The association of different serum IgG4 levels with distinct clinical characteristics and treatment efficacy in patients with IgG4-related disease

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Abstract

Objective

To explore the clinical characteristics and treatment efficacy of IgG4-related disease (IgG4-RD) patients with different levels of serum IgG4.

Methods

A total of 299 patients newly diagnosed with IgG4-RD were enrolled in this study. Patients were classified into four groups according to baseline serum IgG4 levels: Group A: normal concentration; Group B: > normal but <2× the upper reference limit (URL); Group C: between 2× and 5× the URL; Group D: >5× the URL. All patients were followed up for 12 months. The patients' clinical characteristics, laboratory parameters, plasmablasts/plasma cells and treatment efficacy were analysed.

Results

IgG4-RD patients with higher serum IgG4 levels had higher percentages of dacryoadenitis, sialadenitis, and autoimmune pancreatitis and a higher prevalence of allergy history, whereas patients with retroperitoneum and mediastinum lesions usually had lower serum IgG4 levels. In addition, the serum IgG4 re-elevation rate in Group D (19.4%) was higher than those in Group B (4.9%) and Group C (7.7%) (p=0.003 and p=0.020, respectively). Patients suffered fewer clinical relapses with a serum IgG4 reduction ≥50% of baseline serum IgG4 in Group B and ≥40% of baseline serum IgG4 in Group D (p=0.019 and p=0.043, respectively). In addition, the rate of clinical relapse in patients who received combination therapy with glucocorticoids and mycophenolate mofetil was 18.75% in Group D, which was higher than the rates in Groups B and C (0) (p=0.027).

Conclusion

IgG4-RD patients with different levels of serum IgG4 exhibit different clinical characteristics and treatment responses.

Key words

serum IgG4 levels, clinical characteristics, treatment efficacy, IgG4-related disease

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Introduction

Immunoglobulin G4-related disease (IgG4-RD) is an emerging systemic disease characterised by swelling of multiple organs, lymphoplasmacytic infiltration and sclerosis, often combined with elevation of serum IgG4 (1-3). The serum IgG4 level is one of the most important biomarkers in IgG4-RD at present, for both diagnosis and monitoring of the treatment response. A meta-analysis concluded that among various markers, serum IgG4 is a cost-effective, easy and time-efficient assay that can be carried out to detect IgG4-RD(4); other markers include circulating plasmablasts (5, 6), CCL18 (7, 8), follicular T helper cells (9), CD4+ cytotoxic T cells (10, 11), IL2 receptor (12), and autoantibodies such as anti-Annexin A11, anti-Laminin 511 E8 and anti-Galectin-3 (2, 13, 14). In addition, high baseline serum IgG4 and re-elevation of serum IgG4 were reported to be risk factors for disease relapse (15). Although the role of elevated serum IgG4 in IgG4-RD pathogenesis and disease relapse is disputable, until now, there were no better markers for the diagnosis and evaluation of disease stability (16, 17). Serum IgG4 levels are often considered useful in rendering the diagnosis, determining responsiveness to therapy, and predicting the need to adjust treatment (16).

Accumulating studies reported serum IgG4 levels in IgG4-RD and non-IgG4-RD (16), IgG4-related dacryoadenitis/sialadenitis (IgG4-DS) and non-IgG4 DS (16, 18), IgG4-related autoimmune pancreatitis (AIP) and non-IgG4 related pancreatitis (19), and Asian IgG4-RD patients and non-Asian patients (20) and discussed their significance. Paik *et al*. described the clinical and pathological differences between serum IgG4 positivity and negative AIP (21). In a study by Suzuki *et al*., seventy-three patients with AIP were treated with steroids were divided into 3 groups according to their initial serum IgG4 levels: a level 1 group (>2-fold upper limit), a level 2 group (1- to 2-fold upper limit), and a normal group, and they investigated whether a change in serum IgG4 was predictive of AIP relapse during maintenance therapy (22), concluding that a relative rise in serum IgG4 levels after steroid therapy may provide an indication of relapse. However, the above studies did not explore the disease spectrum of IgG4-RD patients with different levels of serum IgG4. Thus, to further understand the association of serum IgG4 with clinical characteristics and treatment response, as well as whether re-elevation of IgG4 level can predict relapse, we classified patients into different groups from low to high according to baseline serum IgG4 levels and analysed the differences in clinical features and treatment efficacy among groups.

Methods

Patient inclusion and exclusion criteria

As shown in Supplementary Figure S1A, 508 patients who fulfilled the 2011 comprehensive diagnostic criteria were enrolled in Peking Union Medical College Hospital. The diagnosis of IgG4-RD was based on the following criteria: (1) a clinical examination showing characteristic diffuse/localised swelling or masses in single or multiple organs; (2) an elevated serum IgG4 concentration (>135 mg/dL); and (3) a histopathologic examination showing (a) marked lymphocytic and plasma cell infiltration and fibrosis or (b) the infiltration of IgG4+ plasma cells (a ratio of IgG4+/IgG+ cells >40% and >10 IgG4+ plasma cells per high-power field) (23). Patients who were treated previously and were followed-up for less than 12 months were excluded.

