Elevated serum IgG4 levels in diagnosis, treatment response, organ involvement and relapse in a prospective IgG4-related disease UK cohort.

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Title Page

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Short Title: Serum IgG4 in a prospective UK IgG4-RD cohort

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Abstract

Background: Elevated serum IgG4 levels have been associated with autoimmune pancreatitis and IgG4-related disease (IgG4-RD) for over a decade. However, an elevated serum IgG4 is not specific for the disease. There have been inconsistent reports of its use in diagnosis, as a marker of disease relapse, and its relationship to organ involvement in retrospective cohorts.

Aims: To ascertain conditions which are associated with an elevated serum IgG4 and to investigate the role of IgG4 in diagnosis, relapse and organ-involvement in a prospective cohort of patients with IgG4-RD.

Methods: We evaluated serum IgG4 measurements in the Oxford Immunology Laboratory over six-years. Patients in whom serum IgG4 was requested to differentiate IgG4-RD from other diseases were recruited into a longitudinal follow-up study to determine final diagnosis. In a prospective cohort of IgG4-RD patients, organ involvement, response to therapy, and disease relapse were determined.

Results: 2067 samples from 1510 patients had serum IgG4 measured. Of these, IgG4 was elevated (≥1.4g/l) in 243 (16.1%) patients. The main indication (85.6%) was to distinguish between IgG4-RD and non-IgG4-RD conditions. Only 5.1% of patients who had serum IgG4 measured for this purpose had a final diagnosis of IgG4-RD. Of those with an elevated serum IgG4, 22.4% met IgG4-RD diagnostic criteria.

Serum IgG4 was elevated in 48 (82.8%) of IgG4-RD patients. An IgG4 cut-off of 1.4g/l gave a sensitivity of 82.8% and specificity of 84.7% to diagnose IgG4-RD. Increasing this to 2.8g/l increased specificity to 96.2% and NPV to 97.7%, with a lower sensitivity of 56.9% and PPV of 44.5%. Serum IgG4 levels fell with corticosteroid therapy, but this was not disease-specific. A serum IgG4 of ≥2.8g/l at diagnosis was associated with multi-organ involvement and risk of relapse.

Conclusion: Serum IgG4 levels are elevated in multiple non-IgG4-RD inflammatory and

malignant conditions, with less than one quarter of those with an elevated IgG4 meeting IgG4-RD diagnostic criteria. A serum IgG4 of ≥2.8g/l is useful in distinguishing between IgG4-RD and non-IgG4-RD diagnoses, predicting multiple organ involvement and risk of relapse in IgG4-RD.

Study Highlights

What is current knowledge?

- Organ manifestations of IgG4-RD are difficult to distinguish from malignant and other inflammatory diseases.
- Elevated serum IgG4 is not specific for a diagnosis of autoimmune pancreatitis or IgG4-RD in retrospective studies.

What is new here?

- Only 5.1% of 1140 patients who had serum IgG4 measured to differentiate IgG4-RD from other malignant and inflammatory conditions had a final diagnosis of IgG4-RD.
- Only 22.4% of 214 patients with an elevated serum IgG4 met IgG4-RD diagnostic criteria.
- There was a male predominance and older age in the elevated serum IgG4 group, independent of final diagnosis.
- Elevated serum IgG4 ≥2.8g/l shows high specificity (>96%) for diagnosis of IgG4-RD in a prospective UK cohort.
- Elevated serum IgG4 ≥2.8g/l at diagnosis predicted multiple organ involvement and risk of relapse in IgG4-RD patients.
- Elevated serum IgG4 levels fall with corticosteroid treatment, but this was not disease-specific.

Keywords: serum IgG4, IgG4-related disease, autoimmune pancreatitis, IgG4-related sclerosing cholangitis, diagnosis, relapse, organ involvement.

Main Text

Introduction

IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition characterised by the development of mass lesions, with similar histopathological findings and an abundant IgG4-positive plasma cell infiltrate in involved organs (1). The disease can mimic malignancy and other inflammatory diseases, resulting in misdiagnosis, delays in treatment and unnecessary surgery. Autoimmune pancreatitis (type I AIP) was the first described pancreatic manifestation of the disease, and IgG4-related sclerosing cholangitis (IgG4- SC) is considered to be the most frequent extra-pancreatic manifestation (2–4). The disease has been described in Asia, the US and throughout Europe, with geographical variations in its clinical presentation, the utility of serum IgG4 in diagnosis, presence of extra-pancreatic manifestations, treatment regimens and outcome (5). Although originally considered a relatively benign disease, we recently provided evidence for high rates of relapse, organ dysfunction and failure, malignancy and mortality in the largest prospective cohort of AIP and IgG4-SC patients reported from Europe (6).

IgG4 is the least prevalent of the four IgG subclasses, representing 3-6% of total IgG in the serum of healthy adults. However, it can account for up to 80% of total IgG after chronic exposure to antigen (7). In the case of IgG4-RD, it can be elevated up to 50 times the upper limit (1). The precise reason and role for this elevated IgG4 is uncertain. Raised serum IgG4 levels are considered important in the diagnosis of IgG4-RD, reflected by their inclusion in current diagnostic criteria (3,8–10). However, studies reviewing routine serum IgG4 level requests in France and the US have shown that raised serum IgG4 can be seen in a variety of other conditions (11–13). There have also been inconsistent reports on the value of serum IgG4 levels in monitoring response to treatment, determining risk of relapse and predicting

organ involvement (5,13,14). All of these studies have been retrospective, and most focused on the pancreatic manifestation of the disease.

In this study, we investigated consecutive serum IgG4 measurements performed at the Oxford University Hospitals Trust over a six-year period to ascertain the disease conditions associated with a raised serum IgG4 level. We subsequently considered all patients who had serum IgG4 concentrations measured to discriminate between a diagnosis of IgG4-RD and a non-IgG4-RD inflammatory, autoimmune or malignant condition. Those with an elevated serum IgG4 level were recruited into a longitudinal follow-up study to determine whether they met IgG4-RD diagnostic criteria and to determine a final clinical diagnosis if not. In the prospective cohort of IgG4-RD patients, the utility of serum IgG4 measurements in diagnosis, monitoring response to corticosteroid therapy, organ involvement, and disease relapse was explored.

Methods

Review of serum immunoglobulin measurements

A review of all serum IgG4 subclass concentrations measured in the clinical immunology department, Churchill Hospital, Oxford, UK from January 2007 until December 2012 (six years) was performed. The indication for IgG4 subclass measurement and the clinical department requesting the test were recorded.

Patient identification and recruitment

We identified those patients in whom the serum IgG4 concentration was measured to discriminate between a diagnosis of IgG4-RD and a non-IgG4-RD inflammatory,

autoimmune or malignant condition, based on test request details and review of the clinical notes. All patients had retrospective evaluation of clinical details, radiological investigations and histopathology. Those patients with an elevated serum IgG4 (225 patients with clinical details available) were recruited into a prospective longitudinal follow-up study to determine final diagnosis and outcome. Written informed consent was obtained (Oxfordshire Research Ethics Council 10/H0604/51). These patients were reviewed in the IgG4 outpatient clinic in Oxford, and were carefully evaluated with attention to clinical presentation, serological values, cross sectional imaging for evidence of pancreatic or other organ involvement, review of morphology and IgG4 immunostaining of liver, bile duct, pancreatic, colonic and other biopsies, where available - 182 specimens in total. The 'final diagnosis' was determined by at least two members of the clinical team (EC, RWC, AE, JC, EB) and discussed at the IgG4 multidisciplinary team meeting if there were conflicts of opinion. The study was approved by the Oxfordshire ethics committee and is registered on the UK NIHR portfolio as study number 10776.

Diagnostic criteria

The diagnoses of type I AIP and IgG4-SC were made using the Mayo HISORt criteria and the International Consensus Diagnostic Criteria (ICDC) (2,4,10). Patients with type II AIP (idiopathic duct centric pancreatitis) are not considered part of the IgG4-RD spectrum, and were excluded (15,16). Patients with extra-pancreatic disease were diagnosed using the Japanese Comprehensive Diagnostic Criteria (JCDC) for systemic IgG4-RD (17). As the Mayo HISORt criteria for AIP and IgG4-SC, and the JCDC for IgG4-RD, included an elevated serum IgG4 level in their algorithms, histology-based diagnostic criteria were applied to those patients with biopsy/resection specimens using the Boston Consensus Histopathological Criteria (18). In accordance with these criteria, 58 patients were defined as

having IgG4-RD (48 with an elevated serum IgG4 and 10 with a normal serum IgG4 level) and 167 patients with an elevated serum IgG4 who did not meet IgG4-RD criteria were identified. In those 10 IgG4-RD patients with a normal serum IgG4, seven were diagnosed by histological criteria and three were diagnosed by imaging characteristics and response to corticosteroids, once malignancy was excluded.

The diagnosis of 'definite IgG4-RD' was made if tissue biopsy or resection specimen demonstrated two of three major histopathological features of the disease (lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis) and immunostaining confirmed IgG4 and IgG staining as per the Boston Consensus Histopathological Criteria (typically >10 IgG4+ plasma cells per high power field (HPF) on biopsy or >50 IgG4+ plasma cells per HPF on resection, with a ratio of IgG4/IgG of >40%, depending on organ sampled) in an appropriate clinical context. The diagnosis of 'probable IgG4-RD' was made with typical organ involvement, characteristic radiographic appearance of those affected organs (for example, sausage-shaped pancreas of AIP), and radiological response to corticosteroid therapy. A patient was defined as 'not IgG4-RD' if they did not meet the diagnostic criteria, even if there were some consistent features (for example, clinical response to corticosteroids or an isolated elevated serum IgG4).

Disease Controls

Patients with known malignant, inflammatory and autoimmune conditions, where serological evaluation and biopsy specimens excluded IgG4-RD, were defined as 'disease controls'. The disease controls included patients with pancreatic disease (chronic pancreatitis and pancreatic carcinoma), biliary disease (primary sclerosing cholangitis and cholangiocarcinoma), liver disease (autoimmune hepatitis), bowel disease (inflammatory bowel disease), renal disease

(tubulointerstitial nephritis) and autoimmune conditions (systemic lupus and rheumatoid arthritis). The diagnostic criteria used for each condition are described in **Supplementary Methods**.

Serum immunoglobulin measurements

Total serum IgG and subclasses (IgG1, IgG2, IgG3 and IgG4) were measured by nephelometry using a BNII analyser (Siemens, Surry, UK) at the Clinical Immunology Department, Churchill Hospital. The normal range for serum immunoglobulin concentrations in the region was determined and validated by Oxford Immunology department, and is closely aligned with national immunoglobulin reference values; IgG 6-16g/l, IgG1 3.2-10.2g/l, IgG2 1.2-6.6g/l, IgG3 0.2-1.9g/l, IgG4 0.1-1.35g/l. For this study, an elevated serum IgG was ≥16g/l and serum IgG4 was ≥1.4g/l, as used in the Mayo HISORt criteria for AIP and in line with other studies. For patients with multiple serum immunoglobulin measurements, the first serum IgG4 level recorded was used. The prozone effect (falsely low serum IgG4 values) was accounted for using serial dilutions where necessary (19).

Organ involvement

Organ involvement was defined as single if only one organ system was involved and multiple if more than one organ system was involved (confirmed radiologically and/or histologically) during follow-up. In patients with AIP, a diffuse enlargement or focal mass at the head of the pancreas and a distal common bile duct stricture was defined as one organ.

Treatment and monitoring

In IgG4-RD patients receiving corticosteroids dosing was standardised in the majority; prednisolone 0.5 mg/kg (30-40mg) for 2-4 weeks and then dose reduction by 5 mg per 1-2

weeks, with expected cessation of treatment by 4-6 months. Patients were assessed at week 0, weeks 2-4, months 3 and 6 after treatment commenced. Subsequent clinical visits were determined by clinical need. Blood tests including renal function, liver function and serum IgG and IgG4 were performed at each clinic visit. Repeat imaging was performed after 4-8 weeks of corticosteroids, upon completion of medical treatment and as dictated by clinical developments. Discontinuation of medication, due to patient preference, intolerance or other side effects, was recorded.

