

Comparison of two subsets of Chinese patients with retroperitoneal fibrosis in terms of IgG4 immunohistochemical staining

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Abstract

Objective. To identify clinical and pathological differences between IgG4-related retroperitoneal fibrosis (IgG4-RPF) and idiopathic RPF (iRPF) in a Chinese population.

Method. Clinical and pathological data of 50 RPF patients from 2006 to 2016 were retrospectively analysed. The presence of at least one characteristic histopathological feature, >30 IgG4⁺ plasma cells per high power field, and an IgG4⁺/IgG⁺ plasma cells ratio cutoff of $>40\%$ were used to define IgG4-RPF.

Results. Patients with IgG4-RPF were significantly more likely to have pain (94.1 vs 68.8%, $P=0.048$), elevated serum IgE concentration (166.1 vs 40.2 IU/ml, $P=0.029$) and tissue eosinophilia (47.1 vs 12.5%, $P=0.018$), compared with patients with iRPF. In the IgG4-RPF subgroup, patients with tissue eosinophilia demonstrated higher levels of CRP (4.3 vs 1.9 mg/dl, $P=0.027$) and ESR (62.1 vs 22.8 mm/h, $P=0.001$). Among the 50 patients with RPF, the average number of tissue IgG4⁺ plasma cells was positively correlated with the number of tissue eosinophils ($r=0.37$, $P=0.009$). Moreover, serum IgG4 concentration and serum IgE concentration showed positive correlation ($r=0.834$, $P=0.000$).

Conclusion. The distinct serological and histopathological features of Chinese patients with IgG4-RPF were elevated serum IgE concentration and tissue eosinophilia, which potentially can aid and support the diagnosis. As serum IgG4 concentration may be normal in patients with IgG4-RPF, serum IgE may represent a useful serological marker in distinguishing IgG4-RPF from iRPF.

Key words: retroperitoneal fibrosis, IgG4, IgE, tissue eosinophilia

Rheumatology key messages

- High serum IgE concentration and tissue eosinophilia were observed in Chinese IgG4-related retroperitoneal fibrosis patients.
- The level of serum IgE was strongly correlated with that of IgG4 in retroperitoneal fibrosis patients.

Introduction

Retroperitoneal fibrosis (RPF) is considered a chronic inflammatory disease of unknown aetiology characterized by lymphoplasmacytic infiltration and fibrosis. The fibrous mass surrounds the aorta, and may compress adjacent

structures, such as the ureter and inferior vena cava [1]. The disease is generally divided into two types: idiopathic RPF and secondary RPF. Secondary RPF often results from drug, surgery, infection and malignancies. Idiopathic RPF (iRPF) accounts for 75% of all RPF cases [2].

IgG4-related disease (IgG4-RD) is a newly recognized clinical entity that was first proposed in 2003 and officially named in 2010 [3, 4]. IgG4-RD has characteristic histopathological features, demonstrating increased number of IgG4⁺ plasma cells within the affected tissue [5]. Multiple organs or tissue can be affected, including the submandibular gland, orbit, lymph nodes, pancreas, abdominal aorta and iliac vessels [6]. A proportion of patients with iRPF meet the diagnostic criteria and fall within the spectrum of IgG4-RD [7]. Therefore, the name IgG4-related

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RPF (IgG4-RPF) was coined. IgG4-RPF is a comprehensive term for retroperitoneal lesions associated with IgG4-RD and mainly manifests as numerous IgG4⁺ plasma cells infiltrating the tissue and various degree of fibrosis.

Subgroup analyses of RPF with respect to IgG4 have been reported by researchers from many countries since 2010. However, the descriptions of the pathologic significance of the IgG4-RPF and iRPF subsets were inconsistent among those studies [8, 9]. Moreover, studies on the clinicopathological distinctions between iRPF and IgG4-RPF in a Chinese population are lacking. The objectives of this study were to examine the IgG4⁺ plasma cells infiltrates within retroperitoneal lesions in Chinese patients with RPF, and determine the clinicopathological features of Chinese patients with IgG4-RPF.

Methods

Patients

We retrospectively reviewed the pathological specimen data of patients with RPF from 2006 to 2016 at the Chinese People's Liberation Army General Hospital. Patients with RPF secondary to malignancy, infections, surgery or drugs were excluded according to the medical records. In total, 50 patients with RPF were included. This study was approved by the Ethical Committee of the Chinese People's Liberation Army General Hospital (Beijing, China) and patient consent forms were signed.

Histopathology and immunohistochemistry

Paraffin blocks of RPF tissues biopsies were available for all 50 patients (see [supplementary data](#), section protocol, available at *Rheumatology* online).

Diagnosis

The diagnosis of IgG4-RPF was established on the basis of three characteristic pathological findings: at least one histopathological feature (dense lymphoplasmacytic infiltrate, storiform fibrosis, obliterative phlebitis); infiltrates of IgG4⁺ plasma cells at counts of >30 cells/high powered field (HPF); and an IgG4⁺/IgG⁺ plasma cell ratio of >40% within the tissue. Patients who did not fulfil these diagnostic criteria were classified as iRPF.