The 299 eligible patients were classified into four groups according to baseline serum IgG4 levels: Group A: normal serum IgG4 concentration {(66-1400] mg/L}, Group B: serum IgG4 > normal but <2× the upper reference limit {URL, (1400-2800] mg/L}, Group C: serum IgG4 between 2× and 5× the URL {(2800-7000] mg/L}, and Group D: serum IgG4 >5× the URL (>7000 mg/L).

There were 14 (4.7%), 45 (15.1%), 83 (27.8%) and 157 (52.5%) patients in the four groups, respectively. The study protocol was approved by the Ethics Committee of Peking Union Medical College Hospital (no. S-442).

Table I. Baseline characteristics of IgG4-RD patients in Group A to Group D.

Characteristics	Group A (n=14)	Group B (n=45)	Group C (n=83)	Group D (n=157)	*p*-value
Age (years)	39.9 ± 16.2	53.1 ± 12.7	54.5 ± 11.6	54.6 ± 11.5	<0.001*
Male/Female	1:1	1.37:1	1.68:1	1.80:1	0.666
History of smoking, n (%)	0 (0%)	26 (57.8%)	41 (49.4%)	78 (49.7%)	0.273
History of allergy, n (%)	7 (50.0%)	18 (40.0%)	42 (50.6%)	96 (61.1%)	0.065
Disease duration (M [Q1-Q3])	5 (2-16)	12 (3-42)	12 (4-36)	12 (6-44)	0.035*
IgG4-RD RI	8.36 ± 2.92	9.27 ± 4.82	10.73 ± 4.29	13.90 ± 5.47	<0.001*

Data shown as mean±SD or number of cases (percentage) or median (Q1, Q3). M (Q1-Q3): median (interquartile range); IgG4-RD RI: IgG4-Related Disease Responder Index. *p<0.05.

All enrolled patients provided written informed consent.

Clinical data and laboratory parameters

The demographic data included age, sex, history of smoking, history of allergy, disease duration and the IgG4-RD responder index (RI)(24). Complete blood counts, urinalysis, liver and renal function tests, erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (hsCRP), C3 and C4, total IgE (T-IgE), serum immunoglobulin G, A and M and IgG subclasses were obtained. The patients' affected organs were determined by clinical symptoms, physical examinations, histological pathology and imaging, including ultrasound scanning, computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography/computed tomography (PET/CT).

Treatment assessment

Treatment response was assessed by evaluating the changes in IgG4-RD RI scores and was divided into three types: complete response (CR), partial response (PR) and no effect (NE, including no improvement or exacerbation). IgG4-RD RI scores <3 and declining ≥2 were recognised as a CR; IgG4-RD RI scores declining ≥2 but remaining ≥3 were recognised as a PR(25, 26). If the patients' IgG4-RD RI score was 3 points at the beginning, PR was considered as a 1-point decrease after the therapy. Patients with a lack of apparent changes in mass sizes and/or clinical manifestations and an IgG4-RD RI score decline <2 were considered to be NE. Patients who achieved an IgG4-RD RI <3 or decline ≥2 points and successfully completed glucocorticoid tapering without relapse were considered to exhibit persistent disease remission.

Serum re-elevation was defined as an elevated serum IgG4 level and a score increased ≥1 after improvement with treatment compared with the previous serum IgG4 level, without clinical symptoms reappearing or imaging findings worsening (27). The score of serum IgG4 was as follows: normal serum IgG4 concentration, score was 0; serum IgG4 > normal but ≤3× the URL, score was 1; serum IgG4>3× the URL but ≤5× the URL, score was 2; serum IgG4>5× the URL but ≤10× the URL, score was 3; serum IgG4>10× the URL, score was 4.

Clinical relapse was used to evaluate disease activity, which was defined as clinical symptoms reappearing or imaging findings worsening with or without elevated serum IgG4 levels (27).

Flow cytometry

PBMCs from IgG4-RD patients were separated by Ficoll gradient centrifugation. B cell subpopulations were stained with PEcy7-anti-CD19, FITC-anti-CD24, and APC-anti-CD38 (BD Bioscience, USA). Plasmablasts/plasma cells were defined as CD19+CD24-CD38^{hi}.

Statistical analysis

Statistical analyses were performed using IBM SPSS (version 22.0) and Prism software (version 6.1). Continuous, normally distributed data are shown as the mean ± standard deviation (SD) and analysed by Student's t-test (two groups), and one-way analysis of variance (ANOVA) was used for comparisons among groups. The continuous non-normally distributed data were presented as the median (first quartile, third quartile) and analysed by the Rank sum test. The categorical variables were described as rates and percentages and assessed by chi-square or Fisher's exact tests. Correlations between variables were assessed using Pearson's rank test (normally distributed) or Spearman's rank correlation test (nonnormally distributed). Odd ratios (OR) were performed to assess the risk of different levels of serum IgG4 for serum re-elevation or clinical relapse. A two-tailed *p*-value <0.05 was considered statistically significant.

