IgG4-Related Disease

Insights in the pathogenesis, clinical presentations, diagnostics and treatment

A.F. Karim

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IgG4-Gerelateerde Ziekte

Inzicht in de pathogenese, klinische presentaties, diagnostiek en behandeling

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Chapter 1

General Introduction

Chapter 1.1 General Introduction and aims of this thesis

The discovery of IgG4-related disease

In 2001, a clinical research group from Japan found that high levels of serum IgG4 in patients distinguished sclerosing pancreatitis from other pancreas or biliary tract conditions (1). However, the ''pathological'' role of an elevated serum IgG4 level appeared not be restricted to the pancreas. In 2003, a new entity of systemic IgG4-related autoimmune disease was reported. The disease was described in the pancreas, bile duct, retroperitoneum and salivary glands (2) and was responsive to glucocorticoids. Histopathological examination revealed typically fibrosis and tissue infiltration of IgG4 positive plasma cells in the affected tissues. After different nomenclatures being purposed for this clinical entity, eventually ''IgG4-related disease'' was chosen as a name for this disease (3). Despite its discovery in 2003, IgG4-related disease (IgG4-RD) is not a new disease. Several previously well-described cases retrospectively might have been IgG4-RD, but these individual diseases were considered as distinct disease entities for years. Examples of these diseases are the Küttner's disease, Riedel's thyroiditis and Mikulicz disease (4).

Why to be aware of IgG4-related disease?

IgG4-RD is a systemic fibro-inflammatory condition with manifestations in virtually all parts of the human body (5). Physicians from different medical fields may be involved with patients who suffer from IgG4-RD. Several tools such as recognition of a typical IgG4-RD patient, serological tests, imaging studies and histology should help a physician to diagnose IgG4-RD, however, awareness is the most important. IgG4-RD can only be diagnosed when specific serological tests and especially, IgG4 staining on histology is performed (6). The pathophysiology is still not completely understood, but disbalance in the immune system leads to the phenotype of IgG4-RD and, if untreated, destructive fibrosis may cause irreversible damage to the affected organ(s) (4). Therefore, early diagnosis may prevent unnecessary and irreversible disease complications.

The clinical manifestations of IgG4-relate disease

Patients with IgG4-RD often present with tumor-like lesions, symptoms are most often restricted to the affected organs (7). Obviously, general symptoms such as fever or weight

loss are usually not seen. The disease can manifest in one organ, but systemic localizations are often present (8). The most frequent affected organs are the lacrimal gland and orbita, lymph nodes, salivary glands and the pancreas. However, almost all organs can be affected. Imaging, especially fluor-18-deoxyglucose positron emission tomography (FDG-PET), can be used to image systemic localizations, while the particular patient may only have symptoms of one affected organ (9) (Figure 1). The list of the affected organs in IgG4-RD is growing. Recently, many case reports and case series were published on IgG4-RD revealing different clinical presentations. Table 1 shows the most frequently affected organs in patients with IgG4-RD and the associated clinical symptoms.

Figure 1

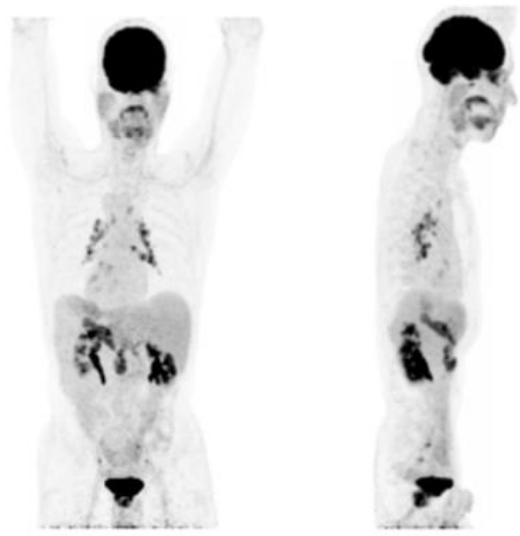

Figure 1. FDG-PET scan of histologically proven systemic IgG4-RD. Multifocal lesions with intense uptake are visualized in the orbital region, mediastinum, hilar and abdominal lymph nodes, pancreas, kidney's and prostate. This patient presented with only periorbital swelling, but extended analysis revealed systemic IgG4 RD.

Table 1. The different organ manifestations and clinical presentations of IgG4-RD.

Organ manifestation	Clinical presentation
Ocular/orbital and lacrimal glands	Lacrimal gland swelling Ocular dryness Proptosis Retro-bulbar mass Scleritis
Salivary glands	Swelling and pseudotumor
Lymph nodes	Lymphadenopathy
Bile duct and liver	Bile duct and liver disease of IgG4-RD with symptoms of hepatic mass, jaundice, pain and abnormal liver biochemistry
Skin	Erythematous, subcutaneous papules or nodules lesions
Retroperitoneum, mesentery, mediastinum	Retroperitoneal fibrosis Mesenteritis Superior vena cava syndrome
Central nervous system	Pachymeningitis Cerebral tumor Cranial nerve palsies Meningeal enhancement Peripheral neuropathy
Lung	Pulmonary nodules or mass Fibrosis Interstitial pneumonia Pleural effusion
Heart and blood vessels	Aortitis, Pericarditis
Kidney	Interstitial nephritis Glomerulonephritis Secondary AA amyloidosis
Ear, nose, throat	Chronic rhinosinusitis IgG4-related skull base disease
Endocrine and exocrine glands	Hypophysitis Riedel's Thyroiditis Prostatitis, prostate hypertrophy Breast manifestation/mastitis Pancreatitis Obstructive jaundice Pancreatic mass

Aims and outline of the thesis

IgG4-RD has gained enormous attention since its discovery. Several study groups in different parts of the world have worked on this disease leading to an evolving understanding of the clinical presentations, pathogenesis and treatment. This attention has led to high quality reports and improved patient care (10, 11). Previous unclassified diseases such as idiopathic pseudotumor of the orbit or in other parts of the human body, idiopathic retroperitoneal fibrosis or Mikulicz disease have now been reclassified as part of the spectrum of IgG4-RD, leading to a better understanding and treatment the clinical features (7).

Currently, IgG4-RD is more often being diagnosed because of increasing awareness, but on the other hand IgG4-RD is still a rare disease often leading to misdiagnosis or delay in diagnosis. Furthermore, the pathophysiology of IgG4-RD is still unraveled despite the significant increase in knowledge about the disease since its discovery in 2003. The current knowledge about epidemiology, pathogenesis, diagnostics, treatment and monitoring of IgG4-RD still needs crucial improvements. With this thesis, I hope to contribute to all of these aspects and to add to the understanding of this recently defined systemic fibro-inflammatory disease.

