Elevated Serum IgG4 Levels in Diagnosis, Treatment Response, Organ Involvement, and Relapse in a Prospective IgG4-Related Disease UK Cohort

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OBJECTIVES: **Elevated serum immunoglobulin G4 (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. The aims of this study were to ascertain conditions that 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 6 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: **Two thousand and sixty-seven samples from 1,510 patients had serum IgG4 measured. Of these, IgG4 was elevated ($\geq 1.4\,g\,l^{-1}$) 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 cutoff of $1.4\,g\,l^{-1}$ gave a sensitivity of 82.8% and specificity of 84.7% to diagnose IgG4-RD. Increasing this to $2.8\,g\,l^{-1}$ increased specificity to 96.2% and negative predictive value to 97.7%, with a lower sensitivity of 56.9% and positive predictive value of 44.5%. Serum IgG4 levels fell with corticosteroid therapy, but this was not disease-specific. A serum IgG4 of $\geq 2.8\,g\,l^{-1}$ at diagnosis was associated with multi-organ involvement and risk of relapse.**

CONCLUSIONS: **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 $\geq 2.8\,g\,l^{-1}$ is useful in distinguishing between IgG4-RD and non-IgG4-RD diagnoses, predicting multiple-organ involvement and risk of relapse in IgG4-RD.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Immunoglobulin G4–related disease (IgG4-RD) is a systemic fibro-inflammatory condition characterized by the development of mass lesions, with similar histopathological findings and an abundant IgG4-positive plasma cell infiltrate in the involved organs (1). The disease can mimic malignancy and other

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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, United States, and throughout Europe, with geographical variations in its clinical presentation, the utility of serum IgG4 in diagnosis, the presence of extra-pancreatic manifestations, and 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 the current diagnostic criteria (3,8–10). However, studies reviewing routine serum IgG4 level requests in France and United States 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 6-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 Ig 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 (6 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 (E.C., R.W.C., A.E., J.C., E.B.) 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 (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, 7 were diagnosed by histological criteria and 3 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 on biopsy or >50 IgG4+ plasma cells per high-power field on resection, with a ratio of IgG4/IgG of >40%, depending on the 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 (PSC) 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** online.

Serum Ig measurements

Total serum IgG and subclasses (IgG1, IgG2, IgG3 and IgG4) were measured by nephelometry using a BNII analyzer (Siemens, Surry, UK) at the Clinical Immunology Department, Churchill Hospital, Oxford, UK. The normal range for serum Ig concentrations in the region was determined and validated by Oxford Immunology Department and is closely aligned with national Ig reference values: IgG 6–16 $g\,l^{-1}$, IgG1 3.2–10.2 $g\,l^{-1}$, IgG2 1.2–6.6 $g\,l^{-1}$, IgG3 0.2–1.9 $g\,l^{-1}$, and IgG4 0.1–1.35 $g\,l^{-1}$. For this study, an elevated serum IgG was ≥16 $g\,l^{-1}$ and serum IgG4 was ≥1.4 $g\,l^{-1}$, as used in the Mayo HISORt criteria for AIP and in line with other studies. For patients with multiple serum Ig 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 standardized in the majority: prednisolone 0.5 $mg\,kg^{-1}$ (30–40 mg) 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, and months 3 and 6 after treatment commenced. Subsequent clinical visits were determined by clinical need. Blood tests, including renal function, liver function, 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, owing 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 endoscopic retrograde cholangiopancreatography. 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 owing to stent dysfunction). Sufficient follow-up to determine relapse was defined as a period of >6 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 centers (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* Dunn's 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 Ig levels. Receiver operator characteristic curves were calculated for sensitivity and specificity. Statistics were calculated using Graphpad Prism v6.0 (GraphPad Software, La Jolla, CA). 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, 2,067 blood samples from 1,510 patients had serum IgG4 subclass levels measured. Of these, 610 samples from 243 unique patients had an elevated serum IgG4 (>1.4 $g\,l^{-1}$). The serum IgG4 was elevated in 16.1% (243/1,510) of the patients in whom IgG4 was requested.