Treatment response

The goals of treatment with corticosteroid therapy are not completely defined in IgG4-RD. In our clinical practice the aims were to (1) improve symptoms, (2) reverse active disease, (3) halt or delay progression of disease including organ failure and additional organ involvement, (4) reduce the need for endoscopic intervention, namely biliary stenting, and (5) achieve long-term maintenance of treatment benefits. Treatment response was defined as a reduction in absolute values in liver function tests, reduction in serum IgG4 level, reduction in size or resolution of mass/stricture/inflammatory change on imaging, reduction or resolution of stricture and stent removal at ERCP. There was no absolute number or percentage used to define a reduction in extent of these lesions.

Disease relapse and treatment

Disease relapse was defined as progression of disease on imaging or deterioration of biochemical parameters (e.g. liver function), after initial treatment had been tapered or discontinued (but not due to stent dysfunction). Sufficient follow-up to determine relapse was defined as a period of greater than six months since initial treatment was discontinued or surgery was performed, and the patient was assessed in outpatient clinic. Relapse was treated

with repeat further courses of steroid therapy, and/or additional second line immunosuppressive therapy, at the discretion of the clinicians. As there are currently no international guidelines for the treatment of relapse, our protocol broadly reflected that used in other major US and European centres (6).

Malignancy and mortality

Malignancy was defined as any evidence of histologically confirmed malignancy, after a diagnosis of IgG4-RD was made. Mortality and its causes were confirmed by cross-referencing with death certificates.

Statistical analysis

A two-tailed Mann-Whitney test and a one-way Kruskal Wallis test with post hoc Dunns test were used to compare individual and multiple groups, respectively. A chi-squared test with Yate's correction was performed for gender differences. Spearman's rank correlation with 95% confidence intervals and Gaussian approximation was calculated for serum immunoglobulin levels. Receiver operator characteristic (ROC) curves were calculated for sensitivity and specificity. Statistics were calculated using Graphpad Prism v6.0. A P-value of <0.05 was considered statistically significant.

Results

Serum IgG4 measurements

A flow diagram showing serum IgG4 measurements over a 6-year period is shown in **Figure 1**. During this time, 2067 blood samples from 1510 patients had serum IgG4 subclass levels measured. Of these, 610 samples from 243 unique patients had an elevated serum IgG4

(>1.4g/l). The serum IgG4 was elevated in 16.1% (243/1510) of the patients in whom IgG4 was requested.

Demographics

The demographics of patients with a normal and elevated serum IgG4 are shown in **Table 1**. Overall, there was a male predominance in the 243 patients with an elevated serum IgG4 (67.9%) and a female predominance in the 1267 patients with normal serum IgG4 group (54.5%) ($p<0.0001$). A higher median age was observed in patients with an elevated (59.4 years) compared to normal (55.0 years) serum IgG4 ($p=0.035$).

Serum Immunoglobulin concentrations

Serum total IgG and IgG1 subclass were higher in the elevated serum IgG4 group, compared to the normal IgG4 group ($p<0.0001$) (**Table 1**).

Clinical indication for serum IgG4 measurements

From 1510 patients, sufficient clinical details were available in 1331 patients (225 patients with an elevated serum IgG4 and 1106 patients with a normal serum IgG4). The indications for serum IgG4 measurement in these patients are shown in **Supplementary Table 1**. The main indication for serum IgG4 measurements was to distinguish between IgG4-RD and other diseases (85.6%; 1140/1331 patients). Only those patients who had serum IgG4 measured when considering a diagnosis of IgG4-RD (214 with elevated serum IgG4 and 926 patients with normal IgG4 levels) are considered for the remainder of this study.

Final diagnosis and disease conditions

The final diagnosis in the 1140 patients in whom serum IgG4 was measured to distinguish between IgG4-RD and other diseases (IgG4-RD versus other inflammatory and autoimmune diseases (994 patients) or malignancy (146 patients) is shown in **Table 2**. The most common conditions in which serum IgG4 was measured were pancreatitis and primary sclerosing cholangitis (to differentiate from AIP and IgG4-SC). Overall, an elevated serum IgG4 was seen in 18.7% (214/1140) of patients in whom it was measured to differentiate IgG4-RD from non-IgG4-RD. Of these, serum IgG4 is elevated in 15.3% of non-IgG4-RD diagnoses versus 82.8% of those confirmed with IgG4-RD ($p<0.0001$). Only 5.1% (58/1140) of patients who had serum IgG4 measured had a final diagnosis of IgG4-RD.

ROC curves for a diagnosis of IgG4-RD

Using the final diagnosis attributed to the 1140 patients, the sensitivity, specificity and predictive values of serum IgG4 to distinguish IgG4-RD from other disease conditions were determined. The serum IgG4 was higher in those patients meeting criteria for IgG4-RD (median 1.74g/l, range 0-54.1g/l) than in those with non-IgG4-RD diagnoses (median 0.42g/l, range 0-15.0g/l) ($p<0.0001$) (**Figure 2A**). At a serum IgG4 cut-off of 1.4g/l (upper limit of normal), the sensitivity was 82.8% (48/58) specificity was 84.7% (916/1082), positive predictive value (PPV) was 22.4% (48/214) and negative predictive value (NPV) was 98.9% (916/926), with an area under the curve (AUC) of 0.90 to diagnose IgG4-RD (**Figure 2B**).

As there were many patients with elevated serum IgG4 who did not meet the diagnostic criteria for IgG4-RD, a higher IgG4 cut-off to diagnose the disease was evaluated. Increasing the IgG4 to 2.8g/l (twice the upper limit), the sensitivity was 56.9% (33/58) specificity was 96.2% (1041/1082), PPV was 44.6% (33/74) and NPV was 97.7% (1041/1066) to diagnose

IgG4-RD. Furthermore, increasing the IgG4 to 5.6g/l (four times the upper limit), the sensitivity was 36.2% (21/58), specificity was 99.5% (1077/1082), PPV was 80.8% (21/26) and NPV was 96.7% (1077/1114). Although 41 patients (3.7%) with non-IgG4-RD diagnoses had a serum IgG4 above 2.8g/l, only 5 patients (0.5%) had a serum IgG4 above 5.6g/l. However, this did include two patients with histologically confirmed malignancy (one resectable CCA and one metastatic with unknown primary). Of the PSC patients, 5 had a serum IgG4 above 2.8g/l but none had a serum IgG4 above 5.6g/l.

Clinical diagnosis in patients with an elevated serum IgG4

Of the 214 patients with an elevated serum IgG4, only 22.4% (48/214) of patients met IgG4-RD criteria. However, IgG4-RD was the most frequent diagnosis made in the high serum IgG4 group (**Table 3**). Amongst other non-IgG4-RD diagnosis, PSC accounted for 16.8%, pancreatitis 14.5% and malignancy 13.1%. The patients who met IgG4-RD criteria were older (65.5 years) than those with non-IgG4-RD diagnoses (57.4 years) (p=0.0006). Both groups had a male preponderance (p=0.14).

Serum immunoglobulins levels and ratios in IgG4-RD and non-IgG4-RD patients with an elevated serum IgG4

Serum immunoglobulin levels and ratios in the IgG4-RD patients and non-IgG4-RD patients with an elevated serum IgG4 were then evaluated (**Figure 3 and Supplementary Table 2)**. Serum total IgG and IgG4 were higher in the IgG4-RD patients (IgG median 16.5g/l, range 8.9-42.3g/l; IgG4 median 4.5g/l, range 1.48-54.1g/l) than in those with non-IgG4-RD diagnoses (IgG median 14.2g/l, range 4.13-47.3; IgG4 median 2.06g/l, range 1.40-15.0g/l) (IgG p=0.043 and IgG4 p<0.0001). There was no difference in IgG1 levels between the two groups (p=0.201.)

Serum IgG/IgG4 and IgG1/IgG4 ratios were lower in patients with IgG4-RD (IgG/IgG4 median 3.78, range 1.04-12.08; IgG1/IgG4 median 2.18, range 0.29-6.55) than non-IgG4-RD diagnoses (IgG/IgG4 median 6.48, range 0.72-16.18; IgG1/IgG4 median 4.04, range 0.29-10.93) (both ratios $p<0.0001$). In those patients with an elevated IgG4, serum electrophoresis demonstrated a polyclonal pattern of increased γ-globulin, with no evidence of monoclonal bands (data not shown).

IgG4-RD patients

From 1140 patients in whom serum IgG4 was measured to distinguish between IgG4-RD and other diseases, 58 patients met probable or definite IgG4-RD diagnostic criteria. The clinical characteristics and serum immunoglobulin values in patients with IgG4-RD are shown in **Supplementary Table 3.** The majority of IgG4-RD patients were male (77.6%) in the seventh decade of life (median 64.3 years, range 24-84 years). Most patients (48/58, 82.8%) had an elevated serum IgG4 level. In the 10 patients with a normal serum IgG4 level, there was lower total IgG ($p<0.001$) and IgG1 ($p=0.015$), and higher IgG/IgG4 ratio ($p=0.0004$) and IgG1/IgG4 ratio ($p=0.038$) compared to the 48 patients with an elevated serum IgG4 **(Supplementary Figure 1)**. In IgG4-RD patients, there was a positive correlation between serum IgG4 levels and serum IgG (rank 0.80, <0.0001) and IgG1 (rank 0.51, $p=0.0003$) levels. There was a negative correlation between serum IgG4 levels and IgG/IgG4 ratio (rank -0.83, $p<0.0001$) and IgG1/IgG4 ratio (rank -0.69, $p<0.0001$) (**Supplementary Figure 2**).

Serum IgG4 and organ involvement in IgG4-RD

The relationship of serum IgG4 and organ involvement in patients with IgG4-RD was evaluated, followed up for a median of 30.7 months (range 1.3 to 73.5 months) shown in

Figure 4. Twenty-five patients (43.1%) had multiple organ involvement. The serum IgG4 level in patients with multi-organ disease (median 6.141g/l, range 0.31-54.1g/l) was higher compared with those with single-organ disease (median 2.12g/l, range 0-21.7g/l) (p=0.0001). At diagnosis, a serum IgG4 of >1.4g/l (p=0.0354) and >2.8g/l (p=0.0004) predicted risk of multiple rather than single organ involvement at follow-up (**Figure 4**). Nine of ten patients with normal serum IgG4 had single organ involvement. Follow-up was comparable in the two groups (multiple-organ disease median 30.9 months, range 1.5 to 73.5 months; single organ disease median 28.8 months, range 1.3 to 68.6 months) (p=0.6801).

Serum IgG4 levels and treatment in IgG4-RD

The relationship of serum immunoglobulin levels and corticosteroid therapy was next examined. Forty-six patients (79.3%) received corticosteroid therapy. There was a decrease in serum total IgG and IgG4, but not IgG1, during corticosteroid treatment (**Supplementary Figure 3 upper panel**). Serum IgG4 levels fell within four weeks of initiation (n=7, p=0.084), and were significantly lower compared with pre-treatment levels at eight weeks (n=11, p=0.033) and 12 weeks (n=15, p=0.018) (**Supplementary Figure 3 lower panel**). The steepest slope of decline was between 0 and 8 weeks at the highest dose of steroids, 20-40mg prednisolone/day, and fluctuated whilst at lower doses 5-10mg prednisolone/day.

A fall in serum IgG4 was not specific to IgG4-RD and other non-IgG4-RD disease conditions when treated with steroids (such as PSC-IBD and AIH) similarly showed a decrease in serum IgG4 (**Supplementary Figure 4**). Serum IgG4 levels also were significantly lower compared with pre-treatment levels at 12 weeks (n=5, p=0.036).