Description of the clinical spectrum of IgG4-RD

Chapter 1.2 gives an overview of the current organ manifestations of IgG4-RD and provides a review on epidemiology, pathogenesis and treatment of IgG4-RD. IgG4-RD is believed to occur in patients aged over 50 years. However, it is not restricted to this patient subgroup. In Chapter 1.3 the manifestations of the disease are described in children. In this systemic review we describe children of all ages with IgG4-RD emphasizing its broad clinical manifestations. In Chapter 2 we highlight important novel clinical manifestations and complications of IgG4-RD. In chapter 2.1 AA amyloidosis due to prolonged untreated IgG4-RD is described for the first time, followed by an analysis of serum amyloid A (SAA) in patients with IgG4-RD. In a retrospective study of patients with idiopathic scleritis (presented in Chapter 2.2), the association between IgG4-RD and scleritis is established in a well-defined cohort of patients with idiopathic scleritis. In patients with previously diagnosed ANCA-negative limited granulomatosis with polyangiitis (GPA), IgG4-RD might be

an alternative diagnosis. Case series of patients with ANCA-negative limited GPA were re-diagnosed as IgG4-RD and are presented in Chapter 2.3. Finally in Chapter 2.4 a rare unexpected presentation of IgG4-RD as a tarsal plate manifestation is presented.

Exploring novel techniques for diagnosis and monitoring of disease

Chapter 3 focuses on improvements in diagnostics and monitoring of IgG4-RD. In Chapter 3.1 the role of expanded IgG4+ B-cells, Th2 cells and Tregulatory (Tregs) cells was studied in patients with IgG4-RD. A new developed ''lymphocyte signature'' based on differential B and T cells subsets in IgG4-RD proved to distinguish patients with IgG4-RD from healthy population and sarcoidosis (another fibrosing disease). Furthermore, due to enhanced T-cell activity in IgG4-RD, the serum levels of soluble interleukin-2 receptor (sIL-2R) were significantly increased in patients with IgG4-RD as compared to healthy population. A significant decrease of sIL-2R was observed after adequate treatment and significant clinical improvement of the disease. The clinical relevance of sIL-2R levels in IgG4-RD is presented in Chapter 3.2.

Studying the pathogenesis of IgG4-RD

In Chapter 4 it is intended to uncover the pathogenesis of IgG4-RD. The role of follicular T-helper 2 cells (Tfh2) are shown to be elevated and the local and systemic B-cell responses are described in Chapter 4.1. In Chapter 4.2 the clinical and functional significance of a variant in the *MTDH* gene in two unrelated families with IgG4-RD are described.

What are the treatment modalities of IgG4-RD?

Chapter 5 highlights the various treatment approaches for IgG4-RD. The successful treatment of a patient with IgG4-RD with anti-TNFα (infliximab) is described in Chapter 5.1. In Chapter 5.2 the results of treatment outcomes in IgG4-related orbital disease are presented by performing a systematic review of the literature and chapter 5.3 focuses on the treatment outcomes of 33 patients with IgG4-RD.

Chapter 6 summarizes the main results of this thesis. In Chapter 7 a Dutch overview on IgG4-RD is presented and Chapter 8 provides a general discussion and the thesis closed with my PhD portfolio including my Curriculum Vitae.

Chapter 1.2 An inflammatory condition with different faces: IgG4-related disease

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ABSTRACT

Background: Immunoglobulin G4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition with involvement of different organs. The pathophysiological mechanism is unclear, but fibrosis is the hallmark of this disease. Early recognition is critical to avoid irreversible organ damage. Recently improved histologic testing boosts the diagnostic yield. We present three cases of patients with IgG4-RD to emphasize the broad clinical presentation of this disease.

Case descriptions: Patient A, a 63-years old male with bilateral orbital swelling, due to IgG4-RD, was shown to suffer from IgG4-RD in a multifocal pattern as demonstrated by PET scanning. Patient B, a 53-years old male with long-standing abdominal mass of unknown origin eventually proved to suffer from IgG4-RD. Patient C, a 32-years old male admitted with pleural effusion and pericardium tamponade. Histological diagnosis after pericardiectomy confirmed IgG4-RD.

Discussion: IgG4-RD has many faces and may mimic other conditions like malignancy and infectious diseases. Knowledge of this disease is necessary to avoid unnecessary diagnostics and delay in the treatment. IgG4-RD may be suspected based on specific clinical findings such as elevated serum IgG4 levels, but the diagnosis can only be established histologically. Although corticosteroids are an effective first choice of therapy, the relapse rate after this treatment remains high. The role of disease-modifying antirheumatic drugs (DMARDs) in the treatment of IgG4-RD has not been outlined yet, but there is increasing evidence that rituximab might be effective second-line therapy.

Conclusion: IgG4-RD is a disease with many faces requiring early recognition and therapy to avoid permanent damage of the organs.

INTRODUCTION

IgG4-RD is a systemic fibro-inflammatory condition with manifestations in almost all parts of the human body (7). It is characterized by tumour-like infiltration of IgG4 positive plasma cells in the tissues, mostly with fibrotic or sclerotic abnormalities, and often elevated serum IgG4 levels (7). IgG4-RD is initially described in patients with sclerosing pancreatitis, but from 2003 recognized as a systemic disease (2). The disease can manifest in one single organ, but it can also occur simultaneously in multiple organs. IgG4-RD mostly occurs in salivary and lacrimal glands, the orbit, the pancreas and the lymph nodes. Other preferential localizations include lungs, kidneys, thyroid, peritoneum and prostate (12). Conditions previously called Mikulicz' s disease, sclerosing sialadenitis, inflammatory orbital pseudotumor, a subset of idiopathic retroperitoneal fibrosis and Riedel's thyroiditis are now reclassified under the umbrella of IgG4-RD (13). IgG4-RD mimics many infectious, inflammatory and malignant disorders often leading to a delay in both diagnosis and treatment potentially progressing into irreversible fibrosis (14). Awareness of this disease is important to avoid unnecessary delay. We therefore present three different cases of patients with IgG4-RD to emphasize the broad clinical presentation of this disease and present a review on pathogenesis, diagnosis and treatment.

CASE PRESENTATIONS

We present briefly three different cases of IgG4-RD. The patient characteristics and the main clinical features are presented in Table 1.