Results

Patient demographics and baseline characteristics

The demographic and baseline characteristics of four groups of patients with IgG4-RD are presented in Table I. Patients in Group A tended to be younger than those in the other three groups. The disease duration in Group D was longer than that in Group A, p=0.006. IgG4-RD RI in Groups A to D was 8.36±2.92, 9.27±4.82, 10.73±4.29 and 13.90±5.47, respectively, gradually increasing from Group A to Group D and highest in Group D (p<0.001). Notably, the prevalence of allergies in Group D was the highest among the four groups, showing a significant difference with Group B (p=0.012). In general, there was a progressive increase in the prevalence of allergies with higher serum IgG4 level.

As presented in Figure 1A, with respect to the organs affected, only 1 (7.1%) patient in Group A had more than three organs affected. Furthermore, the percentage of patients with involvement

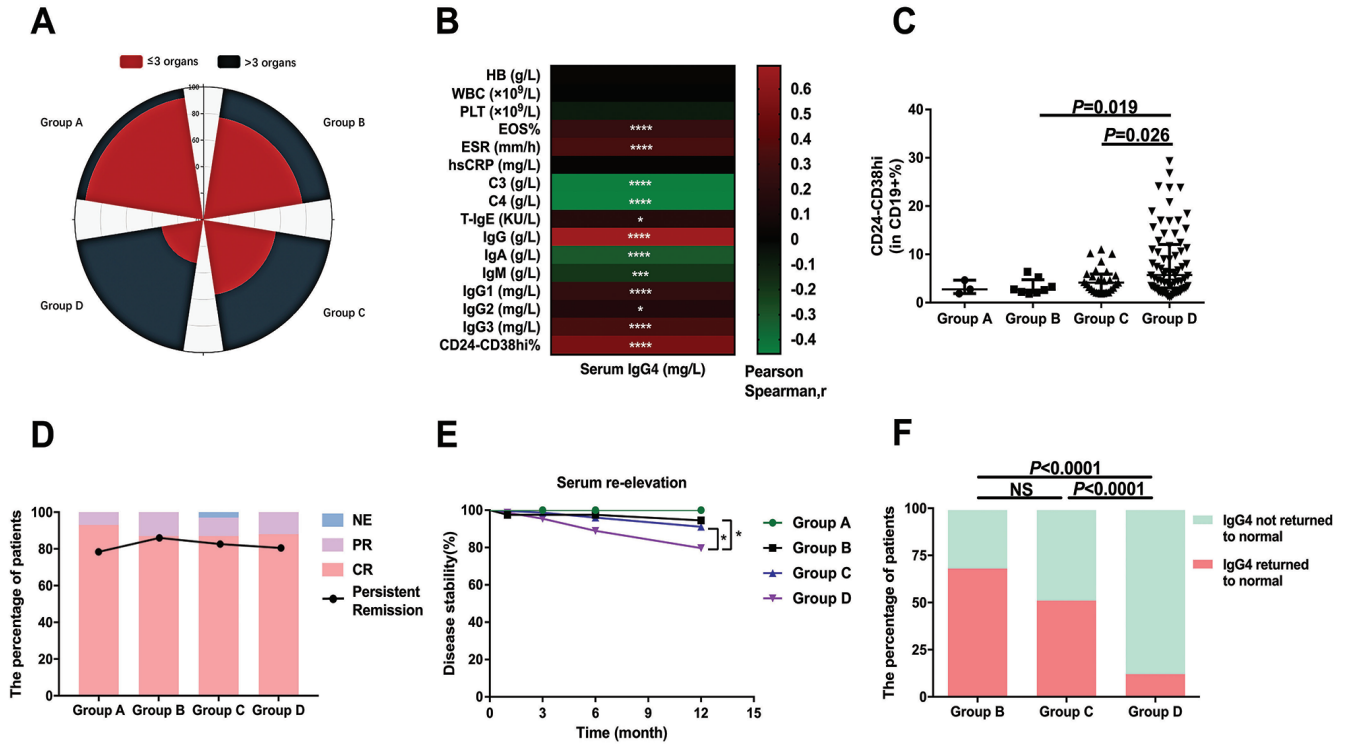

Fig. 1. Baseline characteristics and response to treatment of four groups.
A: The percentage of patients with more than 3 organs affected and ≤3 organs affected in the four groups.
B: Heatmap representation of the correlation of serum IgG4 with laboratory parameters and plasmablasts/plasma cells.
C: The percentage of plasmablasts/plasma cells in the four groups.
D: Treatment response (NE, PR, CR and persistent remission) of four groups.
E: Serum IgG4 re-elevation of patients in each group.
F: The percentage of patients who succeeded or failed to achieve normal serum IgG4 in Group B to Group D at the 12-month follow up.
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 respectively.
HB: haemoglobin; WBC: white blood cell; PLT: platelet; EOS: eosinophil; ESR: erythrocyte sedimentation rate; CRP: high-sensitivity C-reactive protein; Ig: immunoglobulin.; CR: complete response; PR: partial response; NE: no effect.

of more than three organs increased substantially from Group A to Group D; 10 (22.2%) patients in Group B, 36 (43.4%) patients in Group C and 105 (66.9%) patients in Group D had more than three organs affected (p<0.001).

