Khosroshahi and J. H. Stone, "Treatment approaches to IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 67–71, 2011.

[6] A. Khosroshahi, D. B. Bloch, V. Deshpande, and J. H. Stone, "Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease," *Arthritis and Rheumatism*, vol. 62, no. 6, pp. 1755–1762, 2010.

[7] J. H. Stone, G. S. Hoffman, P. A. Merkel et al., "A disease-specific activity index for Wegener's granulomatosis: Modification of the Birmingham Vasculitis Activity Score," *Arthritis and Rheumatism*, vol. 44, no. 4, pp. 912–920, 2001.

[8] P. A. Merkel, D. D. Cuthbertson, B. Hellmich et al., "Comparison of disease activity measures for anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis," *Annals of the Rheumatic Diseases*, vol. 68, no. 1, pp. 103–106, 2009.

[9] W. Cheuk and J. K. C. Chan, "IgG4-related sclerosing disease a critical appraisal of an evolving clinicopathologic entity," *Advances in Anatomic Pathology*, vol. 17, no. 5, pp. 303–332, 2010.

[10] A. Khosroshahi, M. N. Carruthers, V. Deshpande, S. Unizony, D. B. Bloch, and J. H. Stone, "Rituximab for the treatment of IgG4-related disease: Lessons from 10 consecutive patients," *Medicine*, vol. 91, no. 1, pp. 57–66, 2012.

[11] P. Seo, R. A. Luqmani, O. Flossmann et al., "The future of damage assessment in vasculitis," *Journal of Rheumatology*, vol. 34, no. 6, pp. 1357–1371, 2007.

[12] J. H. Stone, A. Khosroshahi, V. Deshpande et al., "IgG4-related disease: recommendations for the nomenclature of this condition and its individual organ system manifestations," *Arthritis & Rheumatism*. Submitted.

Research Article

Increased IgG4-Positive Plasma Cells in Granulomatosis with Polyangiitis: A Diagnostic Pitfall of IgG4-Related Disease

Sing Yun Chang,[1] Karina Keogh,[2] Jean E. Lewis,[1] Jay H. Ryu,[2] and Eunhee S. Yi[1]

[1] *Division of Anatomic Pathology, Mayo Clinic, Rochester, MN 55905, USA*
[2] *Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN 55905, USA*

Correspondence should be addressed to Eunhee S. Yi, yi.joanne@mayo.edu

Granulomatosis with polyangiitis (Wegener's) (GPA) may mimic IgG4-related disease (IgG4-RD) on histologic examination of some biopsies, especially those from head and neck sites. IgG4 immunostain is often performed in this context for differential diagnosis with IgG4-RD. However, the prevalence of IgG4+ cells in GPA has not been explored. We examined the IgG4+ cells in 26 cases confirmed as GPA by a thorough clinical and pathologic assessment. Twenty-six biopsies consisted of 14 sinonasal/oral cavity/nasopharynx, 7 orbit/periorbital, 3 lung/pleura, 1 iliac fossa/kidney, and 1 dura specimens. Eight of 26 (31%) biopsies revealed increased IgG4+ cells (>30/HPF and >40% in IgG4+/IgG+ ratio). The IgG4+ cells and IgG4+/IgG+ ratio ranged 37–137/hpf and 44–83%, respectively. Eight biopsies with increased IgG4+ cells were from sinonasal ($n = 4$) or orbital/periorbital ($n = 4$) sites. In conclusion, increased IgG4+ cells are not uncommonly seen in sinonasal or orbital/periorbital biopsies of GPA, which could pose as a diagnostic pitfall.

1. Introduction

Granulomatosis with polyangiitis (Wegener's) (GPA) is an immune-mediated systemic necrotizing vasculitis often affecting the upper respiratory tract, lung, and kidney [1]. Involvement of GPA is limited to the upper respiratory tract and/or the lung in some cases although virtually anybody site can be involved in GPA such as the eye, skin, joints, heart, and the central nervous system [2]. Necrotizing vasculitis and irregular basophilic parenchymal necrosis with associated palisading granuloma comprise the main histologic characteristics of GPA. Also, neutrophilic microabscesses and fibrosis are commonly found in a background mixed inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, multinucleated giant cells, and macrophages [1, 2].

On histologic examination, GPA can mimic IgG4-related disease (IgG4-RD) since the inflammatory background in GPA may be rich in plasma cells and accompanied by fibrosis and/or obliterated blood vessels as in IgG4-RD [1, 3, 4]. Some biopsies of GPA cases (especially from the upper respiratory tract and orbit) may lack classic morphologic features

such as necrotizing vasculitis, parenchymal necrosis, and palisading granuloma [5, 6]. IgG4 immunostain is now often performed in this context for evaluating the possibility of IgG4-RD. However, the prevalence of IgG4-positive (IgG4+) cells in GPA has not been widely reported in the literature. Therefore, we sought to assess the prevalence of IgG4+ cells in GPA cases that have been confirmed by a thorough clinical and pathologic assessment.

2. Materials and Methods

2.1. Case Selection. Cases with the diagnosis of GPA were identified via electronic search from our surgical pathology file. We initially retrieved 36 biopsies from various body sites obtained during a period of 1999–2011. The sites of biopsies included sinus/nasopharynx ($n = 16$), oral cavity ($n = 1$), orbital region ($n = 11$), lung/pleural ($n = 6$), kidney ($n = 1$), and dura ($n = 1$). Glass slides from each biopsy were reviewed and reassessed for the following histologic features: geographic necrosis, necrotizing granulomas, vasculitis, multinucleated giant cells, microabscesses,
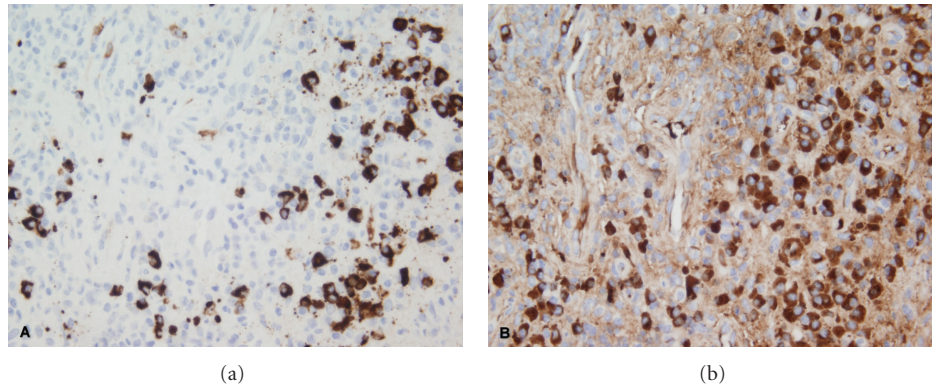
(a)

(b)

Figure 1: (a) Increased IgG4-positive cells with a high IgG4+/IgG+ ratio. (b) IgG+ cells from corresponding hotspot (immunohistochemistry, 400x original magnification).

and fibrosis. Based on these histologic parameters, each case was scored as to the confidence of histopathologic diagnosis of GPA as follows: 0 = nondiagnostic biopsy or not GPA with some findings against the diagnosis of GPA such as infection, 1 = nonspecific (one feature present); 2 = suggestive of GPA (two features present), or 3 = consistent with GPA (three or more features present). A definitive clinicopathologic diagnosis of GPA was made if a case had met the modified clinical criteria of the American College of Rheumatology (ACR) for GPA [7, 8] (modified to include assessment of ANCA status) and a pathology diagnostic score of 1, 2, or 3. Twenty-six of the initial 36 cases were confirmed as the diagnosis of GPA and retained for this study.

2.2. Immunohistochemistry.
Immunohistochemical staining for IgG4 and IgG was performed on all 26 cases using the DAKO dual multimer system or DAKO advance 2 stops multimer system (DAKO, Carpinteria, CA, USA). Monoclonal IgG4 antibody (clone HP6025, dilution 1 : 100; Zymed, San Francisco, CA, USA) and polyclonal rabbit antihuman IgG antibody (dilution 1 : 15,000, DAKO, Carpinteria, CA, USA) were used for the study.

2.3. Quantitation.
IgG4+ and IgG+ cells were counted using the following steps.

   (1) Areas of highest density of IgG4+ plasma cells "hotspot" were identified at low power magnification.

   (2) Using 40x objective lens (Olympus BX50 microscope, 40x objective, 10x eye piece, Olympus DP70 camera, on-screen capture field diameter of 0.34 mm and field area of 0.0884 mm^2), three high-power fields (HPFs) of IgG4+ hot spots, and their corresponding areas of IgG immunostain were photographed.

   (3) All photographs were printed for a manual counting of the absolute number of IgG4+ and IgG+ plasma cells in each HPF.

   (4) Averages of IgG4+ and IgG+ cells in three HPFs were used to determine the IgG4+ cell count and IgG4+/IgG+ ratio for each case. Increase in IgG4+

cells was defined as the average IgG4+ cells greater than 30 per HPF with the IgG4+/IgG+ cell ratio greater than 40%.

2.4. Serum C-Reactive Protein and Immunoglobulin Levels.
Patients' medical records from our institution were reviewed for their serum levels of C-reactive protein (CRP) and immunoglobulin subclasses.

3. Results

Eight of 26 biopsies (31%) showed increased IgG4+ cells with an average IgG4+ cell count and an IgG4+/IgG+ cell ratio ranging from 37 to 137/HPF and from 44 to 83%, respectively (Figure 1). These 8 biopsies were from sinonasal ($n = 4$) and orbital/periorbital ($n = 4$). The IgG4+ cell count ranged from 0 to 28/HPF in the remaining 18 biopsies that were from sinonasal/oral cavity/nasopharynx ($n = 10$), orbit/periorbital ($n = 3$), lung/pleura ($n = 3$), iliac fossa/kidney ($n = 1$), and dura ($n = 1$). The IgG4+ cell counts and IgG4/IgG ratios in the cases without increase in IgG4+ cells ranged from 0 to 28 per HPF and from 0 to 39%, respectively (Table 1). There was no significant difference in the distribution of age at diagnosis or gender between the cases with and without increased IgG4+ cells. Elevated titers of antineutrophil cytoplasmic autoantibodies (ANCA) were present in 25 of 26 cases in this study. All 8 cases with increased IgG4+ cells were positive for ANCA; seven cases were C-ANCA positive, 6 of which were also positive for proteinase 3 (PR-3) ANCA by ELISA. One case was positive for P-ANCA confirmed as myeloperoxidase (MPO) ANCA by ELISA. On histopathologic evaluation, most cases showed characteristic histologic findings of GPA (Figure 2); 2 of 8 cases were graded as pathologic score 3, 2 as score 2, and the remaining 4 as score 1. Details are summarized in Table 1.

The 26 biopsies in this study were obtained from 23 patients. The status of serum CRP level was assessed at the time of the biopsy in 18 patients, and it was elevated in 15 of them (83%) (Table 1). Immunoglobulin levels were evaluated in only three patients (case 7, 9, and 17) and did not reveal polyclonal hypergammaglobulinemia. The serum

Table 1: Clinical features, immunohistochemical findings, ANCA details, and CRP level.

Case no.	Sex	Age at Dx	Biopsy site	IgG4 average	IgG4/IgG ratio	Path Dx score	Clinical Dx (modified ACR criteria)	ANCA (+)	P-ANCA	MPO	C-ANCA	PR3	CRP
1	M	74	Nasal septum	28	87%	3	Generalized	y	n	n	y	y	High
2	F	83	Nasal sinus	20	63%	3	Limited	y	n	n	y	y	High
3	M	54	Nasal septum	62	59%	3	Generalized	y	n	n	y	y	High
4	F	59	Lung RUL RML	27	28%	3	Limited	y	y	y	n	n	NA
5	F	19	Oral cavity	6	19%	3	Generalized	y	n	n	y	y	NA
6	F	18	Nasal sinus	11	79%	1	Generalized	y	y	y	n	n	NA
7*	M	47	Orbit	49	58%	2	Generalized	y	n	n	y	y	High*
8*	M	47	Nasal septum	4	19%	1	Generalized	y	n	n	y	y	*
9**	F	31	Lung, left, pneumonectomy	16	33%	3	Generalized	y	n	n	y	y	NA**
10**	F	31	Kidney	26	39%	3	Generalized	y	n	n	y	y	**
11	M	52	Bilateral orbital mass	1	1%	3	Generalized	n	n	n	n	n	High
12	M	31	Nasal cavity	13	32%	2	Limited	y	n	n	y	y	High
13	F	16	Nasal cavity	1	7%	1	Limited	y	n	n	y	y	High
14	F	47	Orbital soft tissue	24	53%	2	Generalized	y	y	n	y	y	High
15	F	23	Nasopharynx	18	57%	2	Generalized	y	n	n	y	y	High
16	M	44	Nasal cavity	0	0%	2	Generalized	y	n	n	y	y	High
17***	M	36	Nasal cavity	43	47%	1	Generalized	y	n	n	y	y	Normal***
18***	M	36	Eyelid/orbital fat	53	81%	2	Generalized	y	n	n	y	y	***
19	F	64	Nasal cavity	8	13%	2	Generalized	y	y	n	n	n	High
20	F	13	Orbit	15	30%	1	Generalized	y	y	n	n	n	Normal
21	F	23	Orbital mass	69	83%	3	Generalized	y	n	n	y	y	Normal
22	F	71	Periorbital	139	82%	1	Generalized	y	y	y	n	n	High
23	F	31	Nasal cavity	55	73%	1	Generalized	y	n	n	y	y	High
24	F	69	Dura parietooccipital	5	10%	2	Generalized	y	y	n	n	n	High
25	F	40	Pleura	9	34%	3	Generalized	y	n	n	y	y	NA
26	F	49	Sinonasal	37	44%	1	Limited	y	n	n	y	y	High

*, **, *** denote the same patients. Dx: diagnosis; Path: pathology; ACR: American College of Rheumatology; ANCA: antineutrophil cytoplasmic antibody; PR3: proteinase 3; MPO: myeloperoxidase; NA: not available; CRP: c-reactive protein.
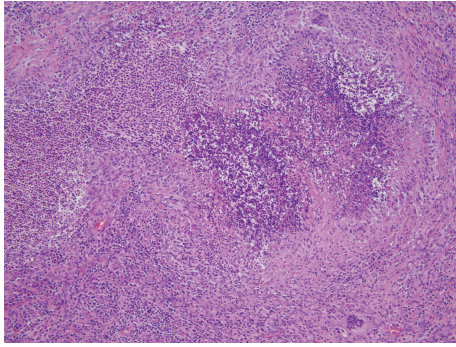
FIGURE 2: Parenchymal necrosis with palisading granuloma, necrotizing vasculitis, and mixed inflammatory infiltrates, characteristic of GPA (hematoxylin and eosin, 100x original magnification).

IgA and IgG4 levels in these three patients were within normal range. The eight biopsies with increased IgG4+ cells were taken from seven patients, five of whom had elevated CRP levels (Table 1). The two patients with normal CRP levels demonstrated classic histopathologic features including geographic necrosis, palisading granulomas, vasculitis, or microabscesses, which supported the clinicopathologic diagnosis of GPA despite the normal CRP level.

4. Discussion

In this study, we sought to determine the prevalence of increased IgG4+ plasma cells in GPA in order to address the role of this finding in the differential diagnosis with IgG4-RD. We believe that this is the largest series of GPA cases to examine IgG4+ cells with application of current criteria and method for evaluation of increased IgG4+ cells in the setting of IgG4-RD. An increase in IgG4+ cells in GPA has been suggested in a recent study by Vaglio et al. [9] in which they reviewed tissue IgG4/IgG ratio in 10 GPA cases along with 9 cases with the Churg-Strauss syndrome (CSS) and 22 cases with chronic sinusitis. However, they did not provide the details on the counting method or results of IgG4+ cells.

In our study, we applied the current criteria and methods in counting IgG4+ cells, which should provide useful and important information in routine diagnostic surgical pathology practice. Also, we made an extra effort to ensure the diagnosis of GPA by a thorough clinicopathologic evaluation and only included the cases with irrefutable diagnosis of GPA. All but one case showed positivity for C- or P-ANCA confirmed with ELISA for PR3 or MPO; the single ANCA negative case demonstrated definite histopathologic findings (pathologic diagnosis score 3) as well as clinical features for GPA, which supported our diagnosis of GPA. Moreover, all 8 cases with increased IgG4+ cells in this study were positive for ANCA, which reiterated our point.

The cutoff number of increased IgG4+ plasma cells has not been well established, and many studies have suggested different cutoff points. Kamisawa et al. and Zhang et al. have used a cutoff point of >30 per HPF [10, 11] (40x objective lens) while Deshpande et al. and Dhall et al. used

>50 HPF (20x or 40x objectives, resp.), and they have reached high sensitivity and specificity in diagnosing autoimmune pancreatitis [12, 13]. In addition to this variable thresholds, the method for counting IgG4+ cells has not been well standardized. We used the same method as in our previous study [14] by enumerating cells on printed images and using averages of three "hot spots" in order to ensure accuracy and reproducibility. We also applied a higher threshold for increased IgG4+ cells by using both the IgG4+ cell count per HPF at >30 and the ratio of IgG4+/IgG+ cells at >40%. Cheuk et al. have proposed that both absolute number of IgG4+ cell higher than 50 per HPF and IgG4+/IgG+ ratio greater than 40% should be present in order to make the diagnosis of IgG4-RD involving lymph nodes and other extranodal sites [3]. Although our cutoffs for IgG4+ status may be slightly lower than the histologic criteria proposed by Cheuk et al. (which also used averages of three different HPFs), the latter used a wider field area than the one in our study (0.196 mm² versus 0.088 mm², resp.). Therefore, the threshold used in this study would be comparable to the one of the most stringent ones in the literature.

The most recent comprehensive diagnostic criteria for IgG4-RD published by Umehara et al. included two major components: (1) serum IgG4 concentration of >135 mg/dL and (2) >10 IgG4+ plasma cells/HPF with a ratio of IgG4+/IgG+ cells >40% [15]. IgG4 serum level was not increased in the 3 cases tested in our study. Instead, most of our GPA cases showed elevated serum CRP which is unusual for IgG4-RD. Based on their criterion for IgG4+ cell count as >10, however, potentially additional five cases in our study would have been considered to have sufficiently increased IgG4+ cells, which will make 50% of our GPA cases with increased IgG4+ cells.

Positive ANCA has been reported in patients with Grave's disease receiving antithyroidal medication such as propylthiouracil (PTU) [16, 17] and may even mimic GPA clinically [18]. However, none of our patients had a history of either Graves's disease or treatment with PTU. The possibility of positive ANCA in patients with IgG4-RD has not been addressed in previous studies, and whether this can occur has yet to be determined. A further study with testing for ANCA in IgG4-RD would be needed to answer this question.

Previous studies have reported the presence of inflammatory infiltrates rich in IgG4+ plasma cells in clinical settings other than IgG4-RD [19–21], usually as an isolated finding. In kidneys, several studies have highlighted a frequent association of IgG4+ plasma cell-rich infiltrates with the glomerular lesions that are typically seen in ANCA-associated angiitis, namely, pauci-immune necrotizing and crescentic glomerulonephritis [19, 20]. In midst of all these findings, questions regarding the nature of relationship between IgG4+ plasma cells and GPA can arise. Interestingly, there have been some data suggesting that ANCA of IgG4 subclass possibly plays a role in the pathogenesis of ANCA-related small vessel vasculitis [22–24].

The predominance of IgG4 as well as IgG1 subclasses of ANCA was first reported in patients with GPA and other clinically related disorders by Brouwer et al. in 1991 [22]. Holland et al. later suggested a possible pathogenic role for

the IgG4 subclass in GPA [23]. *In vitro*, ANCA activated neutrophils by colligating PR3 and FcγRIIa/IIIb receptors [23]. ANCA are predominantly of the IgG isotype, and IgG1, IgG3, and IgG4 subclasses are particularly represented. IgG4 subclass isolated from ANCA-positive sera demonstrated varying abilities to stimulate release of superoxide, which was unrelated to PR3-ANCA titer, neutrophil donor used in their *in vitro* test, or neutrophil FcγRI expression [23]. This study suggested that IgG4 was capable of activating neutrophils via constitutively expressed FcγRIIa/IIIb or colligation of other unidentified cell surface molecules [23]. Liu et al. have reported that MPO-ANCA IgG4 subclass might play a role in the development of GPA [25]. They reported that the titers of anti-MPO IgG4 subclass in patients with GPA was significantly higher than those with microscopic polyangiitis (MPA). The MPO-ANCA in GPA and MPA might recognize overlapping but different epitopes on native MPO molecule. The difference in immunological characteristics of MPO-ANCA might have contributed to different disease entities such as GPA and MPA. Another recent study reported that serum IgG4 levels were markedly elevated in active CSS and also correlated with the disease activity and the number of involved organs [9].