Patient A

This 63-years old male patient was referred to the ophthalmologist because of painless bilateral periorbital swelling and diplopia suspected of lymphoma or recurrence of sarcoidosis. Pulmonary sarcoidosis was diagnosed on basis of clinical symptoms and was not histologically confirmed, and this was stable without medication since 20 years. His history also included levothyroxine for hypothyroidism and alpha-blockers for relapsing lower urinary tract symptoms. Bilateral periorbital swelling with slight proptosis was found on physical examination. Laboratory tests revealed elevated serum IgG4 without other abnormalities. Computed tomography (CT) of the thorax and abdomen was normal. MRI of

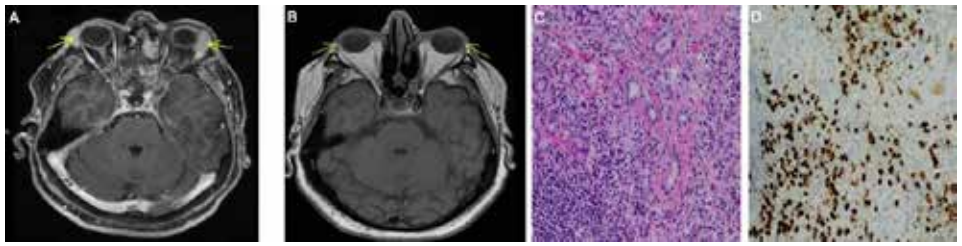
the brain revealed only bilateral enlarged and contrast-enhancing lacrimal glands (Figure 1A). On F-18 FDG PET/CT scan multifocal increased activity was noted in various organs (Table 1). Histology of the lacrimal gland was compatible with IgG4-RD (Figure 1C+D). Prednisone 1mg/kg/day significantly decreased the periorbital swelling, but also resulted in a complete recovery of the urinary tract symptoms within 1 week and recovery of thyroid dysfunction. After 4 weeks, steroids could be tapered and levothyroxine was discontinued without recurrence after 6 months follow-up.

Table 1. characteristics and the main clinical features of the patients.

	Patient A	Patient B	Patient C
Gender	Male	Male	Male
Age	63 years	53 years	32 years
Medical history	- Suspected pulmonary sarcoidosis without histologic confirmation 20 years ago - Hypothyroidism - Lower urinary tract symptoms - No asthma or allergies.	- Unknown abdominal mass for the past 20 years - No asthma or allergies.	- Unremarkable - No asthma or allergies.
Symptoms and duration of symptoms	Progressive bilateral painless periorbital swelling and diplopia since 4 months.	Episodes of malaise, weight loss and an abdominal mass of unknown origin for the last 20 years.	Malaise, dyspnea, pleural and pericardial effusion since couple of weeks. Four weeks after presentation pericardiectomy was performed. Afterwards, persistent pleural effusion in 3 months follow-up, for which prednisone was started.
Diagnosis	Orbital IgG4-RD with multifocal disease manifestation on PET imaging.	Mesenteric IgG4-RD	Pericardial an pleural IgG4-RD
Serum IgG4 pre-treatment	1.65 g/l.	25 g/l.	5.5 g/l (after pericardiectomy).
Serum IgG4 after initiating treatment	0.28 g/l: after prednisone 1mg/kg/d. This value was measured 14 weeks after starting prednisone. Prednisone was at this moment tapered off till 20mg/d from initial doses of 60mg.	4.58 g/l: after prednisone 1mg/kg/d. This value was measured 10 weeks after starting prednisone. Prednisone was at this moment tapered off till 25mg/d from initial doses of 60mg. Azathioprine has been started 2 months after initiating prednisone.	1.69 g/l: after pericardiectomy and prednisone 30mg/d. This value was measured 7 weeks after starting prednisone, prednisone was tapered off till 20mg/d from initial doses of 30mg.
ANA	Negative	Negative	Negative
Other relevant findings	ESR and CRP normal. ACE normal. Anti-TSH receptor absent	Elevated ESR and CRP, decreasing with therapy. Microcytic anaemia very well responding to iron supplements. Gastroscopy, colonoscopy and bone marrow survey normal.	Elevated CRP normalizing after pericardiectomy. ESR not measured.. ANCA, rheumatic factors, lupus anticoagulants absent, complement factors normal.
Imaging	-MRI brain: bilateral enlarged and contrast-enhancing lacrimal glands; - PET scan: multifocal increased activity in various organs, including the lacrimal glands, the parotid gland, the thyroid, the prostate, the right seminal vesicle, the testis and multiple mediastinal and hilar lymph nodes.	CT abdomen: mesenteric mass, decreasing in volume after treatment.	-CT thorax and abdomen: pleural and pericardial effusion; -X-thorax: bilateral pleural effusion on both side, vanishing after starting prednisone; - PET: slight activity of the pleura without other abnormalities (after pericardiectomy)
Histology	Surgical excision of lacrimal gland: -Lymphoplasmacytic infiltration, Storiform fibrosis, obliterative phlebitis, >200 IgG4 positive plasma cells per HPF and IgG4/IgG ratio > 0.5.	Fine needle biopsy mesenteric mass: Lymphoplasmacytic infiltration, storiform fibrosis, >50 IgG4 positive plasma cells per HPF and IgG4/IgG ratio > 0.5. No obliterative phlebitis.	Pericardiectomy: Lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, > 100 IgG4 positive plasma cells per HPF and IgG4/IgG ratio > 0.7.
Treatment	Prednisone 1mg/kg, currently being tapered successfully. No maintenance therapy initiated, because normalization of symptoms, serum IgG4 and MR imaging.	Prednisone 1mg/kg and Azathioprine 150mg/d after tapering prednisone. Azathioprine was initiated because ESR and serum IgG4 were not normalized and persistence of abdominal mass.	Prednisone 30mg daily, currently being tapered successfully. No maintenance therapy was initiated, because serum IgG4 almost normal and pleural effusion disappeared.

CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, ACE = angiotensin converting enzyme, ANCA = anti-neutrophil cytoplasmic antibodies, ANA = antinuclear antibodies, HPF = high power field.

Figure 1. The MRI and histology images of patients 1



A+B: Transverse SE T1 weighted MRI of the orbit. **A:** Note the homogeneously enhancing bilateral enlarged lacrimal glands (yellow arrows). **B:** normalisation of lacrimal gland size and dramatic decrease in enhancement after treatment with prednisone.
C+D: Histology of the lacrimal gland of patient A. **C**: HE-staining demonstrating lymphocytes, plasma cells and local fibrosis. Obliterative phlebitis was also observed. **D:** Immunohistochemical staining for IgG4 (brown color) of the lacrimal gland of patient A showing widely scattered IgG4 positive plasma cells with an average of 240 per HPF out of 2 HPF with a ratio of 0.5 to total IgG plasma cells in the tissue. Figure C and D are at x200 magnification
SE = spin echo, MRI = magnetic resonance imaging.
HE = Hematoxylin and Eosin, HPF = High-power field.

