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Original article

Predictors of disease relapse in IgG4-related disease following rituximab

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Abstract

Objective. IgG4-related disease (IgG4-RD) is a relapsing–remitting condition responsible for fibroinflammatory lesions that can lead to organ damage and life-threatening complications at nearly any anatomical site. The duration of remission following treatment varies and predictors of relapse are unclear. The objectives of this study were to review our experience with rituximab as remission induction in IgG4-RD, to clarify the duration of efficacy and to identify predictors of flare following treatment.

Methods. In this retrospective cohort study, all patients were treated with two doses of rituximab (1 g) separated by 15 days. Clinical, radiographic and laboratory data pertaining to rituximab response and disease relapse were collected from the electronic medical record. Kaplan–Meier curves were constructed to estimate the time to disease relapse. Log-rank analyses were performed to compare times to relapse among subgroups. Potential relapse predictors were evaluated with Cox regression analysis.

Results. Fifty-seven of 60 patients (95%) had clinical responses to rituximab. Forty-one patients (68%) were treated without glucocorticoids. Twenty-one patients (37%) experienced relapses following treatment at a median time from the first infusion of 244 days. Baseline concentrations of serum IgG4, IgE and circulating eosinophils predicted subsequent relapses, with hazard ratios of 6.2 (95% CI: 1.2, 32.0), 8.2 (95% CI: 1.4, 50.0) and 7.9 (95% CI: 1.8, 34.7), respectively. The higher the baseline values, the greater the risk of relapse and the shorter the time to relapse. Only 10% of the patients had elevations of all three major risk factors, underscoring the importance of measuring all three at baseline.

Conclusion. Baseline elevations in serum IgG4, IgE and blood eosinophil concentrations all predict IgG4-RD relapses independently.

Key words: IgG4-related disease, rituximab, IgG4, IgE, eosinophil, biomarker, relapse, remission, IgG4RD Responder Index

CLINICAL SCIENCE

Rheumatology key message

- Measurements of IgG4, IgE and circulating eosinophils are useful predictors of relapses in IgG4-related disease.

Introduction

IgG4-related disease (IgG4-RD) is a systemic fibroinflammatory condition of unclear aetiology that can affect nearly any anatomical site [1, 2]. Untreated disease can lead to permanent organ damage and life-threatening complications [3–7]. The gold standard for the diagnosis of IgG4-RD is its characteristic histopathology accompanied by a significant infiltrate of $IgG4^+$ plasma cells [8].

Both glucocorticoids and rituximab are effective at inducing remission in IgG4-RD [6, 9–12]. The rationale for therapeutic peripheral B cell depletion in IgG4-RD

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stems from several observations, including the finding of elevated circulating plasmablasts in patients with active disease [13, 14]; the swift diminution of plasmablasts following rituximab administration [13, 14]; their subsequent reconstitution with relapse [13]; and some evidence of autoantibody production in IgG4-RD [15].

Although the majority of patients respond to treatment with either glucocorticoids or rituximab, neither approach cures the disease [11]. Rather, IgG4-RD often follows an unpredictable relapsing pattern, and the optimal strategy for maintenance therapy (e.g. low-dose glucocorticoids), retreatment with rituximab, or other is unclear [16, 17]. A better understanding of relapse predictors is important for optimal disease management. We examined predictors of disease relapse in 60 IgG4-RD patients with diverse organ involvement who were treated with rituximab.

Methods

Cohort overview and patient selection

This study was approved by the Massachusetts General Hospital Ethics Committee. All patients signed written informed consent. We included all consecutive patients with biopsy-proven disease who had undergone treatment with rituximab for the first time and had at least 2 months of follow-up (n = 60).

The extent of organ involvement was determined by review of the patient's history, physical examination, imaging and laboratory studies, and biopsies. Multiorgan disease indicates involvement of three or more organs/anatomical sites. Damage was determined through an organ-by-organ assessment of function, radiographic abnormalities, surgical intervention or chronic pain. Disease activity was assessed by the IgG4-RD responder index (RI) [18, 19]. Because of the recent awareness of the shortcomings of serum IgG4 concentration as a biomarker [19], we report RI scores without serum IgG4. An RI of $\geqslant 1$ was classified as active disease.

Pathology methods

All patients had at least one positive tissue biopsy reviewed at our centre. The major histopathology features of IgG4-RD include a dense lymphoplasmacytic infiltrate, fibrosis with a storiform pattern and obliterative phlebitis [8]. At least two of these features were present in all cases. In immunohistochemistry studies, we considered >10 $IgG4^+$ plasma cells/high power field and an $IgG4^+$:IgG^+ ratio $\geqslant 0.40$ to be supportive of the diagnosis. Rigorous clinicopathological correlation was used to exclude alternative diagnoses.

Serum IgG4 assays and other laboratory assessments

Serum IgG4 concentrations were measured by immunonephelometry using standardized procedures to avoid the prozone effect [20]. Circulating $CD19^+$ cells and circulating plasmablasts before and after rituximab treatment were measured by flow cytometry [13]. Laboratory assessments were performed at baseline and at 3–6 months.