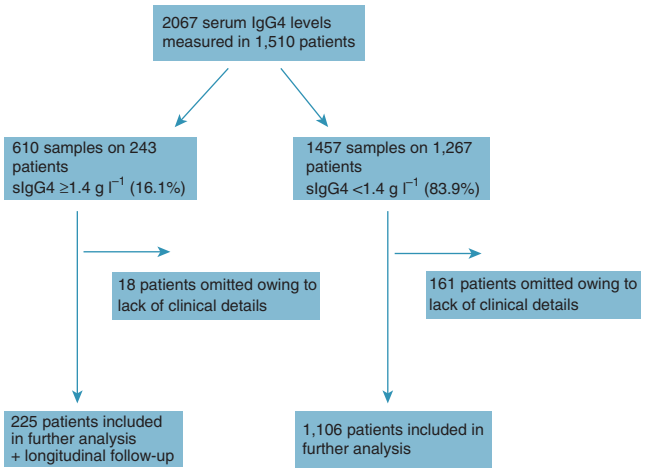

Figure 1. Flow diagram of serum immunoglobulin G4 (IgG4) measurements in the Oxford cohort. A flow diagram illustrating the number of serum IgG4 subclass measurements performed at the Clinical Immunology Department, Churchill Hospital, Oxford, UK over a 6-year period from January 2008 until December 2013.

Table 1. Demographics and serum immunoglobulin levels of patients with a normal and elevated serum IgG4

	High serum IgG4	Normal serum IgG4	*P* value
Samples	610	1,457	
Unique no. of patients	243	1,267	
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) ($g\,l^{-1}$)	14.6 (4.13–47.3)	11 (0.76–43.20)	<0.0001
Serum IgG4, median (range) ($g\,l^{-1}$)	2.24 (1.37–54.1)	0.35 (0–1.39)	<0.0001
Serum IgG1, median (range) ($g\,l^{-1}$)	8.56 (3.59–32.2)	7.19 (0.5–66.0)	<0.0001

F, female; IgG4, immunoglobulin G4; M, male. Mann–Whitney test of continuous variables, where *P* values=**P*<0.05, *****P*<0.0001. Fisher's Exact test of categorical variables, where *P* values=*****P*<0.0001.

Demographics

The demographics of patients with a normal and elevated serum IgG4 are shown in **Table 1**. Overall, there was a male predominance in 243 patients with an elevated serum IgG4 (67.9%) and a female predominance in 1,267 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 with normal (55.0 years) serum IgG4 ($P=0.035$).

Serum Ig concentrations

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

Clinical indication for serum IgG4 measurements

From 1,510 patients, sufficient clinical details were available in 1,331 patients (225 patients with an elevated serum IgG4 and 1,106 patients with a normal serum IgG4). The indications for serum IgG4 measurement in these patients are shown in **Supplementary Table S1**. The main indication for serum IgG4 measurements was to distinguish between IgG4-RD and other diseases (85.6%; 1,140/1,331 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 1,140 patients in whom serum IgG4 was measured to distinguish between IgG4-RD and other diseases (IgG4-RD vs. 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 PSC (to differentiate from AIP and IgG4-SC). Overall, an elevated serum IgG4 was seen in 18.7% (214/1,140) 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 vs. 82.8% of those confirmed with IgG4-RD ($P<0.0001$). Only 5.1% (58/1,140) of patients who had serum IgG4 measured had a final diagnosis of IgG4-RD.

Receiver operator characteristic curves for a diagnosis of IgG4-RD

Using the final diagnosis attributed to the 1,140 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 $1.74\,g\,l^{-1}$, range $0–54.1\,g\,l^{-1}$) than in those with non-IgG4-RD diagnoses (median $0.42\,g\,l^{-1}$, range $0–15.0\,g\,l^{-1}$) ($P<0.0001$) (**Figure 2a**). At a serum IgG4 cutoff of $1.4\,g\,l^{-1}$ (upper limit of normal), the sensitivity was 82.8% (48/58), specificity was 84.7% (916/1,082), 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 cutoff to diagnose the disease was evaluated. Increasing the IgG4 to $2.8\,g\,l^{-1}$ (twice the upper limit), the sensitivity was 56.9% (33/58), specificity was 96.2% (1,041/1,082), PPV was 44.6% (33/74), and NPV was 97.7% (1,041/1,066) to diagnose IgG4-RD. Furthermore, increasing the IgG4 to $5.6\,g\,l^{-1}$ (four times the upper limit), the sensitivity was 36.2% (21/58), specificity was 99.5% (1,077/1,082), PPV was 80.8% (21/26), and NPV was 96.7% (1,077/1,114). Although 41 patients (3.7%) with non-IgG4-RD diagnoses had

a serum IgG4 >2.8 g l^{-1}, only 5 patients (0.5%) had a serum IgG4 >5.6 g l^{-1}. However, this did include two patients with histologically confirmed malignancy (one resectable cholangiocarcinoma and one metastatic with unknown primary). Of the PSC patients, 5 had a serum IgG4 >2.8 g l^{-1} but none had a serum IgG4 >5.6 g l^{-1}.