Patient B

A 53-years old male patient visited several medical specialists for the past 20 years because of an abdominal mass. Extensive diagnostics including biopsies and bone marrow examination did not yield any diagnosis. Patient complained of slowly progressive malaise, weight loss and abdominal pain. After referral to our hospital, IgG4-RD was suspected, also because of elevated serum IgG4 levels. Laboratory tests further revealed elevated ESR, normal ferritin and a microcytic anaemia, known to exist for years. Gastroscopy and colonoscopy were without evidence of malignancy, intraepithelial lymphocytosis, IgG4-RD, villous atrophy, Giardia, Whipple's disease or Helicobacter pylori infection. CT imaging demonstrated a progressively increasing mesenteric mass of 50 mm surrounded by mesenteric lymphadenopathy (Figure 2A). Histology of the mesenteric mass confirmed the diagnosis of IgG4-RD (Figure 2C). Prednisone 1mg/kg/day was initiated. Hereafter, the symptoms subdued, serum IgG4 and ESR decreased and haemoglobin levels almost normalized. The abdominal mass and lymphadenopathy decreased (Figure 2B) and serum IgG4 and ESR levels showed a downward trend . The steroids were tapered after 4 weeks and azathioprine 150mg daily was started after 2 months since the mass had not totally regressed.

Figure 2. The CT and histology images of patients 2

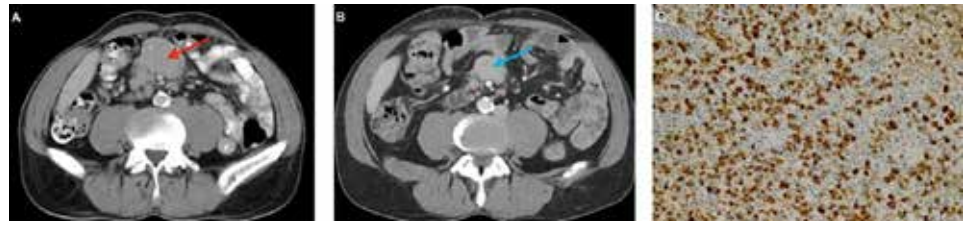


Figure 2. The CT and histology images of patients 2. A+B: CT image of the abdomen after intravenous contrast injection, venous phase. **A:** Pre-treatment: abdominal/mesenteric mass of 50 mm (red arrow) with enlarged mesenteric lymph nodes. **B:** Post-treatment: decrease in size of the mesenteric mass to 36 mm (blue arrow) and decrease in lymph nodes size. **C:** Immunohistochemical staining for IgG4 of mesenteric mass of patient B revealing widely scattered IgG4 plasma cells with an average of 421 per HPF out of 3 HPF with a ratio of 0.5 to total IgG plasma cells in the tumorous tissue. Unfortunately, no HE images were available, but lymphoplasmacytic infiltration and storiform fibrosis were seen and documented.
Figure C is at x200 magnification.

Patient C

This 32-years old male patient was admitted at the department of cardiology because of cardiac tamponade. On a CT of the thorax and abdomen both pleural and pericardial effusion were seen (Figure 3A). Laboratory tests showed elevated CRP, ESR was not measured at that moment. Because of persistent pericardial effusion with constrictive signs, a pericardiectomy was performed and diuretics were given. Hereafter, CRP normalized and ESR was normal. Detailed bacteriological and virological analyses (including serology or viral load determinations of HIV, hepatitis A/B/C, Borrelia burgdorferi, syphilis, mycoplasma, tuberculosis, parvovirus, Cytomegalovirus, Epstein-Barr, Coxiella burnetii, toxoplasmosis, Coxsackievirus and varicella- zoster) were unremarkable. Elevated serum IgG4 and pericardial histology finally offered sufficient evidence for IgG4-RD (Figure 3D+E). Cultures of the pericardial tissue ruled out bacterial pathogens including Mycobacterium Tuberculosis. F-18 FDG PET/CT 3 months after pericardiectomy revealed slight activity of the pleura without other abnormalities. Prednisone 30mg daily led to disappearance of the pleural effusion (Figure 3B+C) and serum IgG4 without requirement for diuretics anymore. Hereafter, prednisone carefully was tapered to 20 mg in 7 weeks without signs of recurrence.

DISCUSSION

We here present 3 cases of patients with formerly unrecognized IgG4-RD, each presenting with a different clinical presentation. The courses of these patients reflect the broad spectrum of clinical faces of IgG4-RD. By demonstrating the variable presentation of Ig4-RD we briefly provide an overview of the spectrum of symptoms and treatment options in this new disease entity.

IgG4-RD is a systemic disease that can be found in almost any organ, but with certain sites of preferences (orbit, salivary tract, pancreas and lymph nodes) that can be guiding to think of this new disease entity. On the other hand, IgG4-RD mimics various benign and malignant disorders. Therefore, careful diagnostics may be applied before setting the diagnosis (7). The vast clinical manifestation range and potentially organ and life threatening situations emphasize that awareness of this relative new entity is pivotal to swiftly set a diagnosis and prevent organ damage (12). This is highlighted by the histories of the presented patients. Patient A presented with a relative short history and was suspected of lymphoma or recurrent sarcoidosis. Extensive diagnostics were conducted to rule out these entities. A typical FDG-uptake pattern led to the diagnosis of IgG4-RD by histology of a lacrimal gland. The abdominal mass resembling retroperitoneal fibrosis seen in patient B is remarkable and rarely described before (15). Multiple diagnostics including biopsies of the abdominal mass excluded conditions such as malignancy and infectious diseases. Eventually after almost 20 years, attention towards IgG4 resulted into the diagnosis of IgG4-RD. Cardiac manifestations of IgG4-RD, such as in patient C, are rare (16). The patient presented with constrictive pericarditis and pleural effusion. It remains a challenge to rule out infectious or malignant disease and think of IgG4-RD.

The diagnosis IgG4-RD is based on the combination of clinical presentation, serological and histological findings, but histology is the gold standard. Although the disease is called IgG4-RD, about 30 to 50% of histologically proven cases show normal IgG4 levels leading to misinterpretation and erroneous rejection of the diagnosis (16). Furthermore, the specificity and positive predictive value of serum IgG4 concentrations are low which make them poor disease markers. In our cases, serum IgG4 levels were elevated in all three patients, but with different ranges (1.65 to 25 g/l). Other, though unspecific, serological findings are ESR and CRP in patients with active disease, but these are elevated

respectively in 53% and 40% of the cases (16). In this study 51% of these patients had elevated serum IgG4 (16). In our patients, not all elevated IgG4 levels corresponded with elevated ESR and CRP. Only in patient A, ESR and CRP were both normal. Although speculative, a longstanding active disease and high serum IgG could lead to elevated ESR and CRP, which applied to case B.