Organ involvement at baseline

Organs involved at baseline in four groups of patients with IgG4-RD are shown in Table II. Of these patients, 102 (65%), 109 (69.4%), 95 (60.5%), and 51 (32.5%) patients in Group D had lymph node, submandibular gland, lacrimal gland and parotid gland involvement, and these values were higher than those in the other three groups and increased greatly with elevation of serum IgG4 (p<0.001). The percentages of AIP were similar in Group C (41.0%) and Group D (41.4%), which were significantly higher than those in Group B (22.2%), p=0.033 and p=0.019, respectively. In addition, the percentage of lung involvement in Group D (32.5%) was the highest among the four groups, with values higher than those in Group A (7.1%, p=0.048), Group B (13.3%, p=0.012), and Group C (15.7%, p=0.005). In addition, the percentages of bile duct lesions in Group D (29.9%) were higher than those in Group B (13.3%), p=0.026. In contrast, the percentage of retroperitoneum lesions in Group D (11.5%) was the lowest, lower than that in Group B (44.4%) and Group C (21.7%) (p<0.001 and p=0.039, respectively). The percentage of mediastinum involvement in Group A (21.4%) was the highest among the four groups, higher than that in Group B (4.4%, p=0.046), Group C (2.4%, p=0.003) or Group D (0.6%, p<0.001). There were no significant differences in prostate, kidney, nasal sinus, thyroid gland, liver, pituitary gland, skin or pachymeningitis involvement between the four groups.

Laboratory parameters

Laboratory tests of the four groups at baseline are listed in Table III. As shown, the percentages of eosinophils, serum IgG, T-IgE, IgG3 and IgG4 in Group D were 4.2 (2.1–7.5), 23.20 (18.38–29.96) g/L, 278.0 (50.7–759.0) KU/L, 517 (280–985) mg/L and 16300 (11550–26500) mg/L, respectively, higher than those in the other three groups (p<0.001, p<0.001, p=0.003, p=0.005 and p<0.001, respectively). Consistently, the percentage of patients with eosinophilia and elevation of serum IgG in Group D (62, 39.5% and 126, 80.3%, respectively) was higher than those in Group A to Group C (p=0.001 and p<0.001, respectively). However, C3 and C4 in Group D were 0.854± 0.309 g/L and 0.142±0.084 g/L, respectively, lower than those in the other three groups (p<0.001). Moreover, compared with the other three groups, more patients in Group D had

Table II. Organ involvement of IgG4-RD patients in Group A to Group D.

Organ involvement, n (%)	Group A (n=14)	Group B (n=45)	Group C (n=83)	Group D (n=157)	*p*-value
Lymph node	9 (64.3%)	16 (35.6%)	37 (44.6%)	102 (65.0%)	0.001*
Submandibular gland	2 (14.3%)	15 (33.3%)	36 (43.4%)	109 (69.4%)	<0.001*
Lacrimal gland	3 (21.4%)	11 (24.4%)	36 (43.4%)	95 (60.5%)	<0.001*
Parotid gland	0 (0%)	2 (4.4%)	12 (14.5%)	51 (32.5%)	<0.001*
Pancreas	0 (0%)	10 (22.2%)	34 (41.0%)	65 (41.4%)	0.097
Bile duct	0 (0%)	6 (13.3%)	16 (19.3%)	47 (29.9%)	0.107
Retroperitoneum	3 (21.4%)	20 (44.4%)	18 (21.7%)	18 (11.5%)	<0.001*
Lung	1 (7.1%)	6 (13.3%)	13 (15.7%)	51 (32.5%)	0.002*
Kidney	0 (0%)	5 (11.1%)	6 (7.2%)	16 (10.2%)	0.720
Prostate	0 (0%)	3 (6.7%)	7 (8.3%)	29 (18.5%)	0.087
Mediastinum	3 (21.4%)	2 (4.4%)	2 (2.4%)	1 (0.6%)	0.002*
Nasal sinus	3 (21.4%)	9 (20.0%)	20 (24.1%)	54 (34.4%)	0.147
Thyroid gland	1 (7.1%)	2 (4.4%)	3 (3.6%)	4 (2.5%)	0.772
Liver	1 (7.1%)	0 (0%)	2 (2.4%)	3 (1.9%)	0.323
Pituitary gland	0 (0%)	0 (0%)	0 (0%)	3 (1.9%)	-
Skin	0 (0%)	0 (0%)	5 (6.0%)	12 (7.6%)	1.000
pachymeningitis	1 (7.1%)	0 (0%)	1 (1.2%)	0 (0%)	0.269

Data shown as number of cases (percentage). *p<0.05.