Given these serological and immunological findings, one can postulate a possible role of tissue infiltrating IgG4+ cells in the pathogenesis of GPA. However, the underlying cause or precise mechanism for increased IgG4+ cells in the tissue as seen in some of our GPA cases has not been completely elucidated and further study would be needed in the future. Also, whether there is any pathogenetic relationship between GPA and IgG4-RD is not entirely clear. Although there have been studies reporting elevated IgG4 in the setting of ANCA-associated systemic diseases, no study demonstrating elevated ANCA in IgG4-RD exists in the English literature to our knowledge. Our anecdotal experiences also indicate that the ANCA is generally not elevated in IgG4-RD.

In conclusion, one should be aware of the fact that IgG4+ cells can be remarkably increased in biopsies of GPA of the sinonasal and orbital/periorbital regions. Since the morphologic and clinical manifestations of GPA and IgG4-RD may overlap, it could be a significant diagnostic pitfall in the differential diagnosis of these two entities. Further study is needed to confirm our observation in a larger number of cases, and a further exploration of potential pathogenetic relationship between GPA and IgG4-RD might be of interest as well.

References

[1] K. O. Leslie and M. R. Wick, *Practical Pulmonary Pathology: A Diagnostic Approach*, Elsevier Saunders, Philadelphia, Pa, USA, 2nd edition, 2011.

[2] E. S. Yi and T. V. Colby, "Wegener's granulomatosis," *Seminars in Diagnostic Pathology*, vol. 18, no. 1, pp. 34–46, 2001.

[3] W. Cheuk and J. K. Chan, "IgG4-related sclerosing disease: a critical appraisal of an evolving clinicopathologic entity," *Advances in Anatomic Pathology*, vol. 17, no. 5, pp. 303–332, 2010.

[4] T. C. Smyrk, "Pathological features of IgG4-related sclerosing disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 74–79, 2011.

[5] P. H. Kalina, J. T. Lie, R. J. Campbell, and J. A. Garrity, "Diagnostic value and limitations of orbital biopsy in Wegener's granulomatosis," *Ophthalmology*, vol. 99, no. 1, pp. 120–124, 1992.

[6] K. O. Devaney, W. D. Travis, G. Hoffman, R. Leavitt, R. Lebovics, and A. S. Fauci, "Interpretation of head and neck biopsies in Wegener's granulomatosis. A pathologic study of 126 biopsies in 70 patients," *American Journal of Surgical Pathology*, vol. 14, no. 6, pp. 555–564, 1990.

[7] "Design of the wegener's granulomatosis etanercept trial (WGET)," *Controlled Clinical Trials*, vol. 23, no. 4, pp. 450–468, 2002.

[8] J. H. Stone, "Limited versus severe Wegener's granulomatosis: baseline data on patients in the Wegener's granulomatosis etanercept trial," *Arthritis and Rheumatism*, vol. 48, no. 8, pp. 2299–2309, 2003.

[9] A. Vaglio, J. D. Strehl, B. Manger et al., "IgG4 immune response in Churg-Strauss syndrome," *Annals of the Rheumatic Diseases*, vol. 71, pp. 390–393, 2012.

[10] T. Kamisawa, N. Funata, Y. Hayashi et al., "A new clinicopathological entity of IgG4-related autoimmune disease," *Journal of Gastroenterology*, vol. 38, no. 10, pp. 982–984, 2003.

[11] L. Zhang, K. Notohara, M. J. Levy, S. T. Chari, and T. C. Smyrk, "IgG4-positive plasma cell infiltration in the diagnosis of autoimmune pancreatitis," *Modern Pathology*, vol. 20, no. 1, pp. 23–28, 2007.

[12] V. Deshpande, S. Chicano, D. Finkelberg et al., "Autoimmune pancreatitis: a systemic immune complex mediated disease," *American Journal of Surgical Pathology*, vol. 30, no. 12, pp. 1537–1545, 2006.

[13] D. Dhall, A. A. Suriawinata, L. H. Tang, J. Shia, and D. S. Klimstra, "Use of immunohistochemistry for IgG4 in the distinction of autoimmune pancreatitis from peritumoral pancreatitis," *Human Pathology*, vol. 41, no. 5, pp. 643–652, 2010.

[14] B. Shrestha, H. Sekiguchi, T. V. Colby et al., "Distinctive pulmonary histopathology with increased IgG4-positive plasma cells in patients with autoimmune pancreatitis: report of 6 and 12 cases with similar histopathology," *American Journal of Surgical Pathology*, vol. 33, no. 10, pp. 1450–1462, 2009.

[15] H. Umehara, K. Okazaki, Y. Masaki et al., "Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011," *Modern Rheumatology*, vol. 22, no. 1, pp. 21–30, 2011.

[16] A. Khosroshahi and J. H. Stone, "A clinical overview of IgG4-related systemic disease," *Current Opinion in Rheumatology*, vol. 23, no. 1, pp. 57–66, 2011.

[17] J. E. Gunton, J. Stiel, P. Clifton-Bligh, E. Wilmshurst, and A. McElduff, "Prevalence of positive anti-neutrophil cytoplasmic antibody (ANCA) in patients receiving anti-thyroid medication," *European Journal of Endocrinology*, vol. 142, no. 6, pp. 587–590, 2000.

[18] M. Pillinger and R. Staud, "Wegener's granulomatosis in a patient receiving propylthiouracil for Graves' disease," *Seminars in Arthritis and Rheumatism*, vol. 28, no. 2, pp. 124–129, 1998.

[19] Y. Raissian, S. H. Nasr, C. P. Larsen et al., "Diagnosis of IgG4-related tubulointerstitial nephritis," *Journal of the American Society of Nephrology*, vol. 22, no. 7, pp. 1343–1352, 2011.

[20] D. C. Houghton and M. L. Troxell, "An abundance of IgG4 plasma cells is not specific for IgG4-related tubulointerstitial nephritis," *Modern Pathology*, vol. 24, no. 11, pp. 1480–1487, 2011.

[21] J. D. Strehl, A. Hartmann, and A. Agaimy, "Numerous IgG4-positive plasma cells are ubiquitous in diverse localised non-specific chronic inflammatory conditions and need to be distinguished from IgG4-related systemic disorders," *Journal of Clinical Pathology*, vol. 64, no. 3, pp. 237–243, 2011.

[22] E. Brouwer, J. W. Tervaert, G. Horst et al., "Predominance of IgG1 and IgG4 subclasses of anti-neutrophil cytoplasmic auto-antibodies (ANCA) in patients with Wegener's granulomatosis and clinically related disorders," *Clinical and Experimental Immunology*, vol. 83, no. 3, pp. 379–386, 1991.

[23] M. Holland, P. Hewins, M. Goodall, D. Adu, R. Jefferis, and C. O. Savage, "Anti-neutrophil cytoplasm antibody IgG subclasses in Wegener's granulomatosis: a possible pathogenic role for the IgG4 subclass," *Clinical and Experimental Immunology*, vol. 138, no. 1, pp. 183–192, 2004.

[24] A. Hussain, T. Pankhurst, M. Goodall et al., "Chimeric IgG4 PR3-ANCA induces selective inflammatory responses from neutrophils through engagement of Fcgamma receptors," *Immunology*, vol. 128, no. 2, pp. 236–244, 2009.

[25] L. J. Liu, M. Chen, F. Yu, M. H. Zhao, and H. Y. Wang, "IgG subclass distribution, affinity of anti-myeloperoxidase antibodies in sera from patients with Wegener's granulomatosis and microscopic polyangiitis," *Nephrology*, vol. 13, no. 7, pp. 629–635, 2008.

Review Article

Treatment of Autoimmune Pancreatitis with the Anecdotes of the First Report

Terumi Kamisawa[1] and Tadashi Takeuchi[2, 3]

[1] *Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo 113-8677, Japan*
[2] *Pancreas Research Foundation of Japan, Tokyo 164-0011, Japan*
[3] *Department of Gastroenterology, Tokyo Women's Medical University, Tokyo 162-8666, Japan*

Correspondence should be addressed to Terumi Kamisawa, kamisawa@cick.jp

The first case that led researchers to put forward a new concept of autoimmune pancreatitis (AIP) was treated with steroids by gastroenterologists in Tokyo Women's Medical University. It is important to differentiate AIP from pancreatic cancer before treatment with steroids is started. Today, steroids are standard therapy for AIP worldwide. In the Japanese consensus guidelines, steroid therapy is indicated for symptomatic AIP. After management of glucose levels and obstructive jaundice, oral prednisolone is initiated at 0.6 mg/kg/day for 2–4 weeks and is gradually tapered to a maintenance dose of 2.5–5 mg/day over 2-3 months. To prevent relapse, maintenance therapy with low-dose prednisolone is used. For relapsed AIP, readministration or increased doses of steroids are effective. The presence of proximal bile duct stenosis and elevated serum IgG4 levels may be predictive of relapse of AIP. It is necessary to verify the validity of the Japanese regimen of steroid therapy for AIP. The necessity, drugs, and duration of maintenance therapy for AIP need to be clarified by prospective studies.

1. Introduction

The first case that led researchers to put forward a new concept of autoimmune pancreatitis (AIP) was treated with steroids by gastroenterologists (Professor Tadashi Takeuchi) in Tokyo Women's Medical University, and the concept was proposed by Yoshida, a member of the group, in 1995 [1]. This paper describes the anecdotes of treatment of the first case that led researchers to put forward a new concept of AIP and reviews current strategies for treatment of this disorder.

2. Anecdotes of Treatment of the First AIP Case

In 1993, a 68-year-old woman who had undergone exploratory laparotomy for jaundice and an abdominal tumor at another hospital, but she was found to have advanced pancreatic cancer that was inoperable, and one month after being discharged she came to Tokyo Women's Medical University Hospital to be treated for pancreatic cancer.

Her general condition was good, and during that 1-month period her jaundice had spontaneously improved without any treatment. Based on the physical examination, the laboratory results, and the findings obtained by diagnostic imaging, AIP would come to mind today, but there was no concept of AIP in those days.

As far as steroid therapy is concerned, we have to pay attention to side effects or complications such as steroid-induced pancreatitis. However, it was fortunate that steroid therapy was dramatically effective without any side effects in this patient; her physical findings, laboratory data, and diagnostic imaging findings became normal, and she was discharged uneventfully.

The 8-week steroid therapy was effective, with the results that hyperglobulinemia and positive autoantibody were normalized, and swelling of the pancreas and irregular narrowing of the main pancreatic duct were also normalized. Those were clearly attributable to an autoimmune mechanism, and we proposed autoimmune pancreatitis.

By the way, the case report was submitted to *Digestive Diseases and Sciences* in the summer of 1993 [1], and it had taken about two years since it had originally been submitted. There was a comment by a reviewer that since it was just a report of a single case, adding the subtitle "Proposal of the Concept of Autoimmune Pancreatitis" may have been an overstatement or an exaggeration. However, retaining the subtitle made a considerable impact, and it might have attracted a great deal attention in the age of the Internet. Obviously, it is difficult to propose anything new.

3. Treatment of AIP

AIP has recently been subclassified into type 1 and type 2 AIP [2, 3]. Type 1 AIP is a classical AIP that shows a histology of lymphoplasmacytic sclerosing pancreatitis and is considered the pancreatic manifestation of IgG4-related systemic disease [4, 5]. Type 2 AIP shows a histology of idiopathic duct-centric chronic pancreatitis and is not related to IgG4 [2, 3]. Although it is reported that type 2 AIP responds well to steroid therapy, similar to type 1 AIP [2], the precise clinical features of type 2 AIP have not been clarified. Only treatment of type 1 AIP is described in this paper.

3.1. Spontaneous Improvement. In some AIP patients, improvement of swelling of the pancreas or irregular narrowing of the main pancreatic duct improves without steroid therapy [6–8]. In our series [6], 3 of 12 AIP patients who were followed for more than 6 months without steroid therapy improved spontaneously, but 2 of them received steroid therapy later. Wakabayashi et al. [7] reported on 4 AIP patients who showed spontaneous regression; they had negative immunoserological tests and no biliary lesions. Kubota et al. [8] reported that seronegative findings for IgG4, no obstructive jaundice, and focal swelling of the pancreas were related to spontaneous remission. Considering that patients who are later treated with steroids due to AIP exacerbation also show steroid responsiveness, asymptomatic segmental AIP cases without biliary lesions may be followed without steroid therapy with periodic laboratory and imaging tests.

3.2. Indication. Because the fibroinflammatory process in AIP responds well to steroid therapy, administration of oral steroids has become standard therapy for AIP. However, it is important to differentiate pancreatic cancer from AIP before starting treatment with steroids in AIP patients. According to a recent international study of AIP [9], steroids are used for AIP patients in all countries. In the Japanese consensus guidelines for the management of AIP [10], indications for steroid therapy in AIP include symptoms such as obstructive jaundice due to associated sclerosing cholangitis and the presence of symptomatic extrapancreatic lesions such as hydronephrosis due to retroperitoneal fibrosis. Because diabetes mellitus (DM) seen in the acute presentation of AIP sometimes improves with steroid therapy, DM coincidental with AIP might be an indication for steroid therapy [10]. Although improvement in clinical findings with steroid therapy may be useful in the differential diagnosis of AIP from pancreatic cancer, facile diagnostic steroid trial should be avoided not to misdiagnose pancreatic cancer as AIP. Diagnostic steroid trials should be conducted carefully by pancreatologists only after a negative workup for cancer including endoscopic ultrasound-guided fine needle aspiration [11, 12]. Serological and imaging tests should be done 2 weeks after commencement of steroid therapy. Rapid response to steroids is reassuring and confirms the diagnosis of AIP. If steroid effectiveness is reduced, the patient should be reevaluated on suspicion of pancreatic cancer.

3.3. Steroid Regimen. In the Japanese guidelines [10], before starting steroid therapy, biliary drainage is usually done in cases with obstructive jaundice. However, as there are some patients whose jaundice is relieved by steroid therapy alone, it is unclear if biliary obstruction can be treated with steroid therapy alone without biliary drainage [13]. In cases with DM, glucose levels must be controlled. The recommended initial oral prednisolone dose is 0.6 mg/kg/day. Serological and imaging tests should be done periodically after commencement of steroid therapy [10]. Magnetic resonance cholangiopancreatography is useful to observe the response to steroids in the pancreaticobiliary ducts noninvasively [14]. Pancreatic size usually normalizes within a few weeks, and biliary drainage becomes unnecessary within about 1 month. Rapid response to steroids is reassuring and confirms the diagnosis of AIP. If steroid effectiveness is reduced, the patient should be reevaluated with a suspicion of pancreatic cancer. The initial dose of steroids should be administered for 2–4 weeks, and the dose should be gradually tapered to a maintenance dose of 2.5–5 mg/day over 2-3 months [10] (Figure 1).

In the Mayo Clinic [15], prednisolone is used at 40 mg/day for 4 weeks and is tapered by 5 mg/week for a total of 11 weeks of therapy. In Korea [16], remission is achieved on a regimen of prednisolone 0.5 mg/kg per day for 1-2 months followed by a gradual tapering of 5–10 mg per month to a maintenance dose of 2.5–7.5 mg/day.

3.4. Remission. Remission is defined as the disappearance of clinical symptoms and resolution of the pancreatic and/or extrapancreatic manifestations on imaging studies [17].

In a multicenter survey of steroid therapy for AIP [17], at remission, the enlarged pancreas returned to near-normal size in 239 of 300 patients (80%) and became atrophic in 58 patients (20%). Elevated serum IgG4 levels decreased in all patients after the start of steroid therapy but failed to normalize (<135 mg/dL) in 115 of 182 patients (63%). At remission, irregularity of the pancreatic ducts and/or some degree of bile duct stenosis remained in 67 of 115 patients (58%) with persistent elevation of serum IgG4 levels, but only 18 of 67 patients (27%) with normalized serum IgG4 levels.

In our other study [18], HbA1c decreased by more than 0.5% in 8 of 21 AIP patients (38%) with DM after 3 months of steroid therapy. One year after the start of therapy, HbA1c decreased compared with levels before steroid therapy in 13 of 15 DM patients (87%). Impaired pancreatic exocrine function improved in all AIP patients and normalized in half of them [19].
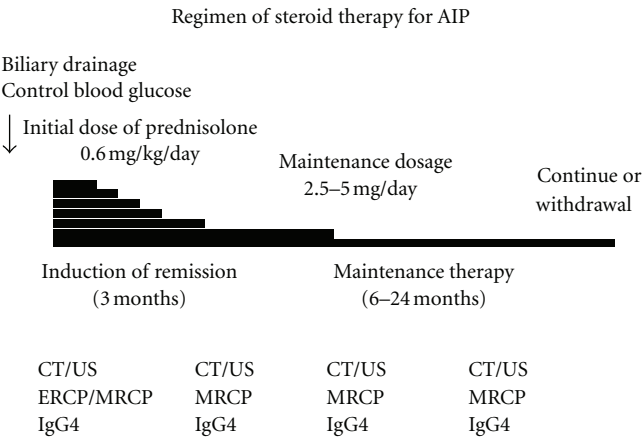
Figure 1: Regimen of oral steroid therapy for AIP.

3.5. Relapse and Maintenance Therapy. Relapse of AIP is defined as reappearance of symptoms with the reappearance of pancreatic and/or extrapancreatic (including bile duct, salivary gland, and retroperitoneum) abnormalities on imaging and/or elevation of serum IgG4 levels [17].

In a multicenter survey [17], the relapse rate of AIP patients was significantly lower in those who received steroid therapy (24%, 110/451) than in those not given steroid therapy (42%, 32/77). There was no correlation between the relapse rate and the initial prednisolone dose (40 mg/day, 19% (31/160) versus 30 mg/day, 23% (65/283)). In patients who received steroid therapy, relapse occurred in the pancreas (n = 57, 52%), bile duct (n = 37, 34%), and extrapancreatic lesions (n = 19; salivary gland swelling (n = 10), interstitial pneumonia (n = 4), and others (n = 5)).

Maintenance steroid therapy (oral prednisolone dose: 2.5 mg–5 mg/day) was given after remission in 377 of 459 patients (82%) treated with steroids. The relapse rate with maintenance therapy was 23% (63/273), which was significantly lower than that of patients who stopped maintenance therapy (34%, 35/104) [17]. In the United States and United Kingdom, where no maintenance therapy was given, relapse rates of patients treated with steroids were reportedly 38–60% [20–22]. In Korea, where maintenance therapy was stopped completely after about 6 months, the relapse rate of AIP patients treated with steroids was 33% (13/40) [16]. In consideration of these findings, maintenance therapy with low-dose prednisolone may prevent relapse. In Japan, maintenance therapy is used for about 1–3 years. However, the optimal duration of maintenance therapy is an issue requiring further investigation, as continued steroid therapy may increase the risk of steroid-induced adverse events. AIP often occurs in the elderly, who are already at heightened risk for osteoporosis and complications of glucose intolerance.

For relapsed AIP, readministration or increasing the dose of steroids is effective. In the United States and United Kingdom, immunomodulatory drugs such as azathioprine were used for maintenance of remission in patients with relapse after steroid withdrawal although azathioprine also has adverse effects such as allergic reactions, bone marrow suppression, and increased risk of infection [20–22]. Recently, it was reported that an AIP patient refractory to steroids and 6 mercaptopurine was successfully treated with rituximab, a monoclonal antibody directed against the CD20 antigen on B lymphocytes [23].

3.6. Predictive Factors for Relapse. For patients with relapsed AIP, maintenance therapy with steroids should be given with longer duration and higher dose than that of the initial maintenance therapy. Furthermore, pancreatic stones may form in some relapsing AIP patients, which might be induced by pancreatic juice stasis from incomplete obstruction of an irreversibly damaged pancreatic duct system [24]. Therefore, relapse of AIP should be avoided as much as possible. Identification of risk factors for relapse may help to identify high-risk patients who would benefit from maintenance therapy up front, and allow short-term therapy in lower-risk patients who may not need long-term therapy.

Hirano et al. [25] reported that obstructive jaundice is a predictive factor for unfavorable events. Kubota et al. [8] reported that diffuse pancreatic swelling independently predicted a relapse of AIP. Raina et al. described that relapse occurred in 7 of 9 patients (78%) with extrapancreatic biliary stenosis after withdrawal of immunosuppressive therapy [21]. Ghazale et al. reported that the relapse rate (64%) of patients with proximal biliary stenosis was significantly higher than that of patients with distal biliary stenosis alone (32%) [22]. In Korean data, relapse rate in patients with intrahepatic or proximal biliary stenosis was 65% compared with 25% in those without proximal biliary disease [26]. In a multicenter study [17], the relapse rate of AIP was significantly higher in patients with persistent elevation of serum IgG4 levels (30%, 34/115) than in those with normalized serum IgG4 levels (10%, 7/69). Although serum IgG4 levels fluctuated by more than 30 mg/dL in 94 of 172 patients (55%) during maintenance therapy, reelevation of serum IgG4 levels was detected in 37 of 54 patients (69%) who relapsed during maintenance therapy. The presence of proximal bile duct stenosis and elevated serum IgG4 levels may be predictive factors of relapse of AIP. Changes in serum IgG4 levels during serial checkups may provide clinically useful information to detect relapse of AIP earlier.