Rituximab administration

All patients were treated with two rituximab infusions separated by ~15 days. To reduce infusion reaction risks, each dose was administered with acetaminophen 650 mg, diphenhydramine 25 mg and methylprednisolone 100 mg. More than two-thirds of the patients were treated without concomitant glucocorticoids except for the methylprednisolone accompanying each infusion. Patients on prednisone at baseline were tapered to discontinuation, generally within 12 weeks.

Assessing treatment response and disease relapse

Treatment response was assessed by evaluating changes in the patient's IgG4-RD RI. Both the provider's objective evaluation (e.g. reduced salivary gland swelling, improved liver function tests) and the patient's subjective report (e.g. reduced pain, reduced narcotic requirement, successful glucocorticoid tapering) contributed to the determination of the clinical response. Clinical responses were defined as a decline in the IgG4-RD RI of at least two points over baseline [9]. Follow-up imaging as appropriate for clinical purposes was assessed for improvement or worsening compared with the baseline study and incorporated into the overall assessment of individual organ activity in the IgG4-RD RI. All patients who had a clinical response to treatment were considered to be at risk for subsequent relapse. Relapse was defined by the new development or return of abnormal findings on physical examination, laboratory tests that reflected IgG4-RD activity within specific organs, or imaging studies. The date of relapse was either the date of symptom onset or new or worse physical examination, laboratory or radiology findings. An isolated increase in the serum IgG4 concentration did not constitute a disease relapse.

Potential predictors of disease relapse

The potential predictors of disease relapses evaluated represent the known clinical, demographic, serological and flow cytometry parameters that were considered most likely to be associated with relapses. Prior studies have suggested that these variables are potential relapse predictors [13, 16, 21].

Statistical analysis

All statistical tests were performed using IBM SPSS Statistics (Version 22, IBM, Armonk, NY, USA). Statistical differences were analysed by Student's *t*-test, the appropriate non-parametric test, or Fisher's exact test, depending on the variable. Kaplan–Meier curves were used to assess time to relapse. Times to relapse in subgroups were compared using the log-rank test. For all analyses of potential predictors of relapse, we included the 57 patients who responded to rituximab therapy. For selected potential predictors of disease relapse, Kaplan–Meier curves were constructed to compare the

time to relapse in the highest quartile with that of the lowest quartile.

Cox regression was used to estimate hazard ratios (HRs) of relapse, adjusted for age, sex and time to censoring. The inclusion of additional variables in the multivariate analysis was limited by the number of relapses observed [22, 23] and the noted co-linearity among candidate biomarkers. Age, number of involved organs, disease duration and RI score were evaluated as continuous variables. Sex and multi-organ disease were evaluated as categorical variables. Serum IgG4 and IgE concentrations, circulating plasmablast and eosinophil levels, and acute phase reactants (ESR and CRP) were divided into quartiles. The lowest quartile was treated as the reference category. Trends in relapse risk across categories of these candidate biomarkers were assessed using Cox proportional hazards models by using the median values of intake for each category to minimize the influence of outliers. A $P < 0.05$ was considered significant for all statistical testing.

Results

Baseline features

The 60 subjects' demographic and baseline features are shown in Table 1. The patients treated with rituximab had a mean (S.D.) age of 55.9 (13.2) years (range: 24–83 years). The majority (75%) were non-Hispanic whites; two-thirds were male.

The mean disease duration prior to treatment was 6.1 (8.2) years. The majority of patients had two or more organs/anatomical sites involved [mean 2.4 (1.4) organs]. The most commonly involved organs/anatomical sites were the salivary glands, orbital structures, pancreas and retroperitoneum (Fig. 1). Damage was present in 60% of patients at baseline. The mean RI score was 6.6 [4.2, range 1 (18)]. Thirty-one (54%) of the patients had an elevated baseline serum IgG4 concentration at the time of treatment with rituximab.

Previous treatment with glucocorticoids

Twenty-three of the 60 patients (38%) had previously untreated disease. Thirty-four (57%) of the patients had been treated previously with glucocorticoids, 19 (57%) of whom improved but relapsed after discontinuing treatment. Eight (23%) relapsed during glucocorticoid tapers. Five (15%) had not improved with glucocorticoid treatment.

Disease response

Forty-one (68%) of the patients were treated without concomitant glucocorticoids. Flow cytometry was performed in 51 of 60 patients at the time of the second rituximab infusion. All 51 were peripherally B cell-depleted based on a quantitative assessment of circulating $CD19^+CD20^+$ lymphocytes. Clinical responses to rituximab treatment were observed in 57 (95%) of the patients. Among the 31 patients with elevated baseline serum IgG4 concentrations, all had declines in IgG4 concentrations after treatment. Serum IgG4 concentrations normalized in 13 (42%) of these patients within 7 months of treatment without additional therapy. However, only 2 (15%) of the 13 patients in the highest quartile of serum IgG4 at baseline normalized their values within this time period. Of the 35 patients with repeat imaging studies following rituximab treatment, 24 (69%) had radiological improvement and 11 (31%) had stability of their lesion(s) described by the original radiology interpretation of the studies.