Table III. Laboratory tests at baseline of IgG4-RD patients in Group A to Group D.

Parameters	Group A (n=14)	Group B (n=45)	Group C (n=83)	Group D (n=157)	*p*-value
WBC ($\times10^9$/L)	8.21 ± 2.92	7.07 ± 2.55	7.15 ± 2.84	7.20 ± 2.32	0.528
HB (g/L)	129 ± 25	127 ± 15	136 ± 20	136 ± 17	0.059
PLT ($\times10^9$/L)	267 ± 87	279 ± 174	228 ± 69	231 ± 65	0.008*
EOS (%)	2.8 (0.7-5.4)	1.6 (0.7-4.1)	3.0 (1.1-4.8)	4.2 (2.1-7.5)	<0.001*
EOS% elevation (n, %)	4 (28.6%)	7 (15.6%)	15 (18.1%)	62 (39.5%)	0.001*
ESR (mm/h)	25 (6-54)	24 (9-46)	16 (4-43)	25 (10-60)	0.040*
hsCRP (mg/L)	2.66 (0.69-21.46)	6.48 (1.43-13.71)	2.08 (0.87-8.35)	1.96 (0.66-5.96)	0.020*
hsCRP elevation (n, %)	6 (42.9%)	27 (60.0%)	29 (34.9%)	49 (31.2%)	0.005*
IgG (g/L)	12.76 (10.89-15.29)	15.4 (12.36-18.04)	14.80 (12.19-18.60)	23.20 (18.38-29.96)	<0.001*
IgG elevation (n, %)	2 (14.3%)	16 (35.6%)	24 (28.9%)	126 (80.3%)	<0.001*
IgA (g/L)	2.66 (1.84-3.50)	2.47 (1.69-3.48)	1.99 (1.40-2.65)	1.66 (1.18-2.12)	<0.001*
IgM (g/L)	1.42 (0.80-1.94)	1.04 (0.64-1.47)	0.79 (0.55-1.25)	0.70 (0.48-1.01)	0.003*
T-IgE (KU/L)	53.5 (25.4-106.5)	136.0 (76.7-301.3)	230.0 (60.0-533.0)	278.0 (50.7-759.0)	0.003*
IgG1 (mg/L)	7820 (6378-9698)	8822 (7660-10000)	7975 (6568-9963)	9040 (7230-10600)	0.091
IgG2 (mg/L)	4010 (2908-6593)	5980 (4388-7385)	6630 (4883-8085)	5805 (4365-7642)	0.454
IgG3 (mg/L)	271 (188-432)	443 (225-560)	406 (227-646)	517 (280-985)	0.005*
IgG4 (mg/L)	505 (259-908)	2070 (1660-2410)	4480 (3440-5480)	16300 (11550-26500)	<0.001*
C3 (g/L)	1.181 ± 0.286	1.086 ± 0.280	1.022 ± 0.264	0.854 ± 0.309	<0.001*
C3 reduction (n, %)	0	3 (9.7%,3/31)	4 (6.7%,4/60)	31 (33.7%,31/92)	<0.001*
C4 (g/L)	0.231 ± 0.129	0.242 ± 0.092	0.187 ± 0.070	0.142 ± 0.084	<0.001*
C4 reduction (n, %)	1 (11.1%,1/9)	0	6 (10%,6/60)	28 (30.4%,28/92)	0.008*

Data shown as mean ± SD or number of cases (percentage) or median (interquartile range).
HB: haemoglobin; WBC: white blood cell; PLT: platelet; EOS: eosinophil; ESR: erythrocyte sedimentation rate; CRP: high-sensitivity C-reactive protein; Ig: immunoglobulin. *p<0.05.

low C3 (31, 33.7%) and low C4 (28, 30.4%) (p<0.001 and p=0.008, respectively). Patients in Group B had higher levels of hsCRP [6.48 (1.43–13.71) mg/L] and PLT (279±174×10^9/L) than those in the other three groups (p=0.020 and p=0.008, respectively). Similarly, more patients in Group B (27, 60%) than in Group A (6, 42.9%), Group C (29, 34.9%) or Group D (49, 31.2%), had an elevation of hsCRP (p=0.005). As shown in Figure 1B, serum IgG4 was positively correlated with EOS%, ESR, serum T-IgE, IgG, IgG1, IgG2 and IgG3 (r=0.2548, p<0.0001; r=0.3283, p<0.0001; r=0.1685, p=0.0136; r=0.6922, p<0.0001; r=0.2362, p<0.0001; r=0.1471, p=0.0123; and r=0.3399, p<0.0001; respectively) and was negatively correlated with C3, C4, serum IgA and IgM (r=-0.4511, p<0.0001; r=-0.4564, p<0.0001; r= -0.313, p=0.0004; and r=-0.1962, p=0.001; respectively).