Serum IgG4 levels and risk of relapse in IgG4-RD

The relationship of relapse to serum IgG4 in the IgG4-RD cohort was next investigated. There were sufficient follow-up data (>6 months since treatment commenced and assessed in outpatient clinic) in 52 of 58 patients with IgG4-RD after treatment; 30 of which relapsed at least once after treatment (57.7%). There was significantly higher serum IgG4 levels at diagnosis in those who relapsed after corticosteroid therapy and/or surgical intervention (median 5.05g/l, range 0.0-54.10) compared to those that did not relapse (median 1.95, range 0.16-16.70) (p=0.0099) (**Figure 5**). There was a significant difference in risk of relapse in IgG4-RD with a serum IgG4 >2.8g/l (p=0.0108) but not with a serum IgG4 >1.4g/l (p=0.0753) (**Figure 5)**. Three of ten patients with normal serum IgG4 experienced disease relapse. There was no association with normalization of elevated serum IgG4 during or after treatment and risk of relapse (p=0.516).

Serum IgG4, malignancy and mortality in IgG4-RD

Five of 58 (8.6%) IgG4-RD patients developed malignancy during follow-up (median 13.5 months, range 2.5 to 42.1 months from IgG4-RD diagnosis to cancer diagnosis). All cancers were histologically confirmed; prostate adenocarcinoma (2), transitional cell carcinoma of the bladder (1), pancreatic adenocarcinoma (1) and cholangiocarcinoma (1). All 5 patients had an elevated serum IgG4 (>2.8g/l) at IgG4-RD diagnosis (median IgG4 5.16g/l, range 2.85-9.84g/l) (**Supplementary Table 4**). An elevated serum IgG4 level itself did not predict risk of malignancy (p=0.277).

Six of 58 (10.3%) IgG4-RD patients died during follow-up (median 13.0 months, range 1.5 to 51.5 months from IgG4-RD diagnosis to date of death). Causes of mortality included pneumonia (2), end-stage pulmonary fibrosis (2), post-operative death following Whipple's

surgery for suspected malignancy (1) and metastatic transitional cell bladder carcinoma (1). All six patients had an elevated serum IgG4 (>1.4g/l) at IgG4-RD diagnosis (median IgG4 2.71g/l, range 1.86-9.84g/l). An elevated serum IgG4 was not predictive of mortality (p=0.99).

An algorithm for the diagnosis of IgG4-RD: Using our data, we have created a diagnostic algorithm to identify clinical features, immunoglobulin values and ratios, which are characteristic of IgG4-RD patients versus non-IgG4-RD conditions (**Figure 6A**). Furthermore, in those patients with IgG4-RD, a second algorithm is used to differentiate normal serum IgG4 (<1.4g/l) and elevated serum IgG4 (divided into ≥1.4g/l and ≥2.8g/l) groups, using immunoglobulin values, ratios and disease characteristics (**Figure 6B**). The diagnosis of IgG4-RD does not depend on serum immunoglobulin levels in isolation however, and should be supported by other criteria, including (1) histological evidence, (2) clinical and/or radiological evidence of organ involvement, and (3) biochemical and/or radiological response to steroids.

Discussion

Using consecutive serum IgG4 measurements performed in a large regional diagnostic laboratory, we evaluated conditions associated with an elevated serum IgG4, investigated the diagnostic utility of IgG4 in differentiating patients with IgG4-RD from other disease conditions, and examined the role of IgG4 serology in a prospective cohort of IgG4-RD patients. Overall, IgG4-RD diagnostic criteria were met in only 5.1% (58/1140) of patients who had serum IgG4 measured for the purpose of discriminating IgG4-RD from other disease conditions. This highlights the clinical challenge in differentiating IgG4-RD from conditions that mimic it, with the majority of IgG4 measurements being performed for non-IgG4-RD

conditions. The high volume of serum IgG4 requests for this purpose (85.6%; 1140/1331 patients) also suggests an increasing awareness of the disease; historically, the main indication for serum IgG subclass measurements was to investigate immunodeficiency, chronic and recurrent infections, which was the case in only 9% of our cohort (11,20). Practice patterns with regard to test ordering during the period of this study may have influenced these results, in particular a referral bias from the gastroenterology and hepatology departments may account for an over-representation of hepatobiliary diseases.

Importantly, IgG4-RD diagnostic criteria were met in only 22.4% (48/214) of patients found to have an elevated serum IgG4. This is particularly noteworthy given the importance placed on a serum IgG4 in diagnosis of the disease, where an elevated serum IgG4 is more likely to identify non-IgG4-RD conditions (in 77.6% of cases), and places more emphasis on the need for accurate interpretation of these levels in the context of disease. Earlier French and US retrospective studies support these findings, where recurrent infection, systemic autoimmune conditions and pancreatobiliary disease, were prominent among the diagnoses of subjects with elevated serum IgG4 levels (11,13). A male predominance in the group with higher serum IgG4 levels and a female predominance in the group with normal levels supports known gender differences in the general population, although the reason for this is currently unexplained (21). This gender balance was observed in almost every disease condition in which an elevated serum IgG4 was found. Given that IgG4 is the predominant subclass in situations of chronic exposure and tolerance, it is possible that the older male distribution in IgG4-RD is explained by a long-standing history to certain environmental or occupational antigens, to which IgG4-RD patients have an aberrant response (22).

The diagnostic utility of serum IgG4 in retrospective single-organ AIP cohorts has been well

described. The original landmark study in Japan suggested that a serum IgG4 level of 1.35g/l had a sensitivity and specificity of 97% to distinguish AIP from pancreatic cancer (23). A subsequent meta-analysis of seven retrospective studies reported sensitivities of 67-96% and specificities of 73–100%, with an AUC of 0.92, to differentiate AIP from pancreatic cancer (24). More recently, a retrospective US study reported that a serum IgG4 of 1.35g/l had a sensitivity of 90% and specificity of 60% to diagnose multi-systemic IgG4-RD and differentiate it from other disease conditions (13). Our results suggest a serum IgG4 of 1.4g/l has a sensitivity of 82.8%, specificity of 84.7%, PPV of 22.4% and NPV of 98.9%, with an AUC of 0.9 to differentiate patients with IgG4-RD from other inflammatory, autoimmune and malignant conditions, followed up prospectively to confirm the diagnosis. Doubling the cut-off value for serum IgG4 (2.8g/l) improved the overall test characteristics, with an increased specificity of 96.2% and PPV of 44.6%, but a fall in sensitivity to 56.9% and NPV to 97.7%. Although it is possible that a diagnosis of IgG4-RD may have been under-recognised by the absence of histology in some patients and strict adherence to the diagnostic criteria, it remains an unsuitable single marker for diagnosis, whereby IgG4 elevations in patients with low pre-test probability of disease are likely to be false-positives.

Failure to understand the test characteristics of serum assays for IgG4 and to employ them effectively in clinical practice can lead to over-diagnosis of IgG4-RD and delays in diagnosis of important malignant and autoimmune conditions. The optimal use of serum IgG4 concentrations in this setting is as an initial diagnostic test that offers important support of an IgG4-RD diagnosis if elevated (≥2.8g/l), as well as a significant argument against the diagnosis if normal. The identification and validation of additional diagnostic markers, such as the serum IgG4:IgG1 ratio in PSC and IgG4-SC patients with an elevated serum IgG4 (25), the presence of IgG4 plasmablasts in those with a normal and elevated serum IgG4 (26),

and IgG/IgG4 mRNA ratio by quantitative PCR (27) thus remains an important objective.

The use of serum IgG4 in determining organ involvement, treatment response and disease relapse seems more promising. In our cohort, 43% of patients had multiple-organ disease at diagnosis, with a serum IgG4 of ≥1.4g/l and ≥2.8g/l at diagnosis predictive of multi rather than single organ involvement. The rate of multi-organ disease in this cohort is lower than that described in some Japanese cohorts, which may be explained by the fact that most patients did not have a PET-CT scan to detect subclinical disease at diagnosis, and that patients with AIP and a distal CBD stricture were classified as having single organ disease (28). In our cohort, 57.7% had at least one episode of disease relapse, with a serum IgG4 of ≥2.8g/l at diagnosis predictive of this. However, normalization of serum IgG4, as suggested by others, did not predict relapse (29). Given that the majority of patients who had multi-organ involvement had eventual disease relapse, the link between these factors requires further exploration.

Corticosteroids are the first-line treatment for IgG4-RD patients with inflammatory disease. We have observed a fall in serum IgG4 whilst on steroid treatment, with significant decline at 8 and 12 weeks of therapy. In some centres, a steroid trial has been used to help differentiate AIP from pancreatic cancer in cases of diagnostic difficulty (30). However we, and others, have shown that serum IgG4 will also fall in non-IgG4-RD conditions (31,32). Hence, reduction in serum IgG4 levels following initiation of steroid therapy cannot be used to distinguish IgG4-RD from other conditions.

Using these data, we recommend that patients who meet diagnostic criteria for IgG4-RD with a serum IgG4 of ≥2.8g/l at diagnosis, should have contrast-enhanced CT chest, abdomen and

pelvis in search for evidence of subclinical disease and clarify the extent of organ involvement at diagnosis. This will then guide the need for and urgency of treatment. The use of PET-CT in IgG4-RD has been suggested, but this strategy is expensive and requires further prospective controlled studies (28). There should also be early consideration of second line immunosuppressive therapy, or prolonged low dose corticosteroid treatment, in these patients in whom relapse risk is high. Conversely, normal serum IgG4 is often seen in patients with single organ involvement and in those whom relapse risk is lower and these patients may need less stringent follow-up after establishing remission with therapy. Importantly, serum IgG4 should not be used in isolation to determine organ involvement and risk of relapse, and should always be interpreted within the clinical context.

To our knowledge, this is the first study where patients with an elevated serum IgG4 were followed up prospectively to determine final diagnosis, and IgG4-RD patients were recruited into a longitudinal study to specifically evaluate serum IgG4 levels in relation to organ involvement, response to treatment and relapse. This is also the largest study in the UK analysing patients with systemic disease. The clinical indication for serum IgG4 measurements has changed and interpreting an elevated serum IgG4 requires knowledge of the clinical, radiological and histopathological scenario. In those patients where there is diagnostic doubt, a multidisciplinary meeting is the appropriate arena to come to a decision and guide management decisions. An elevated serum IgG4 ≥2.8g/l at diagnosis should trigger consideration of further imaging to search for subclinical organ involvement and early consideration of prolonged corticosteroid or second line treatments to prevent relapse.

Figure Legends

Figure 1: Flow diagram of serum IgG4 measurements in the Oxford cohort. A flow diagram illustrating the number of serum IgG4 subclass measurements performed at the clinical immunology department, Churchill Hospital, Oxford, UK over a six-year period from January 2008 until December 2013.

Figure 2: Sensitivity and specificity of serum IgG4 to diagnose IgG4-RD. **(A)** The dot plot shows the serum IgG4 levels in non-IgG4-RD and IgG4-RD diagnoses. On the y-axis is Log 10 scale serum IgG4 in grams per litre (G/L). On the x-axis are patients with non-IgG4-RD diagnoses and IgG4-RD diagnoses. The error bars represent median and inter-quartile range. Mann-Whitney test; NS (not significant) ≥p0.05, *p<0.05, **p<0.005, ***p<0.005, ****P<0.0001. **(B)** The receiver operator characteristic (ROC) curve shows the sensitivity (%) and 1 minus specificity (%) of serum IgG4 for the diagnosis of IgG4-RD. The area under the curve (AUC) is 0.9.

Figure 3: Serum immunoglobulin values and ratios in IgG4-RD and non-IgG4-RD patients with an elevated serum IgG4 level. The dot plot shows serum immunoglobulin levels and ratios in IgG4-RD patients and non-IgG4-RD patients with an elevated serum IgG4 level. On the y-axis is Log 10 scale serum IgG, IgG1 and IgG4 in grams per litre (G/L), IgG1/IgG4 and IgG/IgG4 ratio. On the x-axis are patients with non-IgG4-RD diagnoses and IgG4-RD diagnoses. The error bars represent median and inter-quartile range. Mann-Whitney test; NS (not significant) ≥p0.05, *p<0.05, **p<0.005, ***p<0.005, ****P<0.0001.