Table 1 Characteristics of 60 patients treated with rituximab

Age at evaluation, mean (S.D.), years	55.9 (13.2)
Male, n (%)	40 (67)
% White	75
Baseline responder index, mean (S.D.)	6.6 (4.2)
Number of organs, mean (S.D.)	2.4 (1.4)
Multi-organ disease ($\geqslant$3 organs), n (%)	24 (40)
Disease duration, mean (S.D.), years	6.1 (8.2)
Prior treatment with glucocorticoids, n (%)	34 (57)
Damage at baseline, n (%)	36 (60)
Elevated IgG4 concentration at baseline, n (%)	31 (54)
Elevated IgG4 among untreated patients, n (%)	24 (63)
Serum IgG4, median (IQR), mg/dl	139 (51–459)
Plasmablast level, median (IQR), n/ml	3054 (1111–4988)
$IgG4^+$ plasmablast level, median (IQR), n/ml	2016 (557–4631)
Serum IgE, median (IQR), mg/dl	73 (23–323)
Absolute eosinophil count, median (IQR), n/mm^3	220 (80–510)
Treated without glucocorticoids, n (%)	41 (68)
Median time to glucocorticoid discontinuation, median (IQR), days	87 (65–166)
Response to rituximab, n (%)	
Clinical response, n (%) (n = 60)	57 (95)
Radiographic appearance stable or improved (n = 32)	32 (100)
Serum IgG4 concentration decline (n = 30)	30 (100)
Normal serum IgG4 during follow-up (of those with an elevated baseline)	13 (42)
Median time to censoring, median (IQR), days	274 (180–450)
Flare following first RTX, n (%)	21 (37)

IQR: Interquartile range.

Serum IgG4 concentrations and other follow-up laboratory studies

Table 2 compares the patients' laboratory features before and after rituximab treatment. Follow-up tests were obtained 152 (49) days after treatment. Both the total serum IgG and all IgG subclass concentrations were lower following rituximab treatment, but only the percentage decline in the total serum IgG and serum IgG4 concentrations achieved statistical significance (Table 2). The median IgG4 concentration fell from 258 mg/dl [interquartile range (IQR): 207–828] before treatment to 126 mg/