Plasmablasts/plasma cells

Plasmablasts/plasma cells were evaluated in 109 IgG4-RD patients. The percentage of plasmablasts/plasma cells was the highest in Group D [5.71% (3.04–12.05%)], higher than that in Group B [2.68% (2.11–4.76%), p=0.019] or Group C [4.14% (2.38–5.90%), p=0.026] (Fig. 1C). Interestingly, as shown in Figure 1B, this parameter was positively correlated with serum levels of IgG4 (r=0.532, p<0.0001).

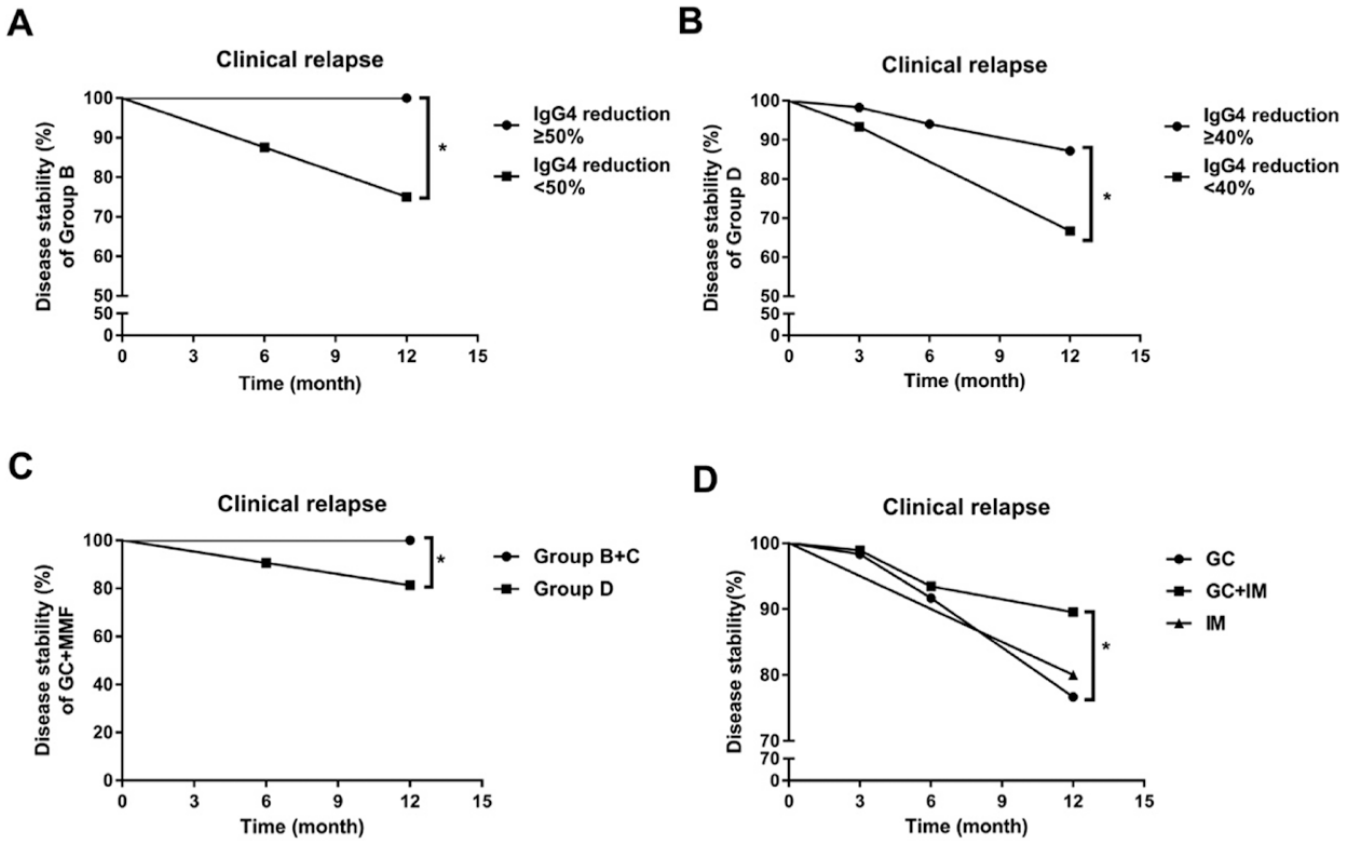

Fig. 2. Treatment strategy of IgG4-RD patients in the four groups and relapse rate after 12 months of treatment.
A: Clinical relapse rate of patients with a serum IgG4 level reduction ≥50% *vs*. <50% of baseline in Group B. **B**: Clinical relapse rate of patients with a serum IgG4 level reduction ≥40% *vs*. <40% of baseline in Group D. **C**: Clinical relapse rate of patients treated with GC+MMF in Group B and C *vs*. Group D. **D**: Clinical relapse rate of patients treated with GC monotherapy, IM monotherapy and GC+IM combination therapy in Groups B, C and D. *p<0.05. GC: glucocorticoids; MMF: mycophenolate mofetil; IM: immunosuppressive agent.