Table 2. Final diagnosis in patients who had serum IgG4 measured to distinguish between IgG4-RD and other diseases

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	1,140	214/1140 (18.7)

AIH, autoimmune hepatitis; IgG4-RD, immunoglobulin G4-related disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis. The proportion (absolute number, percentage) of patients in each group, and with an elevated serum IgG4 in each disease category, is given.

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 the IgG4-RD criteria. However, IgG4-RD was the most frequent diagnosis made in the high serum IgG4 group (**Table 3**). Among 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 Ig levels and ratios in IgG4-RD and non-IgG4-RD patients with an elevated serum IgG4

Serum Ig levels and ratios in the IgG4-RD patients and non-IgG4-RD patients with an elevated serum IgG4 were then evaluated (**Figure 3** and see **Supplementary Table S2**). Serum total IgG and IgG4 were higher in IgG4-RD patients (IgG median 16.5 g l^{-1}, range 8.9–42.3 g l^{-1}; IgG4 median 4.5 g l^{-1}, range 1.48–54.1 g l^{-1}) than in those with non-IgG4-RD diagnoses (IgG median 14.2 g l^{-1}, range 4.13–47.3; IgG4 median 2.06 g l^{-1}, range 1.40–15.0 g l^{-1}) (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 in those with 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 1,140 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 Ig values in patients with IgG4-RD are shown in **Supplementary Table S3**. The majority of IgG4-RD patients were male (77.6%) in the seventh decade

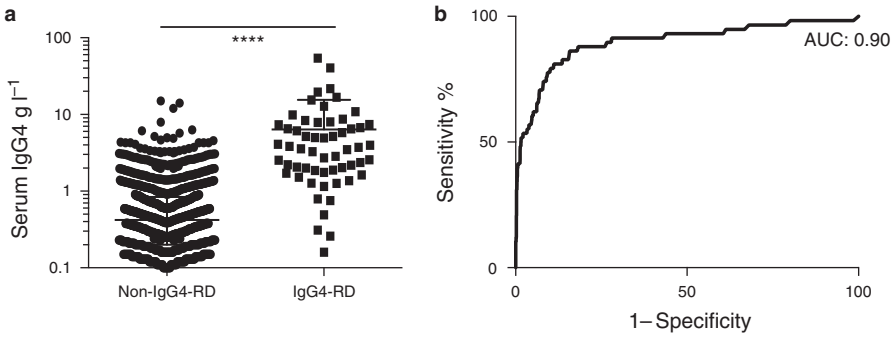

Figure 2. Sensitivity and specificity of serum immunoglobulin G4 (IgG4) to diagnose IgG4-related disease (IgG4-RD). (**a**) The dot plot shows the serum IgG4 levels in non-IgG4-RD and IgG4-RD diagnoses. On the *y* axis is Log 10 scale serum IgG4 in g l^{-1}. On the *x* axis are patients with non-IgG4-RD and IgG4-RD diagnoses. The error bars represent median and inter-quartile range. Mann–Whitney test; NS (not significant) P≥0.05, * P<0.05, ** P<0.005, *** P<0.005, **** P<0.0001. (**b**) The receiver operator characteristic curve shows the sensitivity (%) and 1–specificity (%) of serum IgG4 for the diagnosis of IgG4-RD. The area under the curve (AUC) is 0.9.