Figure 4: The relationship between serum IgG4 and organ involvement. The dot plot shows the relationship of serum IgG4 and organ involvement in patients with IgG4-RD. On the x-

axis are patients with single (n=33) or multiple-organ (n=25) disease. Y-axis, error bars and Mann-Whitney p values as per figure 2. The bar charts show the number of IgG4-RD patients who have single or multiple organ IgG4-RD. One y-value (IgG4=0.0) is excluded as it is below the lower limit of the chart. Fishers Exact test; NS (not significant) ≥p0.05, *p<0.05, **p<0.005, ***p<0.005, ****P<0.0001.

Figure 5: Relapse and serum IgG4 levels in IgG4-RD. The dot plot shows the relationship of serum IgG4 and relapse in patients with IgG4-RD. On the x-axis are patients who have (n=30) or have not (n=22) relapsed. Y-axis, error bars and Mann-Whitney p values as per figure 2. The bar charts show the number of IgG4-RD patients who have relapsed or not relapsed. One y-value (IgG4=0.0) is excluded as it is below the lower limit of the chart. Fishers Exact test p values as per figure 4.

Figure 6: Algorithms in the diagnosis of IgG4-RD (A): Algorithm to differentiate IgG4-RD from non-IgG4-RD conditions. The algorithm identifies clinical features and immunoglobulin values characteristic of IgG4-RD patients, non-IgG4-RD conditions and both conditions. Median values are shown. Immunoglobulin levels have been calculated using the Siemens IgG subclass reagents and Siemens Nephelometer. Abbreviations: LPCI: Lymphoplasmacytic infiltrate; SF: Storiform fibrosis; OP: Obliterative Phlebitis; HPF: high power field; g/l: grams/litre; v: versus. Key: * Criteria to support a diagnosis of IgG4-RD include (1) histological evidence, (2) organ involvement, (3) response to steroids. ± Prominent IgG4-positive plasma cells – the absolute number of IgG4-positive cells depends on the organ involved and specimen taken (Boston Consensus Criteria). For example, in the pancreas and bile duct >10 IgG4 cells/HPF in biopsy specimens and >50 IgG4 cells/HPF in resection specimens. (B): Algorithm in IgG4-RD patients with a normal and elevated serum

IgG4 level. The algorithm identifies immunoglobulin values and disease characteristics in IgG4-RD patients with a normal serum IgG4 <1.4g/l, elevated serum IgG4 ≥1.4g/l and moderately elevated serum IgG4 ≥2.8g/l. Median values are shown. Immunoglobulin levels have been calculated using the Siemens IgG subclass reagents and Siemens Nephelometer.

Table Legends

Table 1: Demographics and serum immunoglobulin levels of patients with a normal and elevated serum IgG4. Mann Whitney test of continuous variables, where p values; *p<0.05, ****P<0.0001. Fishers Exact test of categorical variables, where p values; ****P<0.0001. Abbreviations: M; male, F; female; g/l: grams/litre.

Table 2: Final diagnosis in patients who had serum IgG4 measured to distinguish between IgG4-RD and other diseases. The proportion (absolute number, percentage) of patients in each group, and with an elevated serum IgG4 in each disease category, is given. Abbreviations: PSC; primary sclerosing cholangitis, PBC; primary biliary cirrhosis; AIH; autoimmune hepatitis.

Table 3: Final diagnosis in patients with an elevated serum IgG4, recruited into a prospective longitudinal study at the Oxford Radcliffe Hospital Trust. Abbreviations: PSC; primary sclerosing cholangitis, PBC; primary biliary cirrhosis; AIH; autoimmune hepatitis.

Supplementary Table Legends

Supplementary Table 1: Indication for serum IgG4 measurements. The main indications for measurement of serum IgG4 levels at the clinical immunology department, Churchill Hospital, Oxford. Absolute numbers and percentages are given.

Supplementary Table 2: Serum Immunoglobulin levels and ratios in IgG4-RD and non-IgG4-RD patients with an elevated serum IgG4. Mann-Whitney test p values; NS (not significant) ≥p0.05, *p<0.05, **p<0.005, ***p<0.005, ****P<0.0001. Abbreviations: g/l: grams/litre.

Supplementary Table 3: Clinical characteristics and immunoglobulin levels in IgG4-RD patients. Mann U Whitney test of age and serum immunoglobulin values, p values as per figure 3. Fishers Exact test of gender, organ involvement and relapse; NS (not significant) ≥p0.05, *p<0.05, **p<0.005, ***p<0.005, ****P<0.0001. Abbreviations: G/L: grams/litre.

Supplementary Table 4: Development of Malignancy after IgG4-RD diagnosis. The IgG4-RD organ manifestations, type of histologically confirmed malignancy, serum IgG4 levels at diagnosis and length of time that malignancy was detected after IgG4-RD diagnosis, are given. Abbreviations: AIP: Autoimmune Pancreatitis; SMG: Submandibular Glands (IgG4-Sialoadenitis); IgG4-ILD: IgG4-Interstitial Lung Disease; IgG4-SC: IgG4-Sclerosing Cholangitis; TCC: Transitional Cell Carcinoma; CCA: Cholangiocarcinoma; CA: Carcinoma.

Supplementary Figure Legends

Supplementary Figure 1: Serum immunoglobulin values in IgG4-RD patients. The dot plots show serum immunoglobulin subclass levels in IgG4-RD patients. On the y-axis is linear scale serum IgG and IgG1, and Log 10 scale IgG4 in grams per litre (G/L) IgG1/IgG4 and IgG/IgG4 ratio. The x-axis shows IgG4-RD patients with a high serum IgG4 (>1.4g/l) and a normal serum IgG4 (≤1.4g/l). The error bars and Mann-Whitney p values are as per figure 3.

Supplementary Figure 2: Correlation of serum immunoglobulin levels in IgG4-RD. The correlation plots show the relationship of serum IgG4 and serum IgG and IgG1 levels, and IgG1/IgG4 and IgG/IgG4 ratios in patients with IgG4-RD. The x and y-axes are as per figure 3. Spearman's rank correlation; p values * $p<0.05$, ** $p<0.005$, ***$p<0.001$.

Supplementary Figure 3: Immunoglobulin levels in response to corticosteroid therapy in IgG4-RD. **(Upper panel)** The graphs show serum immunoglobulin levels in relationship to length of time on corticosteroid therapy in IgG4-RD patients. The x-axis plots corticosteroid therapy over time in weeks, where 0 weeks is before initiation of steroids. The Y-axis is per figure 3. The dashed lines represent the upper limit of normal for serum IgG (16g/l), IgG1 (10.2g/l) and IgG4 (1.4g/l) levels. **(Lower panel)** Serum IgG4 concentrations in IgG4-RD patients before and at 4 weeks, 8 weeks, and 12 weeks of corticosteroid therapy. On the y-axis is Log 2 serum IgG4 concentrations KU/L (international units). On the y-axis are time points: before corticosteroids (0 weeks) and 4, 8, 12 weeks into corticosteroid treatment (x weeks). Dashed line is the upper limit of IgG4. Two-tailed paired t-test p values, where NS $p\geq0.05$, *$p<0.05$.

Supplementary Figure 4: IgG4 levels in response to corticosteroids in non-IgG4-RD patients with elevated IgG4. **(A)** The graph shows serum IgG4 levels in relationship to length of time on corticosteroid therapy in non-IgG4-RD patients (6 patients) with an elevated serum IgG4 at diagnosis. The x and y-axes and dashed-line are as per Supplementary Figure 3. **(B)** Serum IgG4 concentrations in non-IgG4-RD patients before and at 12 weeks during corticosteroid therapy. The x and y-axes and dashed-line are as per Supplementary Figure 3.

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Guarantor of the article

Professor Berne Ferry.

Specific author contributions

ELC recruited patients and collected blood samples, collated and analysed the raw data, drafted, edited and approved the final manuscript. RS collected and analysed serological data, edited and approved the final manuscript. DS processed serological samples, collected data and approved the final manuscript. TC collected patient data and approved the final manuscript. MM analysed serological data and approved the final manuscript. ACB reviewed immunostained histological sections and approved the final manuscript. AJE, JC and RWC recruited patients, collected samples and approved the final manuscript. PK edited and approved the final manuscript. EB is the principle investigator for the IgG4-RD NIHR study, edited and approved the final manuscript. BF had the original concept for this study, funded and processed the samples, edited and approved the final manuscript, and is the guarantor for the article.

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Conflicts of Interest

The authors declare no commercial or financial conflict of interest.

Study registration

The study is registered on the UK NIHR portfolio as study number 10776.

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Tables

Table 1: Demographics and immunoglobulin concentrations in patients with an elevated and normal serum IgG4.

	High serum IgG4	Normal serum IgG4	P value
Samples	610	1457	
Unique no. of patients	243	1267	
Gender M/F absolute (%)	165/78 (M 67.9%; F 32.1%)	577/690 (M 45.5%; F 54.5%)	<0.0001
Age median (range) (years)	59.4 (3-93)	55.0 (1-97)	0.035
Serum IgG median (range) (g/l)	14.6(4.13-47.3)	11(0.76-43.20)	<0.0001
Serum IgG4 median (range) (g/l)	2.24(1.37-54.1)	0.35(0-1.39)	<0.0001
Serum IgG1 median (range) (g/l)	8.56(3.59-32.2)	7.19(0.5-66.0)	<0.0001

Table 2: Final diagnosis in patients who had serum IgG4 measured.

Clinical diagnosis	Absolute number and (%) of patients in each group	Absolute number and (%) of patients with an elevated serum IgG4
IgG4-RD criteria met	58 (5.1)	48/58 (82.8)
IgG4-RD criteria not met	1082 (94.9)	166/1082 (15.3)
Malignancy	115	28/115 (24.3)
PSC	234	36/234 (15.4)
Pancreatitis	279	31/279 (11.1)
Cirrhosis	30	12/30 (40.0)
Autoimmune hepatitis	57	7/57 (12.3)
Inflammatory bowel disease	32	5/32 (15.6)
Autoimmune disease	57	14/57 (24.6)
Hepatitis	151	12/151 (8.0)
Biliary disease	77	9/77 (11.7)
Gallstones	11	6/11 (54.5)
Overlap PSC/AIH	9	3/9 (33.3)
PBC	28	1/28 (3.6)
Drug-induced	2	2/2 (100)
Total	1140	214/1140 (18.7)

Table 3: Final diagnosis in patients with an elevated serum IgG4.

Clinical diagnosis (elevated serum IgG4)	Absolute number	% of patients	Male	Female	Age median (range) years
IgG4-RD criteria met	48	22.4	37	11	65.5 (24-83.8)
IgG4-RD criteria not met	166	77.6	109	57	57.4 (2.9-93.0)
Malignancy	28	13.1	12	16	72.1 (42.6-87.7)
PSC	36	16.8	26	10	51.9 (12.4-84.8)
Pancreatitis	31	14.5	25	6	45.9 (15.6-83.0)
Cirrhosis	12	5.6	8	4	60.4 (46.2-84.4)
Autoimmune hepatitis	7	3.3	1	6	62.7 (24.4-73.1)
Inflammatory bowel disease	5	2.3	4	1	30.1 (19.1-71.1)
Autoimmune disease	14	6.5	9	5	57.6 (8.6-75.0)
Hepatitis	12	5.6	10	2	52.8 (22.4-65.6)
Biliary disease	9	4.2	6	3	53.0 (29.2-75.0)
Gallstones	6	2.8	4	2	66.2 (19.4-79.8)
Overlap PSC/AIH	3	1.4	2	1	61.0 (11-64)
PBC	1	0.5	0	1	64
Drug-induced	2	0.9	2	0	49.9 (34.4-65.3)
Total	214	100	146	68	

Figure 1: Flow diagram of serum IgG4 measurements in Oxford, UK.