Therapeutic strategy and treatment efficacy

The therapeutic strategies were classified into four types: no treatment, glucocorticoid (GC) monotherapy, immunosuppressant (IM) monotherapy and GC combined with IM (GC+IM). IM used more than 6 months was defined as IM therapy. The treatment strategies of the four groups with one year of follow-up are shown in Supplementary Figure S1B. There was no significant difference in treatment strategies among the four groups.

As shown in Figure 1D, of 299 patients, 262 (87.6%) patients achieved CR, 34 (11.4%) patients achieved PR, and 3 (1.0%) patients had NE. The number of patients who achieved CR in Group A to Group D was 13 (92.9%), 39 (86.6%), 72 (86.7%) and 138 (87.9%), respectively. The number of patients with PR in Group A to Group D was 1 (7.1%), 6 (13.4%), 8 (9.7%) and 19 (12.0%), respectively. In addition, 3 (3.6%) patients in Group C had NE. By comparison, there was no significant difference in CR and PR among the four groups. During the 12-month follow-up, 246 (82.3%) patients achieved persistent remission, which was 78.6%, 86.7%, 83.1% and 80.9% from Group A to Group D, respectively (p=0.807).

Serum re-elevation and clinical relapse were used to evaluate clinical outcome according to different treatment strategies (patients who did not receive treatment and who experienced disease relapse due to self-withdrawal of drugs were excluded). The serum re-elevation rate and the risk assessment of serum re-elevation in the four groups are presented in Figure 1E and Supplementary Figure S1C. The serum re-elevation rate differed between Group B (4.9%) and Group D (19.4%), p=0.003, as well as between Group C (7.7%) and Group D (19.4%), p=0.020. The OR of serum re-elevation was higher in Group D than in Group B or Group C, with values of 1.159 (95% CI 1.035–1.297, p=0.031) and 1.137 (95% CI 1.026–1.260, p=0.021). There was no significant difference in the clinical relapse rate or the OR of clinical relapse among the four groups (data not shown). As shown in Figure 1F, the percentage of patients whose serum IgG4 returned to normal and those who did not return to normal was evaluated in Group B, Group C and Group D at the 12th month follow up. In a total of 285 patients (Group A was excluded), 33.0% of patients had normal serum IgG4 at the 12th month follow-up. Thirty-one (68.9%) patients in Group B, 43 (51.8%) patients in Group C and 20 (12.7%) patients in Group D had persistent normal serum IgG4 levels during the 12 months of follow-up (p<0.0001). In addition, there were significant differences between Group B and Group D and between Group C and Group D, p<0.0001, respectively.

To further investigate the relationship between the maximum extent of serum

IgG4 reduction and clinical relapse, we compared the clinical relapse rate of patients within each group (Group A excepted) with serum IgG4 level reductions ≥30%, 40%, 50% and 60% *vs*. <30%, 40%, 50% and 60% of baseline, respectively. We found that the maximum extent of serum IgG4 reduction varied among groups. As shown in Figure 2A, the clinical relapse rate of patients with a serum IgG4 level reduction ≥50% in Group B (0%) was lower than that of patients with a serum IgG4 level reduction <50% (25%, p=0.019). Figure 2B shows that the clinical relapse rate of patients with a serum IgG4 level reduction ≥40% in Group D (12.82%) was lower than that of patients with a reduction <40% (33.33%, p=0.043). However, there was no significant difference in clinical relapse in Group C regardless of the alteration of serum IgG4 levels after treatment (data not shown).

To explore whether the serum IgG4 level was related to treatment outcome, we compared the clinical relapse rate of the same treatment strategy in Group B to Group D among patients who received GC, GC+ cyclophosphamide (CTX) and GC+ mycophenolate mofetil (MMF) therapy. As shown in Figure 2C, the clinical relapse rate of patients treated with GC+MMF in Group D (18.75%) was higher than that in Group B and Group C (0%), p=0.027. However, there was no significant difference in the clinical relapse rate in patients treated with GC or GC+CTX therapy (Suppl. Fig. S1D-E).

The clinical relapse rate of 299 IgG4-RD patients treated with different strategies is shown in Figure 2D. The clinical relapse rate in patients who received GC+IM was lower than in patients who received GC monotherapy (p=0.015).

Discussion

In this study, to clarify the differences and similarities between patients with different levels of serum IgG4, we compared the clinical manifestations and treatment responses in IgG4-RD patients grouped by serum IgG4 from a large prospective cohort in China.

Patients with normal serum IgG4 levels were younger than patients with elevated serum IgG4 levels. This result was consistent with a previous American cohort (28). The number of affected organs increased with ascending serum IgG4 levels and was highest in Group D. In addition, patients in Group D presented the highest circulating CD19+CD24-CD38^{hi} plasmablasts/plasma cells, which correlated positively with serum IgG4 levels. In addition, increases in the percentage of allergy history, elevated EOS% and serum levels of IgE were observed from Group B to Group D. Increasing evidence indicated that >5 organs affected (29), dacryoadenitis (29), circulating plasmablasts/plasma cells (5, 6, 30), eosinophilia (29, 31) and high serum IgE (31, 32) were risk factors for disease relapse. Suzuki *et al*., Sasaki *et al*. and our previous study also concluded that IgG4-RD clinical relapse is often associated with higher baseline serum levels of IgG4 (15, 22, 29). This may indicate that patients with baseline higher serum IgG4 levels are more inclined to relapse and need more aggressive treatment strategies to maintain disease stability.