Table 3. Final diagnosis in patients with an elevated serum IgG4 recruited into a prospective longitudinal study at the Oxford Radcliffe Hospital Trust

Clinical diagnosis (elevated serum IgG4)	Absolute number	Percentage 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	

AIH, autoimmune hepatitis; IgG4-RD, immunoglobulin G4-related disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

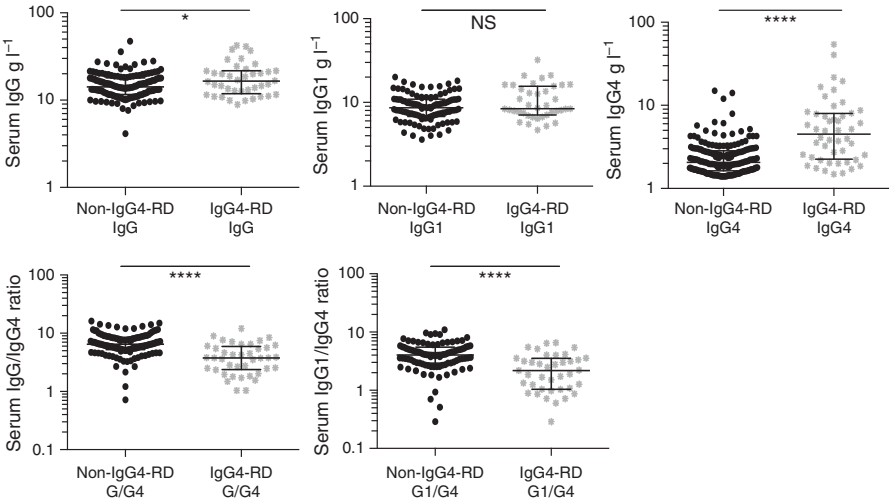

Figure 3. Serum immunoglobulin (Ig) values and ratios in IgG4-related disease (IgG4-RD) and non-IgG4-RD patients with an elevated serum IgG4 level. The dot plot shows serum Ig levels and ratios in IgG4-RD patients and non-IgG4-RD patients with an elevated serum IgG4 level. On the *y* axis is Log 10 scale serum IgG, IgG1, and IgG4 in $g\,l^{-1}$, IgG1/IgG4, and IgG/IgG4 ratio. On the *x* axis are patients with non-IgG4-RD and IgG4-RD diagnoses. The error bars represent median and inter-quartile range. Mann–Whitney test; NS (not significant) $P \geq 0.05$, *$P<0.05$, **$P<0.005$, ***$P<0.005$, ****$P<0.0001$.

of life (median 64.3 years, range 24–84 years). Most patients (48/58, 82.8%) had an elevated serum IgG4 level. In 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 ($P=0.0004$) and IgG1/IgG4 ratios ($P=0.038$) compared with the 48 patients with an elevated serum IgG4 (see **Supplementary Figure S1**). 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 (rank −0.83, $P<0.0001$) and IgG1/IgG4 (rank −0.69, $P<0.0001$) ratios (see **Supplementary Figure S2**).

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 1.3–73.5 months) as shown in **Figure 4**. Twenty-five patients (43.1%) had multiple-organ involvement. The serum IgG4 level in patients with multi-organ disease (median 6.141 $g\,l^{-1}$, range 0.31–54.1 $g\,l^{-1}$) was higher compared with those with single-organ disease (median 2.12 $g\,l^{-1}$, range 0–21.7 $g\,l^{-1}$) (P=0.0001). At diagnosis, a serum IgG4 of >1.4 $g\,l^{-1}$ (P=0.0354) and >2.8 $g\,l^{-1}$ (P=0.0004) predicted risk of multiple- rather than single-organ involvement at follow-up (**Figure 4**). Nine of the 10 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 1.5–73.5 months; single-organ disease median 28.8 months, range 1.3–68.6 months) (P=0.6801).

Serum IgG4 levels and treatment in IgG4-RD

The relationship of serum Ig 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 in IgG1, during corticosteroid treatment (see **Supplementary Figure S3**, upper panel). Serum IgG4 levels fell within 4 weeks of initiation (n=7, P=0.084) and were significantly lower compared with pretreatment levels at 8 weeks (n=11, P=0.033) and 12 weeks (n=15, P=0.018) (see **Supplementary Figure S3**, lower panel). The steepest slope of decline was between 0 and 8 weeks at the highest dose of steroids, 20–40 mg prednisolone per day, and fluctuated while at lower doses 5–10 mg prednisolone per 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–inflammatory bowel disease and AIH) similarly showed a decrease in serum IgG4 (see **Supplementary Figure S4**). Serum IgG4 levels also were significantly lower compared with pretreatment 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 the 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 5.05 $g\,l^{-1}$, range 0.0–54.10) compared with those who 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 >2.8 $g\,l^{-1}$ (P=0.0108) but not with a serum IgG4 >1.4 $g\,l^{-1}$ (P=0.0753) (**Figure 5**). Three of the 10 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 the 58 (8.6%) IgG4-RD patients developed malignancy during follow-up (median 13.5 months, range 2.5–42.1 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 five patients had an elevated serum IgG4 (>2.8 $g\,l^{-1}$) at IgG4-RD diagnosis (median IgG4 5.16 $g\,l^{-1}$, range 2.85–9.84 $g\,l^{-1}$) (see **Supplementary Table S4**). An elevated serum IgG4 level itself did not predict risk of malignancy (P=0.277).