Clinical, serological and imaging data

Information on sex, age, clinical symptoms, disease duration, smoking history and laboratory data were extracted from electronic medical records. Imaging data include the presence of hydronephrosis or renal atrophy and extension of the retroperitoneal mass. Scheel's radiological classification was chosen to assess the extent of the soft-tissue mass [10].

Statistics

All statistical analyses were performed by using SPSS (version 17.0, IBM, Armonk, NY, USA). Descriptive statistical analysis, *t*-test, Mann-Whitney test, χ^2 test, Fisher's exact test and Pearson's correlation analysis were performed. A *P*-value (two-tailed) <0.05 was considered statistically significant.

Results

A total of 20 (40%) pathological specimens were obtained from surgeries, and the other 30 were obtained from fine-needle aspiration (26 were US-guided, 4 were CT-guided). No significant differences were found in the number of IgG4⁺ and IgG⁺ plasma cells and or the IgG4⁺/IgG⁺ plasma cells ratio between the different methods (surgery or fine-needle aspiration) of obtaining specimens.

Among the overall 50 patients with RPF, 34 (68%) patients were identified as having IgG4-RPF and the 16 patients not meeting the diagnostic criteria were classified as having iRPF. The 16 patients in the iRPF group were all men. The average onset age was 51.4 years, and the mean disease course was 84.9 days. Of the 34 patients with IgG4-RPF, 5 were women and 29 were men. The mean age at disease onset was 53.7 years, and the average disease course was 157.7 days. The differences in the patients' age, sex and disease duration between the two groups were not statistically significant (*P* > 0.05). Pain was the most common symptom in both groups, albeit significantly more common in the IgG4-RPF group (94.1 vs 68.8%, *P* = 0.048). The comparisons of other clinical characteristics are shown in detail in Table 1. The baseline serological and imaging characteristics of the two groups were also compared ([supplementary Table S1](#), available at *Rheumatology* online). There were no significant differences in terms of serum eosinophil count, ESR, CRP concentration, hypocomplementaemia, autoantibody, serum IgG subclass concentration, serum creatinine concentration and radiographic class. However, the serum IgE concentration was significantly higher in the IgG4-RPF group (116.1 vs 40.7 IU/ml, *P* = 0.029). The incidences of hydronephrosis and renal atrophy were not statistically significantly different between the two groups.

Histological and immunohistochemical findings

The histopathological features of the 50 RPF samples are summarized in Table 2. Microscopically, lymphoplasmacytic cells were scattered within the tissue. A lymphoid follicles-like structure could be seen in 18 (36%) cases (Fig. 1A). Storiform fibrosis was found in 22 (64.71%) patients with IgG4-RPF (Fig. 1B) and 7 (43.75%) patients with iRPF (Fig. 1C). The percentage of storiform fibrosis seemed to be higher in the IgG4-RPF group; however, the difference was not significant (*P* = 0.161). Obliterative phlebitis, a characteristic feature of IgG4-RD, was observed in only one patient in the IgG4-RPF group in this study. Eosinophils were sparsely distributed in the tissue (Fig. 1D). Tissue eosinophilia was observed in 16 (47.06%) patients in the IgG4-RPF group and 2 (12.50%) patients in the iRPF group, with the difference being statistically significant (*P* = 0.018). The eosinophil counts within tissue varied among the 50 specimens. The average number of eosinophils was 17.82 (range 0–43) in the IgG4-RPF group and 6.88 (range 0–28) in the iRPF group, with the difference also being statistically significant (*P* = 0.022). Infiltration of fat cells was seen in 26 (76.47%) patients in the IgG4-RPF group and 9 (56.25%) patients in the iRPF group.

TABLE 1 Comparison of clinical characteristics of histological IgG4-positive and -negative RPF patients

Variables	iRPF	IgG4-RPF	P-value
Patients, <i>n</i>	16	34	
Male, <i>n</i> (%)	16 (100)	29 (85.29)	0.266
Age, mean (range), years	51.4 (23–72)	53.7 (12–77)	0.550
Disease duration, mean (range), days	84.9 (14–270)	157.7 (1–1095)	1.000
Smoking, <i>n</i> (%)	9 (56.25)	14 (41.18)	0.318
Alcohol intake, <i>n</i> (%)	5 (31.25)	6 (17.65)	0.473
Initial symptoms, <i>n</i> (%)			
Fever	1 (6.25)	4 (11.76)	0.920
Pain	11 (68.75)	32 (94.12)	0.048
Arthralgia	1 (6.25)	0	0.320 ^a
Vomiting	1 (6.25)	7 (20.59)	0.381
Constipation	0	5 (14.71)	0.266
Diarrhea	0	2 (5.88)	1.000 ^a
Low extremity oedema	3 (18.75)	5 (14.71)	1.000

Data are reported as *n* (%) unless stated otherwise. ^aFisher's exact test. iRPF: idiopathic retroperitoneal fibrosis; RPF: retroperitoneal fibrosis; IgG4-RPF: IgG4-related retroperitoneal fibrosis.