In the future, it will be necessary to verify the validity of the Japanese regimen of steroid therapy for AIP. The necessity, drugs, and duration of maintenance therapy need to be clarified by prospective studies.

As recent interesting reports have shown that pancreatic cancer was complicated with AIP [27, 28], we have to pay attention not only AIP but also pancreatic cancer even in a follow-up period.

4. Conclusion

It is important to differentiate AIP from pancreatic cancer before starting therapy. Steroids are the standard therapy for AIP, but this regimen should be evaluated in prospective studies.

Conflict of Interests

Acknowledgment

References

[1] K. Yoshida, F. Toki, T. Takeuchi, S. I. Watanabe, K. Shiratori, and N. Hayashi, "Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis," *Digestive Diseases and Sciences*, vol. 40, no. 7, pp. 1561–1568, 1995.

[2] R. P. Sah, S. T. Chari, R. Pannala et al., "Differences in clinical profile and relapse rate of type 1 versus type 2 autoimmune pancreatitis," *Gastroenterology*, vol. 139, no. 1, pp. 140–148, 2010.

[3] T. Kamisawa, K. Notohara, and T. Shimosegawa, "Two clinicopathologic subtypes of autoimmune pancreatitis: LPSP and IDCP," *Gastroenterology*, vol. 139, no. 1, pp. 22–25, 2010.

[4] T. Kamisawa, N. Funata, Y. Hayashi et al., "A new clinicopathological entity of IgG4-related autoimmune disease," *Journal of Gastroenterology*, vol. 38, no. 10, pp. 982–984, 2003.

[5] T. Kamisawa, K. Takuma, N. Egawa, K. Tsuruta, and T. Sasaki, "Autoimmune pancreatitis and IgG4-related sclerosing disease," *Nature Reviews Gastroenterology and Hepatology*, vol. 7, no. 7, pp. 401–409, 2010.

[6] T. Kamisawa, H. Anjiki, K. Takuma, N. Egawa, and N. Kubota, "The natural course of autoimmune pancreatitis," *Hepato-Gastroenterology*, vol. 56, no. 91-92, pp. 866–870, 2009.

[7] T. Wakabayashi, Y. Kawaura, Y. Satomura, H. Watanabe, Y. Motoo, and N. Sawabu, "Long-term prognosis of duct-narrowing chronic pancreatitis: strategy for steroid treatment," *Pancreas*, vol. 30, no. 1, pp. 31–39, 2005.

[8] K. Kubota, H. Iida, T. Fujisawa et al., "Clinical factors predictive of spontaneous remission or relapse in cases of autoimmune pancreatitis," *Gastrointestinal Endoscopy*, vol. 66, no. 6, pp. 1142–1151, 2007.

[9] T. Kamisawa, S. T. Chari, S. A. Giday et al., "Clinical profile of autoimmune pancreatitis and its histological subtypes: an international multicenter survey," *Pancreas*, vol. 40, no. 6, pp. 809–814, 2011.

[10] T. Kamisawa, K. Okazaki, S. Kawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: III. Treatment and prognosis of AIP," *Journal of Gastroenterology*, vol. 45, no. 5, pp. 471–477, 2010.

[11] K. Okazaki, S. Kawa, T. Kamisawa et al., "Japanese clinical guidelines for autoimmune pancreatitis," *Pancreas*, vol. 38, no. 8, pp. 849–866, 2009.

[12] T. Shimosegawa, S. T. Chari, L. Frulloni et al., "International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the international association of pancreatology," *Pancreas*, vol. 40, no. 3, pp. 352–358, 2011.

[13] S. T. Chari, "Current concepts in the treatment of autoimmune pancreatitis," *Journal of the Pancreas*, vol. 8, no. 1, pp. 1–3, 2007.

[14] K. Takuma, T. Kamisawa, T. Tabata, Y. Inaba, N. Egawa, and Y. Igarashi, "Utility of pancreatography for diagnosing autoimmune pancreatitis," *World Journal of Gastroenterology*, vol. 17, no. 18, pp. 2332–2337, 2011.

[15] S. T. Chari, T. C. Smyrk, M. J. Levy et al., "Diagnosis of autoimmune pancreatitis: the mayo clinic experience," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 8, pp. 1010–1016, 2006.

[16] D. H. Park, M. H. Kim, H. B. Oh et al., "Substitution of Aspartic Acid at Position 57 of the DQβ1 Affects Relapse of Autoimmune Pancreatitis," *Gastroenterology*, vol. 134, no. 2, pp. 440–446, 2008.

[17] T. Kamisawa, T. Shimosegawa, K. Okazaki et al., "Standard steroid treatment for autoimmune pancreatitis," *Gut*, vol. 58, no. 11, pp. 1504–1507, 2009.

[18] T. Kamisawa, A. Okamoto, T. Wakabayashi, H. Watanabe, and N. Sawabu, "Appropriate steroid therapy for autoimmune pancreatitis based on long-term outcome," *Scandinavian Journal of Gastroenterology*, vol. 43, no. 5, pp. 609–613, 2008.

[19] T. Kamisawa, N. Egawa, S. Inokuma et al., "Pancreatic endocrine and exocrine function and salivary gland function in autoimmune pancreatitis before and after steroid therapy," *Pancreas*, vol. 27, no. 3, pp. 235–238, 2003.

[20] N. S. Sandanayake, N. I. Church, M. H. Chapman et al., "Presentation and management of post-treatment relapse in autoimmune pancreatitis/immunoglobulin G4-associated cholangitis," *Clinical Gastroenterology and Hepatology*, vol. 7, no. 10, pp. 1089–1096, 2009.

[21] A. Raina, D. Yadav, A. M. Krasinskas et al., "Evaluation and management of autoimmune pancreatitis: experience at a large us center," *American Journal of Gastroenterology*, vol. 104, no. 9, pp. 2295–2306, 2009.

[22] A. Ghazale, S. T. Chari, L. Zhang et al., "Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy," *Gastroenterology*, vol. 134, no. 3, pp. 706–715, 2008.

[23] M. Topazian, T. E. Witzig, T. C. Smyrk et al., "Rituximab therapy for refractory biliary strictures in immunoglobulin G4-associated cholangitis," *Clinical Gastroenterology and Hepatology*, vol. 6, no. 3, pp. 364–366, 2008.

[24] K. Takuma, T. Kamisawa, T. Tabata, Y. Inaba, N. Egawa, and Y. Igarashi, "Short-term and long-term outcomes of autoimmune pancreatitis," *European Journal of Gastroenterology and Hepatology*, vol. 23, no. 2, pp. 146–152, 2011.

[25] K. Hirano, M. Tada, H. Isayama et al., "Long-term prognosis of autoimmune pancreatitis with and without corticosteroid treatment," *Gut*, vol. 56, no. 12, pp. 1719–1724, 2007.

[26] S. H. Moon, M. H. Kim, and D. H. Park, "Treatment and relapse of autoimmune pancreatitis," *Gut and Liver*, vol. 2, no. 1, pp. 1–7, 2008.

[27] T. Fukui, T. Mitsuyama, M. Takaoka, K. Uchida, M. Matsushita, and K. Okazaki, "Pancreatic cancer associated with autoimmune pancreatitis in remission," *Internal Medicine*, vol. 47, no. 3, pp. 151–155, 2008.

[28] A. Ghazale and S. Chari, "Is autoimmune pancreatitis a risk factor for pancreatic cancer?" *Pancreas*, vol. 35, no. 4, p. 376, 2007.

Clinical Study

Clinical Aspects of IgG4-Related Orbital Inflammation in a Case Series of Ocular Adnexal Lymphoproliferative Disorders

Masayuki Takahira,[1] Yoshiaki Ozawa,[1] Mitsuhiro Kawano,[2] Yoh Zen,[3] Shoko Hamaoka,[1] Kazunori Yamada,[2] and Kazuhisa Sugiyama[1]

[1] *Department of Ophthalmology, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 9208640, Japan*
[2] *Division of Rheumatology, Kanazawa University, Kanazawa, Ishikawa 9208640, Japan*
[3] *Institute of Liver Studies, King's College Hospital, London, SE5 9RS, UK*

Correspondence should be addressed to Masayuki Takahira, takahira@med.kanazawa-u.ac.jp

The most frequent ocular adnexal tumors and simulating lesions are lymphoproliferative disorders (LPDs), including malignant lymphomas and orbital inflammation with lymphoid hyperplasia or infiltration. IgG4-related orbital inflammation (IgG4-ROI) often involves lacrimal glands and other orbital tissues and is an important differential diagnosis. The present study evaluated clinical aspects of IgG4-ROI in a case series of orbital LPD. Sixty-two consecutive cases of orbital LPD, pathologically diagnosed from November, 2004, through March, 2011, were investigated. Histological types were 22 cases with MALT lymphoma, 11 cases with diffuse large B-cell lymphoma (DLBCL), 3 cases with other malignant lymphomas, 16 cases with IgG4-ROI, and 10 cases with non-IgG4-ROI. Ages of the IgG4-ROI group (56 ± 10 yrs) were significantly lower than the MALT lymphoma (71 ± 12 yrs) and DLBCL (75 ± 14 yrs) groups. Orbital lesions other than lacrimal glands were present in six cases including extraocular muscle swelling, mass lesions surrounding the optic nerve, and supraorbital and infraorbital nerves enlargements. Although none of the malignant lymphomas were related to IgG4, previous evidence suggested that malignant lymphomas can arise from IgG4-ROI. Based on this study (26%) and another report (33%), it is likely that nearly a quarter of orbital LPD are IgG4-ROI.

1. Introduction

The most frequent tumors and simulating lesions in ocular adnexa are lymphoproliferative disorders (LPDs), including malignant lymphomas and orbital inflammation with lymphoid hyperplasia or infiltration, some of which are historically called "orbital pseudotumors." In Japanese studies, Goto [1] reported that the rate of LPD among 409 cases with orbital tumors and simulating lesions was 43%, while Ohtsuka et al. [2] described that the rate was 49% out of 213 cases. In the United States, LPD is a common orbital disease although the rate seems to be somewhat lower: 24% of LPD out of 703 cases with orbital lesions was reported by Shields et al. [3], and 26% of LPD out of 268 cases was reported by Shinder et al. [4].

When patients with suspected orbital LPD are encountered, tissue biopsy is preferred since image examinations alone cannot distinguish inflammatory lesions from malignant lymphomas. Pathological examination can also detect whether the lesion is related to IgG4 or not. IgG4-related disease (IgG4-RD) often involves lacrimal glands, which is now known as IgG4-related Mikulicz's disease [5, 6] or IgG4-related dacryoadenitis [7, 8] by many recent reports over several years. Recently, it was also elucidated that IgG4-related orbital inflammatory lesions include other ocular adnexal tissues such as extraocular muscles [9] and periorbital membrane [10]. Therefore, IgG4-RD is a differential diagnosis in orbital LPD. The question is then raised as to what percentage of orbital LPD is related to IgG4. In this study, an orbital LPD case series was investigated and clinical

aspects of IgG4-related orbital lymphoproliferative disorders were evaluated.

2. Patients and Methods

In Kanazawa University Hospital in Japan, a 47-year-old woman with IgG4-immunopositive histopathology and an elevated serum IgG4 level of 1000 mg/dL was diagnosed as a first case of IgG4-related dacryoadenitis in November, 2004 [8]. From that time through March, 2011, sixty-two cases (27 men and 35 women; mean age, 66 ± 14 yrs; range 32– 89 yrs) were pathologically diagnosed with orbital lympho-proliferative diseases (LPD) from surgical samples of ocular adnexal tissue. The two main categories of orbital LPD were malignant lymphomas and orbital inflammations: the latter included reactive lymphoid hyperplasia, lymphoid infiltrated lesions, and inflammatory pseudotumor. This consecutive 62 case series was investigated retrospectively. Conjunctival lesions were not enrolled in this study because conjunctival involvement in IgG4-RD has never been experienced in previous reports [9, 11] or in the author's institution. Intraocular lymphoma belongs to CNS lymphoma and thus was also excluded in this study. In most cases, immunoglob-ulin heavy chain gene rearrangement in surgical samples was also examined to support the differential diagnosis of malignant lymphoma. Diagnostic criterion for positive IgG4-immunostaining in orbital tissue (IgG4-related orbital disease) was either (1) the ratio of IgG4-positive cells to IgG-positive cells (IgG4+/IgG+ cells) was more than 40% [12], or (2) the number of IgG4-positive cells was more than 30 per high power microscopy field [13]. Mouse monoclonal antibody anti-human IgG4 (05-3800, ZYMED, USA) and rabbit polyclonal antibody anti-human IgG (A0423, Dako, USA) were used for immunostaining. Serum IgG and IgG4 were measured in all of the cases with an IgG4-positive pathological diagnosis. IgG4-related orbital lesions including lacrimal gland swelling, extraocular muscle enlargement, and other mass lesions were evaluated using computed tomogra-phy (CT) and/or magnetic resonance imaging (MRI).

3. Results

Histological types of the 62 orbital LPD were 22 cases (35%) with extranodal marginal zone lymphoma of mucosa-associated lymphoid-tissue type (MALT lymphoma), 11 cases (18%) with diffuse large B-cell lymphoma (DLBCL), 3 cases (5%) with other malignant lymphomas (1 mantle cell lymphoma, 1 NK/T cell lymphoma, 1 small lym-phocytic lymphoma), 16 cases (26%) with IgG4-related orbital inflammation (IgG4-ROI), and 10 cases (16%) with non-IgG4-related orbital inflammation (non-IgG4-ROI) (Figure 1). None of the malignant lymphomas showed a relationship with IgG4 in this case series. Figure 2 depicts the ages of the four groups of DLBCL, MALT lymphoma, IgG4-ROI, and non-IgG4-ROI. Ages of the IgG4-ROI group averaged 56 ± 10 yrs, which was significantly lower than those of the MALT lymphoma (71 ± 12 yrs, $P = 0.00027$ in a Student's t-test) and DLBCL (75 ± 14 yrs, $P = 0.00107$)
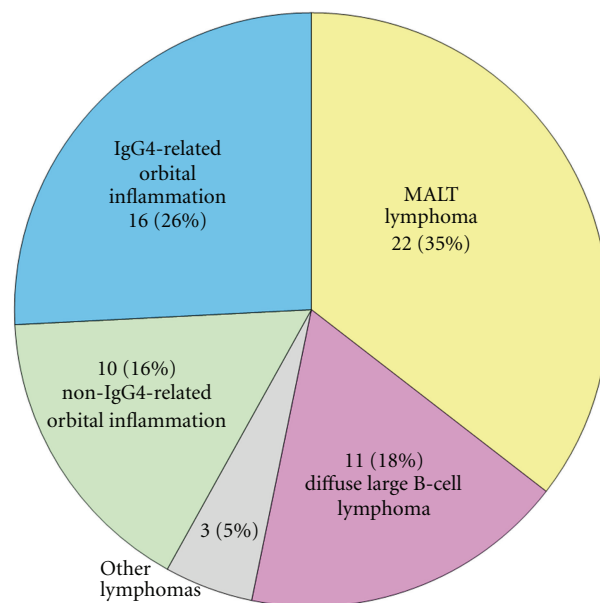

Figure 1: Breakdown of diagnosis in orbital lymphoproliferative disorders. Patients were a consecutive 62 case series with or-bital lymphoproliferative disorders pathologically diagnosed from November, 2004, to March, 2011, in Kanazawa University Hos-pital, Japan. Cases with conjunctival and intraocular lesions were excluded. MALT lymphoma is an abbreviation for extranodal mar-ginal zone B-cell lymphoma of mucosa-associated lymphoid tissue.
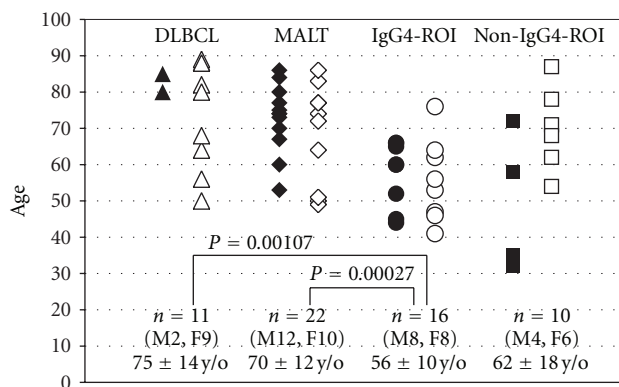

Figure 2: Ages of orbital lymphoproliferative disorders. Each plot depicts age at diagnosis in groups of MALT lymphoma, DLBCL (diffuse large B-cell lymphoma), IgG4-related orbital inflammation and non-IgG4-related orbital inflammation. Closed and open plots represent men and women, respectively.

groups. There were no significant age differences between other combinations of the four groups.

Clinical data of sixteen cases of IgG4-ROI are summa-rized in Table 1, which were sorted in ascending order of serum IgG4 level. The age ranged between 41 to 76 yrs (mean ± SD; 56 ± 10 yrs, median; 58 yrs), and there was no sex difference (8 men and 8 women). These cases except one (number 1, detailed in the discussion) were accompanied by elevated serum IgG4 concentration (549 ± 293 mg/dL,

TABLE 1: Clinical data in 16 cases with IgG4-related orbital disease.

Case number	Age	Sex	Orbital lesions	Lesions other than orbit	Serum IgG (mg/dL)	Serum IgG4 (mg/dL)	IgG4/IgG (%)	IgG4 + cells /HPF	Serum IgE (IU/mL)	Therapy
1	62	F	LG	None	1690	29	2	43	14	None
2	66	M	LG	SG	1960	164	8	143	1621	None
3	56	F	LG	Lung lesion	1671	194	12	46	254	Steroid
4	46	F	LG, EOM	SG, retroperitoneal fibrosis	1350	209	15	92	170	Steroid
5	41	F	LG	Sinusitis	1554	260	17	62	178	None
6	53	F	LG	SG	2260	359	16	58	611	Steroid
7	65	M	LG, EOM, ION	Sinusitis, submandibular lymphadenopathy	1448	404	28	98	3374	Steroid
8	60	M	LG, EOM, OPN, SON, ION	SG, neck skin nodule	1220	463	38	82	4608	Steroid
9	64	F	LG	SG, neck lymph nodes TB	1820	486	27	83	90	Steroid
10	66	M	LG	SG, lung lesion, axillary lymphadenopathy	1900	575	30	48	151	Steroid
11	44	M	LG, OPN, ION	None	1322	599	45	63	351	Steroid
12	76	F	LG	Nephritis	2860	769	27	58	428	Steroid
13	60	M	LG	SG,	1952	886	45	48	575	Steroid
14	45	M	LG, EOM, ION	Lung lesions, sinusitis	2260	914	40	74	72	Steroid
15	52	M	LG	SG	3440	949	28	50	973	Steroid
16	47	F	LG, SON, ION	SG	2350	1000	43	55	183	Steroid
				Normal range	870–1700	<135	<7	<10	<250	

LG: lacrimal gland, EOM: extraocular muscle, OPN: optic nerve, SON: supraorbital nerve, ION: infraorbital nerve, SG: salivary gland.

$n = 15$, normal range < 135 mg/dL) and an increased ratio of serum IgG4/IgG ($28 \pm 12\%$, $n = 15$, normal range < 7%). In 9 patients, the serum IgE was also elevated. In all 16 cases, diagnosis was made by lacrimal gland biopsy. In pathology, these were characterized by lymphoplasmacytic infiltration, forming lymphoid follicles (germinal center) and sclerosing fibrosis [8]. IgG4-positive plasma cells were observed around lymphoid follicles and in intraglandular areas [8]. Orbital lesions other than lacrimal glands were detected by MRI in six cases (cases numbers 4, 7, 8, 11, 14, and 16 in Table 1) as shown representatively in Figure 3. Swollen extraocular muscles were seen in four cases (numbers 4, 7, 8 and 14). Supraorbital nerve (frontal nerve), and/or infraorbital nerve enlargements were observed in 4 cases (numbers 7, 8, 11, and 14). A mass lesion surrounding the optic nerve was detected in two patients (numbers 8 and 11).

Steroid therapy was performed for all the IgG4-ROI cases except three patients because their eyelid swellings decreased in size after biopsy surgery (case numbers 1, 2, and 5) and because of normal serum IgG4 (case number 1) and diabetes mellitus (case number 2). Eleven cases underwent oral prednisolone tapering therapy with an initial dose of 20, 30, or 40 mg per day. In two cases with diabetes mellitus, lower doses of oral prednisolone (10 mg daily in case number 10 and 8 mg daily in case number 15) were administered initially and reduced to a maintenance dose. All of the 11 cases essentially responded well to initial doses of oral prednisolone, but in 5 cases (numbers 4, 8, 10, 11, and 14) ocular symptoms such as eyelid swelling deteriorated during tapering, and thus increase in the dosage was required. In case number 11 with retrobulbar mass (Figure 3(c)), oral prednisolone therapy alone (30 mg daily initially) failed to diminish his exophthalmos, and then intravenous methyl-prednisolone pulse (500 mg for 3 days) was additionally performed three times.