Six of the 58 (10.3%) IgG4-RD patients died during follow-up (median 13.0 months, range 1.5–51.5 months from IgG4-RD diagnosis to date of death). Causes of mortality included pneumonia (2), end-stage pulmonary fibrosis (2), postoperative death following Whipple's surgery for suspected malignancy (1), and metastatic transitional cell bladder carcinoma (1). All six patients had an elevated serum IgG4 (>1.4 $g\,l^{-1}$) at IgG4-RD diagnosis (median IgG4 2.71 $g\,l^{-1}$, range 1.86–9.84 $g\,l^{-1}$). 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, Ig values, and ratios, which are characteristic of IgG4-RD patients vs. non-IgG4-RD conditions (**Figure 6a**). Furthermore, in those patients with IgG4-RD, a second algorithm is used to differentiate normal serum IgG4 (<1.4 $g\,l^{-1}$) and elevated serum IgG4 (divided into ≥1.4 and ≥2.8 $g\,l^{-1}$) groups, using Ig values, ratios, and disease characteristics (**Figure 6b**). The diagnosis of IgG4-RD does not depend on serum Ig 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% (58/1,140) 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%; 1,140/1,331 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 overrepresentation of hepatobiliary diseases.

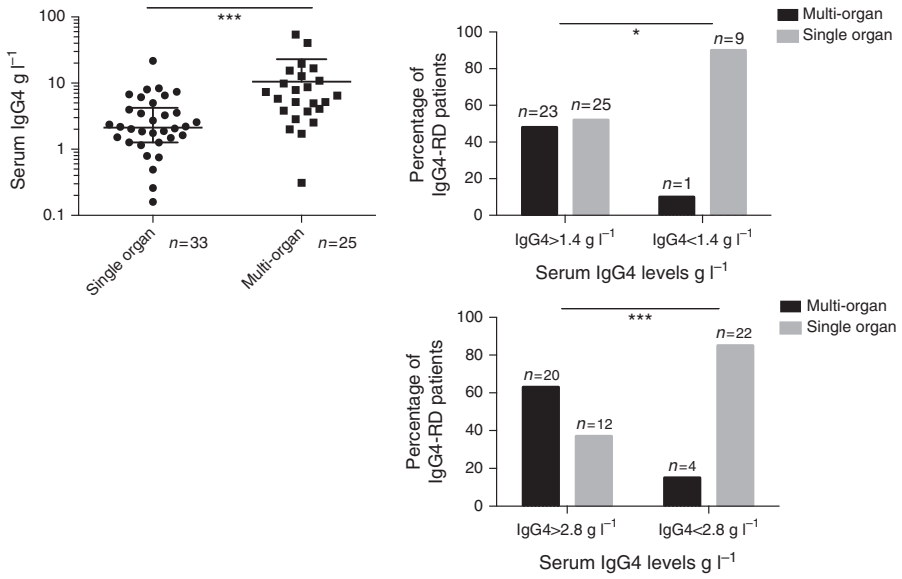

Figure 4. The relationship between serum immunoglobulin G4 (IgG4) and organ involvement. The dot plot shows the relationship of serum IgG4 and organ involvement in patients with IgG4-related disease (IgG4-RD). On the *x* axis are patients with single- (*n*=33) or multiple-organ (*n*=25) disease. *y* 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. Fisher's Exact test; NS (not significant) $P \geq 0.05$, *$P<0.05$, **$P<0.005$, ***$P<0.005$, ****$P<0.0001$.