Measuring plasmablasts originating from CD20+ B cells is a superior alternative to measuring IgG4 concentrations in serum (17), but so far this technique has not widely been introduced for clinical application. So far, imaging studies play a crucial role in the diagnostic of IgG4-RD, however, imaging is not specific for this disease and several conditions such as malignancy should be excluded. Radionuclear imaging in patient A revealed more sensitive than conventional CT. Several studies have shown the usefulness of FDG-PET/CT scan for diagnosis, staging and the degree of organ involvement and monitoring of therapy response, and this imaging method seems to detect more lesions than conventional methods like ultrasonography and CT (18).

Figure 3. Radiology and histology images of patient 3.

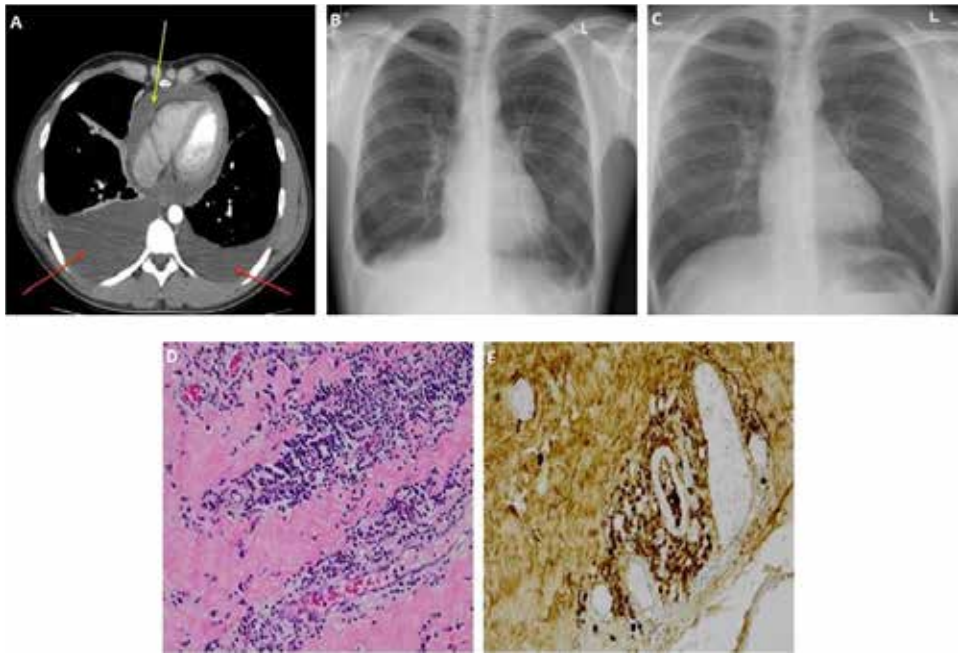


Figure 3. Radiology and histology images of patient 3. A: CT scan of the thorax showing bilateral pleural effusion (red arrows) and pericardial effusion (yellow arrow). **B:** Pleural effusion was evident on plain film of the thorax as well. **C:** Disappearance of pleural effusion six weeks after starting prednisone. **D:** Histology of pericardium of patient C. HE-staining showing lymphocytes, plasma cells and fibrosis. Obliterative phlebitis was also observed. **E:** Immunohistochemical staining for IgG4 consists of IgG4 plasma cells with an average of 136 per HPF out of 3 HPF with a ratio of 0.7 tot total IgG plasma cells in the tissue. Figures D and E are at x200 magnification.

This emphasizes the utility of PET scanning in IgG4-RD. However, histology remains crucial for the diagnosis of IgG4-RD. The histological abnormalities should meet the Boston consensus about the IgG4-RD (6). The characteristic histological features of IgG4-RD are dense lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis. The ratio of IgG4/IgG positive plasma cells in tissues should be greater than 0.4 and the numbers of IgG4 positive plasma cells per high power field (HPF) should be greater than the numbers agreed in the consensus (6). The absolute numbers of IgG4 positive plasma cells and the thresholds for disease differ for the diverse organs. Our patients had histologically confirmed IgG4-RD according to criteria, however, in case B no obliterative phlebitis was seen.

The pathogenesis of IgG4-RD is unclear (3). Generally, the disease is characterised by a decreased T-helper cells 1/T-helper cells 2 ratio and increased numbers of regulatory T-cells most probably as a result from a certain antigen triggering the immune system. Production of different cytokines such as interleukin (IL)-4, IL-5, IL-10, IL-13 and transforming growth factor (TGF)-beta leads to co-activations of B-cells, production of IgG4 expressing B-cells and fibrosis. Still, the role of IgG4 antibodies is unclear, but in the pathophysiology of IgG4-RD, these antibodies most probably play an anti-inflammatory role as response to an unknown trigger (19). Patient C presented with constrictive pericarditis and pleural effusion. Plasma cell manifestation of pericardium has also been described in multiple myeloma (20), whereby infiltration of plasma cells in the pericardium is suggested to be the reason. Maybe some viral infection has led to IgG4 positive plasma cell infiltration in the serosal cavity leading to the clinical manifestation of this disease, but this remains a speculative hypotheses. The pleural effusion was most probably also because of infiltration by lymphoplasmacytic cells, as it was slightly PET positive and disappeared after starting prednisone. However, secondary pleural effusion because of restricted heart function due to constrictive pericarditis could also have contributed to the development of pleural effusion.