4. Discussion

In this study, none of the malignant lymphoma cases was related to IgG4. However, some previous reports suggested that orbital malignant lymphomas can be related to IgG4. Cheuk reported three cases of ocular adnexal lymphoma (2 MALT lymphoma and 1 follicular lymphoma) arising in IgG4-related dacryoadenitis, and that the rate of transformation of malignant lymphoma in the background of IgG4-ROI was approximately 10% [12]. Sato et al. first detected IgH gene rearrangement in two cases of ocular adnexal IgG4-related disease [11] and later reported seven patients with ocular adnexal MALT lymphoma arising from IgG4-related orbital disease [14]. On the other hand, he described a case of IgG4-producing MALT lymphoma of the lymph node [15]. Also in the orbital region, Oyama reported a case of IgG4-expressing MALT lymphoma in the lacrimal gland [16]. Based on these findings, there could be two possibilities of
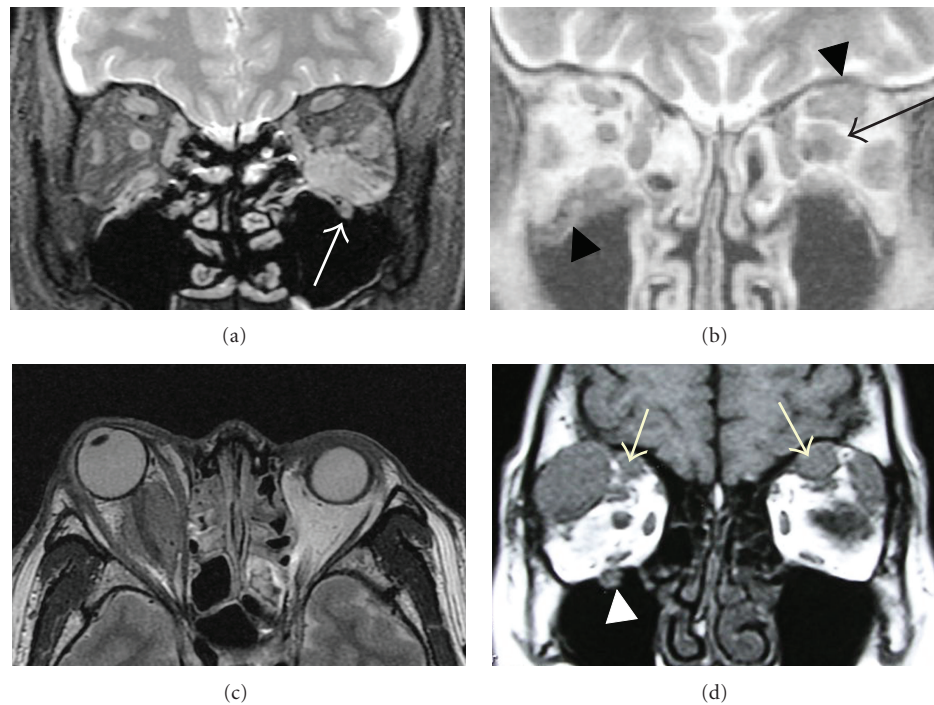
(a)

(b)

(c)

(d)

FIGURE 3: Representative cases with IgG4-related orbital inflammatory lesions other than lacrimal glands. (a) Enlargements in the left inferior rectus muscle and infraorbital nerve (arrow) in a 65-year-old man with a serum IgG4 of 404 mg/dL (case number 7 in Table 1). (b) Swelling of the left superior and lateral rectus muscles, a mass lesion around the left optic disc (arrow), and enlargements of the left supraorbital nerve and the right infraorbital nerve (arrow heads) were seen in a 60-year-old man with a serum IgG4 of 463 mg/dL (case number 8). (c) Mass lesion around the right optic disc was observed in a 44-year-old man with a serum IgG4 of 599 mg/dL (case number 11). (d) Bilateral supraorbital nerve enlargements (arrows) and right infraorbital nerve enlargement were observed in a 47-year-old woman with a serum IgG4 of 1000 mg/dL (case number 16). Magnetic resonance images are T1-weighted in (b) and (d) and T2-weighted in (a) and (c).

orbital MALT lymphoma arising from preexisting IgG4-ROI and de novo IgG4-positive orbital MALT lymphoma [12]. In any case, orbital biopsy should be mandatory to evaluate malignancy before treatment even if serological examination already detected elevated serum IgG4.

There is no doubt that IgG4-ROI most frequently involves the lacrimal gland, which is reported as IgG4-related dacryoadenitis and Mikulicz's disease [5–8]. In addition, lesions of IgG4-ROI other than the lacrimal glands were reported previously. Sato et al. reported that orbital masses other than the lacrimal gland were detected in 7 out of 21 cases with IgG4-ROI [11]. Kubota et al. reported a case with bilateral multiple extraocular muscle enlargement [9]. Mehta et al. presented a case of IgG4-ROI with enlargement of the infraorbital canal and periorbital membrane involvement [10]. Similar lesions were observed in the present case series of IgG4-ROI (Figure 3). In addition, mass lesions around the optic nerve were seen in two cases, indicating that involvement of the optic nerve sheath may not be rare in IgG4-ROI.

Among 16 cases of IgG4-ROI, one patient (case number 1) presented normal levels of serum IgG4. In this case, the ratio of IgG4+/IgG+ cells in immunohistochemistry was around 30%, somewhat lower than values in diagnostic criteria [6, 12], but IgG4-positive cells were more than 30 per high power microscopy field. The pathological findings that lymphoplasmacytic infiltration with germinal centers and

dense sclerosing fibrosis was characteristic for IgG4-ROI. So far, we cannot resolve the reason for this discrepancy between pathological and serological findings. However, Kubota previously reported a patient with an inverse discrepancy who had negative findings on the IgG4 immunostaining despite presumably typical IgG4-ROI with elevated serum IgG4 [9].

The rate of IgG4-RD in 62 cases with orbital LPD was 26% in this study and was reported to be 33% out of 58 orbital LPD cases from another Japanese institute [17]. Thus, it is likely that nearly a quarter of orbital lymphoproliferative disorders are estimated to be related to IgG4. Further multicenter studies will be required to confirm this rate and to evaluate the frequency and location of IgG4-ROI other than the lacrimal glands.

References

[1] H. Goto, "Review of ocular tumor," in *Practical Ophthalmology*, H. Goto, Ed., pp. 24–29, Bunkodo, Tokyo, Japan, 2008.

[2] K. Ohtsuka, M. Hashimoto, and Y. Suzuki, "A review of 244 orbital tumors in Japanese patients during a 21-year period: origins and locations," *Japanese Journal of Ophthalmology*, vol. 49, no. 1, pp. 49–55, 2005.

[3] J. A. Shields, C. L. Shields, and R. Scartozzi, "Survey of 1264 patients with orbital tumors and simulating lesions: the 2002

Montgomery Lecture, part 1," *Ophthalmology*, vol. 111, no. 5, pp. 997–1008, 2004.

[4] R. Shinder, N. Al-Zubidi, and B. Esmaeli, "Survey of orbital tumors at a comprehensive cancer center in the United States," *Head and Neck*, vol. 33, no. 5, pp. 610–614, 2011.

[5] M. Yamamoto, H. Takahashi, S. Sugai, and K. Imai, "Clinical and pathological characteristics of Mikulicz's disease (IgG4-related plasmacytic exocrinopathy)," *Autoimmunity Reviews*, vol. 4, no. 4, pp. 195–200, 2005.

[6] Y. Masaki, L. Dong, N. Kurose et al., "Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders," *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1310–1315, 2009.

[7] W. Cheuk, H. K. L. Yuen, and J. K. C. Chan, "Chronic sclerosing dacryoadenitis: part of the spectrum of IgG4-related sclerosing disease?" *American Journal of Surgical Pathology*, vol. 31, no. 4, pp. 643–645, 2007.

[8] M. Takahira, M. Kawano, Y. Zen, H. Minato, K. Yamada, and K. Sugiyama, "IgG4-related chronic sclerosing dacryoadenitis," *Archives of Ophthalmology*, vol. 125, no. 11, pp. 1575–1578, 2007.

[9] T. Kubota, S. Moritani, M. Katayama, and H. Terasaki, "Ocular adnexal IgG4-related lymphoplasmacytic infiltrative disorder," *Archives of Ophthalmology*, vol. 128, no. 5, pp. 577–584, 2010.

[10] M. Mehta, F. Jakobiec, and A. Fay, "Idiopathic fibroinflammatory disease of the face, eyelids, and periorbital membrane with immunoglobulin G4-positive plasma cells," *Archives of Pathology and Laboratory Medicine*, vol. 133, no. 8, pp. 1251–1255, 2009.

[11] Y. Sato, K. I. Ohshima, K. Ichimura et al., "Ocular adnexal IgG4-related disease has uniform clinicopathology," *Pathology International*, vol. 58, no. 8, pp. 465–470, 2008.

[12] W. Cheuk, H. K. L. Yuen, A. C. L. Chan et al., "Ocular adnexal lymphoma associated with IgG4+ chronic sclerosing dacryoadenitis: a previously undescribed complication of IgG4-related sclerosing disease," *American Journal of Surgical Pathology*, vol. 32, no. 8, pp. 1159–1167, 2008.

[13] T. Kamisawa, N. Funata, Y. Hayashi et al., "Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis," *Gut*, vol. 52, no. 5, pp. 683–687, 2003.

[14] Y. Sato, K. Notohara, M. Kojima, K. Takata, Y. Masaki, and T. Yoshino, "IgG4-related disease: historical overview and pathology of hematological disorders: review article," *Pathology International*, vol. 60, no. 4, pp. 247–258, 2010.

[15] Y. Sato, K. Takata, K. Ichimura et al., "IgG4-producing marginal zone B-cell lymphoma," *International Journal of Hematology*, vol. 88, no. 4, pp. 428–433, 2008.

[16] T. Oyama, J. Takizawa, N. Nakamura, S. Aoki, Y. Aizawa, and H. Abe, "Multifocal mucosa-associated lymphoid tissue lymphoma associated with IgG4-related disease: a case report," *Japanese Journal of Ophthalmology*, vol. 55, no. 3, pp. 304–306, 2011.

[17] K. I. Ohshima, "Ocular adnexal IgG4-related disorders: do they overlap with Mikulicz's disease or MALT lymphoma?" *Neuro-Ophthalmology Japan*, vol. 25, no. 4, pp. 466–474, 2008.

Review Article

Regulatory T Cells in Type 1 Autoimmune Pancreatitis

**Kazushige Uchida,[1] Takeo Kusuda,[2] Masanori Koyabu,[1] Hideaki Miyoshi,[2]
Norimasa Fukata,[2] Kimi Sumimoto,[1] Yuri Fukui,[1] Yutaku Sakaguchi,[1] Tsukasa Ikeura,[2]
Masaaki Shimatani,[2] Toshiro Fukui,[1] Mitsunobu Matsushita,[2] Makoto Takaoka,[2]
Akiyoshi Nishio,[1] and Kazuichi Okazaki[1, 2]**

[1] *Department of Gastroenterology and Hepatology, Kansai Medical University, 10-15 Fumisono Cho,
Moriguchi, Osaka 570-8506, Japan*
[2] *The Third Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kansai Medical University, 2-3-1
Shinmachi, Hirakata, Osaka 573-1191, Japan*

Correspondence should be addressed to Kazuichi Okazaki, okazaki@hirakata.kmu.ac.jp

Autoimmune pancreatitis (AIP) is a newly recognized pancreatic disorder. Recently, International Consensus Diagnostic Criteria for AIP (ICDC) was published. In this ICDC, AIP was classified into Type 1 and Type 2. Patients with Type 1 AIP have several immunologic and histologic abnormalities specific to the disease, including increased levels of serum IgG4 and storiform fibrosis with infiltration of lymphocytes and IgG4-positive plasmacytes in the involved organs. Among the involved organs showing extrapancreatic lesions, the bile duct is the most common, exhibiting sclerosing cholangitis (IgG4-SC). However, the role of IgG4 is unclear. Recently, it has been reported that regulatory T cells (Tregs) are involved in both the development of various autoimmune diseases and the shift of B cells toward IgG4, producing plasmacytes. Our study showed that Tregs were increased in the pancreas with Type 1 AIP and IgG4-SC compared with control. In the patients with Type 1 AIP and IgG4-SC, the numbers of infiltrated Tregs were significantly positively correlated with IgG4-positive plasma cells. In Type 1 AIP, inducible costimulatory molecule (ICOS)+ and IL-10+ Tregs significantly increased compared with control groups. Our data suggest that increased quantities of ICOS+ Tregs may influence IgG4 production via IL-10 in Type 1 AIP.

1. Introduction

In 1961, Sarles et al. observed the first case of idiopathic chronic pancreatitis with hypergammaglobulinemia, in which an autoimmune mechanism was supposedly involved [1]. In 1991, Kawaguchi et al. reported two cases of an unusual lymphoplasmacytic sclerosing inflammatory disease involving the entire pancreas, common bile duct, gallbladder, and, in one patient, the lip [2]. In addition, two patients presented mass-like enlargement of the pancreatic head. Histopathological characteristics included diffuse lymphoplasmacytic infiltration, marked interstitial fibrosis, acinar atrophy, and obliterative phlebitis of the pancreatic and portal veins, which was termed as lymphoplasmacytic sclerosing pancreatitis (LPSP). In 1995, Yoshida et al. first proposed the concept of "autoimmune pancreatitis (AIP)," in which patients showed a diffusely enlarged pancreas, a narrowing pancreatogram, increased serum IgG, the presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and steroidal efficacy [3]. In 2001, Hamano et al. reported that elevated serum IgG4 levels were highly specific and sensitive for the diagnosis of AIP [4]. In 2003, Kamisawa et al. suggested that AIP is a systemic disease, based on the findings that the pancreas and other involved organs have abundant infiltration of IgG4-positive plasma cells [5]. Thereafter, Japanese investigators reported numerous AIP cases, and AIP has been accepted as a new clinical entity [6–9].

Human regulatory T cells (Tregs) were first isolated from peripheral blood and characterized as CD4+CD25high T cells

by several groups in 2001 [10–12], based on the finding in 1995 that mouse Tregs constitutively express CD25 [13]. We now know that these cells play a critical role in preventing autoimmune diseases by suppressing self-reactive T cells—which are present in all healthy individuals—through incompletely understood mechanisms that involve cell contact and secretion of inhibitory cytokines [14]. Although the role of Tregs in AIP remains unclear, we will discuss Tregs and the mechanism of IgG4 related AIP.

2. Type 1 and Type 2 AIP

Reports from Europe [15] and the United States [16] described unique histological patterns in the resected pancreases of patients with mass-forming chronic nonalcoholic pancreatitis with epithelial destruction by granulocytes, which is now supposed to be distinguishable from Type 1 AIP, IgG4-related AIP or LPSP, and called idiopathic duct centric pancreatitis (IDCP), AIP with granulocyte epithelial lesions (AIP with GEL), or Type 2 AIP [17]. In 2011, the International Consensus Diagnostic Criteria for Autoimmune Pancreatitis (ICDC) was published. In this ICDC, AIP was classified into Type 1 and Type 2 [18]. Most of the Japanese AIP cases are LPSP, whereas those concerning IDCP are very few. Although we recently reported the first case of IDCP in Japan with full radiological and histopathological findings [19], it still remains unclear whether the clinical manifestations of the Japanese patients with IDCP are similar to those of Western countries or not. Therefore, Japanese consensus clinical guidelines have focused on Type 1 AIP (IgG4-related AIP) [20–23]. An overlap in the histological features of the two patterns may exist in some patients. Although the pathogenesis is still unclear, the most important issue in managing AIP is to differentiate it from pancreas and biliary malignancy.

3. Other Organ Involvement (OOI) in Type 1 AIP

Type 1 AIP often shows other organ involvement (OOI) such as AIP, sclerosing cholangitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis [24–28]. Moreover, sialoadenitis is also major complication with Type 1 AIP. The patients with Mikulicz's disease (MD) usually have bilateral, painless, and symmetrical swelling of the lachrymal, parotid, and submandibular glands [29]. This disease is originally classified as an atypical type of Sjögren's syndrome. Recently, MD has been considered to be completely different from Sjögren's syndrome because of the lack of anti-SS-A/Ro or anti-SS-B/La antibodies, elevated serum levels of IgG4, infiltration of IgG4-positive plasma cells into the glands, and recovery of secretion with steroid treatment [25]. Sclerosing cholangitis with Type 1 AIP shows various cholangiographic features similar to those of primary sclerosing cholangitis (PSC). However, the steroid responses and the prognoses of Type 1 AIP patients with sclerosing cholangitis differ from these features in patients with PSC, which suggests different

pathogenesis. These findings led us to the concept of "IgG4-related disease" [30] such as IgG4-related systemic sclerosing disease [5, 31], systemic IgG4-related plasmacytic syndrome (SIPS) [32], and IgG4-positive multiorgan lymphoproliferative syndrome (IgG4-MOLPS) [33].

4. Regulatory T Cells (Tregs)

Tregs expressing the transcription factor forkhead box P3 (FOXP3) originally identified by Hori et al. have key roles in the immune system, which are indispensable for the maintenance of dominant self-tolerance and immune homeostasis [34]. Dysfunction of FOXP3 is known to cause fatal autoimmune diseases, immunopathology, and allergy [35]. Most of FOXP3$^+$ Tregs are CD4$^+$ T cells strongly expressing CD25 (the interleukin-2 (IL-2) receptor α-chain), can suppress the activation, proliferation and effector of immune cells, including CD4$^+$ and CD8$^+$ T cells, natural killer (NK) and NKT cells, B cells, and antigen-presenting cells (APCs) in vitro and in vivo [36]. This unique ability can make FOXP3$^+$ Tregs to control immune responses to prevent development of autoimmune disease, immunopathology, and allergy, as well as to maintain allograft tolerance and fetal-maternal tolerance during pregnancy [37].

Increased CD4$^+$CD25$^+$ T cells were observed in the periphery and inflamed tissues of the patients with rheumatoid arthritis, Sjögren's syndrome, and psoriasis, compared with healthy controls [38]. The increased numbers of Tregs suggest that the reason for failed regulation in the inflamed tissue may be insufficient or defective Tregs function due to either cell-intrinsic or cell-extrinsic factors. However, the precise mechanism is still unclear.

5. Subtypes of Regulatory T Cells

CD4$^+$ T-cell differentiation into the conventional T-cell and Tregs lineages can be identified by phenotypic markers. All T-cell lineages originate in the thymus and emigrate as naïve CD45RA$^+$ T cells, and activation of naive T cells in the periphery induces their differentiation into both conventional and regulatory subsets. Conventional T cells further differentiate into memory T cells, which can be reactivated. CD45RA$^+$ naïve Tregs also differentiate into CD45RA$^-$ effector Tregs. On the other hand, the CD45RA$^-$ peripheral Tregs compartment is converted Tregs-like cells, which are derived from conventional T cells. These converted Tregs-like cells have cell surface marker expression similar to that expressed by natural Tregs [39].

The effector Tregs subsets are heterogeneous in the expression of inducible costimulatory molecule (ICOS) [40]. In the periphery, two functionally different subsets of effector Tregs, ICOS$^+$ or ICOS$^-$ effector Tregs actively produce the suppressive cytokines IL-10 or TGF-β, respectively [40]. It is reported that the growth of Tregs secreting IL-10 required dendritic cells (DCs) expressing high-level ICOS-ligand and was prevent by blockade of ICOS-ICOS-ligand signaling [41]. DC is important in the proliferation of ICOS$^+$ or ICOS$^-$ Tregs. While activated plasmacytoid DCs

(pDCs) preferentially promote the proliferation of the ICOS[+] Tregs through ICOS-ligand, activated myeloid DCs (mDCs) preferentially promote the proliferation of the autologous ICOS[−] Tregs through B7 signaling [40].

6. Tregs in Type 1 AIP

6.1. Flow Cytometric Analysis of Peripheral Blood in the Patient with Type 1 AIP. To clarify the role of Tregs in IgG4-related diseases, we analyzed Tregs showing CD4[+]CD25[high] and CD4[+]CD25[+]CD45RA[+] (naïve) from peripheral blood by flow cytometry in the patients with Type 1 AIP. For comparison, we also analyzed patients with other pancreatic disease (idiopathic or alcoholic pancreatitis) and healthy subjects as controls. In Type 1 AIP, in addition to increased soluble CTLA4, circulatory naïve (CD45RA[+]) Tregs were significantly decreased in the peripheral blood of the patients with AIP, whereas the major population of effector (CD45RA[−]) Tregs was significantly increased [42]. In addition, we studied infiltrating cells in the pancreas by immunohistochemistry and analyzed ICOS[+] Tregs and IL-10[+] Tregs in the peripheral blood by flow cytometry. ICOS[+] Tregs were significantly higher in AIP patients than in the patients with other pancreatic diseases and the healthy control group. IL-10[+] Tregs were significantly higher in AIP patients than in the healthy control group [43]. We previously investigated three types of DCs: mDC (Lin-HLA-DR[+]CD11c[+]), pDC (Lin-HLA-DR[+]CD11c[−]), and CD123[+] pDC (Lin-HLA-DR[+]CD11c[−]CD123[+]). There were no significant differences in the three kinds of DCs among the healthy control, alcoholic and idiopathic CP, and AIP groups. However, mDC and CD123[+] pDC significantly decreased in patients with AIP with steroid therapy, compared with other groups [41]. The relationship between DC and Tregs in AIP is still unclear.