TABLE 2 Histopathologic features of RPF samples separated according to IgG4/total IgG ratio

Pathologic finding	IgG4-RPF	iRPF	P-value
IgG4 ⁺ plasma cells, mean (range)	92.6 (55–259)	6.7 (0–32)	<0.001
IgG ⁺ plasma cells, mean (range)	115.2 (80–325)	13.1 (30–64)	<0.001
IgG4 ⁺ /IgG ⁺ ratio, mean (range)	80.18 (43.13–164.26)	7.1 (0–29.9)	<0.001
Storiform fibrosis, <i>n</i> (%)	22 (64.71)	7 (43.75)	0.161
Obliterative phlebitis, <i>n</i> (%)	1 (2.94)	0	1.000 ^a
Tissue eosinophilia ^b , <i>n</i> (%)	16 (47.06)	2 (12.50)	0.018
Fat cells infiltrate, <i>n</i> (%)	26 (76.47)	9 (56.25)	0.261

Data are reported as *n* (%) unless stated otherwise. ^aFisher exact test. ^bTissue eosinophilia defined as ≥ 25 eosinophils per five HPFs. iRPF: idiopathic retroperitoneal fibrosis; RPF: retroperitoneal fibrosis; IgG4-RPF: IgG4-related retroperitoneal fibrosis; HPF: high powered field.

All samples from the IgG4-RPF group showed focal or diffuse IgG4⁺ plasma cell infiltration and various degrees of fibrosis within the tissue (Fig. 2A and B). The numbers of IgG4⁺ and IgG⁺ plasma cells and the IgG4/IgG ratio are listed in Table 2. The number of IgG4⁺ plasma cells per HPF in the IgG4-RPF group ranged from 55 to 259. As expected, fewer IgG4⁺ plasma cells were seen in the iRPF group, ranging from 0 to 32 (Fig. 2C and D). Of the 16 patients with iRPF, 10 (62.50%) had no tissue IgG4⁺ plasma cell infiltrates. The number of IgG4⁺ plasma cells per HPF ranged from 0 to 32. The IgG4/IgG ratio was significantly higher in the IgG4-RPF group than in the iRPF group (80.18 and 7.10%, respectively, $P < 0.01$). As serum IgG4 measurements were not available at our hospital prior to 2010, only 16 patients with IgG4-RPF had data on serum IgG4 levels. Of these, only five (31.3%) showed elevated concentration.

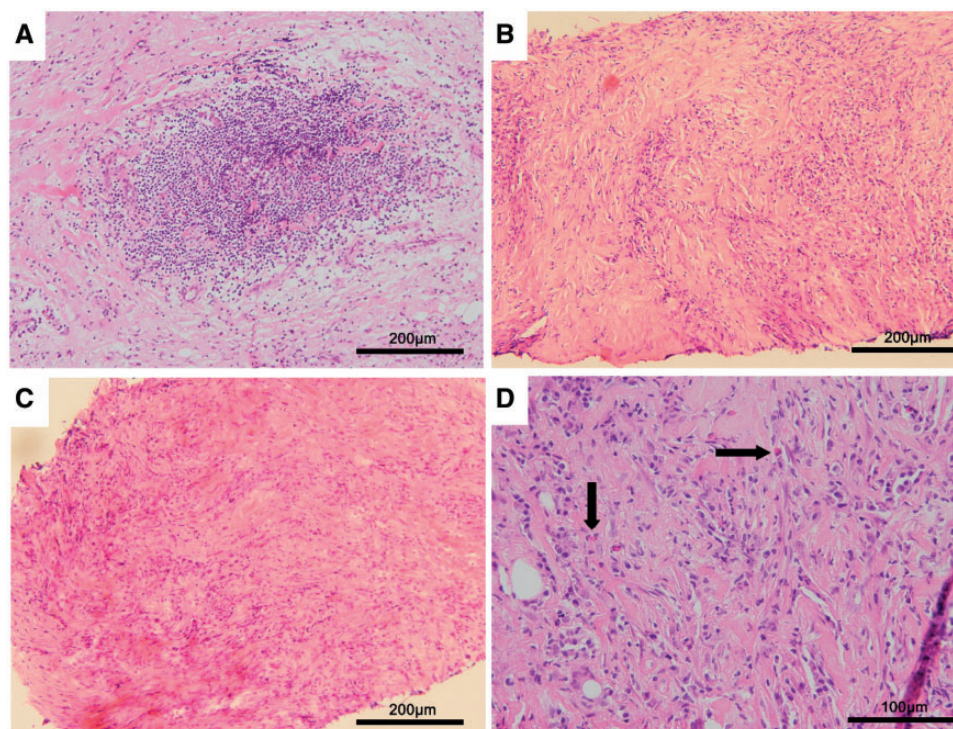
Subgroup analysis based on tissue eosinophilia in IgG4-RPF patients