IgG4-RD can cause significant morbidity and even lead to organ damage. Aggressive treatment is therefore necessary, especially when vital organs are at risk (3). Glucocorticoids are the first choice of treatment for most types of IgG4-RD and are mostly effective at a prednisone dosage of 30-40 mg/day and should be adjusted on body weight or in cases of aggressive disease (8). This treatment dose is, in most cases, rapidly effective, but should be maintained for 2-4 weeks after initiation. Thereafter, prednisone can be tapered according to clinical responses. The clinical response of prednisone is dependent upon the organ system involved and the degree of fibrosis. Pancreatic function and lacrimal gland function for example will respond better to this treatment than retroperitoneal disease or sclerosing mesenteritis (8). This phenomenon highlights the need for earlier treatment of this disease (14). About 25% of patients demonstrate relapse after tapering prednisone necessitating steroid-sparing agents. Patient A responded very well to prednisone. His symptoms, serum IgG4 and MRI imaging normalized and remained so during

tapering. Patient C responded also very well to prednisone. His symptoms disappeared, serum IgG4 reached almost normal levels and a recent X-thorax demonstrated no pleural effusion anymore. Therefore, we decided not to initiate maintenance therapy in cases A and C. According to international consensus, a steroid-sparing agent is appropriate when the glucocorticoid dosage cannot be tapered due to persistently active disease (8). Azathioprine was for this reason initiated in case B. Conventional steroid-sparing agents such as mycophenolate mofetil, azathioprine and methotrexate have all been used in treatment of IgG4-RD, but management of further immunosuppressive therapy with these DMARDs has not been outlined (8) and there are no studies confirming the superiority of one of these agents in the treatment of IgG4-RD. There is improving evidence for the efficacy of rituximab in the treatment of IgG4-RD, even as single therapy (21). This B-cell ablative therapeutic agent has induced clinical remission in patients with different organ involvement of IgG4-RD (12). More case series or prospective studies with different DMARDs and rituximab are required in order to define the (long-term) effect of these agents in the treatment of IgG4-RD.

CONCLUSION

In conclusion, IgG4-RD is a rare and new clinical entity with many faces and manifestations in different parts of the body. Early recognition is critical to start treatment and to avoid permanent damage of the organs. Diagnosis is based on histology, while serum IgG4 could be supportive. Glucocorticoids are the first choice of treatment, but there is often a need for maintenance therapy. Several DMARDs as well as rituximab are used in the treatment of IgG4-RD, with growing evidence for the latter.

Chapter 1.3 IgG4-Related Disease: a systematic review on this unrecognized disease in Pediatrics

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ABSTRACT

Background: Immunoglobulin G4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition with an unclear pathophysiological mechanism affecting different parts of the body. If untreated, the disease can lead to fibrosis and irreversible organ damage. IgG4-RD mostly has been described in adults, hence it is generally unknown among pediatricians. This systematic search of the literature provides an overview of all reports published on IgG4-RD in children in order to create awareness of IgG4-RD in pediatrics and to emphasize the broad clinical presentation of this disease.

Methods: A systematic literature search of Embase, Medline, Web-of-Science, PubMed publisher, Cochrane and Google Scholar was performed for case reports on IgG4-RD in children.

Results: of total 740 articles identified by the search, 22 case reports including 25 cases of IgG4-RD in children were found. The median age of the children was 13 years, of which 64% were girls. IgG4-related orbital disease (44%) and autoimmune pancreatitis type 1 /IgG4-related pancreatitis (12%) predominantly occurred. Less frequently, other manifestations as pulmonary manifestation, cholangitis and lymphadenopathy were also found. Almost all cases were histologically proven. Prednisone was the first choice of treatment leading to favorable clinical response in 83% of the cases. Maintenance therapy with steroid sparing agents was required in 43% of the cases needing therapy. Rituximab was successful in all 4 cases, whereas, the disease modifying rheumatic drugs (DMARDs) mycophenolate mofetil, azathioprine and methotrexate were effective in almost 50% of the cases.

Conclusion: IgG4-RD in children is a generally unknown disease among pediatricians, but several pediatric cases have been described. Prednisone is the first choice of treatment leading to disease remission in the majority of the cases. DMARDs and rituximab are alternative effective steroid sparing agents with more positive evidence for the latter.

BACKGROUND

IgG4-RD is a systemic fibro-inflammatory disease affecting different parts of the body (7). The disease is characterized by tumour-like infiltrations of IgG4 positive plasma cells in the tissues, mostly with fibrotic abnormalities and often elevated serum IgG4 levels (7). The underlying pathophysiological mechanism of IgG4-RD is still unclear, but when untreated, the disease can lead to irreversible organ damage because of the fibrosis. Early recognition and therapy are therefore critical (12, 22). In recent time there has been a lot of attention to IgG4-RD in adult care leading to evolving knowledge about pathogenesis, diagnosis and treatment of this disease. However, further studies are required to provide more insight into this disease, in particular, the underlying pathogenesis has yet to be clarified. The average age at which IgG4-RD can occur, is estimated to be older than 50 years (7, 23). Although case reports are available on IgG4-RD in children (24, 25), no pediatric studies or reviews about this disease have been published yet. Knowledge and awareness of this disease is essential to prevent missing the diagnosis and subsequent delay of treatment, especially in children. We performed a systematic literature search in order to make an overview of all the case reports that have been published regarding IgG4-RD in children. The main purpose of this study was to create awareness of IgG4-RD in pediatrics and to emphasize the broad clinical presentation of this disease. Furthermore, with the current knowledge about the disease we wanted to provide an overview on epidemiology, pathogenesis and treatment of this disease for the pediatricians.

METHODS

A systematic literature search was conducted to provide an overview of all case reports and (if available) case series regarding IgG4-RD in pediatrics. The study was performed and reported in accordance with the PRISMA statement for systematic reviews.

Data source, study selection and data extraction

Relevant articles on IgG4-RD in children were retrieved from Embase.com, Medline (Ovid), Web-of-Science, and the Cochrane Library from inception to last date of inclusion July 16th 2015. Additional references were obtained from PubMed (the subset as supplied by publisher, containing references not yet indexed in Medline) and Google Scholar (the most relevant citations). No filters for date or language were used in the search strategy. See the additional appendix for the full search strategies for all databases. Two authors (Karim and Westenberg) reviewed and extracted the data independently.

RESULTS

Of a total of 740 articles identified by the search, 34 articles on IgG4-RD in pediatrics were eligible (Figure 1). After screening, 22 case reports on IgG4-RD in children were identified. Three articles described two pediatric patients leading to a total of 25 cases of IgG4-RD (26-28). The main outcomes of this study are demonstrated in Table 1.