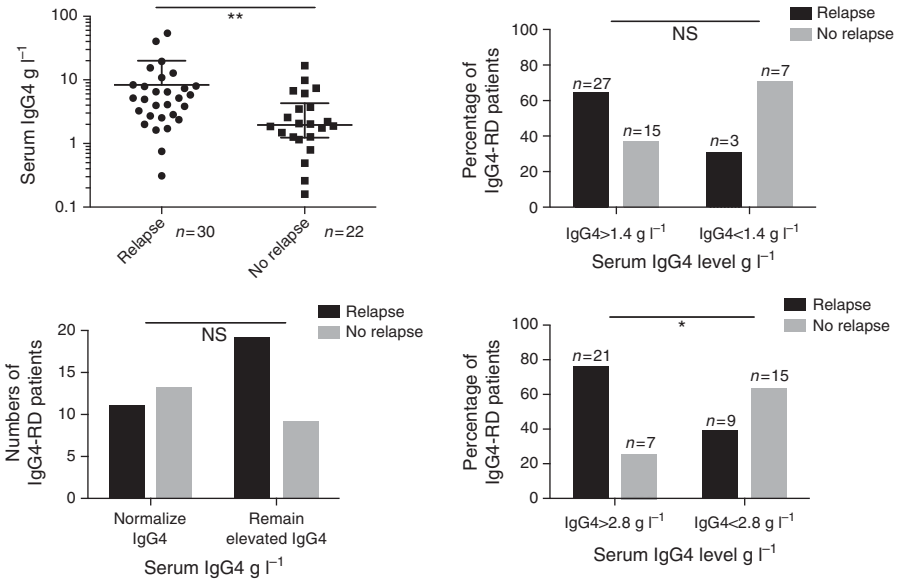

Figure 5. Relapse and serum immunoglobulin G4 (IgG4) levels in IgG4-related disease (IgG4-RD). The dot plot shows the relationship of serum IgG4 and relapse in patients with IgG4-RD. On the *x* axis are patients who have (*n*=30) or have not (*n*=22) relapsed. *y* Axis, error bars and Mann–Whitney *P* 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. Fisher's Exact test *P* values as per **Figure 4**.