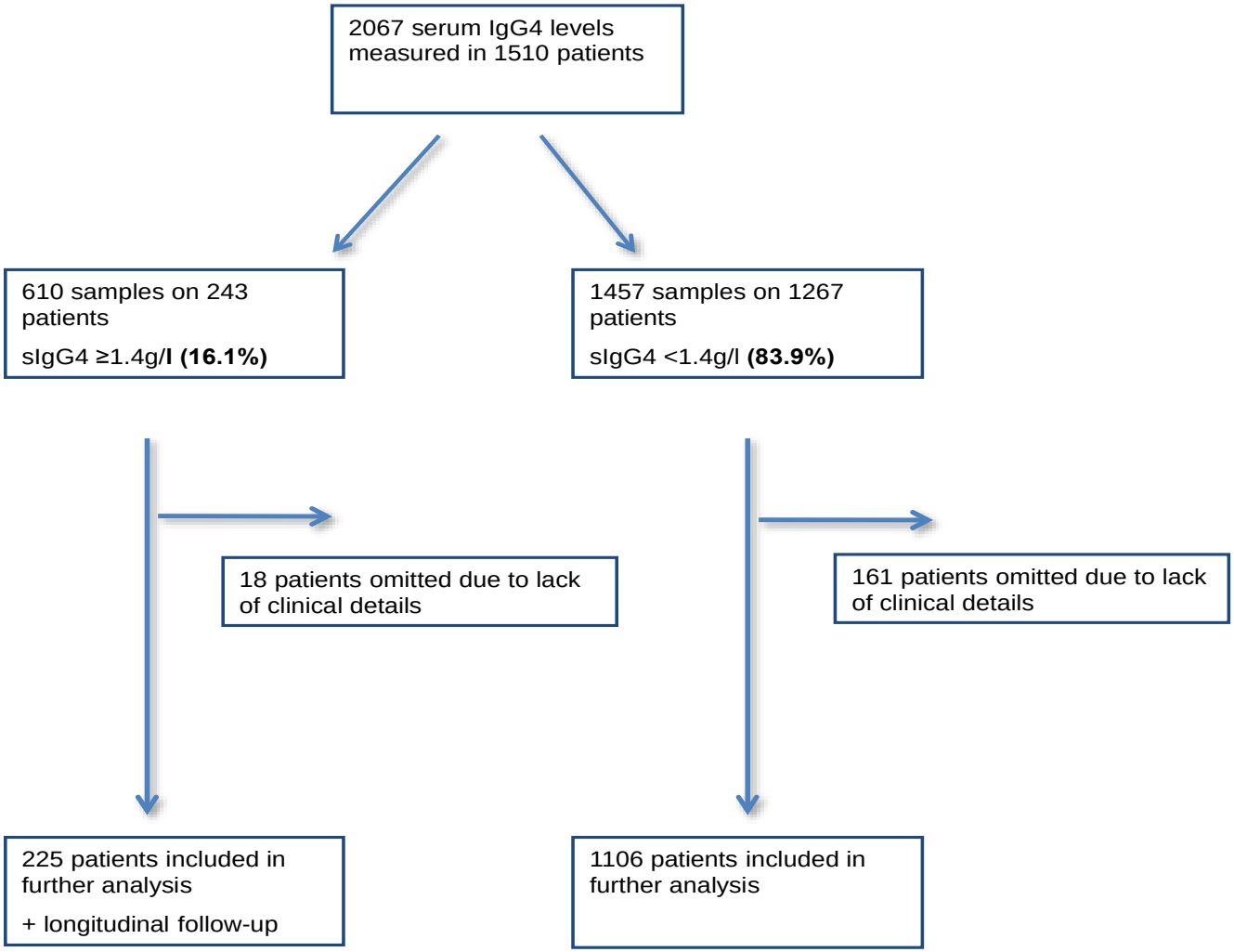


Figure 2: ROC curve for sensitivity and specificity of serum IgG4 to diagnose IgG4-RD.

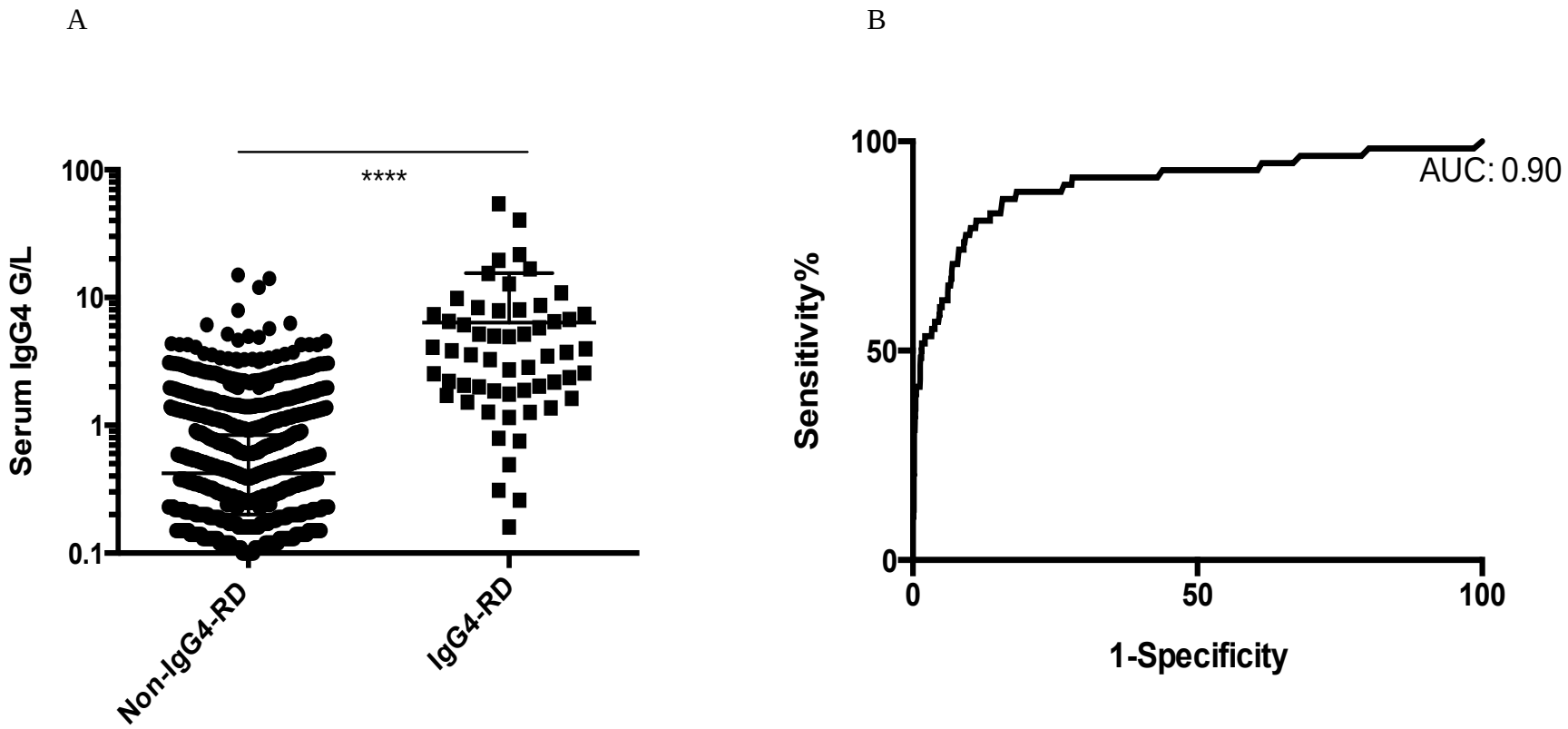


Figure 3: Serum immunoglobulin values and ratios in IgG4-RD and non-IgG4-RD patients with an elevated serum IgG4 level

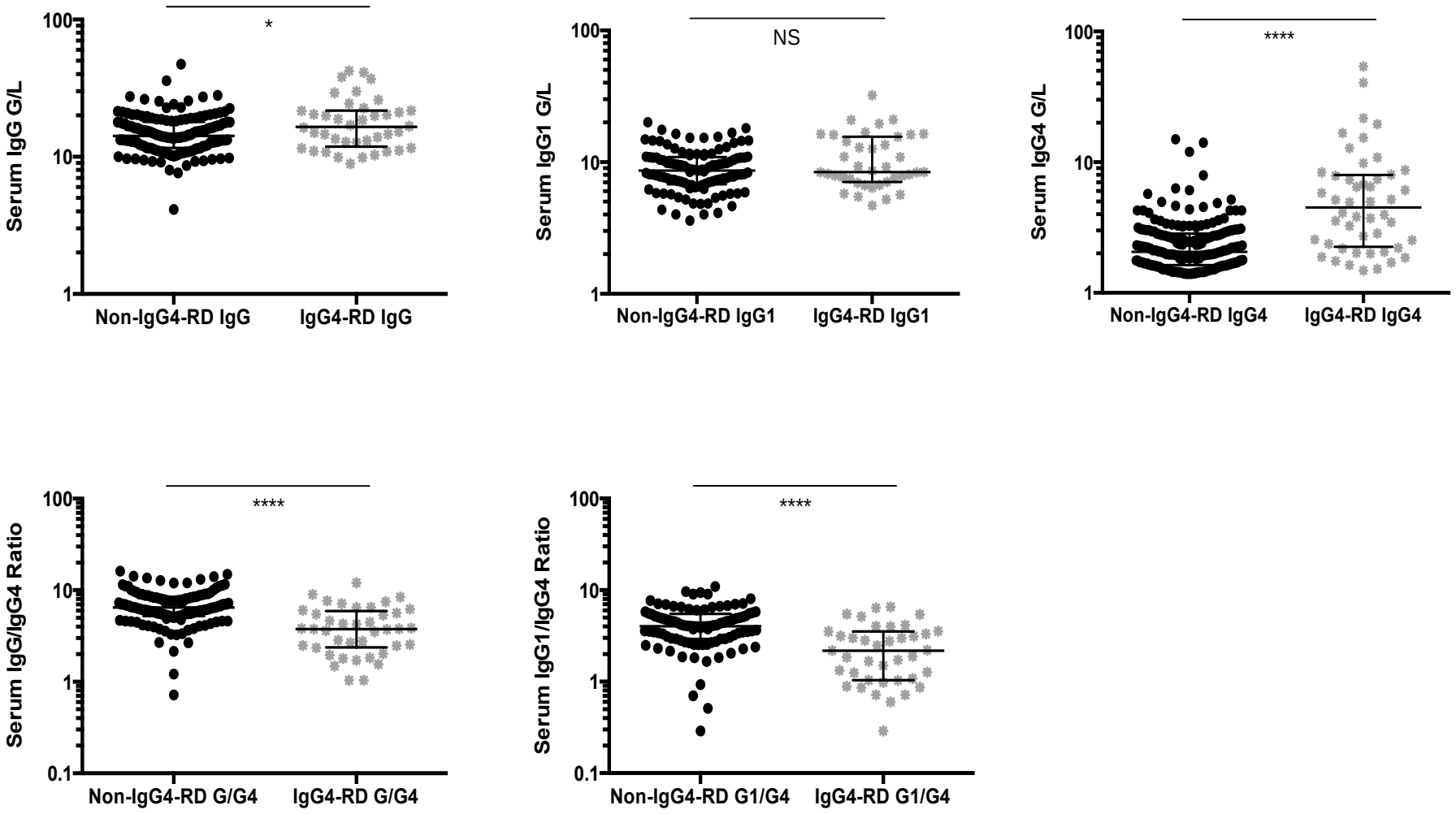


Figure 4: The relationship between serum IgG4 and organ involvement.