6.2. Immunohistochemical Analysis in Type 1 AIP. IgG4-related sclerosing cholangitis (SC) was recognized as a disease entity characterized by sclerosing inflammation with abundant IgG4-positive plasma cells; Type 1 AIP was associated in most cases. We analyzed Tregs in the pancreas and liver of Type 1 AIP. In the pancreas of Type 1 AIP, the ratio of Foxp3-positive cells to infiltrated mononuclear cells (Foxp3/Mono) was significantly higher than in patients with alcoholic chronic pancreatitis. In Type 1 AIP, Foxp3/Mono and IgG4/Mono were positively correlated. This data is similar to flow cytometric analysis.

We compared the laboratory and immunohistochemical findings of the liver biopsy specimens in the patients with IgG4-SC and primary sclerosing cholangitis (PSC). After staining these specimens with anti-IgG1, anti-IgG4, and anti-Foxp3 antibodies, we compared the ratio of IgG4-, IgG1-, and Foxp3-positive cells to infiltrated mononuclear cells (IgG4, IgG1, Foxp3/Mono) among specimens. The ratio of IgG4/G1-positive plasma cells was significantly higher in IgG4-SC than in PSC. The Foxp3/Mono ratio in patients with IgG4-SC was significantly increased compared with PSC. In the patients with IgG4-SC, Foxp3-positive cells were

significantly correlated with IgG4-positive cells. In the other groups, there was no correlation [44].

7. Animal Models of Type 1 AIP

We previously reported several autoantibodies in the patients with Type 1 AIP. Among the 26 patients with Type 1 AIP, antilactoferrin (LF) was detected in the sera of 19 (73.1%), antinuclear antibody (ANA) in 18 (69.2%), anticarbonic anhydrase (CA)-II in 14 (53.8%), RF in 6 (23.1%), antismooth muscle antibody in 4 (15.4%), antiglutamic acid decarboxylase antibody in 1 (3.8%), and antiislet cell antibody in 1 (3.8%). However, AMA was not found in the sera of any of these patients [45]. We hypothesized that LF or CA-II may be one of the candidates of target antigen in Type 1 AIP. We established animal models using LF or CA-II as antigen. In neonatal thymectomized (nTx)-BALB/c mouse models immunized with CA-II or LF, CD4[+] T cells, rather than B cells, there are the predominant infiltrates in pancreas and salivary gland, and around bile duct, which are similar to human Type 1 AIP [46]. The results from these animal models suggest that the depletion of thymus-derived Tregs in the periphery [46] and major histocompatibility complex (MHC) class II restricted autoreactive CD4[+] T cells that escape from positive selection in the thymus may be of importance to the induction of target organs. In the early stage of Type 1 AIP, these CD4[+] T cells probably induce proinflammatory reactions as direct cytotoxic effects through Fas ligand [47]. In the MHC class II-deficient mouse [48] and in WBN/Kob rat models [49], CD8[+] T cells may play roles as effector cells. WBN/Kob rats spontaneously develop sialoadenitis, thyroiditis, sclerotic cholangitis, and tubulointestinal nephritis because of congenitally decreased peripheral Tregs. CD8[+] T cells also seem to be effector cells in spite of unknown target antigens. WBN/Kob rats showed deposition of tissue-specific IgG2b in the injured pancreas and lachrymal glands [49]. Although details of the IgG subclass in rodents remain unclear, rat IgG2b, a minor subclass of IgG, is separated in a similar position to human IgG4 by electrophoresis. In view of the results from animal models, even though CD8[+] T cells may be partially involved, CD4[+] T cells play major roles in the development of systemic inflammation, which are similar to the lesions in human IgG4-related diseases [9, 50]. As TGF-β is an important regulatory factor in maintaining immune homeostasis, TGF-β-dominant negative mutant mice suggest that the loss of TGF-β signaling may contribute to AIP [51].

8. Our Hypothesis for the Pathogenesis of AIP as IgG4-Related Disease

Zen et al. reported that Th2 and regulatory cytokines, and Tregs had an important role in IgG4-SC [52]. In the patients with asthma, high-dose allergen exposure during immunotherapy results in both immune deviation of Th2 responses in favor of a Th0/Th1 response and in the generation of IL-10- and TGF-β-producing Tregs. Additionally, these cytokines induce preferential switching of B-cell

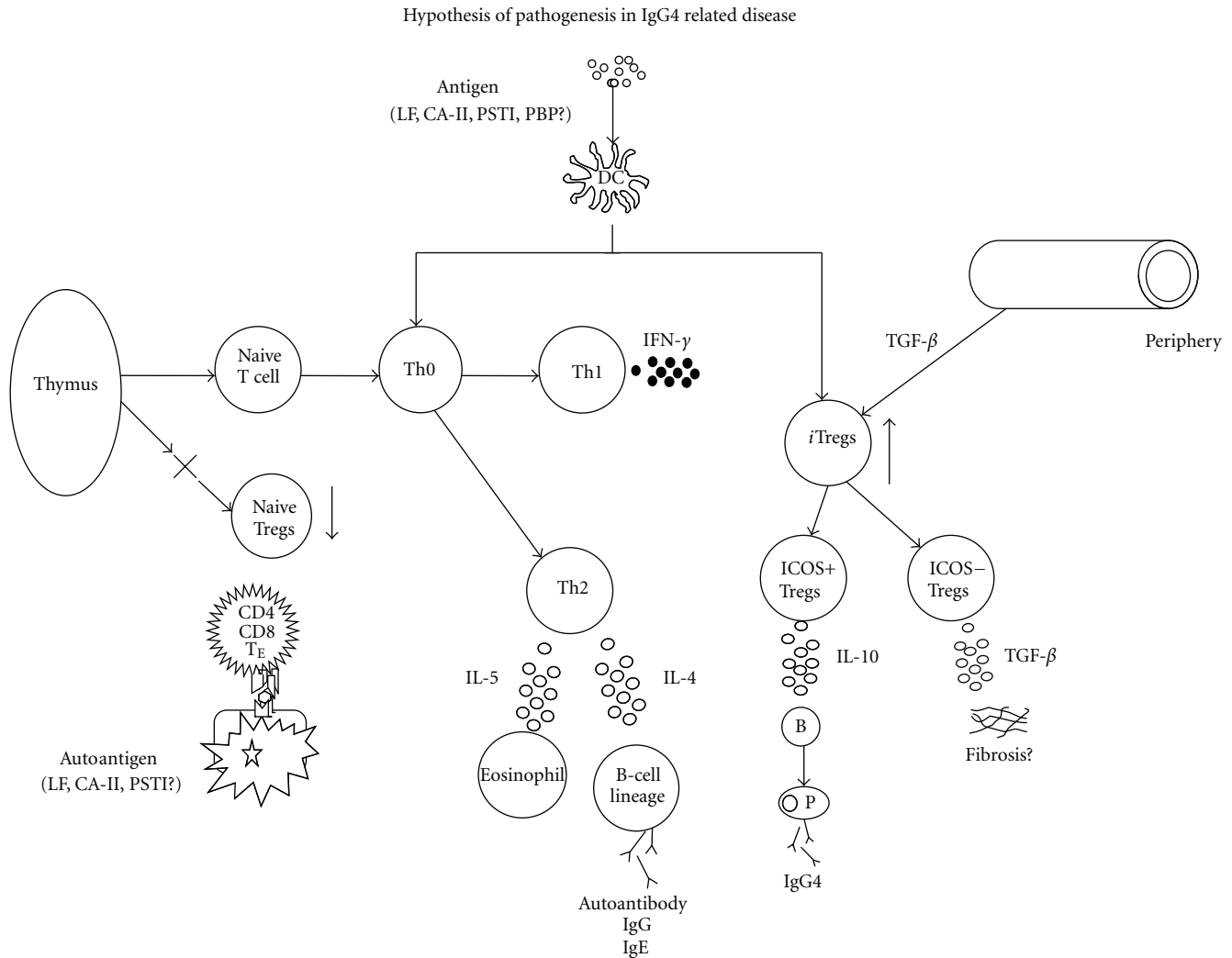Hypothesis of pathogenesis in IgG4 related disease

FIGURE 1: Hypothesis for the pathogenesis of Type 1 autoimmune pancreatitis (AIP) in IgG4-related disease. In regard to central tolerance, naïve and natural regulatory T cells (Tregs) derived from the thymus suppress autoreactive CD4 or CD8 cells in the normal state. In IgG4-related disease, the basic concept is a biphasic mechanism of "induction" and "progression". Initial response to self-antigens (e.g., lactoferrin (LF), carbonic anhydrase II (CA-II), CA-IV, pancreatic secretory trypsin inhibitor (PSTI), amylase-alpha, and plasminogen binding protein (PBP) peptide of *Helicobacter pylori*) might be induced by decreased naïve Tregs. Th2 immune responses are followed by a Th1-type immune response with the release of proinflammatory cytokines (interferon-γ (IFN-γ), interleukin (IL)-1β, IL-2, tumor necrosis factor-α (TNF-α)). Th2-type immune responses, producing IgG, IgG4, and autoantibodies may be involved in the pathophysiology of progression. The production of IgG4 may be regulated by increased IL-10 secreted from inducible co-stimulatory molecule (ICOS)$^+$ effector Tregs. Fibrosis may be regulated by TGF-β secreted from ICOS$^-$ Tregs. This figure modified from Okazaki et al. [54]. DC: dendritic cell, TE: effector T cell, nTreg: natural Tregs.

responses in favor of IgG and IgG4 antibodies [53]. We proposed a hypothesis for the pathogenesis of Type 1 AIP (Figure 1). This concept is based on a biphasic mechanism of "induction" and "progression." An initial response to self-antigens (e.g., LF, CA-II, CA-IV, pancreatic secretory trypsin inhibitor (PSTI), amylase-alpha, and plasminogen binding protein (PBP) peptide of *Helicobacter pylori*) might be induced by decreased naïve Tregs.

Decreased naive Tregs may be pathogenetic. In the early stage of Type 1 AIP, proinflammatory cytokines (IFN-γ, IL-1-β, IL-2, TNF-α) are released via Th1-type immune response. In the chronic stage, IgG, IgG4, and autoantibodies

are produced via Th2-type immune responses. Particularly, increased numbers of ICOS$^+$ Tregs may affect IgG4 production via IL-10 in Type 1 AIP. On the other hand, fibrosis may be regulated by TGF-β secreted from ICOS$^-$ Tregs.

9. Conclusion

In conclusion, Tregs seem to be important in the production of IgG4 as well as the induction of IgG4-related disease. However, further studies are necessary to clarify the pathogenesis, including genetic backgrounds, disease-specific antigens, and the role of IgG4.

Acknowledgments

References

[1] H. Sarles, J. C. Sarles, R. Muratore, and C. Guien, "Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease?" *The American Journal of Digestive Diseases*, vol. 6, no. 7, pp. 688–698, 1961.

[2] K. Kawaguchi, M. Koike, K. Tsuruta, A. Okamoto, I. Tabata, and N. Fujita, "Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas," *Human Pathology*, vol. 22, no. 4, pp. 387–395, 1991.

[3] K. Yoshida, F. Toki, T. Takeuchi, S. I. Watanabe, K. Shiratori, and N. Hayashi, "Chronic pancreatitis caused by an autoimmune abnormality: proposal of the concept of autoimmune pancreatitis," *Digestive Diseases and Sciences*, vol. 40, no. 7, pp. 1561–1568, 1995.

[4] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[5] T. Kamisawa, N. Funata, Y. Hayashi et al., "A new clinicopathological entity of IgG4-related autoimmune disease," *Journal of Gastroenterology*, vol. 38, no. 10, pp. 982–984, 2003.

[6] T. Ito, I. Nakano, S. Koyanagi et al., "Autoimmune pancreatitis as a new clinical entity: three cases of autoimmune pancreatitis with effective steroid therapy," *Digestive Diseases and Sciences*, vol. 42, no. 7, pp. 1458–1468, 1997.

[7] A. Horiuchi, S. Kawa, T. Akamatsu et al., "Characteristic pancreatic duct appearance in autoimmune chronic pancreatitis: a case report and review of the Japanese literature," *The American Journal of Gastroenterology*, vol. 93, no. 2, pp. 260–263, 1998.

[8] K. Uchida, K. Okazaki, Y. Konishi et al., "Clinical analysis of autoimmune-related pancreatitis," *The American Journal of Gastroenterology*, vol. 95, no. 10, pp. 2788–2794, 2000.

[9] K. Okazaki, K. Uchida, and T. Chiba, "Recent concept of autoimmune-related pancreatitis," *Journal of Gastroenterology*, vol. 36, no. 5, pp. 293–302, 2001.

[10] C. Baecher-Allan, J. A. Brown, G. J. Freeman, and D. A. Hafler, "CD4$^+$ CD25high regulatory cells in human peripheral blood," *Journal of Immunology*, vol. 167, no. 3, pp. 1245–1253, 2001.

[11] L. S. Taams, J. Smith, M. H. Rustin, M. Salmon, L. W. Poulter, and A. N. Akbar, "Human anergic/suppressive CD4$^+$CD25$^+$ T cells: a highly differentiated and apoptosis-prone population," *European Journal of Immunology*, vol. 31, no. 4, pp. 1122–1131, 2001.

[12] H. Jonuleit, E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A. H. Enk, "Identification and functional characterization of human CD4$^+$CD25$^+$ T cells with regulatory properties isolated from peripheral blood," *Journal of Experimental Medicine*, vol. 193, no. 11, pp. 1285–1294, 2001.

[13] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, "Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases," *Journal of Immunology*, vol. 155, no. 3, pp. 1151–1164, 1995.

[14] C. M. Cosantino, C. M. Baecher-Allan, and D. A. Hafler, "Human regulatory T cells and autoimmunity," *European Journal of Immunology*, vol. 38, no. 4, pp. 921–924, 2008.

[15] G. Zamboni, J. Lüttges, P. Capelli et al., "Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: a study on 53 resection specimens and 9 biopsy specimens," *Virchows Archiv*, vol. 445, no. 6, pp. 552–563, 2004.

[16] K. Notohara, L. J. Burgart, D. Yadav, S. Chari, and T. C. Smyrk, "Idiopathic chronic pancreatitis with periductal lymphoplasmacytic infiltration: clinicopathologic features of 35 cases," *The American Journal of Surgical Pathology*, vol. 27, no. 8, pp. 1119–1127, 2003.

[17] S. T. Chari, G. Kloeppel, L. Zhang, K. Notohara, M. M. Lerch, and T. Shimosegawa, "Histopathologic and clinical subtypes of autoimmune pancreatitis: the honolulu consensus document," *Pancreas*, vol. 39, no. 5, pp. 549–554, 2010.

[18] T. Shimosegawa, S. T. Chari, L. Frulloni et al., "International consensus diagnostic criteria for autoimmune pancreatitis: guidelines of the international association of pancreatology," *Pancreas*, vol. 40, no. 3, pp. 352–358, 2011.

[19] T. Kusuda, K. Uchida, S. Satoi et al., "Idiopathic duct-centric pancreatitis (IDCP) with immunological studies," *Internal Medicine*, vol. 49, no. 23, pp. 2569–2575, 2010.

[20] K. Okazaki, S. Kawa, T. Kamisawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: I. Concept and diagnosis of autoimmune pancreatitis," *Journal of Gastroenterology*, vol. 45, no. 3, pp. 249–265, 2010.

[21] S. Kawa, K. Okazaki, T. Kamisawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: II. Extrapancreatic lesions, differential diagnosis," *Journal of Gastroenterology*, vol. 45, no. 4, pp. 355–369, 2010.

[22] T. Kamisawa, K. Okazaki, S. Kawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: III. Treatment and prognosis of AIP," *Journal of Gastroenterology*, vol. 45, no. 5, pp. 471–477, 2010.

[23] K. Okazaki, S. Kawa, T. Kamisawa et al., "Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 626–631, 2006.

[24] T. Pickartz, J. Mayerle, and M. M. Lerch, "Autoimmune pancreatitis," *Nature Clinical Practice Gastroenterology and Hepatology*, vol. 4, no. 6, pp. 314–323, 2007.

[25] K. Uchida, S. Yazumi, A. Nishio et al., "Long-term outcome of autoimmune pancreatitis," *Journal of Gastroenterology*, vol. 44, no. 7, pp. 726–732, 2009.

[26] K. Uchida, K. Okazaki, M. Asada et al., "Case of chronic pancreatitis involving an autoimmune mechanism that extended to retroperitoneal fibrosis," *Pancreas*, vol. 26, no. 1, pp. 92–94, 2003.

[27] T. Saeki, S. Nishi, N. Imai et al., "Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis," *Kidney International*, vol. 78, no. 10, pp. 1016–1023, 2010.

[28] Y. Li, E. Nishihara, M. Hirokawa, E. Taniguchi, A. Miyauchi, and K. Kakudo, "Distinct clinical, serological, and sonographic characteristics of Hashimoto's thyroiditis based with

and without IgG4-positive plasma cells," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 3, pp. 1309–1317, 2010.

[29] J. Mikulicz, "Uber eine eigenartige symmetrische erkrankung der thranen- und mundspeicheldrusen," in *Beitrage zur Chirurgie Festschrift Gewidmet*, T. Billroth, Ed., pp. 610–630, Ferdinand Enke, Stuttgart, Germany, 1892.

[30] H. Umehara, K. Okazaki, Y. Masaki et al., "A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details," *Modern Rheumatology*, vol. 22, no. 1, pp. 1–14, 2011.

[31] T. Kamisawa and A. Okamoto, "Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 613–625, 2006.

[32] M. Yamamoto, H. Takahashi, M. Ohara et al., "A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease," *Modern Rheumatology*, vol. 16, no. 6, pp. 335–340, 2006.

[33] Y. Masaki, L. Dong, N. Kurose et al., "Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders," *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1310–1315, 2009.

[34] S. Hori, T. Nomura, and S. Sakaguchi, "Control of regulatory T cell development by the transcription factor Foxp3," *Science*, vol. 299, no. 5609, pp. 1057–1061, 2003.

[35] E. Gambineri, T. R. Torgerson, and H. D. Ochs, "Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis," *Current Opinion in Rheumatology*, vol. 15, no. 4, pp. 430–435, 2003.

[36] S. Sakaguchi, T. Yamaguchi, T. Nomura, and M. Ono, "Regulatory T cells and immune tolerance," *Cell*, vol. 133, no. 5, pp. 775–787, 2008.

[37] C. Baecher-Allan and D. A. Hafler, "Human regulatory T cells and their role in autoimmune disease," *Immunological Reviews*, vol. 212, pp. 203–216, 2006.

[38] J. H. Buckner, "Mechanisms of impaired regulation by CD4+ CD25+ FOXP3+ regulatory T cells in human autoimmune diseases," *Nature Reviews Immunology*, vol. 10, no. 12, pp. 849–859, 2010.

[39] M. Miyara, Y. Yoshioka, A. Kitoh et al., "Functional delineation and differentiation dynamics of human CD4+ T cells expressing the foxP3 transcription factor," *Immunity*, vol. 30, no. 6, pp. 899–911, 2009.

[40] T. Ito, S. Hanabuchi, Y. H. Wang et al., "Two functional subsets of FOXP3+ Regulatory T Cells in Human Thymus and Periphery," *Immunity*, vol. 28, no. 6, pp. 870–880, 2008.

[41] O. Akbari, G. J. Freeman, E. H. Meyer et al., "Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity," *Nature Medicine*, vol. 8, no. 9, pp. 1024–1032, 2002.

[42] H. Miyoshi, K. Uchida, T. Taniguchi et al., "Circulating naïve and CD4+CD25high regulatory T cells in patients with autoimmune pancreatitis," *Pancreas*, vol. 36, no. 2, pp. 133–140, 2008.

[43] T. Kusuda, K. Uchida, H. Miyoshi et al., "Involvement of inducible costimulator- and interleukin 10-positive regulatory T cells in the development of IgG4-related autoimmune pancreatitis," *Pancreas*, vol. 40, no. 7, pp. 1120–1130, 2011.

[44] M. Koyabu, K. Uchida, H. Miyoshi et al., "Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases," *Journal of Gastroenterology*, vol. 45, no. 7, pp. 732–741, 2010.