Patients

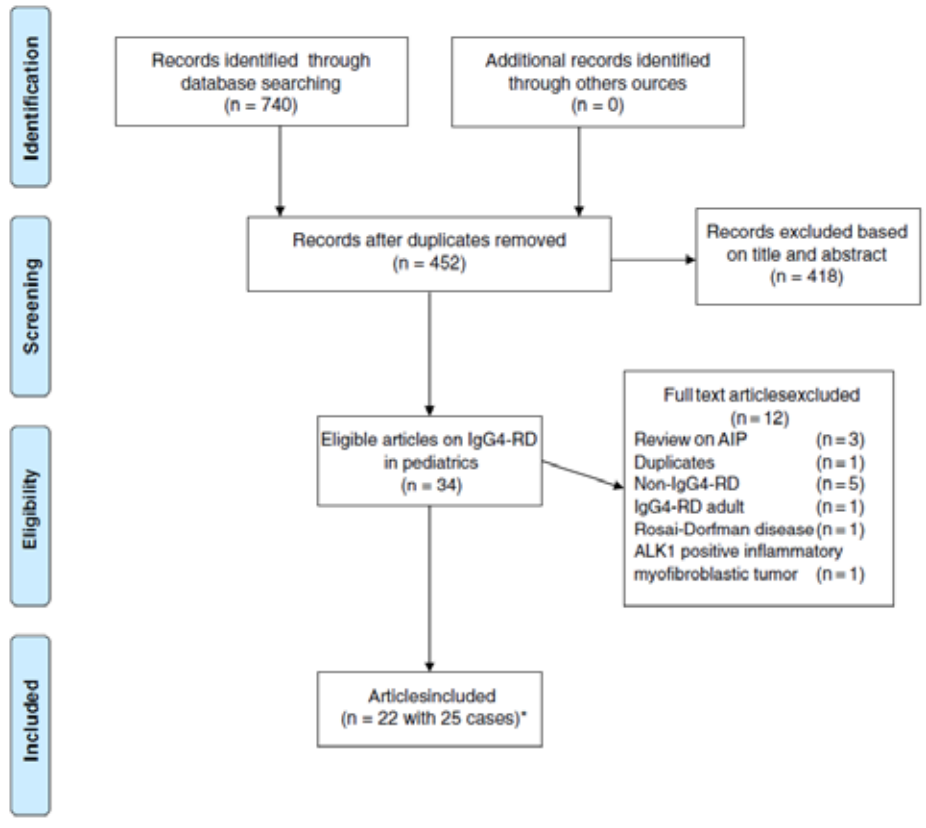
With this systematic literature review we identified 22 case reports of IgG-RD in children. Identified studies were published over a 5-year span (2010-2015). The case reports included patients aged ranging from 22 months to 17 years of age. The median age of the children in this study was 13 years and 64% of the children were girls.

Organ manifestation

The cases described in this study show a spectrum of different organ manifestations (Figure 2) of IgG4-RD. However, most of the cases report IgG4-related orbital disease (IgG4-ROD) (44%) (24-32). Other manifestations were IgG4-related pancreatitis/autoimmune pancreatitis type 1 (AIP 1) (12%), IgG4-related cholangitis (8%), IgG4-related

pulmonary disease (8%), and the remaining cases (28%) were single cases of Riedel's thyroiditis/IgG4-related thyroid disease, IgG4-related sialadenitis, IgG4-related mesenteritis, IgG4-related lymphadenopathy, IgG4-related dacryoadenitis, IgG4-related sinonasal disease and IgG4-related hepatic mass. Kidney involvement was seen in three cases in combination with

Figure 1. Search strategy and selection of the articles



* Three articles demonstrated each two cases of IgG4-RD in children. Therefore, a total of 25 cases were available for this study.

other organ manifestations (30, 32, 33). Systemic IgG4-RD (two or more organ manifestations) occurred in 40% of the cases (27, 30-38).

Diagnosis

In this study, all cases of IgG4-RD were histologically confirmed, except one case of Riedel's thyroiditis (39), whereby histology was performed without IgG4 staining. Riedel's thyroiditis is recently included in the spectrum of IgG4-RD (40), therefore we decided to include this case report in this study. Furthermore, despite the presence of IgG4 positive plasma cells in the tissue, two case reports concerning Rosai-Dorfman disease and ALK-1 positive inflammatory myofibroblastic tumor (41, 42) were excluded, because according to Boston consensus these diseases should not be considered as IgG4-RD. Serum IgG4 was measured in 23 of the 25 cases, and was found to be elevated in 16 cases (24, 25, 27, 29, 31, 33-38, 43-46) (70%).

Therapy

Prednisone was the first choice of treatment in 23 of the 25 cases (24-30, 33-39, 43-49). In one case no treatment was initiated or mentioned (31), and in another case surgery alone resulted in complete remission (26).

The doses of prednisone that was used were not mentioned in all cases, but when specified was usually between 0.5 and 2 mg/kg/day. Prednisone therapy resulted in a rapid response in 19 of the 23 cases treated (24-30, 32, 33, 36-39, 43-48). Prednisone alone induced remission and could be tapered and discontinued without relapse in 10 of the cases (43%), and thus was the sole agent used (25, 27, 37-39, 43, 45-48). Second line therapy was initiated in the 4 cases (17%) that did not respond completely to prednisone and in the 9 cases where prednisone alone did not induce permanent remission. In 3 of 4 cases not responding to prednisone, the prednisone doses were adequate, however, in 1 case the dosage was not mentioned. DMARDs were attempted as steroid-sparing agents in 11 cases. Mycophenolate mofetil was successful as a steroid-sparing agent in 3 of the 5 cases in which it was used (26, 28, 29, 33, 35). Azathioprine was a successful as a steroid sparing agent in 2 of 4 cases in which it was used (24, 34, 36, 44), while methotrexate was successful in 1 of 2 cases (28). Because of disease relapse despite azathioprine, one patient achieved clinical remission with 5mg prednisone after high doses induction of prednisone (17).