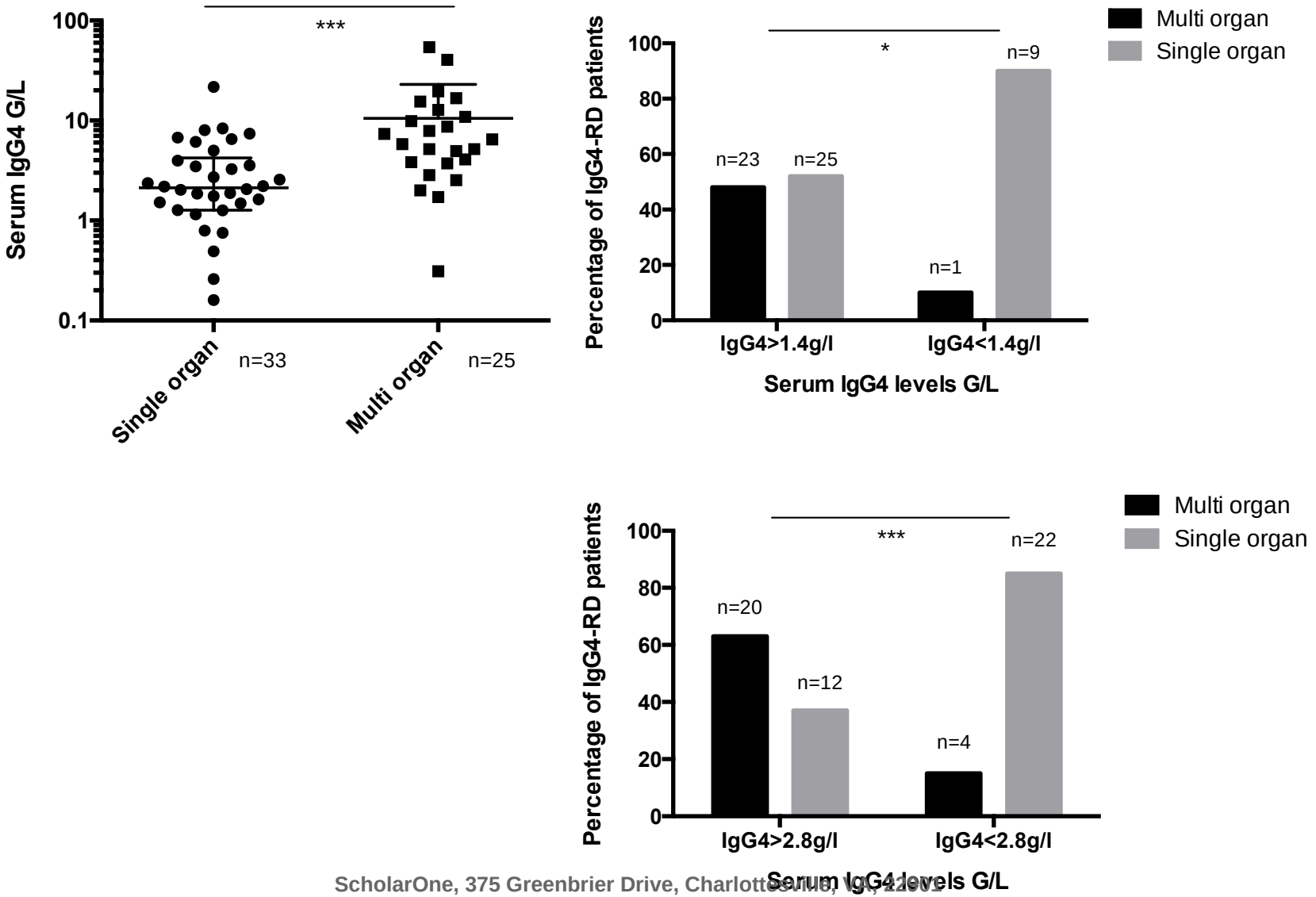


Figure 5: Relapse and serum IgG4 levels in IgG4-RD

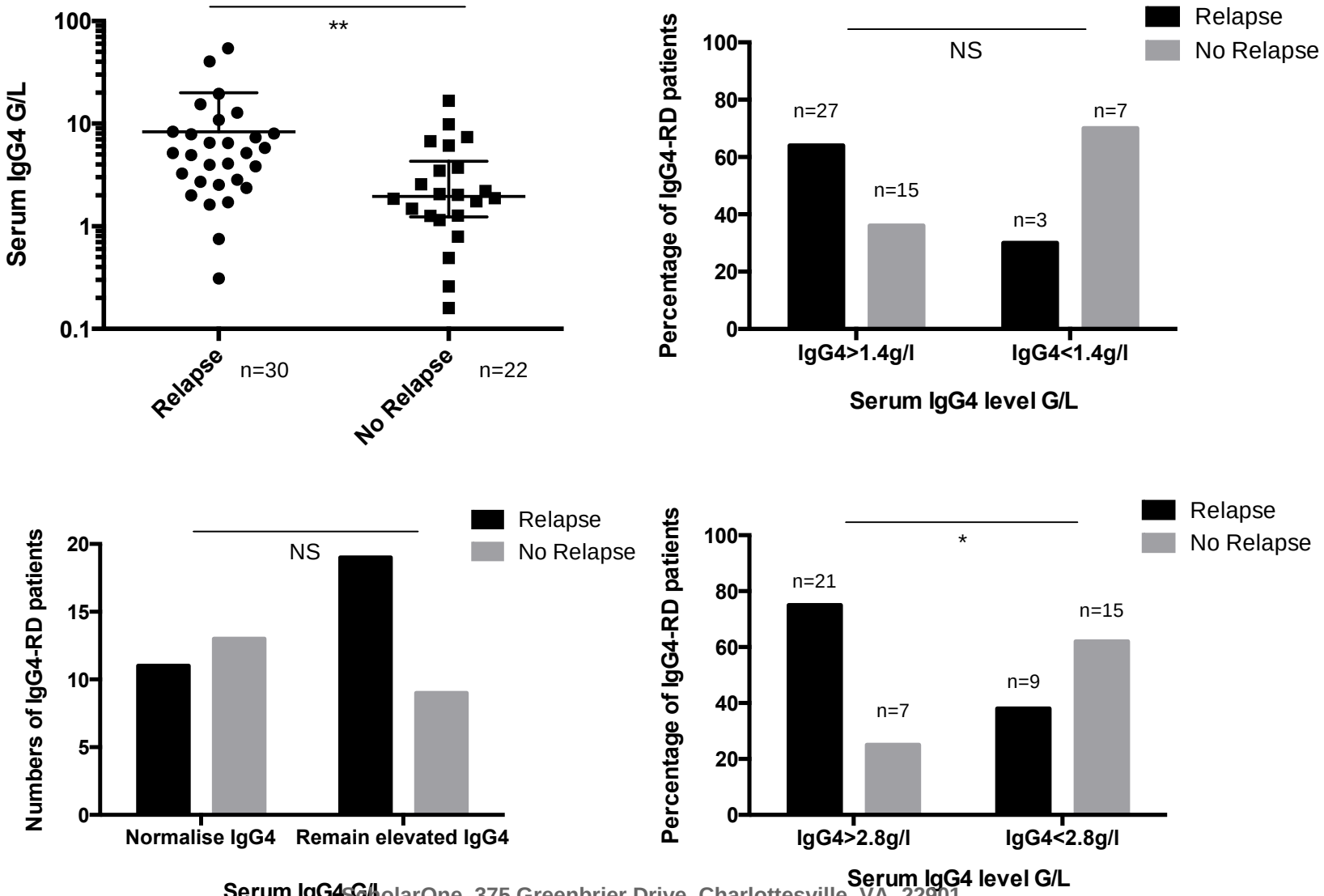
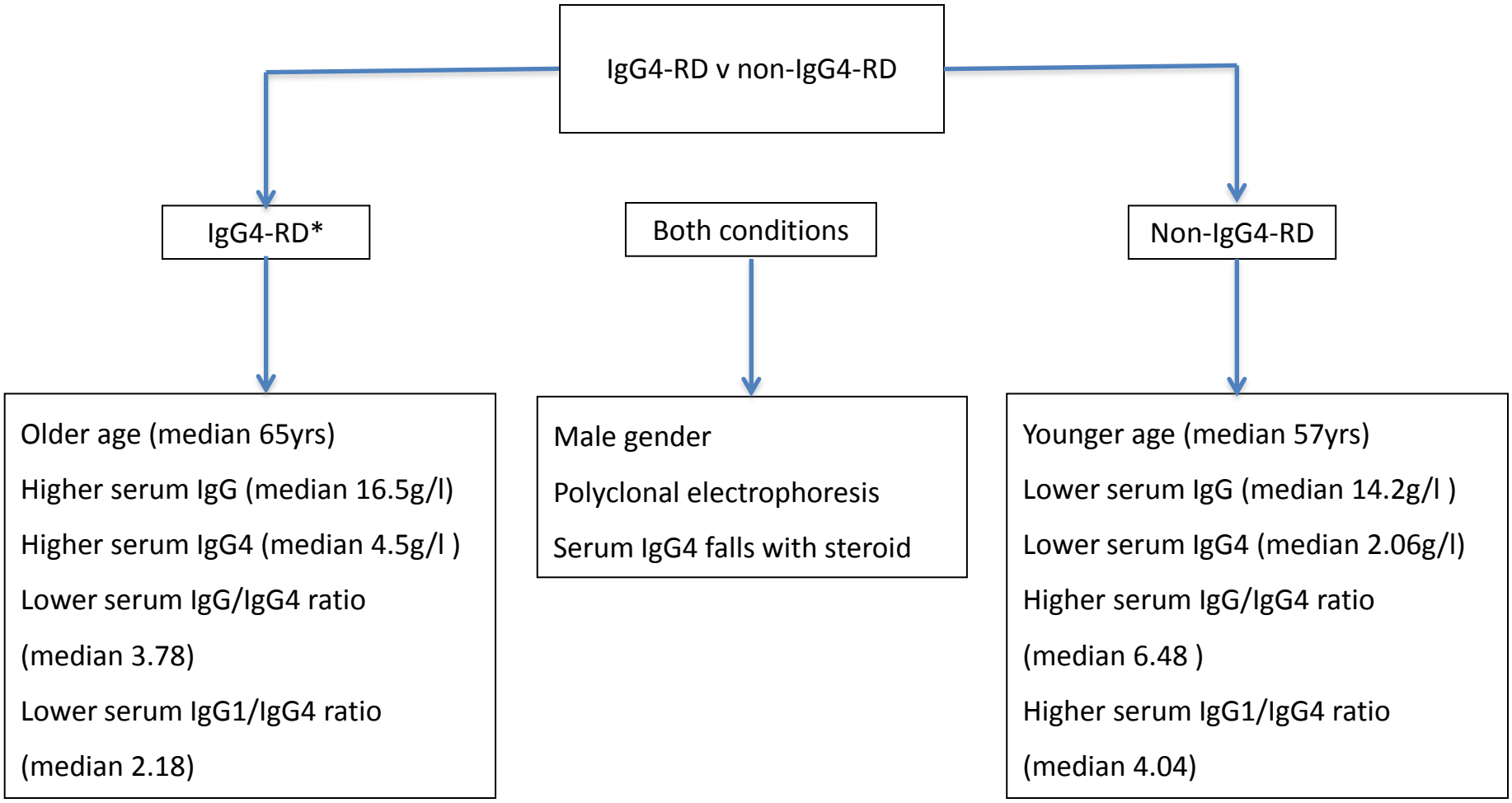
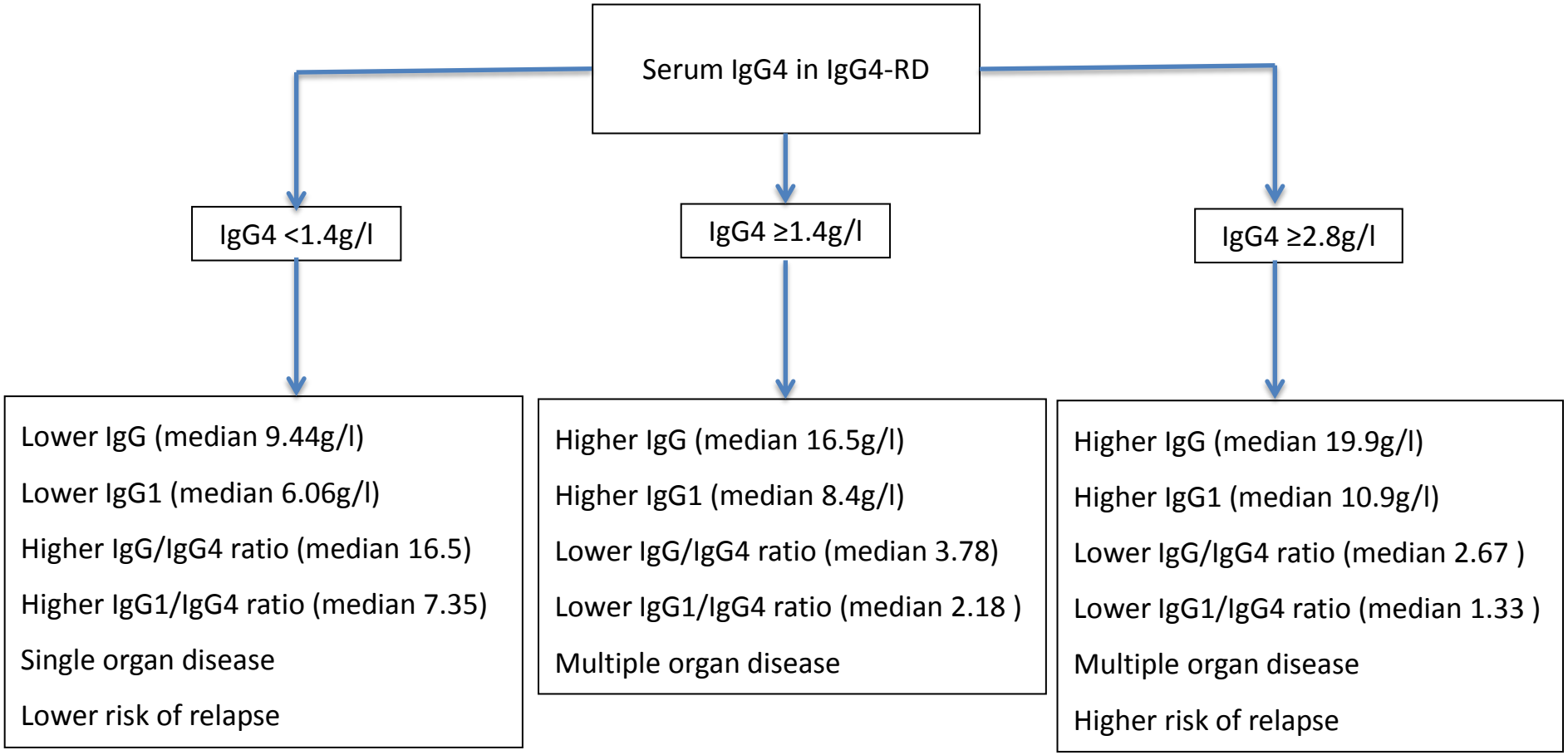