A total of 16 (47.06%) IgG4-RPF patients were observed to have tissue eosinophilia (Table 3), and were divided into

the tissue eosinophilia-positive (TEP) group. The number of eosinophils in the TEP group was 33 (range 25–43). Eighteen (52.94%) IgG4-RPF patients were divided into the eosinophilia-negative (TEN) group. The number of eosinophils in the TEN was 4.33 (range 0–18). Male ratio, age of disease onset and clinical symptoms were not found to be significantly different between the two groups. Serological findings were also compared between the two groups. We found that the concentrations of serum ESR and CRP in TEP group were significantly higher than those in TEN group. On the other hand, the TEN group had significant higher levels of serum IgE than the TEP group (257.0 vs 84.3, $P = 0.043$). No significant differences were observed in serum creatinine concentration between the two groups.

Correlation among tissue IgG4⁺ plasma cells, eosinophils counts, serum IgG4 and IgE concentration

Comparative analyses of pathological features were carried out between IgG4-RPF and iRPF cases. The results indicated that patients with IgG4-RPF had a significantly high prevalence of tissue eosinophilia. Thus, eosinophils

Fig. 1 Histopathology of idiopathic RPF

(A) Numerous plasma cells and lymphocytes infiltrate tissue and form lymphoid follicles-like structure (10 × 20). (B, C) Storiform fibrosis is seen in IgG4-RPF and iRPF, respectively (10 × 20). (D) High power view showing eosinophiles (arrows) scattering within the tissue (10 × 40). IgG4-RPF: IgG4-related retroperitoneal fibrosis.

in the tissue were counted in the 50 samples and correlation analysis was performed. The average number of IgG4⁺ plasma cells in three HPFs was positively correlated with the sum of eosinophil counts in five HPFs within the tissue ($r=0.37$, $P=0.009$) in the 50 samples. Among the 50 patients with RPF, the serum IgG4 concentration was positively correlated with serum IgE concentration ($r=0.834$, $P<0.001$). However, no linear correlation was found between the number of IgG4⁺ plasma cell infiltrates within the tissue and serum IgG4 concentration.

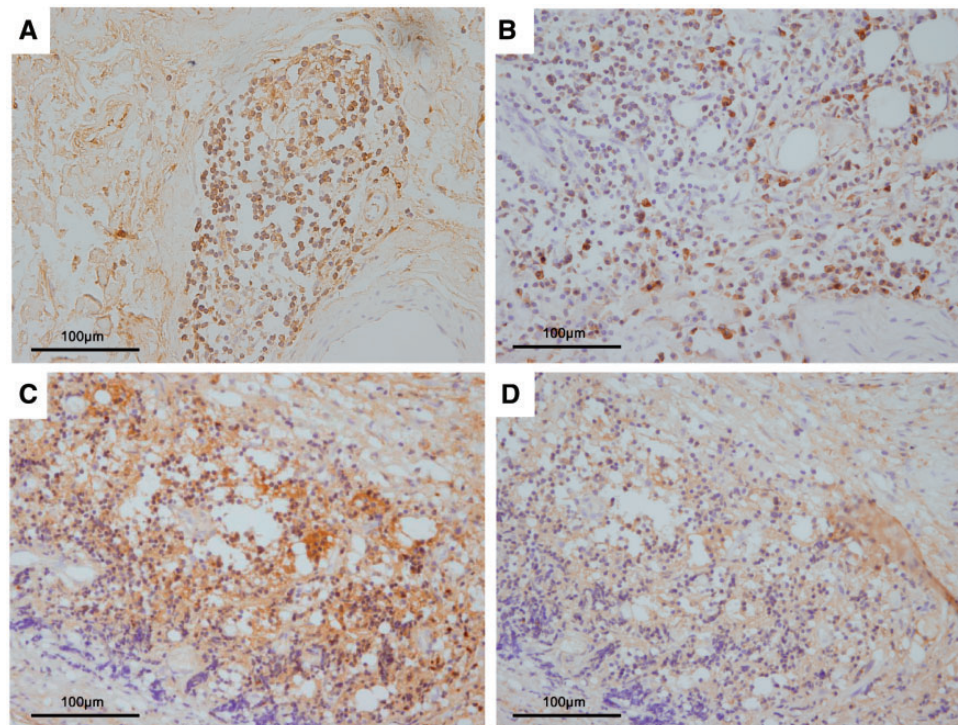
Discussion

Dense lymphoplasmacytic infiltration, storiform fibrosis and obliterative phlebitis are considered the three major histopathological features of IgG4-RD [7]. However, formulating a single set of diagnostic criteria covering every affected organ has proven difficult, as the presence of storiform fibrosis and obliterative phlebitis differs between the affected organs [5, 7, 10–13].