[45] M. Asada, A. Nishio, K. Uchida et al., "Identification of a novel autoantibody against pancreatic secretory trypsin inhibitor in patients with autoimmune pancreatitis," *Pancreas*, vol. 33, no. 1, pp. 20–26, 2006.

[46] K. Uchida, K. Okazaki, T. Nishi et al., "Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydrase II and lactoferrin," *Laboratory Investigation*, vol. 82, no. 4, pp. 411–424, 2002.

[47] A. Demols, O. Le Moine, F. Desalle, E. Quertinmont, J. L. Van Laethem, and J. Devière, "CD4+ T cells play an important role in acute experimental pancreatitis in mice," *Gastroenterology*, vol. 118, no. 3, pp. 582–590, 2000.

[48] B. A. Vallance, B. R. Hewlett, D. P. Snider, and S. M. Collins, "T cell-mediated exocrine pancreatic damage in major histocompatibility complex class II-deficient mice," *Gastroenterology*, vol. 115, no. 4, pp. 978–987, 1998.

[49] Y. Sakaguchi, M. Inaba, M. Tsuda et al., "The Wistar Bonn Kobori rat, a unique animal model for autoimmune pancreatitis with extrapancreatic exocrinopathy," *Clinical and Experimental Immunology*, vol. 152, no. 1, pp. 1–12, 2008.

[50] K. Okazaki, K. Uchida, M. Ohana et al., "Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response," *Gastroenterology*, vol. 118, no. 3, pp. 573–581, 2000.

[51] T. Marth, W. Strober, and B. L. Kelsall, "High dose oral tolerance in ovalbumin TCR-transgenic mice: systemic neutralization of IL-12 augments TGF-β secretion and T cell apoptosis," *Journal of Immunology*, vol. 157, no. 6, pp. 2348–2357, 1996.

[52] Y. Zen, T. Fujii, K. Harada et al., "Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis," *Hepatology*, vol. 45, no. 6, pp. 1538–1546, 2007.

[53] D. S. Robinson, M. Larché, and S. R. Durham, "Tregs and allergic disease," *Journal of Clinical Investigation*, vol. 114, no. 10, pp. 1389–1397, 2004.

[54] K. Okazaki, K. Uchida, M. Koyabu, H. Miyoshi, and M. Takaoka, "Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease," *Journal of Gastroenterology*, vol. 46, no. 3, pp. 277–288, 2011.

Review Article

The Utility of Serum IgG4 Concentrations as a Biomarker

Shigeyuki Kawa,[1] Tetsuya Ito,[2] Takayuki Watanabe,[2] Masahiro Maruyama,[2] Hideaki Hamano,[3] Masafumi Maruyama,[2] Takashi Muraki,[2] and Norikazu Arakura[2]

[1] *Center for Health, Safety, and Environmental Management, Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*
[2] *Department of Gastroenterology, School of Medicine Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*
[3] *Department of Medical Information, School of Medicine Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*

Correspondence should be addressed to Shigeyuki Kawa, skawapc@shinshu-u.ac.jp

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IgG4-related disease is a new disease entity involving IgG4 in its clinical presentation and having 6 characteristic features: (1) systemic involvement; (2) solitary or multiple lesions showing diffuse or localized swelling, masses, nodules, and/or wall thickening on imaging; (3) high serum IgG4 concentration >135 mg/dL; (4) abundant infiltration of lymphoplasmacytes and IgG4-bearing plasma cells; (5) a positive response to corticosteroid therapy; and (6) complications of other IgG4-related diseases. To date, most IgG4-related diseases have been recognized as extrapancreatic lesions of autoimmune pancreatitis. This paper will discuss the utility of IgG4 as a biomarker of IgG4-related diseases, including in the diagnosis of autoimmune pancreatitis and its differentiation from pancreatic cancer, in the prediction of relapse, in the long-term follow-up of patients with autoimmune pancreatitis and normal or elevated IgG4 concentrations, and in patients with autoimmune pancreatitis and extrapancreatic lesions, as well as the role of IgG4 in the pathogenesis of IgG4-related disease.

1. Introduction

IgG4-related disease is a new disease entity involving IgG4 in its clinical presentation. To date, 6 characteristic features of IgG4-related disease have been identified: (1) systemic involvement; (2) solitary or multiple lesions showing diffuse or localized swelling, masses, nodules, and/or wall thickening on imaging; (3) high serum IgG4 concentrations >135 mg/dL; (4) abundant infiltration of lymphoplasmacytes and IgG4-bearing plasma cells; (5) a positive response to corticosteroid therapy; (6) complications of other IgG4-related diseases [1–4].

IgG4-related disease involves organs throughout the entire body. The major manifestations of IgG4-related disease include autoimmune pancreatitis, lacrimal and salivary gland lesions known as Mikulicz's disease, sclerosing cholangitis, retroperitoneal fibrosis, lung disease, and tubulointerstitial nephritis. In addition, many minor lesions have been reported in patients with IgG4-related disease, including hypophysitis, thyroiditis, hepatopathy, and prostatitis. At present, it is not clear whether these lesions are caused by the same etiology or merely show clinical and pathological findings associated with IgG4. Imaging modalities have shown diffuse or localized swelling in the pancreas and the lacrimal and salivary glands, masses in patients with retroperitoneal fibrosis, nodules in patients with lung pseudotumors, and wall thickening in the bronchi and bile ducts.

Most patients with IgG4-related disease have high serum IgG4 concentrations, over 135 mg/dL, [5] a finding both sensitive and specific for this disease, as well as useful for its diagnosis. Characteristic pathological findings include the infiltration of large numbers of lymphoplasmacytes and IgG4 bearing plasma cells [6]. Although storiform or swirling fibrosis and obstructive phlebitis are also characteristics of IgG4-related disease, they are rarely observed in specific lesions, such as those of the salivary glands. Most lesions, except for those that are predominantly fibrotic, respond positively to corticosteroid therapy. For example, patients with autoimmune pancreatitis show reduced swelling, products with lung pseudotumors show the disappearance of nodules, and patients with sclerosing cholangitis show the

disappearance of bile duct strictures after corticosteroid treatment. At present, most IgG4-related diseases have been recognized as extrapancreatic lesions of autoimmune pancreatitis [2, 7, 8].

This paper will discuss the utility of serum IgG4 concentrations as a biomarker of the major IgG4-related disease, autoimmune pancreatitis. Topics will include IgG4 concentration and the diagnosis of autoimmune pancreatitis, as well as its differentiation from pancreatic cancer; IgG4 and the prediction of relapse; long-term followup of patients with autoimmune pancreatitis and either normal or elevated IgG4 concentration; IgG4 and extrapancreatic lesions in patients with autoimmune pancreatitis; and the role of IgG4 in the pathogenesis of IgG4-related disease.

2. IgG4 and the Diagnosis of Autoimmune Pancreatitis

The sera of patients with autoimmune pancreatitis have a polyclonal band in the rapidly migrating fraction of γ-globulins, resulting in β–γ bridging. Immunoprecipitation assays have confirmed that this band is always due to elevation of IgG4 concentration [5]. IgG is composed of 4 subclasses, IgG1, IgG2, IgG3, and IgG4. In normal subjects, IgG4 constitutes only 3–7% of total serum IgG. However, serum IgG4 concentrations are over 10-fold higher in patients with autoimmune pancreatitis. Elevated serum IgG4 has also been observed in individuals with allergic disorders, parasite infestations, and pemphigus. High serum IgG4 concentrations have been observed in 90% of patients with autoimmune pancreatitis, but rarely in patients with pancreatic cancer, chronic pancreatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and Sjögren's syndrome, suggesting that IgG4 is a sensitive and specific marker of autoimmune pancreatitis and may be diagnostic for this disease [5]. Corticosteroid therapy significantly reduces serum IgG4 concentration and the IgG4/IgG ratio [5]. The utility of IgG4 for the diagnosis of autoimmune pancreatitis has been evaluated worldwide, with a sensitivity ranging from 50% to 92% and a specificity over 90%. Serum IgG4 concentration is therefore considered a reliable marker for the diagnosis of autoimmune pancreatitis and has been included in various diagnostic criteria [9–12]. Differences in sensitivity and specificity may be partly due to the use of different assays to measure serum IgG4 and different cutoffs for the upper limit of normal around the world, as well as variations in diagnostic criteria used in individual countries, which may be associated with histological differences between lymphoplasmacytic sclerosing pancreatitis (LPSP) [13] and idiopathic duct-centric chronic pancreatitis (IDCP) [14].

Clinical features of autoimmune pancreatitis have been reported to differ based on the serum concentration of IgG4. Compared with patients having normal serum IgG4 levels, those with elevated IgG4 are regarded as being in a highly active state, with a higher incidence of jaundice at onset, more frequent diffuse pancreatic enlargement on imaging, significantly higher 18F-2-fluoro-2-deoxy-d-glucose uptake by pancreatic lesions, more frequent extrapancreatic lesions, and more frequent requirement for maintenance therapy [15].

In addition, infiltration of IgG4 bearing plasma cells is a histological hallmark of autoimmune pancreatitis and is used in pathologic diagnoses [6].

3. IgG4 and the Differentiation of Autoimmune Pancreatitis from Pancreatic Cancer

Lymphoplasmacytic sclerosing pancreatitis (LPSP), which is similar pathologically to autoimmune pancreatitis, has been observed in 2.5% of patients undergoing the Whipple resection [16]. Therefore, it is necessary to differentiate autoimmune pancreatitis from pancreatic cancer. We reported that IgG4 had a sensitivity of 90%, a specificity of 98%, and an accuracy of 95% in differentiating between these conditions, [5] indicating that IgG4 is useful both for the diagnosis of autoimmune pancreatitis and for differentiating it from pancreatic cancer. Other reports have also shown the usefulness of IgG4 in differential diagnosis [17–19]. The sensitivity and specificity of IgG4 were superior to those of IgG, ANA, and RF, although the additional measurement of ANA and RF further increased the sensitivity and negative predictive value of IgG4 [8].

4. IgG4 and Prediction of Relapse

Some patients with autoimmune pancreatitis experience relapse during their clinical course. For effective management, it is necessary to determine the frequency of relapse and its prevention. During the period from 1992 to 2011, a total of 93 patients with autoimmune pancreatitis were examined and treated at Shinshu University Hospital. Of the 84 patients followed up for more than 1 year, 28 (33%) experienced relapse. In Japanese patients, the relapse rate has been estimated to vary from 30 to 50%, [20–22] although corticosteroid therapy significantly reduced relapse rates [22]. Japanese consensus guidelines for the management of autoimmune pancreatitis have stated that the indications for corticosteroid therapy include symptoms such as obstructive jaundice, abdominal pain, and back pain, and the presence of symptomatic extrapancreatic lesions. The major lesions at relapse included autoimmune pancreatitis ($n = 26$), sclerosing cholangitis ($n = 18$), lachrymal and salivary gland lesions ($n = 5$), and retroperitoneal fibrosis ($n = 4$). In addition, the involvement of other organs and symptoms were seen at relapse. We failed to identify any serum markers at diagnosis that could predict relapse, although we observed elevated concentrations of IgG and immune complex in the relapse compared with the nonrelapse group, although these differences were not significant. Serial changes in IgG4 and immune complexes in a 69-year-old woman with autoimmune pancreatitis who experienced 3 relapses showed that these markers were elevated in serum several months before clinically evident relapse, suggesting that regular measurements of these markers in an out-patient clinic may predict relapse [23].

TABLE 1: IgG4 concentrations, age and complications of more than 3 extrapancreatic lesions in patients with autoimmune pancreatitis and major extrapancreatic lesions.

	n (male/female)	Age, yr Median (range)	IgG4, mg/dL Median (range)	Complications of more than 3 extrapancreatic lesions, n (%)
Autoimmune pancreatitis	92 (72/20)	66 (38–85)	545 (4–2970)	11(12%)
Mikulicz's disease	41 (31/10)	65 (43–82)	697 (18–2970)	13 (32%)
Sclerosing cholangitis	71 (53/18)	67 (38–84)	500 (4–2970)	13 (18%)
Retroperitoneal fibrosis	27 (23/4)	66 (50–80)	1110 (247–2970)	12 (44%)
Kidney lesion	18 (14/4)	67 (56–82)	1313 (156–2970)	9 (50%)

5. Long-Term Followup of Patients with Autoimmune Pancreatitis and Normal or Elevated IgG4

We followed 2 patients with autoimmune pancreatitis for 10 years each, a 55-year-old man with a serum IgG4 concentration of 1135 mg/dL and a 65-year-old woman with a serum IgG4 concentration of 42 mg/dL [24]. The first patient experienced several recurrences, developing a pancreatic stone and pancreatic duct stenosis, whereas the latter patient showed no duct changes over time. These findings suggest that autoimmune pancreatitis accompanied by normal IgG4 concentrations may represent lower activity and a nonprogressive state [24].

6. IgG4 and Extrapancreatic Lesions in Autoimmune Pancreatitis

Extrapancreatic lesions in patients with autoimmune pancreatitis may involve organs throughout the entire body [7, 8]. Serum IgG4 concentrations were well correlated with the number of extrapancreatic organs involved, indicating a correlation between increased serum IgG4 and extrapancreatic involvement. Among the 5 types of extrapancreatic involvement, lachrymal and salivary gland lesions and hilar lymph adenopathy have been significantly associated with high serum IgG4 concentrations, suggesting that patients with high serum IgG4 should be assessed for the occurrence of these lesions [7]. However, a recent study of large numbers of patients with autoimmune pancreatitis and extrapancreatic lesions showed different results, as shown in Table 1. Patients with IgG4-related retroperitoneal fibrosis and kidney lesions had higher IgG4 concentrations than other patients, probably because these lesions were complications of many other IgG4-related diseases (Table 1).

7. Role of IgG4

IgG4 in these patients may be (1) pathogenic, (2) anti-inflammatory, or (3) as a rheumatoid factor. For example, anti-desmoglein3 IgG4 autoantibody has been reported pathogenic for pemphigus vulgaris [25]. Transfer of an anti-desmoglein3 IgG4 autoantibody from a pemphigus vulgaris

patient to BALB/C mice resulted in a pemphigus vulgaris like lesion, suggesting the involvement of an IgG4 autoantibody directed against an unknown target antigen. Similarly, IgG4 deposits have been detected in tissues of patients with autoimmune pancreatitis [26]. In contrast, IgG4 was found to have anti-inflammatory effects against allergic reactions. IgG4 antibodies can bind to soluble antigens, blocking the interaction between these antigens and IgE on mast cells and inhibiting allergic reactions. A dynamic Fab arm exchange of IgG4 can occur, resulting in bispecific activity, loss of monospecific cross-linking activity, and loss of the ability to form immune complexes, resulting in anti-inflammatory effects [27].

IgG4 may act as an autoantibody against IgG or have rheumatoid factor activity. Western blotting has shown that IgG4 from the sera of patients with autoimmune pancreatitis can bind to IgG1, IgG2, IgG3, and IgG Fc [28]. Furthermore, IgG4 Fc, but not IgG4 Fab, was found to bind to IgG Fc [28], indicating that IgG4 binding to IgG Fc is via an Fc-Fc interaction, not via rheumatoid activity. ELISA showed that IgG4 from the serum of each patient with autoimmune pancreatitis could bind to IgG1 coated onto microplates, a binding well correlated with serum IgG4, but not rheumatoid factor, concentration [28]. The role of IgG4 Fc-IgG Fc is unclear, but it may have physiological and/or pathological effects, suggesting the need for further studies.

8. Conclusion

The utility of serum IgG4 concentration as a biomarker of the major IgG4-related disease, autoimmune pancreatitis, includes its ability to diagnose autoimmune pancreatitis as well as to differentiate this disease from pancreatic cancer. Moreover, serum IgG4 concentration may be a marker for predicting relapse and for the evaluation of extrapancreatic lesions. It remains unclear, however, whether IgG4 and its rheumatoid factor-like activity may be beneficial or pathogenic in these patients.

References

[1] S. Kawa and S. Sugai, "History of autoimmune pancreatitis and Mikulicz's disease," *Current Immunology Reviews*, vol. 7, no. 2, pp. 137–143, 2011.

[2] S. Kawa, Y. Fujinaga, M. Ota, H. Hamano, and S. Bahram, "Autoimmune pancreatitis and diagnostic criteria," *Current Immunology Reviews*, vol. 7, no. 2, pp. 144–161, 2011.

[3] H. Umehara, K. Okazaki, Y. Masaki et al., "A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details," *Modern Rheumatology*, vol. 22, no. 1, pp. 21–30, 2012.

[4] S. Kawa, H. Hamano, and K. Kiyosawa, "Pancreatitis," in *The Autoimmune Diseases*, N. Rose and I. MacKay, Eds., pp. 779–786, Academic Press, St Louis, Mo, USA, 4th edition, 2006.

[5] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[6] H. Hamano, S. Kawa, Y. Ochi et al., "Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis," *The Lancet*, vol. 359, no. 9315, pp. 1403–1404, 2002.

[7] H. Hamano, N. Arakura, T. Muraki, Y. Ozaki, K. Kiyosawa, and S. Kawa, "Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis," *Journal of Gastroenterology*, vol. 41, no. 12, pp. 1197–1205, 2006.

[8] S. Kawa, K. Okazaki, T. Kamisawa, T. Shimosegawa, and M. Tanaka, "Japanese consensus guidelines for management of autoimmune pancreatitis: II. Extrapancreatic lesions, differential diagnosis," *Journal of Gastroenterology*, vol. 45, no. 4, pp. 355–369, 2010.

[9] K. Okazaki, S. Kawa, T. Kamisawa et al., "Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal," *Journal of Gastroenterology*, vol. 41, no. 7, pp. 626–631, 2006.

[10] K. P. Kim, M. H. Kim, J. C. Kim, S. S. Lee, D. W. Seo, and S. K. Lee, "Diagnostic criteria for autoimmune chronic pancreatitis revisited," *World Journal of Gastroenterology*, vol. 12, no. 16, pp. 2487–2496, 2006.

[11] S. T. Chari, T. C. Smyrk, M. J. Levy et al., "Diagnosis of autoimmune pancreatitis: the mayo clinic experience," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 8, pp. 1010–1016, 2006.

[12] M. Otsuki, J. B. Chung, K. Okazaki et al., "Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea symposium on autoimmune pancreatitis," *Journal of Gastroenterology*, vol. 43, no. 6, pp. 403–408, 2008.

[13] K. Kawaguchi, M. Koike, K. Tsuruta, A. Okamoto, I. Tabata, and N. Fujita, "Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas," *Human Pathology*, vol. 22, no. 4, pp. 387–395, 1991.

[14] K. Notohara, L. J. Burgart, D. Yadav, S. Chari, and T. C. Smyrk, "Idiopathic chronic pancreatitis with periductal lymphoplasmacytic infiltration: clinicopathologic features of 35 cases," *American Journal of Surgical Pathology*, vol. 27, no. 8, pp. 1119–1127, 2003.

[15] H. Matsubayashi, H. Sawai, H. Kimura et al., "Characteristics of autoimmune pancreatitis based on serum IgG4 level," *Digestive and Liver Disease*, vol. 43, no. 9, pp. 731–735, 2011.

[16] S. C. Abraham, R. E. Wilentz, C. J. Yeo et al., "Pancreaticoduodenectomy (Whipple resections) in patients without malignancy: are they all "chronic pancreatitis"?" *American Journal of Surgical Pathology*, vol. 27, no. 1, pp. 110–120, 2003.

[17] A. M. Morselli-Labate and R. Pezzilli, "Usefulness of serum IgG4 in the diagnosis and follow up of autoimmune pancreatitis: a systematic literature review and meta-analysis," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 1, pp. 15–36, 2009.

[18] E. K. Choi, M. H. Kim, T. Y. Lee et al., "The sensitivity and specificity of serum immunoglobulin G and immunoglobulin G4 levels in the diagnosis of autoimmune chronic pancreatitis: Korean experience," *Pancreas*, vol. 35, no. 2, pp. 156–161, 2007.

[19] A. Ghazale, S. T. Chari, T. C. Smyrk et al., "Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1646–1653, 2007.

[20] M. Takayama, H. Hamano, Y. Ochi et al., "Recurrent attacks of autoimmune pancreatitis result in pancreatic stone formation," *American Journal of Gastroenterology*, vol. 99, no. 5, pp. 932–937, 2004.

[21] K. Hirano, Y. Asaoka, M. Tada et al., "No significant relation between relapse of autoimmune pancreatitis and substitution of aspartic acid at position 57 of DQβ1," *Journal of Gastroenterology*, vol. 44, no. 7, pp. 799–800, 2009.

[22] K. Kubota, S. Watanabe, T. Uchiyama et al., "Factors predictive of relapse and spontaneous remission of autoimmune pancreatitis patients treated/not treated with corticosteroids," *Journal of Gastroenterology*, vol. 46, no. 6, pp. 834–842, 2011.