FIG. 1 Baseline organ involvement in the treatment cohort

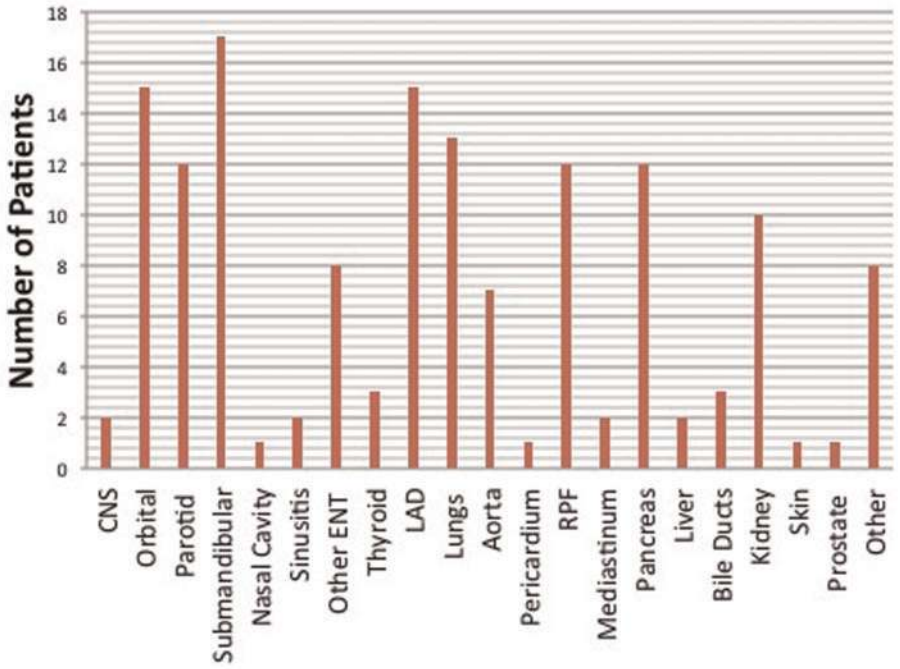


TABLE 2 Laboratory findings before and after rituximab

Test	Normal	Baseline among all treated, median (IQR)	Pre-RTX, median (IQR)[a]	Post-RTX, median (IQR)[a]	P-value[b]	Average % change (S.D.)[c]	95% confidence interval	P-value[b]
IgG4, mg/dl	2.4–135	139 (51–459)	258 (207–827.9)	126 (86–342)	<0.001	−42.2 (30.7)	−51.2, −33.4	<0.001
Total IgG	767–1590	1290 (1029–1690)	1,383 (1110–1973)	1360 (1130–1430)	0.001	−13.2 (23.9)	−20.4, −5.7	0.001
IgG1	341–894	628 (521–990)	756 (521–1120)	761 (550–784)	0.005	+9.0 (131.3)	−30.9, +48.9	0.65
IgG2	171–632	411 (310–587)	411 (341–1000)	473 (202–521)	0.01	−4.7 (59.9)	−13.5, +23	0.60
IgG3	18.4–106	74.8 (45–114)	76.6 (40.2–141.0)	36.5 (23–92.2)	0.02	+16.8 (202.5)	−44.8, +78.3	0.59
IgE	0–100	72.5 (23–322.5)	122 (118–479)	79 (47–269)	<0.001	−48.6 (27.2)	−61.7, −35.5	<0.001
Plasmablast, n/mm^3	N/A		3572 (2160–4785)	351 (235–1287)	<0.001	−75.4 (20.6)	−84.9, −65.9	<0.001
ESR, mm/h	<20	19.5 (8.5–60)	60 (31–72)[d]	17 (14–39)[d]	<0.001	−51.8 (32.4)	−66.2, −37.4	<0.001
CRP, mg/l	<8.0	5.4 (1.7–12.1)	44.2 (12.1–94)[d]	5.7 (2.4–11.8)[d]	0.001	−62.5 (36.7)	−84.7, −40.2	<0.001
C3, mg/dl	86–184	114 (90–129)	100 (74–120)	124 (99–132)	0.94			
C4	16–38	23 (14–28)	23 (13–27)	26 (22–32)	0.21			
Abs eos, n/mm^3	0.0–900	220 (80–510)	400 (110.0–770.0)	210 (160–560)	0.04			

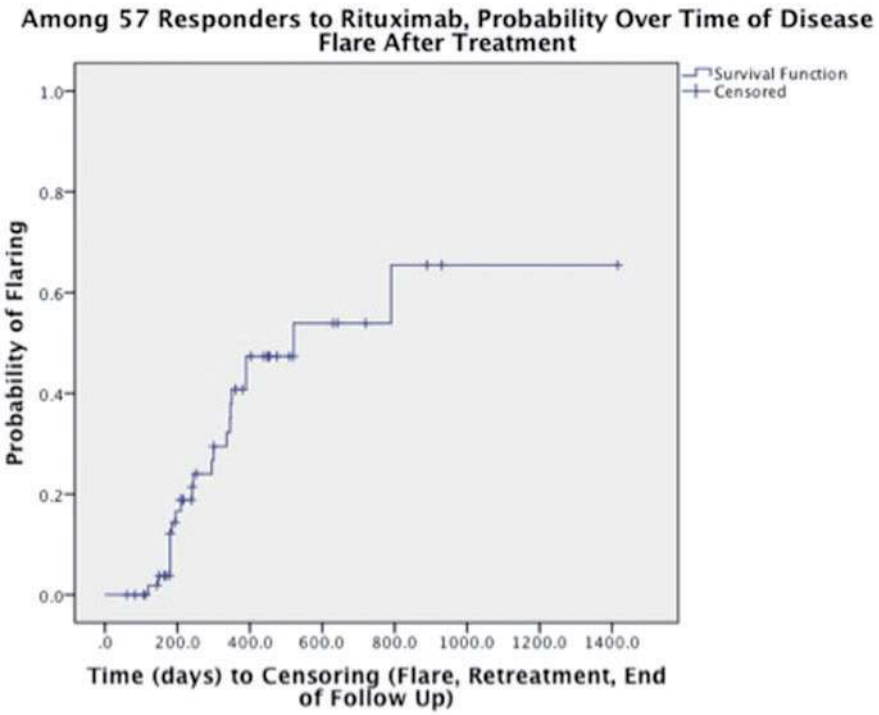
Abs eos: absolute eosinophil count. n: number. [a]Includes only those with pre- and post-values. [b]Comparison of pre- with post-RTX median by Wilcoxon signed rank test and of percentage change by *t*-test. [c]Using paired-sample *t*-test, difference in percentage change between IgG4 and IgG was significant ($P < 0.0001$). The degree of blood plasmablast decline was significantly greater than that of the serum IgG4 concentration ($P = 0.0001$). [d]Includes only those with baseline elevations of ESR or CRP.

dl (IQR: 86–342) after treatment. The percentage change in the serum IgG4 concentration was steep [−42% (31%)], greater than that observed for the total IgG concentration [−13% (24%); $P < 0.0001$].

The plasmablast level (n = 19) declined even more steeply than the serum IgG4 concentration. The median baseline plasmablast level fell from 3572/mm^3 (IQR: 2160–4785) to 351/mm^3 (IQR: 235–1287) after rituximab, for a mean decline of 76% (21%) ($P = 0.0001$ compared with percentage change in the serum IgG4 concentration).