This study investigated the clinicopathological differences between iRPF and IgG4-RPF in 50 Chinese patients. Similar studies have been carried out in other countries (Table 4) [8, 9, 14, 15]. Although slightly different diagnostic criteria were used in these studies, an IgG4⁺/IgG⁺ plasma cells ratio >40% in retroperitoneal lesions was generally used [10, 16, 17]. The mean age at disease onset in patients with IgG4-RPF in our study was

53.7 years, lower than the mean age reported in other studies [8, 9, 14]. The youngest patient in our study was 12 years old. With respect to the results of immunohistochemistry analysis, the mean IgG4⁺/IgG⁺ plasma cells ratio in patients with IgG4-RPF was 80.18% in our study. This was similar to the findings from the USA [13], but higher than the ratios reported in studies from Japan (62%) and the Czech Republic (63%) [14, 15]. While storiform fibrosis was the most common histopathological feature, obliterative phlebitis was only found in one of the patients with IgG4-RPF, which is lower than the studies from South Korea and the USA [8, 9]. Tissue eosinophilia was found significantly more frequent in patients with IgG4-RPF. Similarly, in the study from the USA, patients with IgG4-RPF were statistically more likely to demonstrate evidence of storiform fibrosis and tissue eosinophilia on retroperitoneal biopsies compared with patients with iRPF [9].

IgG4-RPF patients were subgrouped according to the number of eosinophils within the tissue, into TEP and TEN groups. Patients in the IgG4-RPF TEP group had higher levels of ESR and CRP than the TEN group. This may suggest that tissue eosinophilia is a marker of disease activity in patients with IgG4-RPF. Furthermore, it seemed that the higher the number of eosinophils infiltrating the tissue, the higher the number of tissue IgG4⁺ plasma cells observed. Similar findings are reported in patients with IgG4-related pancreatitis [18, 19].

Fig. 2 Immunohistochemical features of RPF

(A, B) Immunohistochemical stain for IgG4 (10 × 40). IgG4⁺ plasma cells are focally distributed (A) or diffusely distributed (B). Immunohistochemical stain for IgG (C) and IgG4 (D) (10 × 40). The IgG4⁺/IgG⁺ plasma cell ratio was 18%.

TABLE 3 Clinical characteristics of IgG4-RPF patients with or without eosinophil infiltration

Characteristics	Tissue eosinophilia-positive ^a	Tissue eosinophilia-negative	P-value
Patients	16	18	
Male, <i>n</i> (%)	12 (75)	17 (94.44)	0.266
Age, mean (s.d.), years	52.13 (14.85)	53.06 (10.63)	0.505
IgG4 ⁺ plasma cells ^b	310.25 (102–777)	248.94 (55–522)	0.281
Eosinophil counts	33 (25–43)	4.33 (0–18)	<0.001
Initial symptoms, <i>n</i> (%)			
Fever	2 (12.5%)	2 (11.1)	1.000
Abdominal pain	11 (68.75)	12 (66.66)	1.000
Flank pain	10 (62.5)	13 (72.22)	0.717
Back pain	3 (18.75)	3 (16.67)	1.000
Vomiting	3 (18.75)	4 (22.22)	1.000
Hydronephrosis	14 (87.5)	15 (83.33)	1.000
Renal atrophy	2 (12.5)	2 (11.1)	1.000
Serological findings			
Serum ESR concentration, mean (range), mm/h	62.09 (25–105)	22.8 (0–69)	0.001
Serum CRP concentration, mean (range), mg/dl	4.382 (0.506–8.9)	1.92 (0.296–1.79)	0.027
Serum creatinine, mean (range), µmol/l	154.64 (59.7–143.5)	128.65 (64.4–282.3)	0.880
Serum IgE concentration, mean (range), IU/ml	84.307 (5.35–117)	257 (37.1–618)	0.043

Data are reported as *n* (%) unless otherwise stated. ^aTissue eosinophilia defined as ≥25 eosinophils per five HPFs. ^bDefined as average IgG4⁺ plasma cells number in three HPFs. IgG4-RPF: IgG4-related retroperitoneal fibrosis; HPF: high powered field.

Eosinophilic granulocytes, members of the innate immune system, are considered culprit cells in allergic diseases. There have been reported cases in which some patients with IgG4-RD had longstanding allergies,

such as sinusitis, atopic dermatitis and bronchial asthma [20]. Torre *et al.* [21] first evaluated the epidemiology of atopy and peripheral blood eosinophilia, as well as the serum IgE concentration in 70 cases of IgG4-RD in

TABLE 4 Comparison of our study with the published studies on RPF respect to IgG4