[23] S. Kawa and H. Hamano, "Clinical features of autoimmune pancreatitis," *Journal of Gastroenterology*, vol. 42, no. 18, pp. 9–14, 2007.

[24] S. Kawa, H. Hamano, Y. Ozaki et al., "Long-term follow-up of autoimmune pancreatitis: characteristics of chronic disease and recurrence," *Clinical Gastroenterology and Hepatology*, vol. 7, no. 11, supplement, pp. S18–S22, 2009.

[25] B. Rock, C. R. Martins, A. N. Theofilopoulos et al., "The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem)," *The New England Journal of Medicine*, vol. 320, no. 22, pp. 1463–1469, 1989.

[26] S. Aoki, T. Nakazawa, H. Ohara et al., "Immunohistochemical study of autoimmune pancreatitis using anti-IgG4 antibody and patients' sera," *Histopathology*, vol. 47, no. 2, pp. 147–158, 2005.

[27] M. van der Neut Kolfschoten, J. Schuurman, M. Losen et al., "Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange," *Science*, vol. 317, no. 5844, pp. 1554–1557, 2007.

[28] S. Kawa, K. Kitahara, H. Hamano et al., "A novel immunoglobulin-immunoglobulin interaction in autoimmunity," *Plos ONE*, vol. 3, no. 2, Article ID e1637, 2008.

Clinical Study

IgG4-Related Perineural Disease

Dai Inoue,[1, 2] Yoh Zen,[3] Yasuharu Sato,[4] Hitoshi Abo,[2] Hiroshi Demachi,[2] Akio Uchiyama,[5] Toshifumi Gabata,[1] and Osamu Matsui[1]

[1] *Department of Radiology, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8641, Japan*

[2] *Department of Radiology, Toyama Prefectural Central Hospital, Toyama 930-8550, Japan*

[3] *Institute of Liver Studies, King's College Hospital, London 5E5 9RS, UK*

[4] *Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan*

[5] *Department of Pathology, Toyama Prefectural Central Hospital, Toyama 930-8550, Japan*

Correspondence should be addressed to Dai Inoue, d-inoue@lake.ocn.ne.jp

Aims. To elucidate characteristics of IgG4-related disease involving the peripheral nervous system. *Methods.* Retrospective review of 106 patients with IgG4-related disease identified 21 peripheral nerve lesions in 7 patients. Clinicopathological and radiological features were examined. *Results.* Peripheral nerve lesions were commonly identified in orbital or paravertebral area, involving orbital ($n = 9$), optic ($n = 4$), spinal ($n = 7$), and great auricular nerves ($n = 1$). The predominant radiological feature was a distinct perineural soft tissue mass, ranging 8 to 30 mm in diameter. Histologically, the epineurium was preferentially involved by massive lymphoplasmacytic infiltration rich in IgG4+ plasma cells. All lesions were neurologically asymptomatic and steroid-responsive at the first presentation, but one recurrent lesion around the optic nerve caused failing vision. *Conclusion.* IgG4-related disease of the peripheral nervous system is characterized by orbital or paravertebral localization, perineural mass formation, and rare neurologic symptoms. The term "IgG4-related perineural disease" seems appropriate to describe this entity.

1. Introduction

IgG4-related disease is a newly designated disease entity, which can be defined as an idiopathic fibroinflammatory condition rich in IgG4+ plasma cells. This disease affects a variety of organs including the salivary gland [1], pancreas [2], bile duct [3], lung [4], kidney [5], and aorta/artery [6, 7]. IgG4-related disease shares clinicopathological characteristics irrespective of the affected organs. Clinical features can be summarized as occurring predominantly in adult male patients, elevated serum IgG4 concentrations, responsive to steroid therapy, and synchronous or metachronous association with IgG4-related disease in other organs [8, 9]. IgG4-related disease is histologically characterized by diffuse lymphoplasmacytic infiltration rich in IgG4+ plasma cells, storiform fibrosis, obliterative phlebitis, and moderate tissue eosinophilia [1, 3–6, 10]. IgG4-related disease predominantly

develops in glandular organs, but nonglandular tissue like retroperitoneum can be affected as well [11, 12].

The diagnosis of IgG4-related disease needs a multidisciplinary approach, in which radiological examination plays an important role. Unique imaging features of IgG4-related disease are of help in recognizing this disease. Radiological examination is regarded as a necessary component for the diagnosis of type 1 autoimmune pancreatitis [13, 14]. Imaging features of renal, pulmonary, or arterial lesions have been also well characterized [15–18]. Recently, a few papers have described the peripheral nerve involvement in IgG4-related disease [19–21]. However, the radiological features of IgG4-related peripheral nerve lesions remain to be clarified.

In this study, we retrospectively examined IgG4-related disease involving the peripheral nervous system. The purpose of this study is to elucidate the clinicopathological and

radiological characteristics of IgG4-related peripheral nerve lesions.

2. Patients and Methods

2.1. Case Selection. This retrospective study was approved by the institutional review board, and the informed consent requirement was waived. We selected consecutive 105 patients (87 men and 18 women; median 68 years, range 38–86 years) that showed radiological features consistent with IgG4-related disease in our hospitals and related institution in the period between September 1998 and May 2011. Another case with IgG4-related peripheral nerve disease (case 6) was obtained from a radiology consultation file. The hospitals involved in this study are university or community-based general hospitals, which have departments of rheumatology, gastroenterology, ophthalmology, otolaryngology, and nephrology so that patients suspected of having IgG4-related disease were referred there. Doctors including radiologists in those hospitals have sufficient knowledge of IgG4-related disease. For most patients, radiological examination first raised a possibility of IgG4-related disease, which was confirmed by serological or pathological examination. Minor exceptions were patients presented with bilateral proptosis with or without submandibular gland enlargement, in which IgG4-related disease was suspected on physical examinations.

We retrospectively reviewed radiology and pathology data of 106 patients with IgG4-related disease collected regarding the presence or absence of macroscopic peripheral nerve abnormalities. A total of 7 patients with peripheral nerve involvement were found and enrolled in this study. All patients were male with a median age of 58 years (range 44–74 years). Clinical features of these patients are summarized in Table 1. Followup data were also examined particularly in terms of the presence or absence of recurrences and neurological symptoms by reviewing inpatient and outpatient records and followup imaging.

2.2. Diagnosis of IgG4-Related Disease. The diagnosis of IgG4-related disease was made based on serological, imaging, and histological examinations. Serum IgG4 concentrations were elevated in all patients (median 1280 mg/dL; range 325–3440 mg/dL, normal range <135 mg/dL). Five patients (cases 3–7) had histological examination for specimens taken from the lacrimal gland (surgical biopsy; cases 3, 4, and 6), kidney (needle biopsy; cases 5, 6, and 7), hepatic mass (needle biopsy; case 5), and cervical lymph node (surgical biopsy; case 6). Cases 1 and 7 had surgical biopsies from peripheral nerve lesions. Case 2, who did not have histological examination, was diagnosed as IgG4-related ophthalmic disease based on the clinical presentation with left proptosis, imaging features, and a high serum IgG4 concentration (372 mg/dL).

All resected or biopsied specimens were reviewed by a pathologist and confirmed features consistent with IgG4-related disease including diffuse lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, occasional eosinophils, numerous IgG4$^+$ plasma cell infiltrates, and high IgG4$^+$/IgG$^+$ plasma cell ratios (>40%) [1, 3, 5, 6].

2.3. Radiological Examinations. All images were reviewed by two radiologists, and decisions were reached by consensus. Because of the retrospective nature of this study, the imaging examinations performed were not consistent. Imaging examinations evaluable for neural lesions performed were CT in six patients (cases 1–6), MRI in five (cases 1–4, 6), and 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) in one (case 5). Case 7 did not undergo radiological examination for the neural lesion. Images were reviewed in terms of the location, shape, and size of each lesion, configuration of the border to surrounding adipose tissue (well circumscribed or infiltrative), and the presence or absence of radiologically detectable nerve fibers within the lesion. The size of lesions was defined as the maximum diameter measured in the vertical imaging plane to the peripheral lesions.

3. Results

3.1. Clinical Characteristics

3.1.1. Location. A total of 21 peripheral nerve lesions were identified in 7 patients. Six of them were found in a consecutive cohort of 105 patients with IgG4-related disease in radiology database, suggesting the prevalence of the peripheral nerve involvement in IgG4-related disease to be 5.8% with a 95% confidence interval of 2.1% to 12.0%. For the remaining one, only clinicopathological and radiology data were provided for a second opinion from a referring hospital. Locations and radiological features are summarized in Table 3. Of 7 patients, 5 (71%) had two or more lesions simultaneously. Twenty lesions (95%) were located in orbital or paravertebral area, involving infraorbital ($n = 5$), supraorbit-al ($n = 4$), optic ($n = 4$), lumbar spinal ($n = 3$), sacral spinal ($n = 3$), and cervical spinal nerves ($n = 1$). The remaining lesion was involvement of great auricular nerve in a cervical mass in case 7. Peripheral nerve lesions in ophthalmic area were always associated with IgG4-related dacryoadenitis or orbital disease, whereas spinal nerve lesions were isolated without IgG4-related disease in adjacent tissue. Among the consecutive cohort of 105 patients, 18 patients (17%) had ophthalmic lesions and 6 of them (6/18) appeared to have peripheral nervous involvement, suggesting the prevalence of 33.3% with a 95% confidence interval of 13.3% to 59.0%.

3.1.2. Other Organs Involvement. All patients were found to have IgG4-related lesions in other organs, all but one of which were identified at the same time as the neural lesions (Table 2). Enlargement of bilateral lacrimal glands, which had been present for 10 years before the episode of the peripheral nerve lesion, in case 7 was retrospectively diagnosed as IgG4-related dacryoadenitis.

3.1.3. Symptoms. All patients presented symptomatically (Table 1). Symptoms were related to other organ lesions or mass effects of peripheral nerve lesions. Real neurological signs such as paralysis were not evident in any cases at the first presentation. Interestingly, cases 1, 2, and 4 had

TABLE 1: Clinical characteristics, laboratory data, and initial dose of steroid.

Case	Age	Gender	Symptom	IgG (mg/dL)*	IgG4 (mg/dL)†	ANA (titer)	IgE (IU/mL)‡	Initial dose of steroid
1	55	Male	Double vision, subcutaneous nodule in cheek	4845	2050	×40	988	30 mg/day
2	53	Male	Left proptosis, double vision	NA	372	×40	NA	30 mg/day
3	61	Male	Lacrimal gland enlargement	1450	463	<40	4608	20 mg/day
4	44	Male	Right proptosis, double vision, lacrimal gland enlargement	1146	325	<40	151	30 mg/day
5	58	Male	Epigastralgia	2850	1280	NA	NA	30 mg/day
6	74	Male	Lacrimal gland enlargement, cervical lymph node enlargement	6024	2550	NA	NA	30 mg/day
7	61	Male	Palpable left cervical mass, lacrimal gland enlargement,	7364	3440	×2560	1146	40 mg/day

NA: not analyzed; *normal range < 1600 mg/dL; †normal range < 135 mg/dL; ‡normal range < 170 IU/mL.

TABLE 2: IgG4-related disease identified in other organs.

Case	Other organ manifestations
1	Enlargement of the left extraocular muscle
2	Enlargement of the left extraocular muscle
3	Dacryoadenitis, sialadenitis
4	Dacryoadenitis
5	Hepatic inflammatory pseudotumor, tubulointerstitial nephritis
6	Dacryoadenitis, mediastinal lymphadenopathy, tubulointerstitial nephritis, enlargement of the bilateral extra-ocular muscle
7	Dacryoadenitis,*lung lesions, tubulointerstitial nephritis

*A single lesion identified before the episode of perineural disease.

double vision due to abnormal ocular movement. This attributed to mass effects of IgG4-related ophthalmic disease including dacryoadenitis, myossitis, and perineural lesions, because peripheral nerve lesions in these patients involved supraorbital, infraorbital, and optic nerves, all of which are sensory nerves unlikely to cause abnormal ocular movement. The cause of epigastralgia in case 5 was not identified by serological and endoscopic examinations. This symptom spontaneously resolved.

3.1.4. Treatment and Recurrence. All patients had followup images of at least either CT or MRI for peripheral nerve lesions. Both chest and abdominal CTs, which were also available for all patients, were reviewed with regard to recurrent lesions at other sites. Steroid therapy at an initial dose of 20 to 40 mg/day was effective for all patients, making peripheral nerve lesions decrease in size in conjunction with decreased size of other organ lesions. Recurrent perineural lesions were confirmed during steroid taper in two patients (cases 2 and 4). In case 2, the recurrent lesion, which compressed the left optic nerve in the optic bony canal, caused failing vision and papilledema. The recurrent lesion promptly responded to an increased dose of steroid. Visual acuity and papilledema were fully recovered. Although the

perineural mass focally remained in the most recent followup MRI, he is currently free of neurological symptoms. Recurrent lesions involving right optic and infraorbital nerves in case 4 also decreased in size with an increased dose of steroid.

3.2. Imaging Characteristics. Radiological examination was performed for peripheral nerve lesions in 6 patients (cases 1–6). All lesions were radiologically characterized by distinct masses along the affected nerve fascicles (Figures 1–4). The size of the lesions ranged from 8 to 30 mm (median 13 mm). In case 1, bilateral orbital masses extended to the subcutis of the cheek along infraorbital nerves and their branches (Figures 1(a), 1(c)–1(f)). Spinal nerve lesions in cases 5 and 6 involved nerve fascicles mainly within and distal to intervertebral foramen, and portions proximal to the foramen, which anatomically correspond to nerve roots, were affected only in cervical nerve lesion of case 5. All lesions were well circumscribed with a round or lobular shape. The latter was common in optic nerve lesions (Table 3). On CT images, all lesions showed isodensity compared with those of skeletal muscles. Peripheral nerve lesions showed isointensity in T1-weighted MRI images and iso- to slightly high-intensity in T2-weighted images compared with those of skeletal muscles (Figures 1–4). All lesions were homogeneously enhanced (Figures 2(c), 2(d), and 3(c)). Calcification or necrosis was not a feature in any lesion.

Although nerve fibers involved in masses were not identifiable in CT images (Figure 2(a)), MRI demonstrated optic nerves penetrating the lesions in cases 1, 2, 3, and 4 (Figures 1(b), 1(d), 2(b), 2(d), and 3(c)). Intralesional nerves did not show any abnormalities regarding the signal intensity or pattern of contrast enhancement. FDG-PET, which was performed in only case 5, showed high uptakes in perineural lesions with SUV max 5.6 in C6, 5.2 in right L5, and 4.6 in right S1 (Figures 4(b), 4(d), 4(f), and 4(g)).

3.3. Histopathological Features of the Perineural Lesions. Histopathological examinations in perineural lesions were performed in cases 1 and 7. Case 1 had a surgical biopsy from the peripheral nerve lesion involving the infraorbital nerve. The pathology specimen consisting of perineural

TABLE 3: Characteristics of perineural lesions.

Case	Affected nerve	Performed imaging examinations	Size	Shape	Involved nerve
1	Right supraorbital nerve	CT (P), MRI (P)	15 mm	Round	Not visible
	Right infraorbital nerve	CT (P), MRI (P)	25 mm	Lobular	Not visible
	Left infraorbital nerve	CT (P), MRI (P)	26 mm	Lobular	Not visible
	Left optic nerve	CT (P), MRI (P)	12 mm	Lobular	Identifiable (MRI)
2	Left optic nerve	CT (P), MRI (CE)	14 mm	Lobular	Identifiable (MRI)
3	Left supraorbital nerve	CT (P), MRI (P)	10 mm	Round	Not visible
	Left optic nerve	CT (P), MRI (P)	10 mm	Lobular	Identifiable (MRI)
4	Right infraorbital nerve	CT (P), MRI (CE)	8 mm	Round	Not visible
	Right optic nerve	CT (P), MRI (CE)	30 mm	Lobular	Identifiable (MRI)
5	Left C6 nerve	CT (P), FDG-PET	9 mm	Round	Not visible
	Right L5 nerve	CT (CE), FDG-PET	13 mm	Round	Not visible
	Right S1 nerve	CT (CE), FDG-PET	13 mm	Round	Not visible
6	Right supraorbital nerve	CT (P), MRI (CE)	8 mm	Round	Not visible
	Right infraorbital nerve	CT (P), MRI (CE)	12 mm	Round	Not visible
	Left supraorbital nerve	CT (P), MRI (CE)	13 mm	Round	Not visible
	Left infraorbital nerve	CT (P), MRI (CE)	12 mm	Round	Not visible
	Right L5 nerve	CT (CE)	14 mm	Round	Not visible
	Left L5 nerve	CT (CE)	19 mm	Round	Not visible
	Right S1 nerve	CT (CE)	9 mm	Round	Not visible
	Left S1 nerve	CT (CE)	13 mm	Round	Not visible
7	Left greater auricular nerve	None	15 mm*	Round*	Not analyzed

P: plain; CE: contrast-enhanced; *macroscopically examined on the resected specimen.

soft tissue showed severe lymphoplasmacytic infiltration, irregular fibrosis, and occasional eosinophils. Obliterative phlebitis was not identified. Immunostaining revealed a large number of IgG4+ plasma cells (107 cells/hpf (0.237 square mm)) and an IgG4/IgG ratio was 61.8%. The relationship between nerve fascicles and inflammation could not be assessed because no nerve fibers were sampled.

Case 7, who did not undergo radiological examination of the peripheral nerve lesion, had IgG4-related disease in bilateral lacrimal glands, lung (interstitial pneumonia), and kidney. He noticed a palpable mass in the left neck, which was surgically resected on suspicion of an enlarged lymph node. Macroscopically, the resected lesion was a well-circumscribed spherical mass measuring 15 mm in the maximum diameter. Histologically, the specimen was not a lymph node but an inflammatory nodule centered on large nerve fascicles (Figures 5(a) and 5(b)), which were considered to be the great auricular nerve. The epineurium was extensively enlarged with massive lymphoplasmacytic infiltration rich in IgG4+ plasma cells (IgG4+ plasma cells 215 cells/hpf; IgG4/IgG 58.9%) (Figures 5(c) and 5(d)). Fibrosis or eosinophilic infiltration was not conspicuous. Penetrating nerve fibers, which were separated from the inflammatory process by the perineurium, were histologically unremarkable with scarce intraneural inflammatory cell infiltration (Figure 5(b)). He eventually noticed sensory dysfunction in the left retroauricular region after the surgical biopsy probably because the nerve was surgically transected.

Compared to radiological features and histological findings, perineural masses corresponded to the expanded perineurium affected by the IgG4-related inflammatory process. Homogeneous enhancement on imagings was supposed to represent massive inflammatory cell infiltration in the perineurium.

4. Discussion

The obtained results can be summarized as follows: (1) IgG4-related disease can rarely involve peripheral nerves particularly in ocular or paravertebral area, the former usually associated with ophthalmic lesions. (2) IgG4-related neural lesions are radiologically characterized by nerve-centered distinct soft tissue masses. (3) Histological features are massive lymphoplasmacytic infiltrates rich in IgG4+ plasma cells predominantly affecting the epineurium, suggesting "IgG4-related perineural disease" to be an appropriate term to describe these lesions. (4) Neurological symptoms are only rarely noted in this retrospective study.