In recent years, about 20–60% of IgG4-RD patients were reported to have a history of allergy (33, 34), however, the relationship among allergy, serum IgE and serum IgG4 levels still require further investigation. Saeki *et al*. revealed that high serum levels of IgE and IgG4 were common features of IgG4-RD with/without allergy (35). However, our study indicated that patients with higher serum IgG4 levels had a higher prevalence of allergies. Both Liu *et al*. and our previous study have found that the prevalence of allergies was higher in IgG4-DS patients, among which group the high level of serum IgG4 and IgE could be noticed (18, 36). Serum IgE tended to increase simultaneously with serum IgG4 and IgG4 was proved to act as an inhibitor of IgE in some allergic responses (37). In addition, serum IgE might be potentially used in diagnosis (86% specificity, 36% sensitivity) and predicting relapse of IgG4-RD (88% specificity, 64% sensitivity), highlighting that an IgE-mediated allergic response occurs in some patients(32). Therefore, we speculate that treatment targeting allergy may be beneficial in IgG4-RD patients with a history of allergy. Moreover, further research should be undertaken to further elucidate the mechanism of allergic reactions in IgG4-RD.

Patients in Group D had a higher percentage of submandibular gland, lacrimal gland and parotid gland involvement. In addition, patients in Group D also had a higher percentage of pancreas/bile duct, lung and prostate involvement, consistent with our previous finding that patients with IgG4-DS with higher levels of serum IgG4 had a higher possibility of lung and prostate involvement (18). However, patients with lower serum IgG4 had a higher prevalence of retroperitoneum and mediastinum involvement, indicating that different pathogenic subtypes of IgG4-RD have different spectra of affected organs.

A total of 27.4–67% patients were reported to have elevated hsCRP levels (25, 28, 33, 34, 38). In our study, 37% of patients had an elevated hsCRP level, which was more common in patients with retroperitoneal involvement, illustrating more prominent inflammation in the circulation of patients with an affected retroperitoneum. More patients in Group D had C3 and C4 reduction. Fukui S *et al*. also observed that serum C5a levels were high during active disease and low during remission in patients with IgG4-RD (39). Further studies are required to determine whether complement could be a biological marker or a therapeutic target.

IgG4-RD patients in Group D than in Group B or Group C had a higher prevalence of re-elevation of serum IgG4 during GC tapering. After 1 year of treatment, approximately one-third of our patients achieved normal serum IgG4, and it was more difficult for patients with higher baseline serum IgG4 to achieve normal serum IgG4. After treatment, serum IgG4 in Group D posed a major challenge to achieve normal level, and patients with a reduction of serum IgG4 ≥40% of the baseline level in the maintenance stage were less likely to suffer clinical relapse. Our previous study indicated IgG4-RD patients with higher baseline serum IgG4 had difficulty in achieving normal level after treatment(18). More prospective studies

with larger sample sizes and multicentre are needed to verify the significance of serum re-elevation in the future.

In general, glucocorticoids were recognised as the first-line treatment strategy for IgG4-RD. However, Hart *et al*. and our previous studies indicated a lower clinical relapse rate in patients treated with combination therapy with GC+IM than in those treated with GC monotherapy (26, 27, 40). In IgG4-RD patients treated with the GC+MMF strategy, patients with higher baseline levels of serum IgG4 were more inclined to suffer clinical relapse, indicating that higher baseline serum IgG4 levels were a risk factor for relapse that needed a more aggressive therapy strategy. Because the therapy strategy in Group D was more aggressive than those in the other groups, there was no significant difference in CR and PR among the four groups. In addition, IgG4-RD patients with long-term GC maintenance therapy combined with/without IM have been reported to have a lower recurrence rate than those who withdraw treatment (41, 42). Therefore, to avoid relapse, maintenance therapy was an option. To date, the regimens of maintenance therapies for IgG4-RD patients include GC monotherapy and GC combined with IMs and rituximab therapy (43, 44). IgG4-RD patients in our cohort had maintenance therapy, especially in Group D, for clinical relapse, and the serum re-elevation rate was high.

There are some limitations of this study. First, this is a single-centre study. Second, the number of patients in our study was relatively small, especially in Group A. Third, the follow-up duration was 12 months. A longer follow-up period is needed to evaluate disease relapse more accurately and comprehensively.

In conclusion, distinct clinical characteristics occurred among patients with different serum IgG4 levels. In addition, the serum re-elevation rate was higher in patients with higher baseline serum IgG4 levels, and aiming to decrease serum IgG4 levels ≥40% may be beneficial for disease stability. Finally, combined therapy with glucocorticoids and immunosuppressants led to a lower relapse rate in IgG4-RD.

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