	Khosroshahi et al. [9]	Zen et al. [14]	Laco et al. [15]	Koo et al. [8]	Our study
Country	USA	Japan	Czech Republic	South Korea	China
Year	2013	2009	2013	2014	2016
IgG4-RPF/total cases	13/23	10/17	2/6	9/19	34/50
Age of IgG4-RPF patients, mean (range), years	57 (38–71)	66.5 (52–78)	–	59.4	53.7 (12–77)
Male ratio, %	79	100	–	55.6	85.29
Diagnosis criteria	IgG4 ⁺ /IgG ⁺ plasma cell ratio >40%	Diffuse IgG4 ⁺ plasma cell infiltration and an IgG4 ⁺ /IgG ⁺ cell ratio >30%	Organ swelling or masses; serum IgG4 concentration >135 mg/dl; IgG4 ⁺ /IgG ⁺ plasma cell >40%; IgG4 ⁺ plasma cells >10/HPF	Media positive plasma cell count of >30/HPF; in at least three fields; and IgG4 ⁺ /IgG ⁺ plasma cell >40%	At least one characteristic histopathologic feature; IgG4 ⁺ plasma cells that number >30/HPF; IgG4 ⁺ /IgG ⁺ plasma cell >40%
IgG4-RPF vs non-IgG4-RPF					
Serum IgG4 concentration, mean (range), mg/dl	107 (3–386) vs 17 (16–18) ^b	694.7 (154–2330) vs 30.33 (10–53) ^a	–	–	178.08 (28.1–665) vs 95.73 (30.9–135)
IgG4 ⁺ plasma cells/HPF, mean (range)	13 (2–55) vs 1 (0–8) ^a	68 (24–144) vs 3 (0–12) ^a	114 (48–238) vs 0	80 (35–170) vs 4.2 (3–28) ^a	92.6 (55–259) vs 6.7 (0–32) ^a
IgG ⁺ plasma cells/HPF, mean (range)	–	109 (41–206) vs 53 (3–743)	180 (114–373) vs 0	140.7 (55–200) vs 78.3 (11–210)	115.2 (80–325) vs 13.1 (30–64) ^a
IgG4 ⁺ /IgG ⁺ ratio, %	81 vs 8 ^a	62 vs 4 ^a	63 vs NA	57.4 vs 3.4 ^a	80.18 vs 7.1 ^a
Storiform fibrosis, %	92 vs 30 ^a	50 vs 14	50 vs 25	88.9 vs 70	64.71 vs 43.75
Tissue eosinophilia, %	77 vs 0 ^a	100 vs 25 ^a	100 vs 50	44.4 vs 10	47.06 vs 12.50 ^a
Phlebitis, %	31 vs 0	–	100 vs 0	44.4 vs 10	2.94 vs 0

^aP <0.05. ^bP-value not reported. IgG4-RPF: IgG4-related retroperitoneal fibrosis; HPF: high powered field; NA: not available.

the USA. Their results demonstrated that the majority of patients (68.6%) were non-atopic, and that some non-atopic patients demonstrated peripheral eosinophilia and elevated levels of IgE. They concluded that processes inherent to IgG4-RD itself rather than atopy *per se* contribute to the development of eosinophilia in the absence of atopy. Thus, the hypothesis that eosinophils involved in allergic reactions are associated with the onset of the disease remains to be verified. Nevertheless, eosinophils are known to be an important source of TGF- β , which can promote fibrosis [22–24].

In our study, serum IgE concentration was strongly correlated with serum IgG4 concentration. Although the serum IgG4 concentration was not routinely tested at our hospital before 2010, data were only available for 16 of the 34 patients with IgG4-RPF and 4 of the 16 patients with iRPF. The results showed that IgG4 concentration was elevated in only five (31.25%) of the patients in the IgG4-RPF group. These patients were all men with a mean age of 63.2 years. Although serum IgG4 concentration is frequently used in the diagnosis of IgG4-RD, it is important to emphasize that it can be normal in up to 40% of patients with biopsy-proven IgG4-RD [25]. For patients with normal serum IgG4 concentration, serum IgE levels may potentially prove helpful in the diagnosis of IgG4-RPF, as well as possibly serving as a marker of disease activity and relapse. Culver *et al.* [26] investigated the role of IgE in the diagnosis of IgG4-RD, as well as its use in identifying risk of future relapse. They found that a serum IgE concentration >480 kIU/l distinguished IgG4-RD from healthy controls with a 36% sensitivity, 86% specificity, and a positive likelihood ratio of 3.2. A serum IgE concentration >380 kIU/l at diagnosis identified patients with disease relapse with 64% sensitivity, 88% specificity and a positive likelihood ratio of 5.4. Based on the results of Culver *et al.* and our findings, we believe that serum IgE is a potential dynamic marker for monitoring the disease process.

Zen *et al.* [14] proposed that some cases of iRPF represented burned-out IgG4-RPF. Our results demonstrated higher serum IgE concentration and tissue eosinophilia in patients with IgG4-RPF compared with iRPF. The patients with IgG4-RPF who also had tissue eosinophilia had higher levels of CRP and ESR, indicating that tissue eosinophilia may reflect disease activity. Since iRPF samples were less likely to show lymphoplasmacytic and eosinophilic tissue infiltration, local inflammation tended to be quiescent. Therefore, we share the opinion that iRPF might be burned-out IgG4-RPF.