Fifty-two patients had serum IgE concentrations measured at baseline and the IgE concentrations were elevated in 24 (46%). Among the 24 patients with IgE elevations, 75% also had elevations of serum IgG4. Among the patients with IgE elevations at baseline, the median concentration fell following treatment from 122 mg/dl (118–479) at baseline to 79 mg/dl (47–269)

FIG. 2 Probability of disease relapse over time following rituximab response



($P < 0.001$). Absolute peripheral eosinophilia (>500/mm^3) was present in 26% of patients, more than half of whom (53%) had both eosinophilia and an elevated serum IgG4 concentration. Among the patients with absolute peripheral eosinophilia at baseline, the median absolute eosinophil count did not change significantly after treatment, actually increasing from 747/mm^3 (616–840) to 790/mm^3 (560–1120) ($P = 0.59$) afterwards.

Finally, changes in acute phase reactants occurred following treatment. Among the 28 patients with baseline ESR elevations, the median ESR declined from 60 mm/h (31–72) to 17 mm/h (14–39) ($P < 0.001$). Among the 16 patients with elevated CRP levels, the median baseline CRP declined from 44.2 mg/l (12.1–94) to 5.7 mg/l (2.4–11.8) following rituximab ($P = 0.001$).

Patients who failed to respond to rituximab

Three patients did not respond to rituximab. All three appeared to have a low *a priori* likelihood of responding because of their clinical characteristics. One was a 39-year-old woman with a history of multi-organ disease who was treated for Riedel's thyroiditis. The thyroid examination at baseline revealed an apple-sized gland with a woody hardness to palpation. Rituximab was administered as a final effort to obviate complex surgery, which was ultimately required because of advanced fibrosis compressing the trachea that failed to respond. The second was a 63-year-old woman who had a 4-year history of painful swelling of the orbit that did not respond to glucocorticoids. The patient underwent surgical debulking of the orbital pseudotumour after two courses of rituximab did not lead to improvement. Finally, a 27-year-old woman with 3 years of midline destructive disease experienced enlargement of her palate defect following treatment.

Disease flares and time to disease relapses

The incidence rate of relapse was 0.39 per person-year. Figure 2 shows the cumulative probability of having a disease relapse following rituximab. Following the first rituximab infusion, patients were followed for a median of 452 days (range: 61–1952; IQR: 240–880). The median time to censoring (relapse, retreatment or end of follow-up) was 252 days (IQR: 180–444). Over this period, 21 (37%) of the 57 responders experienced a relapse. Two of these (10%) relapsed with organ involvement that had not been present at baseline. The median time to relapse following first infusion was 244 days (95% CI: 158–330 days; range: 120–1052 days). The median time to disease relapse or retreatment (n = 28) was 252 days (95% CI: 191–313 days; range: 120–1052 days). The median time to prophylactic retreatment (n = 7) was 300 days (range: 143–1052 days).

No significant differences were observed in time to disease relapse according to gender or whether the following were present: multiorgan disease, organ damage at baseline, baseline serum IgG4 concentration elevation or normalization of the serum IgG4 concentration following treatment. The longest follow-up period without a disease relapse was 1416 days, and the shortest time to a relapse was 120 days. That patient's baseline clinical organ involvement included thoracic and abdominal aortic aneurysms [3].

Serum IgG4 concentrations, serum IgE concentrations, eosinophilia and disease relapse

Twenty of the 21 patients who relapsed had pre-treatment serum IgG4 concentrations measured. The baseline serum IgG4 concentrations were elevated in 14 (70%). Of these, only 10 (71%; 50% overall) had elevated serum IgG4 concentrations at relapse. No patient with a normal baseline serum IgG4 concentration and a subsequent disease relapse (n = 4) had an elevated serum IgG4 concentration at relapse.

Ten patients had IgE concentrations checked at baseline and at the time of flare. Eight (80%) had elevated baseline serum IgE concentrations, all of whom had IgE elevations at relapse. Fourteen patients had peripheral eosinophil levels checked at baseline and at the time of flare. Five had peripheral eosinophilia at baseline, four of whom had eosinophilia at relapse. One patient with normal eosinophil levels at baseline developed eosinophilia at the time of relapse.

B cell detectability and disease relapse

The quantity of circulating B cells was assessed in 16 patients at the time of relapse. $CD19^+$ B cells were in the normal range (6–25% of total lymphocytes) in three of these patients. In 12 patients, circulating B cells were detectable below the normal range. In one patient, the number of circulating B cells was below the level of quantification in our clinical laboratory.