Figure 6 (A): Algorithm to differentiate IgG4-RD from non-IgG4-RD conditions



* Criteria to support the diagnosis of IgG4-RD
1. Histological evidence - Boston Consensus Histological Criteria for IgG4-RD
Morphology: LPCI; SF; OP + Tissue IgG4/IgG ratio >40% + Prominent tissue IgG4-positive cells±
2. Organ involvement (Clinical / Radiological evidence)
3. Response to steroids (Biochemical / Radiological)

Figure 6 (B): Algorithm in IgG4-RD patients with a normal and elevated serum IgG4 level



Supplementary Methods

Diagnosis of disease controls

A diagnosis of PSC was made in accordance with the European Association for the Study of the Liver Guidelines in those with a cholestatic biochemical profile, characteristic cholangiography and/or consistent liver histology when secondary sclerosing cholangitis had been excluded (33). A diagnosis of AIH was made using the International AIH Group's simplified scoring system (34), and of overlap syndrome when patients had clinical, laboratory, and histological features of both AIH and one of the cholestatic liver diseases primary biliary cirrhosis (cholangitis) or PSC. A diagnosis of IBD was based on the European Crohn's and Colitis Organisation guidelines for ulcerative colitis (35) and Crohn's disease (36).

Acute pancreatitis was classified according to the revised Atlanta classification (37) and chronic pancreatitis according to the TIGAR-O system of aetiologies (38). Pancreatic and/or biliary malignancy suggested by cross-sectional imaging was then confirmed histologically. Tubulointerstitial nephritis was defined in patients with compatible renal profile with supportive histology from renal biopsy. Autoimmune diseases included patients with systemic lupus, coeliac disease, and rheumatoid arthritis who had positive autoantibody profiles and supportive histology where necessary. Lastly, a diagnosis of allergy and atopy was made in accordance with the European Academy of Allergy and Clinical Immunology classification, with clinical symptoms and a positive IgE-specific allergen test (39).

Supplementary References

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Supplementary Table 1: Indication for serum IgG4 measurements

Indication for serum IgG4	Serum high IgG4	Serum normal IgG4
Distinguish between IgG4-RD and inflammatory, autoimmune or other disease mimic (%)	155 (68.9%)	839 (75.8%)
Distinguish between IgG4-RD and malignancy (%)	59 (26.2%)	87 (7.9%)
Immunodeficiency: recurrent or persistent infections (%)	6 (2.7%)	130 (11.8%)
Part of an autoimmune work-up including for Addison's, myasthenia gravis, vasculitis, rheumatoid, lupus (%)	5 (2.2%)	50 (4.5%)
Total patients (%)	225	1106

Supplementary Table 2: Serum Immunoglobulin levels and ratios in IgG4-RD and non-IgG4-RD patients with an elevated serum IgG4.

Immunoglobulin serum levels	**IgG4-RD**	**Non-IgG4-RD**	**p value**	
IgG g/l median (range)	16.50 (8.90-42.30)	14.20 (4.13-47.30)	0.043	*
IgG1 g/l median (range)	8.40 (4.68-32.20)	8.62 (3.59-20.10)	0.201	NS
IgG4 g/l median (range)	4.51 (1.48-54.10)	2.06 (1.40-15.00)	<0.0001	****
Ratio IgG1/IgG4 median (range)	2.18 (0.29-6.55)	4.04 (0.29-10.93)	<0.0001	****
Ratio IgG/IgG4 median (range)	3.78 (1.04-12.08)	6.48 (0.72-16.18)	<0.0001	****
Ratio IgG4/IgG1 median (range)	0.46 (0.15-3.40)	0.25 (0.09-3.44)	<0.0001	****
Ratio IgG4/IgG median (range)	0.27 (0.08-0.96)	0.15 (0.06-1.38)	<0.0001	****

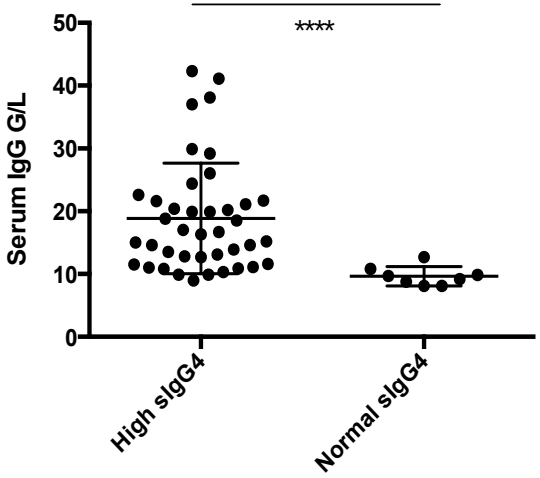
Supplementary Table 3: Clinical characteristics and immunoglobulin levels in IgG4-RD.

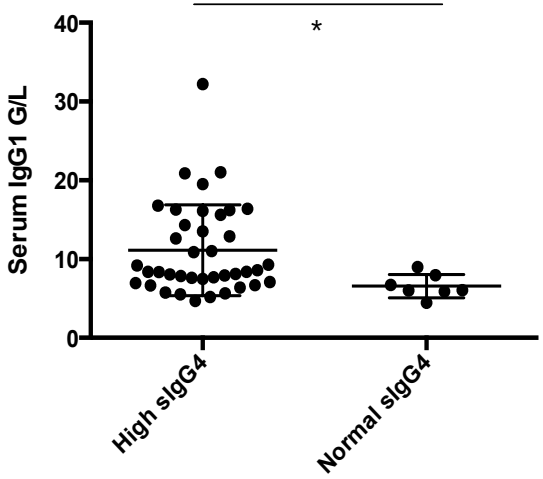
	All IgG4-RD	IgG4-RD with normal IgG4	IgG4-RD with high IgG4	P value	
Patient number	58	10	48		
Age median(range)	64.3 (24-83.8)	62.2 (27-80.5)	65.5 (24-83.8)	0.2732	NS
Gender Male(Female)	43(15)	6(4)	37(11)	0.2651	NS
Organs Single(Multiple)	34(24)	9(1)	25(23)	0.0354	*
Relapse yes(no)	30(22) +	3(7)	27(15) +	0.0753	NS
Serum IgG median(range)	14.60 (8.09-42.30)	9.44 (8.09-12.70)	16.5 (8.97-42.30)	<0.0001	****
Serum IgG1 median(range)	8.23 (4.46-32.2)	6.06 (4.46-8.98)	8.40 (4.68-32.20)	0.0153	*
Serum IgG4 median(range)	3.52 (0.0-54.10)	0.62 (0.0-1.27)	4.51 (1.48-54.10)	<0.0001	****
Serum IgG/IgG4 ratio median(range)	4.06 (0.0-50.56)	16.5 (0.0-50.56)	3.78 (1.04-12.08)	0.0004	***
Serum IgG1/IgG4 ratio median(range)	2.47 (0.0-27.88)	7.35 (0.0-27.88)	2.18 (0.29-6.55)	0.038	*

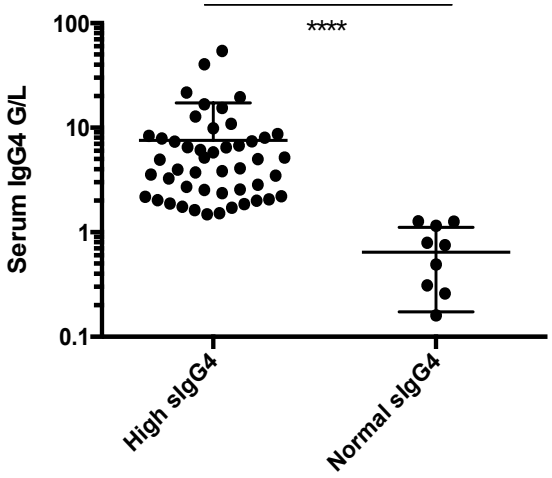
Supplementary Table 4: Development of Malignancy after IgG4-RD diagnosis.

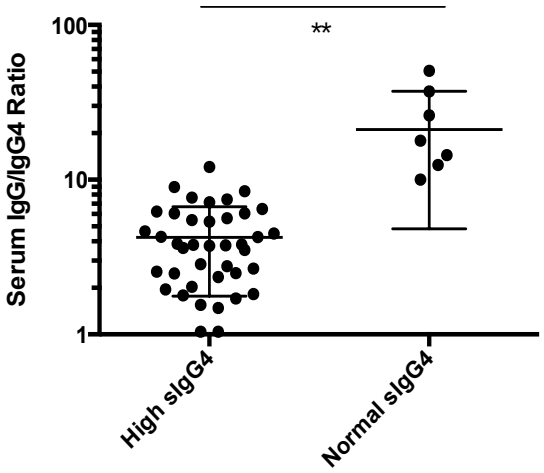
IgG4-RD Organ Involvement	Malignancy confirmed	Serum IgG4 level at diagnosis (g/l)	Months after IgG4 diagnosis
AIP, SMG, IgG4-ILD, LN	TCC Bladder	9.84	15
Bile duct	CCA	6.1	3
AIP, IgG4-SC, IgG4-ILD, LN	Prostate Adenocarcinoma	5.16	42
AIP, Parotids	Pancreas CA	4.09	6
AIP, IgG4-SC, Lungs	Prostate Adenocarcinoma	2.85	14

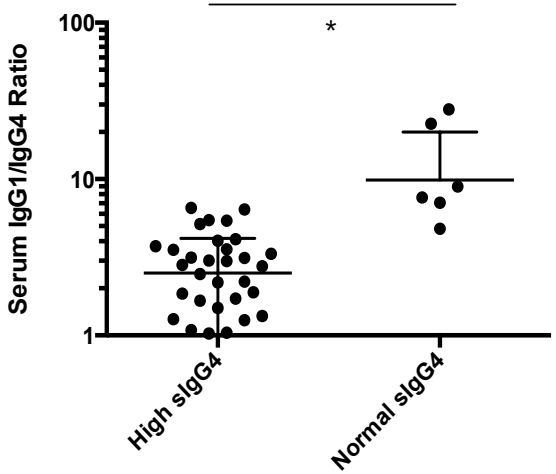
Supplementary Figure 1: Serum immunoglobulin values in IgG4-RD patients.