To our knowledge, this is the largest study to compare the clinicopathological features of IgG4-RPF and iRPF. Owing to the retrospective analysis of the study, data regarding the presence of extraretroperitoneal involvement were incomplete, preventing further comparisons based on organ involvement between patients with IgG4-RPF and iRPF.

In conclusion, the most prominent distinctions between the two subsets of RPF were the presence of tissue eosinophilia and serum IgE concentrations. The serum IgG4

concentration may not be elevated in patients with IgG4-RPF. Serum IgE might be a useful marker for diagnosing and monitoring the disease, especially for patients with normal serum IgG4 concentration.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

References

- Vaglio A, Salvarani C, Buzio C. Retroperitoneal fibrosis. *Lancet* 2006;367:241–51.
- Rossi GM, Rocco R, Accorsi Buttini E, Marvisi C, Vaglio A. Idiopathic retroperitoneal fibrosis and its overlap with IgG4-related disease. *Intern Emerg Med* 2017;12:287–99.
- Takahashi H, Yamamoto M, Suzuki C *et al.* The birthday of a new syndrome: IgG4-related diseases constitute a clinical entity. *Autoimmun Rev* 2010;9:591–4.
- Lian L, Wang C, Tian JL. IgG4-related retroperitoneal fibrosis: a newly characterized disease. *Int J Rheum Dis* 2016;19:1049–55.
- Umehara H, Okazaki K, Masaki Y *et al.* Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 2012;22:21–30.
- Wallace ZS, Deshpande V, Mattoo H *et al.* IgG4-related disease: clinical and laboratory features in one hundred twenty-five patients. *Arthritis Rheumatol* 2015;67:2466–75.
- Deshpande V, Zen Y, Chan JK *et al.* Consensus statement on the pathology of IgG4-related disease. *Mod Pathol* 2012;25:1181–92.
- Koo BS, Koh YW, Hong S *et al.* Clinicopathologic characteristics of IgG4-related retroperitoneal fibrosis among patients initially diagnosed as having idiopathic retroperitoneal fibrosis. *Mod Rheumatol* 2015;25:194–8.
- Khosroshahi A, Carruthers MN, Stone JH *et al.* Rethinking Ormond's disease: "idiopathic" retroperitoneal fibrosis in the era of IgG4-related disease. *Medicine (Baltimore)* 2013;92:82–91.
- Zen Y, Inoue D, Kitao A *et al.* IgG4-related lung and pleural disease: a clinicopathologic study of 21 cases. *Am J Surg Pathol* 2009;33:1886–93.
- Umehara H, Okazaki K, Nakamura T *et al.* Current approach to the diagnosis of IgG4-related disease—combination of comprehensive diagnostic and organ-specific criteria. *Mod Rheumatol* 2017;27:381–91.
- Kamisawa T, Zen Y, Pillai S, Stone JH. IgG4-related disease. *Lancet* 2015;385:1460–71.
- Deshpande V, Gupta R, Sainani N *et al.* Subclassification of autoimmune pancreatitis: a histologic classification with clinical significance. *Am J Surg Pathol* 2011;35:26–35.

- 14 Zen Y, Onodera M, Inoue D *et al.* Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4. *Am J Pathol* 2009;33:1833–9.
- 15 Laco J, Podhola M, Kamarádová K *et al.* Idiopathic vs. secondary retroperitoneal fibrosis: a clinicopathological study of 12 cases, with emphasis to possible relationship to IgG4-related disease. *Virchows Arch* 2013;463:721–30.
- 16 Cheuk W, Chan JK. IgG4-related sclerosing disease: a critical appraisal of an evolving clinicopathologic entity. *Adv Anat Pathol* 2010;17:303–32.
- 17 Sato Y, Kojima M, Takata K *et al.* Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease. *Mod Pathol* 2009;22:589–99.
- 18 Zen Y, Harada K, Sasaki M *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? *Am J Surg Pathol* 2004;28:1193–203.
- 19 Chang DH, Wu PS, Wang YC *et al.* Clinicopathology of immunoglobulin G4-related chronic sclerosing sialadenitis: a single-center study. *Otolaryngol Head Neck Surg* 2016;155:974–81.
- 20 Kamisawa T, Anjiki H, Egawa N, Kubota N. Allergic manifestations in autoimmune pancreatitis. *Eur J Gastroenterol Hepatol* 2009;21:1136–9.
- 21 Torre ED, Mattoo H, Mahajan VS *et al.* Prevalence of atopy, eosinophilia, and IgE elevation in IgG4-related disease. *Allergy* 2014;69:269–72.
- 22 Sterba PM, Alexander V, Eckman JA *et al.* Relationship between allergen-induced basophil histamine release and CD203c stimulation during omalizumab treatment. *J Allergy Clin Immunol* 2009;123:S198.
- 23 Chang HT, Tseng LJ, Hung TJ *et al.* Inhibition of the interactions between eosinophil cationic protein and airway epithelial cells by traditional Chinese herbs. *BMC Syst Biol* 2010;4:S8.
- 24 Imai Y, Yasuda K, Sakaguchi Y *et al.* Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci USA* 2013;110:13921–6.
- 25 Sah RP, Chari ST. Serologic issues in IgG4-related systemic disease and autoimmune pancreatitis. *Curr Opin Rheumatol* 2011;23:108–13.
- 26 Culver EL, Sadler R, Bateman AC *et al.* Increases in IgE, eosinophils, and mast cells can be used in diagnosis and to predict relapse of IgG4-related disease. *Clin Gastroenterol Hepatol* 2017;15:1444–52.