Predictors of disease flare

In univariate analyses, patients treated with rituximab but without glucocorticoids were not at an increased risk of relapse [16 (42%) *vs* 5 (26%), P = 0.4]. Similarly, specific types of organ involvement (e.g. renal, pancreas) were not associated with relapse. We examined other potential predictors of disease relapses using Cox regression adjusted for time to censoring as well as age and sex (Table 3). In Cox regression analyses adjusted only for time to censoring, the highest quartiles of baseline serum IgG4 (HR = 5.3, 95% CI: 1.2, 24.7), IgE (HR = 6.9, 95% CI: 1.4, 33.0) and absolute eosinophil levels (HR = 5.5, 95% CI: 1.4, 22.0) were associated with relapse. The hazard ratios for subsequent relapse for patients with the highest quartiles of IgG4, IgE and eosinophil levels were all higher following adjustment for age and sex: IgG4 6.2 (95% CI: 1.2, 32.0), IgE 8.2 (95% CI: 1.4, 50.0) and eosinophil level 7.9 (95% CI: 1.8, 34.7). Despite the strong associations between high baseline IgG4, IgE and eosinophil concentrations and the risk of subsequent relapse, only six patients (10%) had all three of these risk factors at baseline. Age, gender, number of organs involved, disease duration, the RI, and upper quartiles of plasmablast levels and inflammatory markers were not associated with relapse after adjusting for time to censoring.

In Fig. 3, Kaplan–Meier curves compare the probability of relapse-free survival in the highest quartiles with the lowest quartiles of potential predictors during follow-up. By log-rank testing, there was a significant difference in the time to relapse comparing the highest and lower quartiles of the serum IgG4 concentration (P = 0.01), serum IgE concentration (P = 0.008) and circulating eosinophil levels (P = 0.03). There was no significant difference in the time to relapse comparing the highest and lowest quartiles of ESR, CRP or plasmablast levels (P = 0.55, 0.96 and 0.26, respectively).

Discussion

A better understanding of risk factors for disease relapses and when those relapses are likely to occur mark important advances in the management of IgG4-RD. This study of IgG4-RD patients treated with rituximab not only expands knowledge regarding the efficacy of B cell depletion substantially, it is also the first to evaluate relapses in patients treated with rituximab over a lengthy period of follow-up. Most importantly, it provides novel information about risk factors for disease relapse [6, 11, 16, 21]. Two-thirds of the patients were treated with rituximab alone and 95% had a clinical response to this treatment. However, 37% of the patients relapsed following successful treatment. High baseline values of IgG4, IgE and the total eosinophil count all predicted subsequent disease relapses within the follow-up period of this study.

The patients studied represent the full spectrum of IgG4-RD. Patients with highly inflammatory, multi-organ disease associated with elevated serum IgG4 concentrations were included, as were patients with single-organ involvement and normal serum IgG4 concentrations. Our findings suggest that B cell depletion is an effective treatment strategy, yet highlight the relapsing nature of this condition [9, 10]. The timing of relapse was variable, ranging from 120 days to >1400 days after treatment. The median time to relapse—244 days—is longer than the time typically required for the start of B cell repopulation following rituximab. Indeed, the majority of patients who relapsed had begun to reconstitute their peripheral B lymphocytes, highlighting the importance of cells in the B cell lineage to the pathogenesis of IgG4-RD. Only 71% of those with elevated baseline serum IgG4 concentrations had elevated concentrations at relapse. This suggests that even though baseline elevations in serum IgG4 predict future flares, the use of serum IgG4 concentrations in the longitudinal care of patients is an imperfect means of assessing for the presence of relapse [11, 24, 25]. Blood plasmablast levels appear to be more useful for that purpose [13].

We used Cox regression to evaluate potential relapse predictors in the 57 treatment responders. Disease severity, as captured by the number of organs involved, the RI score and inflammatory markers, was not associated with relapse. Similarly, age and gender were not associated with relapse, in contrast to prior reports that linked older age and male gender to risk of relapse [21]. Rather, baseline serum concentrations of IgG4 and IgE as well as circulating levels of eosinophils were associated with an increased risk of relapse. Further, for each of these predictors, the higher the baseline value, the greater the risk of relapse and the shorter the time to relapse. Yet only 10% of the patients in the study had elevations of all three major risk factors

Table 3 Hazard ratio for risk of flare: unadjusted and age-, sex-adjusted Cox regressions