This study demonstrated that the peripheral nervous system is one of the organs that can be involved in IgG4-related disease. It is unclear how IgG4-related perineural disease was interpreted before the entity of IgG4-related disease was recognized. Orbital lesions that involve both peripheral nerves and adjacent ophthalmic tissue might be referred to as orbital inflammatory pseudotumors [22]. Perineuritis is another possibility particularly for an isolated perineural disease as seen in cases 2 and 7.
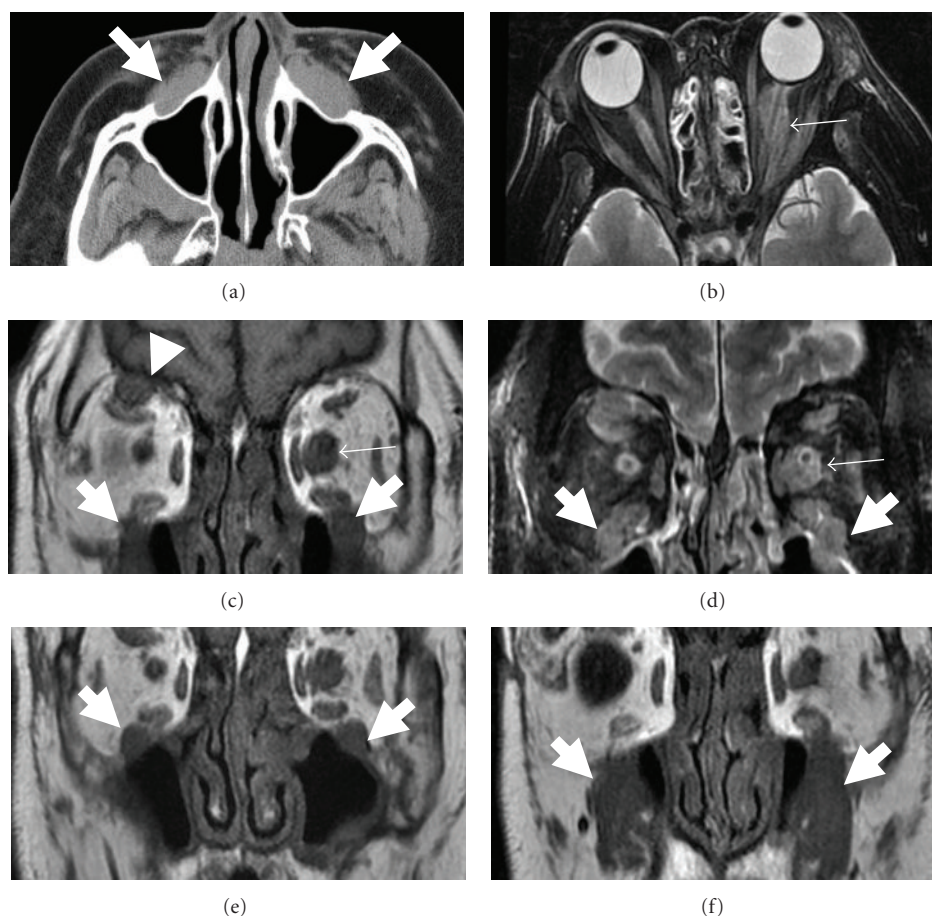
FIGURE 1: CT (a) and MRI ((b and d): T2-weighted images; (c, e, and f): T1-weighted images) of orbital space in 55-year-old man (case 1). CT image reveals soft tissue around the bilateral infra orbital nerve (a; arrows). MRI shows soft tissue around the left orbital nerve (b–d; small arrows), bilateral infraorbital nerve (c–f; arrows) and right supraorbital nerve (c and d; arrowheads). Left orbital nerve can be detected in the lesion (b and d).

The predominant radiological feature of IgG4-related perineural disease was a well-circumscribed mass, seen as soft tissue intensity on MRI. Interestingly, MRI demonstrated unremarkable optic nerves penetrating the perineural masses, suggesting little, if any, damage to the nerve fascicles themselves. This is in keeping with the fact that nerve fibers entrapped in the lesion were histologically unremarkable and neurological symptoms were rare. However, smaller nerves, such as the infraorbital nerve, were difficult to visualize on MRI. It is not yet conclusive to what extent FDG-PET is useful in detecting IgG4-related perineural disease like as reported in other organs [23], but this functional imaging helped us to recognize spinal nerve lesions, which were overlooked by other modalities, in case 5.

IgG4-related perineural disease needs to be differentiated from a variety of other diseases including malignant lymphoma, neurolymphomatosis [24], neurogenic tumors, and non IgG4-related inflammatory diseases such as sarcoidosis [25] or idiopathic inflammatory pseudotumor. The presence or absence of IgG4-related disease in other organs seems most helpful for this differential diagnosis, given that all cases in this study had other organ manifestations. Serum

IgG4 concentrations are also useful for the diagnosis, as is true with IgG4-related disease in other organs. However, histological examination is still necessary for patients with unusual clinical or radiological features. It is still an open question whether IgG4-related perineural disease is always associated with other organ lesions or can develop as a solitary IgG4-related lesion.

It is interesting that 13 of 21 (62%) perineural lesions were seen in the orbital area, where branches of the trigeminal nerve were commonly involved (43%). Affected nerves described in previous papers were also trigeminal or optic nerve branches [19–21]. One possible explanation of this site predilection is that IgG4-related ophthalmic disease more commonly shows perineural extension than other organ manifestations. In fact, all ophthalmic perineural lesions were associated with IgG4-related orbital disease. Ophthalmic perineural lesions were identified in 33% (6/18) of patients with IgG4-related orbital disease in our cohort. Another interesting point is that all perineural lesions identified were rather proximal and localize to where nerves encounter foramen. Molecules that can be targeted by IgG4-related disease may be more abundantly present in proximal
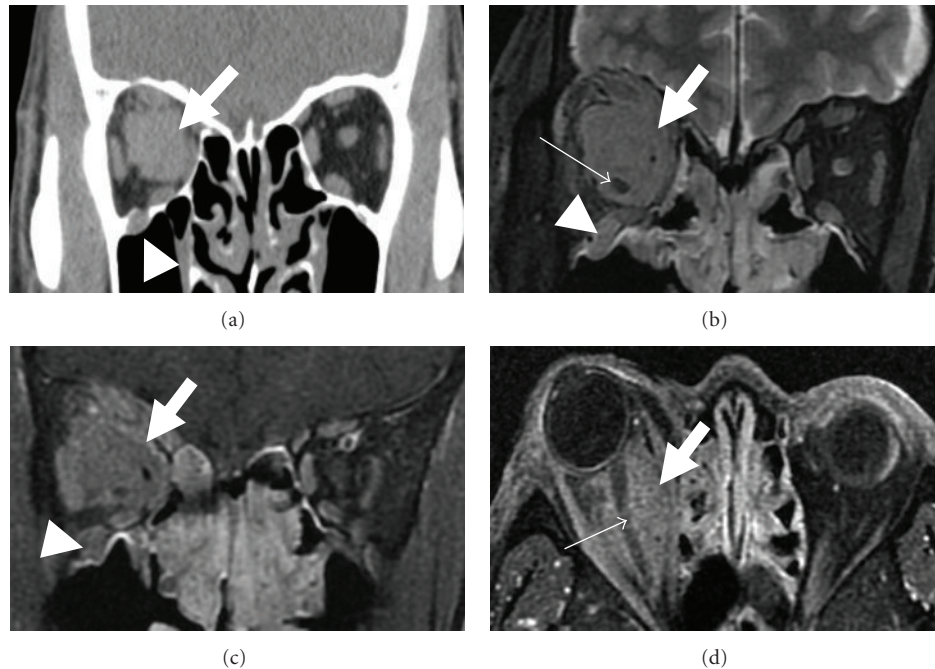
FIGURE 2: CT (a) and MRI (b: T2-weighted image; (c and d): contrast-enhanced T1-weighted images) of orbital space in 44-year-old man (case 4). Soft tissue mass is detected in the right orbital space (a; arrow). This lesion extends along the right optic nerve in MRI (b–d; arrows). The right optic nerve penetrating the lesion is detectable in MRI (b and d; small arrows). Soft tissue mass along the right infraorbital nerve is also noted (a–c; arrowheads).
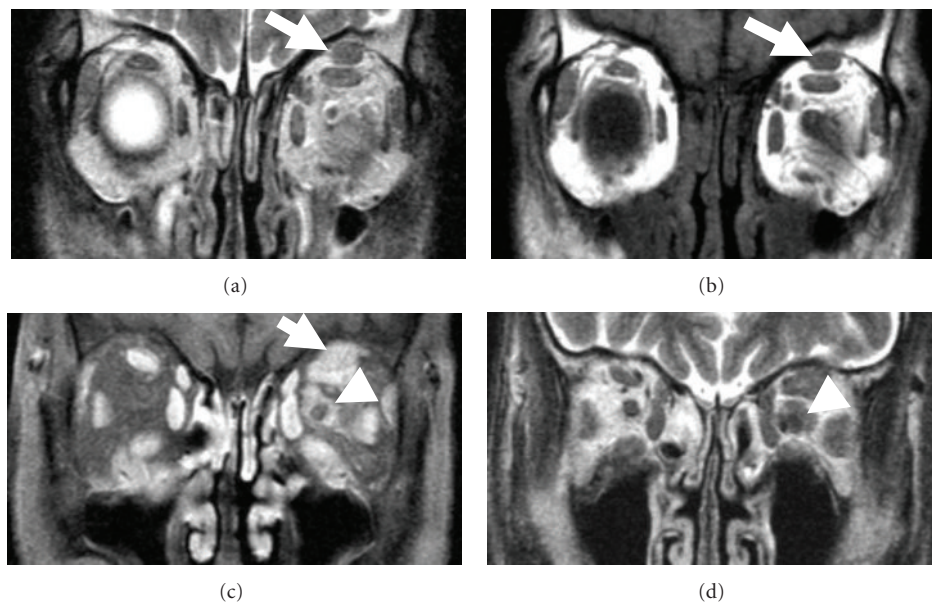

FIGURE 3: MRI ((a and d): T2-weighted images; b: T1-weighted image; c: contrast-enhanced T1-weighted image) of orbital space in 61-year-old man (case 3). MRI shows the soft tissue around the left supraorbital nerve (a–c; arrows) and optic nerve (c and d; arrowheads). The left optic nerve can be detected in the lesion.

parts of peripheral nerves. Another possible explanation is that distal perineural masses may be difficult to recognize as being along small nerves leading to false recognition as other anatomical structures like enlarged lymph nodes on images. In fact, microscopic perineural inflammation is commonly seen in surgically resected specimens of IgG4-related disease.

This study had a few limitations. Because of the retrospective nature of this study, imaging examinations underwent for each cases were not consistent. Neurological symptoms might not be extensively examined because a neurological involvement was not suspected at diagnosis. A prospective study seems necessary to conclude how often
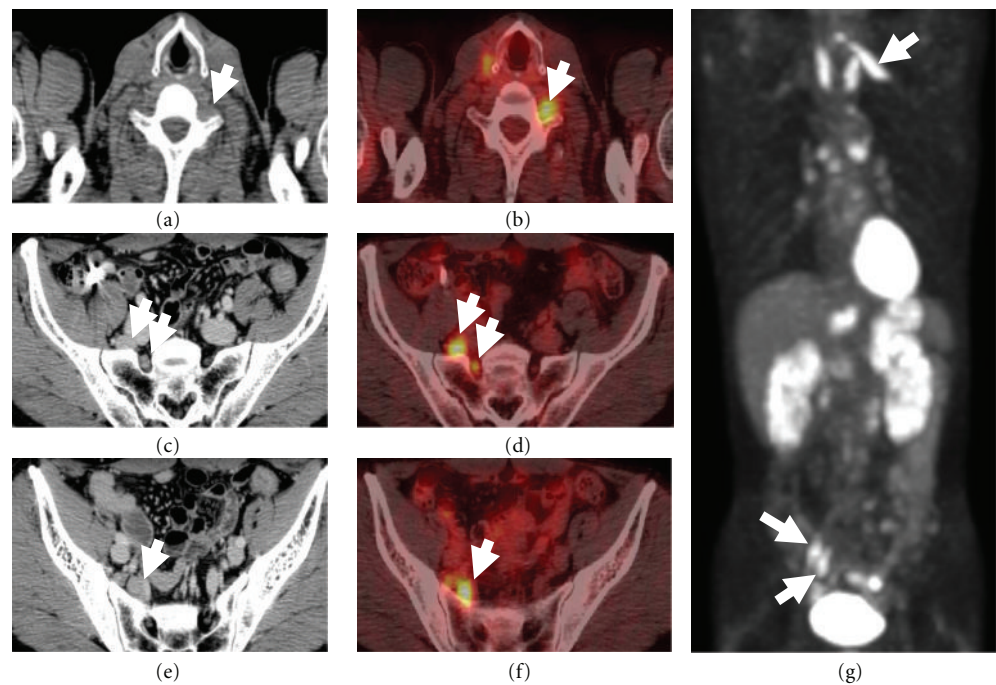
Figure 4: CT (a, c, and e) and FDG-PET (b, d, and f: fusion images; g: coronal MIP image) of whole body in 58-year-old man (case 5). CT shows soft tissue density around the left C6 (a; arrow), right L5 (c; arrows), and S1 nerves (e; arrow). High FDG uptakes are identified as perineural masses (b, d, f, and g; arrows).
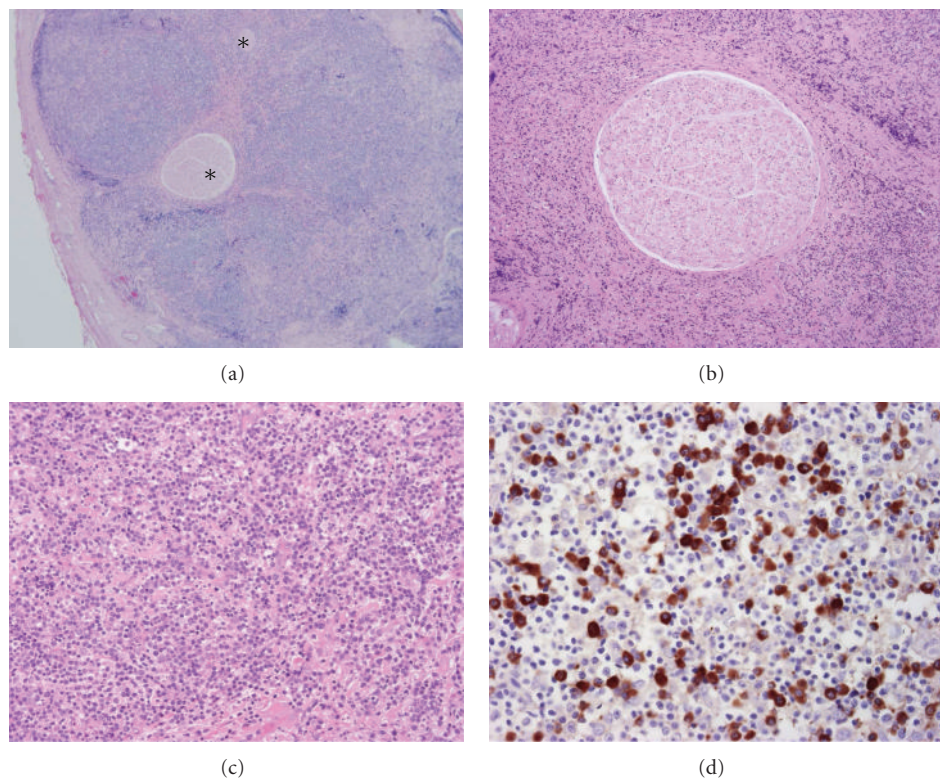

Figure 5: Histological features of resected IgG4-related perineural disease in 61-year-old man (case 7). (a) The epineurium is involved in a massive inflammatory process, where nerve fascicles (∗) are embedded. (b) The endoneurium is unremarkable without inflammatory cell infiltration. (c) Inflammatory cells consist predominantly of lymphocytes and plasma cells. (d) Immunostaining for IgG4 reveals a large number of IgG4+ plasma cells.

and to what extent IgG4-related perineural disease can cause neurological symptoms. Someone might also argue that IgG4-related inflammation was confirmed in only 2 cases. However, perineural lesions in the other patients most likely attributed to IgG4-related disease given the fact that they were present at diagnosis and responded to steroid therapy and the radiological features would not fit with other known neuropathies.

In conclusion, IgG4-related disease of the peripheral nervous system, which can be called IgG4-related perineural disease, is characterized by orbital and paravertebral localization, perineural mass formation detectable as soft tissue intensity in MRI images, and rare neurological symptoms.

Abbreviations

CT: Computed tomography
MRI: Magnetic resonance imaging
FDG-PET: 18F-fluorodeoxyglucose positron emission tomography
H&E: Hematoxylin and eosin.

Conflict of Interests

None of the authors declare any competing interests.

Acknowledgments

References

[1] S. Kitagawa, Y. Zen, K. Harada et al., "Abundant IgG4-positive plasma cell infiltration characterizes chronic sclerosing sialadenitis (Küttner's tumor)," *American Journal of Surgical Pathology*, vol. 29, no. 6, pp. 783–791, 2005.

[2] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[3] Y. Zen, K. Harada, M. Sasaki et al., "IgG4-related sclerosing cholangitis with and without hepatic inflammatory pseudotumor, and sclerosing pancreatitis-associated sclerosing cholangitis: do they belong to a spectrum of sclerosing pancreatitis?" *American Journal of Surgical Pathology*, vol. 28, no. 9, pp. 1193–1203, 2004.

[4] T. Taniguchi, M. Ko, S. Seko et al., "Interstitial pneumonia associated with autoimmune pancreatitis," *Gut*, vol. 53, no. 5, pp. 770–771, 2004.

[5] L. D. Cornell, S. L. Chicano, V. Deshpande et al., "Pseudotumors due to IgG4 immune-complex tubulointerstitial nephritis associated with autoimmune pancreatocentric disease," *American Journal of Surgical Pathology*, vol. 31, no. 10, pp. 1586–1597, 2007.

[6] S. Kasashima, Y. Zen, A. Kawashima et al., "Inflammatory abdominal aortic aneurysm: close relationship to IgG4-related periaortitis," *American Journal of Surgical Pathology*, vol. 32, no. 2, pp. 197–204, 2008.

[7] J. H. Stone, A. Khosroshahi, A. Hilgenberg, A. Spooner, E. M. Isselbacher, and J. R. Stone, "IgG4-related systemic disease and lymphoplasmacytic aortitis," *Arthritis and Rheumatism*, vol. 60, no. 10, pp. 3139–3145, 2009.

[8] R. Pannala and S. T. Chari, "Autoimmune pancreatitis," *Current Opinion in Gastroenterology*, vol. 24, no. 5, pp. 591–596, 2008.

[9] T. Shimosegawa and A. Kanno, "Autoimmune pancreatitis in Japan: overview and perspective," *Journal of Gastroenterology*, vol. 44, no. 6, pp. 503–517, 2009.

[10] T. Kamisawa, N. Funata, Y. Hayashi et al., "Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis," *Gut*, vol. 52, no. 5, pp. 683–687, 2003.

[11] H. Hamano, S. Kawa, Y. Ochi et al., "Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis," *Lancet*, vol. 359, no. 9315, pp. 1403–1404, 2002.

[12] Y. Zen, M. Onodera, D. Inoue et al., "Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4," *American Journal of Surgical Pathology*, vol. 33, no. 12, pp. 1833–1839, 2009.

[13] S. T. Chari, N. Takahashi, M. J. Levy et al., "A diagnostic strategy to distinguish autoimmune pancreatitis from pancreatic cancer," *Clinical Gastroenterology and Hepatology*, vol. 7, no. 10, pp. 1097–1103, 2009.

[14] D. V. Sahani, S. P. Kalva, J. Farrell et al., "Autoimmune pancreatitis: imaging features," *Radiology*, vol. 233, no. 2, pp. 345–352, 2004.

[15] N. Takahashi, A. Kawashima, J. G. Fletcher, and S. T. Chari, "Renal involvement in patients with autoimmune pancreatitis: CT and MR imaging findings," *Radiology*, vol. 242, no. 3, pp. 791–801, 2007.

[16] D. Inoue, Y. Zen, H. Abo et al., "Immunoglobulin G4-related lung disease: CT findings with pathologic correlations," *Radiology*, vol. 251, no. 1, pp. 260–270, 2009.

[17] D. Inoue, Y. Zen, H. Abo et al., "Immunoglobulin G4-related periaortitis and periarteritis: CT findings in 17 patients," *Radiology*, vol. 261, no. 2, pp. 625–633, 2011.

[18] Y. Fujinaga, M. Kadoya, S. Kawa et al., "Characteristic findings in images of extra-pancreatic lesions associated with autoimmune pancreatitis," *European Journal of Radiology*, vol. 76, no. 2, pp. 228–238, 2010.

[19] T. Watanabe, Y. Fujinaga, S. Kawakami et al., "Infraorbital nerve swelling associated with autoimmune pancreatitis," *Japanese Journal of Radiology*, vol. 29, no. 3, pp. 194–201, 2011.

[20] M. Katsura, A. Morita, H. Horiuchi, K. Ohtomo, and T. Machida, "IgG4-related inflammatory pseudotumor of the trigeminal nerve: another component of IgG4-related sclerosing disease?" *American Journal of Neuroradiology*, vol. 32, no. 8, pp. E150–E152, 2011.

[21] Z. S. Wallace, A. Khosroshahi, F. A. Jakobiec et al., "IgG4-related systemic disease as a cause of "idiopathic" orbital inflammation, including orbital myositis, and trigeminal nerve involvement," *Survey of Ophthalmology*, vol. 57, no. 1, pp. 26–33, 2012.

[22] Z. X. Ding, G. Lip, and V. Chong, "Idiopathic orbital pseudotumour," *Clinical Radiology*, vol. 66, no. 9, pp. 886–892, 2011.

[23] Y. L. Tae, M. H. Kim, H. P. Do et al., "Utility of 18F-FDG PET/CT for differentiation of autoimmune pancreatitis with atypical pancreatic imaging findings from pancreatic cancer,"

American Journal of Roentgenology, vol. 193, no. 2, pp. 343–348, 2009.

[24] S. Grisariu, B. Avni, T. T. Batchelor et al., "Neurolymphomatosis. An International Primary CNS Lymphoma Collaborative Group report," *Blood*, vol. 115, no. 24, pp. 5005–5011, 2010.

[25] R. Shah, G. H. Roberson, and J. K. Curé, "Correlation of MR imaging findings and clinical manifestations in neurosarcoidosis," *American Journal of Neuroradiology*, vol. 30, no. 5, pp. 953–961, 2009.