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

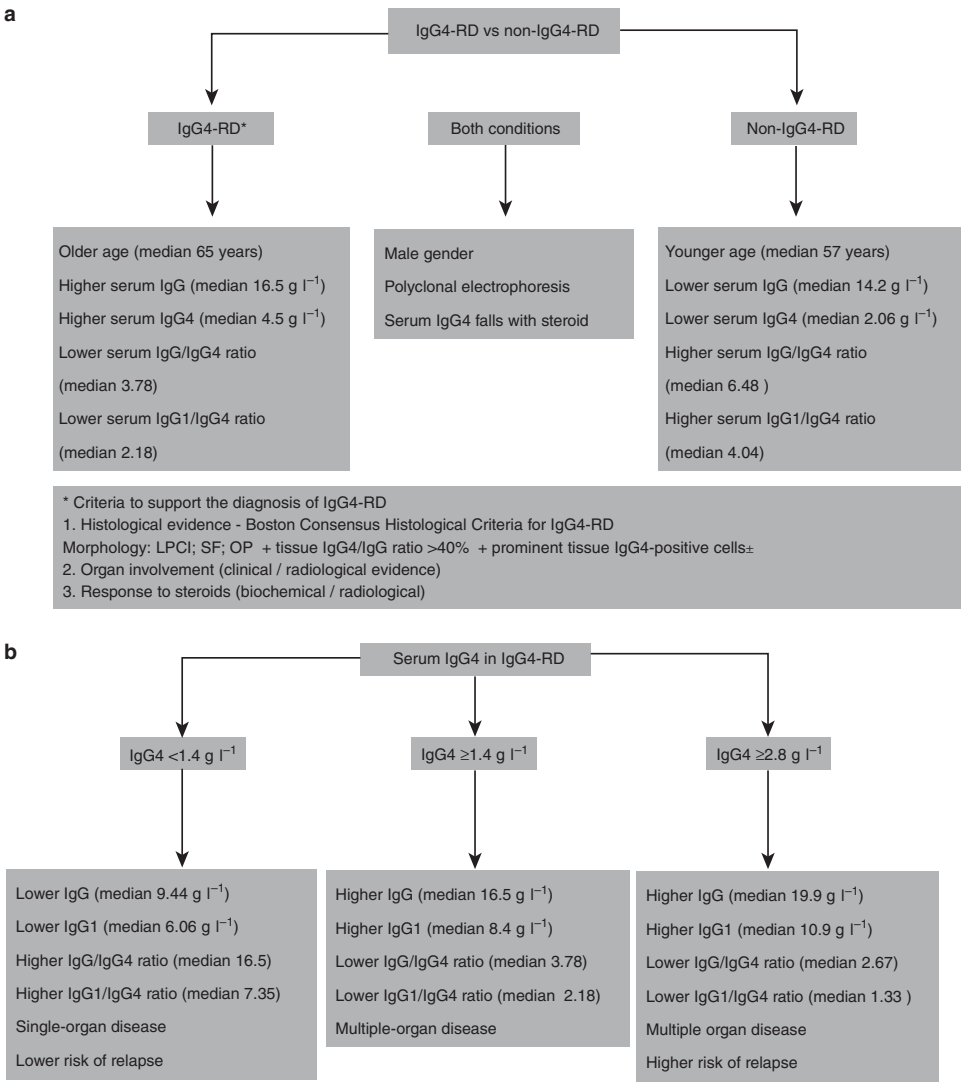

Figure 6. Algorithms in the diagnosis of immunoglobulin G4–related disease (IgG4-RD). (**a**) Algorithm to differentiate IgG4-RD from non-IgG4-RD conditions. The algorithm identifies clinical features and Ig values characteristic of IgG4-RD patients, non-IgG4-RD conditions, and both conditions. Median values are shown. Ig levels have been calculated using the Siemens IgG subclass reagents and Siemens Nephelometer. 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 Ig values and disease characteristics in IgG4-RD patients with a normal serum IgG4 $<1.4\,g\,l^{-1}$, elevated serum IgG4 $\geq 1.4\,g\,l^{-1}$, and moderately elevated serum IgG4 $\geq 2.8\,g\,l^{-1}$. Median values are shown. Ig levels have been calculated using the Siemens IgG subclass reagents and Siemens Nephelometer. HPF, high-power field; LPCI, lymphoplasmacytic infiltrate; OP, obliterative phlebitis; SF, storiform fibrosis.

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 $1.35\,g\,l^{-1}$ 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 $1.35\,g\,l^{-1}$ 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 that a serum IgG4 of 1.4 g l^{-1} has a sensitivity of 82.8%, specificity of 84.7%, PPV of 22.4%, and an 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 cutoff value for serum IgG4 (2.8 g l^{-1}) 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-recognized 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 pretest 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 overdiagnosis 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 (≥2.8 g l^{-1}), 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 ≥1.4 and ≥2.8 g l^{-1} 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 (positron emission tomography and computed tomography) 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 ≥2.8 g l^{-1} 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 while on steroid treatment, with significant decline at 8 and 12 weeks of therapy. In some centers, 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 ≥2.8 g l^{-1} at diagnosis should have contrast-enhanced CT of 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 United Kingdom analyzing 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 ≥2.8 g l^{-1} 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.

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CONFLICT OF INTEREST

Guarantor of the article: Berne Ferry, PhD, FRCPath.

Specific author contributions: E.L.C. recruited patients and collected blood samples, collated and analyzed the raw data, and drafted, edited, and approved the final manuscript. R.S. collected and analyzed serological data, edited, and approved the final manuscript. D.S. processed serological samples, collected data, and approved the final manuscript. T.C. collected patient data and approved the final manuscript. M.M. analyzed serological data and approved the final manuscript. A.C.B. reviewed immunostained histological sections and approved the final manuscript. A.J.E., J.C., and R.W.C. recruited patients, collected samples, and approved the final manuscript. P.K. edited and approved the final manuscript. E.B. is the principle investigator for the IgG4-RD NIHR study, edited. and approved the final manuscript. B.F. had the original concept for this study, funded and processed the samples, edited and approved the final manuscript, and is the guarantor of the article.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE?

- ✓ Organ manifestations of immunoglobulin G4–related disease (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 1,140 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 $\geq 2.8\,g\,l^{-1}$ shows high specificity (>96%) for diagnosis of IgG4-RD in a prospective UK cohort.
- ✓ Elevated serum IgG4 $\geq 2.8\,g\,l^{-1}$ 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.

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