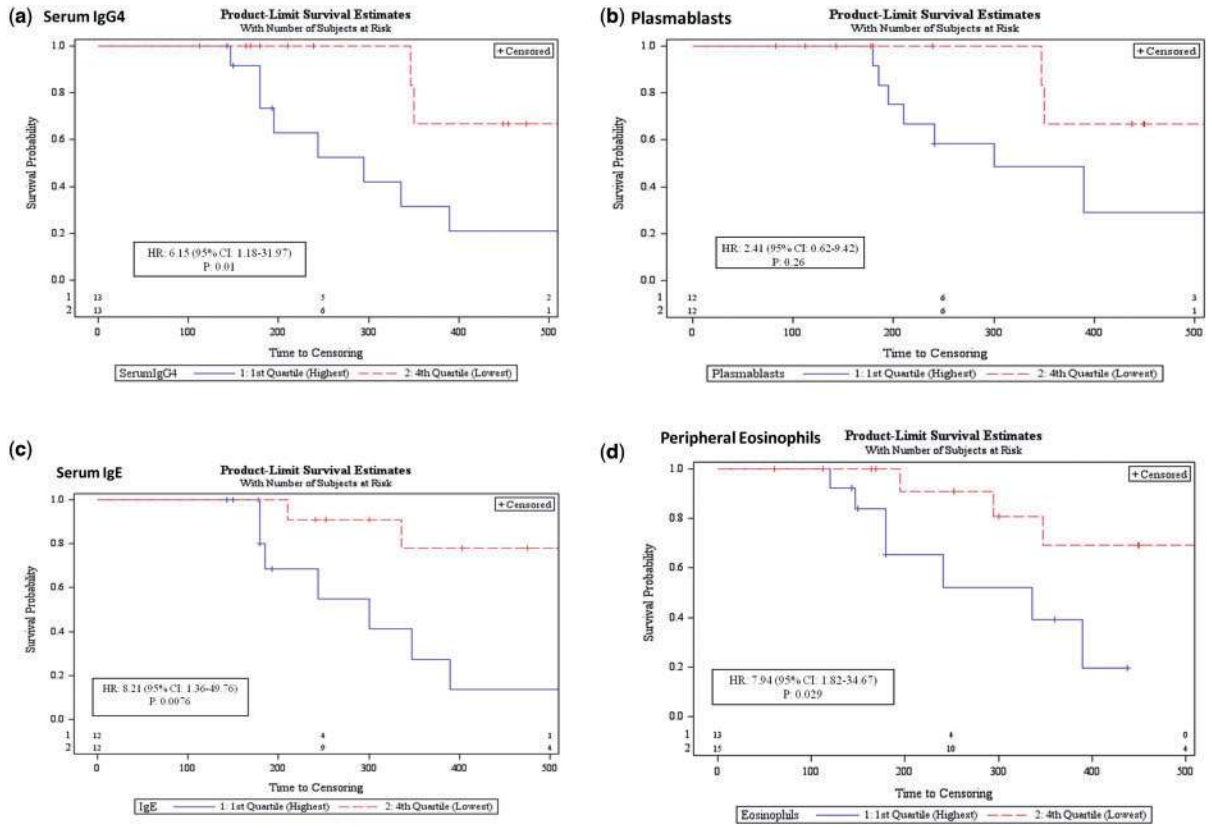
Variable	Hazard ratio (95% CI)	Age-, sex-adjusted hazard ratio (95% CI)
Demographics		
Age, continuous	1.0 (0.98, 1.06)	1.0 (0.97, 1.06)
Male, gender	1.3 (0.42, 3.86)	1.1 (0.33, 3.65)
Disease severity and response index		
No. of involved organs (per 1 organ)	1.2 (0.87, 1.62)	1.2 (0.85, 1.60)
Multi-organ cases (i.e. $\geqslant$3 organs)	1.3 (0.49, 3.19)	1.2 (0.47, 3.11)
Disease duration (continuous)	1.0 (0.99, 1.06)	1.0 (0.99, 1.06)
Responder index (continuous)	1.1 (0.97, 1.17)	1.1 (0.96, 1.16)
Antibody concentrations		
Serum IgG4 concentration		
First quartile (median 858.0 mg/dl)	**5.3 (1.15, 24.74)**	**6.2 (1.18, 31.97)**
Second quartile (median 313.0 mg/dl)	1.9 (0.36, 9.59)	2.3 (0.41, 12.84)
Third quartile (median 123.0 mg/dl)	2.0 (0.39, 10.50)	2.5 (0.45, 13.84)
Fourth quartile (median 34.0 mg/dl)	Ref	Ref
P for trend	0.01	0.02
Serum IgE concentration		
First quartile (median 560.5 mg/dl)	**6.9 (1.44, 33.00)**	**8.2 (1.36, 49.76)**
Second quartile (median 140.5 mg/dl)	2.9 (0.56, 14.93)	2.5 (0.46, 13.67)
Third quartile (median 35.0 mg/dl)	1.7 (0.28, 10.32)	1.6 (0.24, 10.78)
Fourth quartile (median 3.0 mg/dl)	Ref	Ref
P for trend	$<$0.01	$<$0.01
Inflammatory markers		
CRP		
First quartile (median 70.5 mg/l)	0.9 (0.22, 3.26)	0.5 (0.12, 2.29)
Second quartile (median 7.5 mg/l)	1.1 (0.33, 3.62)	0.8 (0.21, 2.82)
Third quartile (median 2.0 mg/l)	0.5 (0.12, 1.85)	0.4 (0.08, 1.52)
Fourth quartile (median 0.9 mg/l)	Ref	Ref
P for trend	0.85	0.86
ESR		
First quartile (median 88.0 mm/h)	1.6 (0.46, 5.49)	1.4 (0.34, 5.34)
Second quartile (median 39.0 mm/h)	1.2 (0.37, 3.99)	1.1 (0.29, 4.18)
Third quartile (median 13.0 mm/h)	1.4 (0.37, 5.29)	1.4 (0.36, 5.16)
Fourth quartile (median 6.0 mm/h)	Ref	Ref
P for trend	0.54	0.75
Circulating cells		
Plasmablasts		
First quartile (median 8175.5/ml)	2.2 (0.59, 8.49)	2.4 (0.62, 9.42)
Second quartile (median 4016.0/ml)	2.1 (0.52, 8.92)	2.3 (0.53, 10.11)
Third quartile (median 1583.0/ml)	0.8 (0.16, 3.96)	0.7 (0.14, 3.76)
Fourth quartile (median 511.0/ml)	Ref	Ref
P for trend	0.10	0.11
Eosinophils		
First quartile (median 770.0/mm^3)	**5.5 (1.35, 21.98)**	**7.9 (1.82, 34.67)**
Second quartile (median 400.0/mm^3)	2.3 (0.56, 9.08)	2.3 (0.57, 9.48)
Third quartile (median 140.0/mm^3)	1.5 (0.33, 6.70)	1.9 (0.41, 8.44)
Fourth quartile (median 50.0/mm^3)	Ref	Ref
P for trend	$<$0.01	$<$0.01