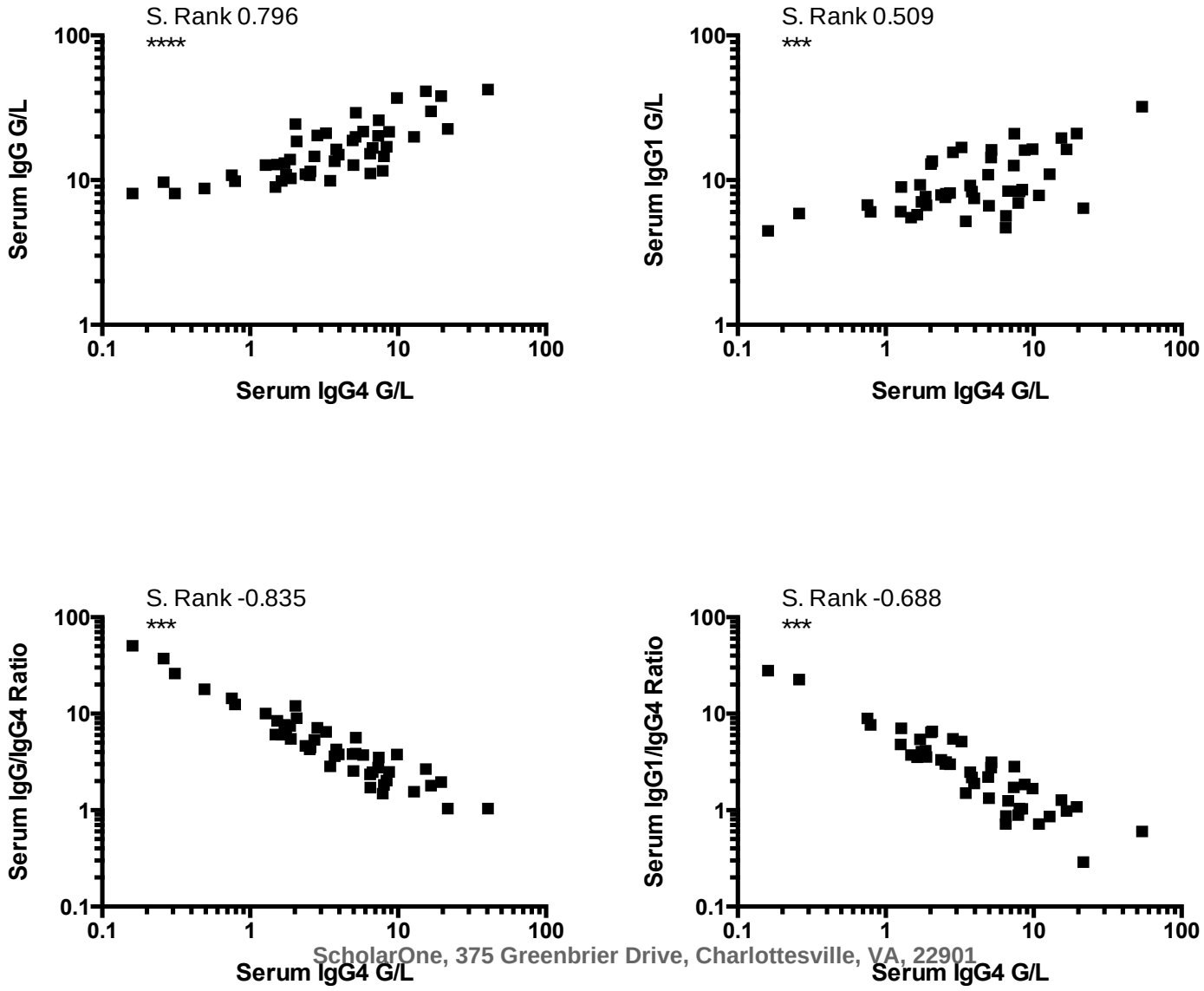




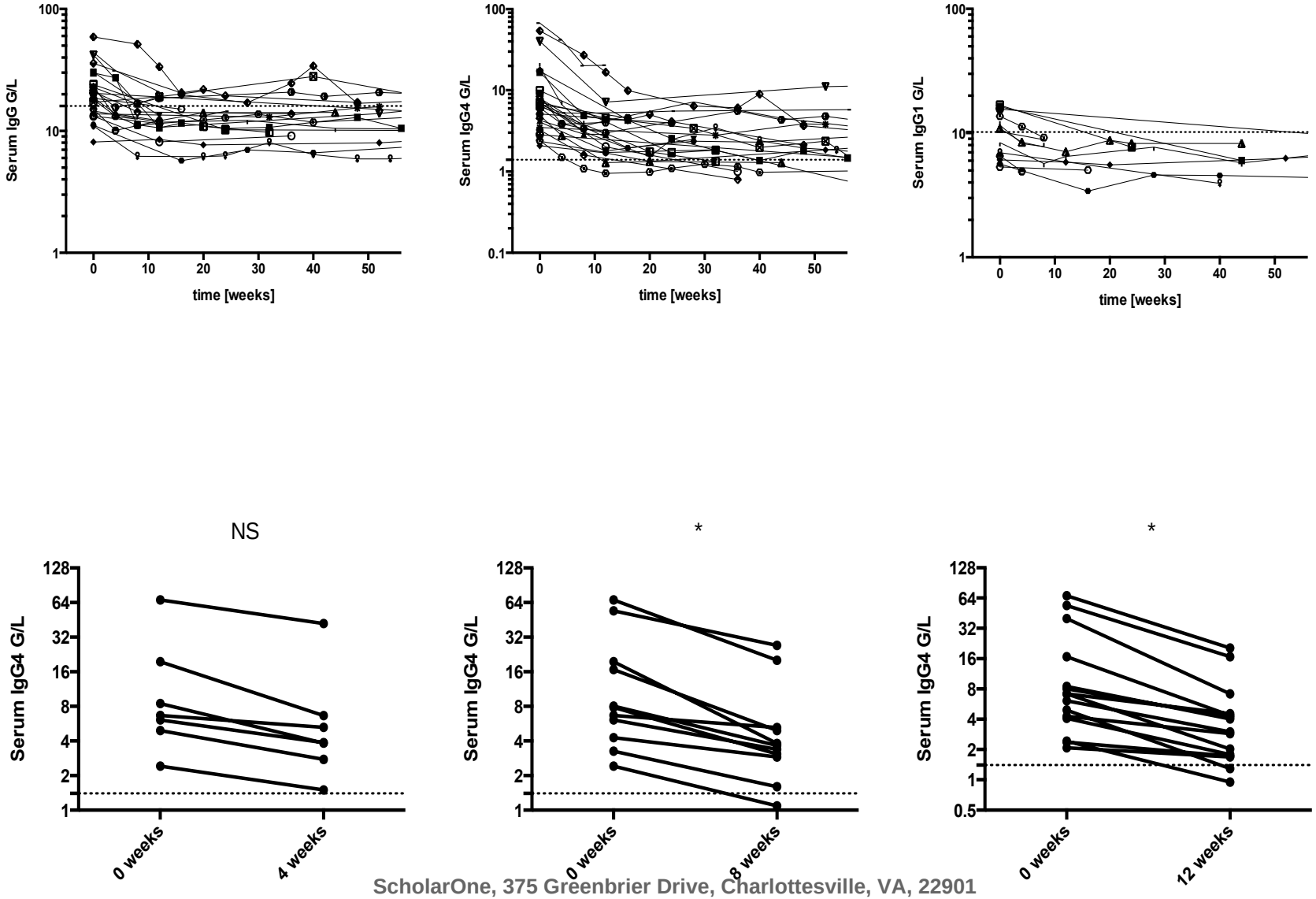




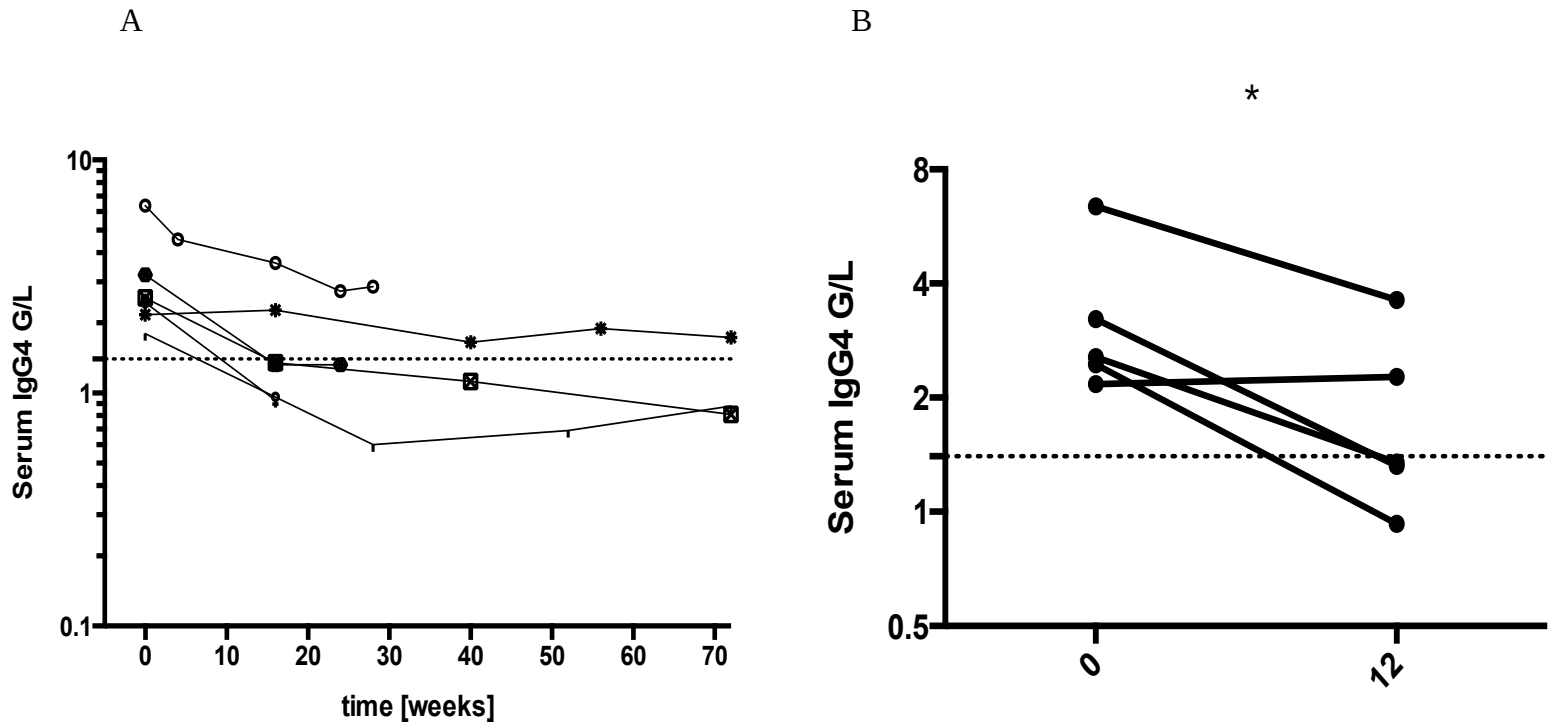
Supplementary Figure 2: Correlation of serum immunoglobulin levels in IgG4-RD.



Supplementary Figure 3: Immunoglobulin levels in response to corticosteroid therapy in IgG4-RD.



Supplementary Figure 4: IgG4 levels in response to corticosteroids in non-IgG4-RD with elevated IgG4.



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