SUPPLEMENTARY DATA

Protocol section

Serial 3- μ m sections were cut from these blocks and stained with hematoxylin-eosin (HE). Observation of storiform fibrosis, obliterative phlebitis and tissue eosinophilia [>5 per HPF (10 $\times$ eyepiece and 40 $\times$ objective lens)] were assessed by two experienced pathologists without knowing any patients' clinical information. In addition the numbers of eosinophil in 5 high power fields was individually counted. Eosinophils counts of ≥ 25 in 5 HPFs were defined as tissue eosinophilia-positive (TEP) group. Eosinophils counts of < 25 in 5 HPFs were defined as tissue eosinophilia-negative (TEN) group.

Immunohistochemistry was performed on paraffin serial sections for IgG⁺ and IgG4⁺ plasma cells. Tissue sections underwent deparaffinized in xylene, rehydrated in ethanol, and immersed in 3% hydrogen peroxide for inhibiting endogenous peroxidase activity. Microwave-heating for antigen retrieval was performed in citrate buffer. Goat serum was used as a blocking agent before adding primary rabbit monoclonal antibodies against human IgG (Abcam, ab109489, 1/300 dilution) and IgG4 (Abcam, ab109493, 1/1000 dilution). After incubated with the primary antibodies at 4 $^{\circ}$ C for 20 hours, secondary antibodies were added in the tissue and react for 40 minutes. Peroxidase-conjugated streptavidin was then applied. Finally, the tissue was visualized using diaminobenzidine and were counterstained with hematoxylin. The number of IgG⁺ and IgG4⁺ plasma cells were counted on the corresponding regions in 3 HPFs (10 $\times$ eyepiece and 40 $\times$ objective lens) with high

density of positive cells, and the IgG4⁺/IgG⁺ plasma cell ratio was calculated.

Supplementary table S1 Serological and imaging differences between histological immunoglobulin G4-positive and negative retroperitoneal fibrosis (RPF) patients

Variables	iRPF	IgG4-RPF	<i>P</i> value
Eosinophils, mean (range)	0.032(0.01-0.22)	0.023(0-0.1)	0.655
Elevated ESR, n (%)	5(31.25%)	16(47.06%)	0.291
Serum ESR concentration,mm/h	37(2-140)	43.39(0-105)	0.320
Elevated CRP, n (%)	6(37.50%)	15(44.12%)	0.763
Serum CRP concentration, mean (range), mg/dl	1.49(0.297-4.1)	2.94(0.296-8.9)	0.408
hypocomplementemia, n (%)	2(12.50%)	2(5.88%)	0.421
Autoantibody, n (%)	1(6.25%)	4(11.76%)	0.920
Serum IgE concentration, mean (range), mg/dl	40.27(7.01-135)	166.11(5.35-618)	0.029
Serum IgG concentration, mean (range), mg/dl			
IgG	1352.50(1000-1850)	1528(1000-1960)	0.222
IgG1	866(497-1170)	831.69(497-1160)	0.671
IgG2	535.5(387-671)	636.13(284-971)	0.369
IgG3	43.93(25.3-66.8)	53.33(8.73-198)	0.887
IgG4	95.73(30.9-135)	178.08(28.1-665)	0.603
Serum creatinine, mean, (range),	198.61(61.7-961.6)	139.91(59.7-703.3)	0.555

μmol/l			
Radiographic Class, n (%)			
I n (%)	6(37.5%)	14(41.18%)	0.804
I + II n (%)	0	1(2.94%)	1.000†
I +III n (%)	7(43.75%)	11(32.35%)	0.434
I +IV n (%)	1(6.25%)	1(2.94%)	0.542†
I , II ,III n (%)	1(6.25%)	1(2.94%)	0.542†
I , II ,III,IV n (%)	1(6.25%)	0	0.320†
I ,III,IV n (%)	0	2(5.88%)	1.000†

Data are reported as n (%) unless otherwise stated. †Fisher exact test. Class I: Soft-tissue density surrounding the infrarenal aorta and/or iliac vessels; Class II: Soft-tissue density surrounding the infrarenal vena cava; Class II: Soft-tissue density surrounding the infrarenal vena cava; Class III: Lateral extension of the inflammation/fibrosis with compression of 1 or both ureters; Class IV: Extension of inflammation/fibrosis to include the renal hilum with compression of the renal artery and/or renal vein. RPF: retroperitoneal fibrosis; iRPF: idiopathic retroperitoneal fibrosis.