Values in bold show statistical significance. Ref: reference category.

detected—IgG4, IgE and peripheral blood eosinophil elevations at baseline—underscoring the importance of measuring all three. Among patients who had a value in the highest quartile in at least one of these three variables (n = 24), however, the risk of disease flare was associated with an 8-fold increased risk of relapse.

Although autoimmune disease is often associated with a Th1-polarized immune state, the relative polarization toward a Th1 or Th2 phenotype in IgG4-RD remains controversial [26–28]. Our group has previously described the fact that for a significant number of patients with IgG4-RD, elevations in pre-treatment eosinophil levels and IgE concentrations are independent of atopic disease history, suggesting that these elements reflect mechanisms inherent to the immune response driving IgG4-RD [26]. Moreover, we previously demonstrated that a Th2-polarized immune state in IgG4-RD was associated with antecedent atopic disease as opposed to a reflection of IgG4-RD activity [28]. The findings

FIG. 3 Kaplan–Meier plots of the risk of disease relapse for the highest and lowest quartiles of potential predictors



in the current study suggest that although the precise aetiologies of serum IgG4 and IgE elevations and peripheral eosinophilia remain uncertain, they may reflect differences in immunopathogenesis between patient subsets or variability in disease severity that predisposes patients to an increased risk of relapse.

Our group recently identified the close correlation between elevated levels of circulating oligoclonal plasmablasts and disease activity in IgG4-RD [13, 14]. This study is the first to consider elevated levels of circulating plasmablasts as a potential predictor of relapse. Our failure to demonstrate a significant risk of relapse associated with the baseline plasmablast level may have been an issue of power, given the relatively small number of relapses observed. The conduct of a larger study that includes more detailed analyses of plasmablasts and $IgG4^+$ plasmablasts will be an important complement to this work. The sum of our work related to serum IgG4 concentrations and plasmablast levels to date suggests that baseline serum IgG4 concentrations are a better predictor of future flare risk, but plasmablast levels constitute a superior biomarker for the assessment of disease activity on a longitudinal basis [13, 14].

Our study has certain limitations. First, the retrospective nature of the study creates the potential for recall and misclassification bias with regard to some variables, for example, those based on patient report and physician documentation. As a specific example, the lack of follow-up imaging studies in some patients because they could not be justified from the standpoint of clinical care may have limited detection of asymptomatic lesions that either did not respond to treatment or worsened following treatment. Second, because all patients in this study received targeted B cell depletion as their strategy for remission induction, the generalizability of these observations to patients treated only with glucocorticoids is unclear. Third, while we tried to be as inclusive as possible when assessing candidate predictors of disease flare, we were unable to accurately capture the number of prior disease flares which may be associated with subsequent flare. However, we tried to include covariates such as age, disease extent and disease duration that may account for unmeasured confounders, including the number of prior relapses. Fourth, our cohort is predominantly white, limiting our ability to assess differences in flare risk by race and ethnicity. In addition, the IgG4-RD RI was used to assess disease activity but this instrument has not been fully validated; such efforts are currently underway and include investigators at sites around the world. Finally, observation of a greater number of disease relapses would have permitted a fuller evaluation of the role of plasmablasts, including $IgG4^+$ plasmablasts, and other variables in predicting disease exacerbations.

In conclusion, rituximab appears to be an effective agent for the treatment of IgG4-RD, but disease relapses occur frequently following treatment responses that are often excellent. Baseline serum IgG4 and IgE concentrations as well as eosinophil levels are important predictors of relapse. These data raise important questions about the underlying immunological mechanisms responsible for IgG4-RD, the subject of ongoing investigations in the field.

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