Table 1. Outcomes reported in case reports on IgG4-RD in pediatrics

Reference	Age	Sex	Organ manifestation	Serum IgG4	Therapy	Comments
Miglani 2010 (43)	13y	M	AIP-1 *H+*	El (603mg/dl)	Pred 20mg/d	Initially suspected of malignancy. Pred tapered and stopped in 4 months
Ibrahim 2010 (44)	3y	F	IgG4-R cholangitis *H+*	El (258mg/dl)	Pred 2mg/kg/d and Aza 1.5mg/kg	Relapse after tapering pred and required a low (2mg/d) maintenance dose of pred and Aza
Mannion 2010 (33)	13y	F	AIP-1 and IgG4-R fibrosing mediastinitis, renal and hepatic manifestation *H+*	El (73.4 mg/dl)	Pred and MMF	Good results by MMF, pred tapered and stopped successfully
Zakeri 2011 (39)	17y	M	Riedel's thyroiditis H+*	NM	Pred 40mg/d	Pred tapered and stopped in 3 months
Melo 2012 (47)	11y	M	IgG4-R sialadenitis *H+*	NM	Pred	
Griepentrog 2013 (26)	10y	F	IgG4-ROD *H+*	N (L U)	Lateral orbitotomy	No further treatment was required
Griepentrog 2013 (26)	14y	F	IgG4-ROD *H+*	N (L U)	Pred, dosage unknown, and MMF	MMF because of relapse after tapering pred, successful
Kalapesi 2013 (29)	5y	F	IgG4-ROD *H+*	El (1.52 g/l)	Pred 1mg/kg and MMF (600mg/m2)	Weaned off pred and maintained on MMF successfully
Naghibi 2013 (34)	16y	F	IgG4-related colitis, in the past AIP-1 *H+*	El (210 mg/dl)	Adalimumab	Refractory disease to pred 0.5mg/kg, Aza and infliximab. Adalimumab successful
Pifferi 2013 (45)	15y	M	IgG4-R pulmonary disease *H+*	El (1090mg/dl)	Pred 0.6mg/kg/day	Treatment for 4 weeks.
Sane 2013 (30)	12y	F	IgG4-ROD and nephrotic syndrome *H+*	N (L U)	Methylpred and rituximab	The nephrotic syndrome also resolved. Initial good response to pred 40mg, but relapse occurred
Pasic 2013 (31)	10y	F	Mikulicz disease/IgG-ROD *H+*	EL 9.02 g/l	NM	
Caso 2013 (35)	17y	M	IgG4-R lymphad and scleritis *H+*	El (4.43 g/l)	Rituximab and pred 10mg daily	Refractory to MMF, good results with rituximab
Hasosah 2014 (36)	7y	F	IgG4-R mesenteritis and pericarditis *H+*	El (149 mg/dl)	Pred, aza and colchicine (doses unknown)	Relapsed despite aza, further treatment with 5mg prednisone as maintenance therapy
Jariwala 2014 (24)	7y	M	IgG4-ROD *H+*	El (109.3 mg/dl)	Pred 1mg/kg/d and Aza 2mg/kg/d	Good clinical results
Mittal 2014 (25)	14y	M	IgG4-ROD *H+*	El (4.3 g/l)	Pred 0.6mg/kg/d	Initial improvement, but lost to follow-up
Notz 2014 (48)	13y	F	IgG4-R dacryoadenitis *H+*	N (23.9 mg/dl)	Pred 40mg/d for 3 months	

Prabhu 2015 (27)	15y	F	IgG4-ROD and sinonasal disease *H+*	El (579 mg/dl)	Rituximab	Insufficient response to prednisone
Prabhu 2015 (27)	15 y	F	IgG4-R sinonasal disease *H+*	El (206 mg/dl)	Pred (dosage unknown)	
Batu 2015 (28)	14y	F	IgG4-ROD *H+*	N (7.5 g/l) (0-12.5 g/l)	Pred (dosage unknown)	Pred was tapered and stopped, MTX as maintenance therapy
Batu 2015 (28)	9y	F	IgG4-ROD *H+*	N (3.7 g/l)	Methylpred and cyclophosphamide	No response to pred, MTX or MMF. Now stable disease
Corujeira 2015 (37)	22Mo	F	IgG4-R pulmonary disease and IgG4-R lymphad *H+*	El (805 mg/dl)	Pred 2mg/kg/d	Pred tapered over period of 6 months.
Gillispie 2015 (32)	7y	F	IgG4-ROD, nerve and renal disease *H+*	N (L U)	Pred and rituximab	Refractory to pred, responsive to rituximab
Nada 2015 (38)	10y	M	IgG4-R hepatic mass and coagulopathy *H+*	El (420mg/dl)	Pred 2mg/kg/day	Coagulopathy also resolved after treatment
Rosen 2015 (46)	17y	M	IgG4-R cholangitis *H+*	El (242 mg/dl)	Pred 30mg/d	Pred weaned in 3 months.

Y, year; IgG4-R, IgG4-related; IgG4-ROD, IgG4-related orbital disease; Mo, months; H+, histology performed; Mikulicz disease, IgG4-related orbital and submandibular disease; M, male; F, female; AIP-1, autoimmune pancreatitis type 1; Pred, prednisone; Aza, azathioprine; EL, elevated; MMF, mycophenolate mofetil; LU, level unknown; N, normal; NM, not measured; Methylpred, Methylprednisolone; Lymphad, Lymphadenopathy.

Figure 2. Organ manifestation of IgG4-RD in children

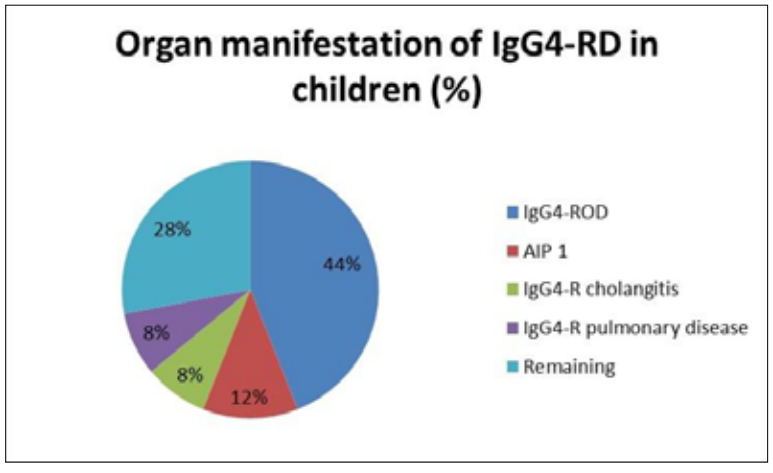


Figure 2. **Organ manifestation of IgG4-RD in children**. Remaining: Riedel's thyroiditis/IgG4-related thyroid disease, IgG4-related sialadenitis, IgG4-related mesenteritis, IgG4-related lymphadenopathy, IgG4-related dacryoadenitis, IgG4-related sinonasal disease and IgG4-related hepatic mass.

Rituximab was initiated in 4 cases (27, 30, 32, 35) of therapy refractory diseases leading to positive clinical outcomes in all these cases. Two of these cases initiated rituximab single therapy (27, 32), in one case methylprednisolone was combined with rituximab (30) and in another case prednisone 10mg daily was used as maintenance therapy beside rituximab (35). Adalimumab (34) and cyclophosphamide (28) were both successfully used in therapy refractory cases.

DISCUSSION

In this systematic search of the literature we describe 25 published cases of IgG4-RD in children. The cases demonstrate different organ manifestations of the disease with different clinical outcomes emphasizing the broad clinical spectrum of this disease.

Epidemiology

IgG4-RD is a rare and recently recognized fibro-inflammatory condition of which the diagnosis is often delayed or unrecognized because of unawareness. Generally, it occurs in middle aged patients, more often in men than women (7). However, in this study we identified more female patients than male patients. In children IgG4-RD is even more uncommon and will subsequently lead to significant delayed or unrecognized disease. All cases identified with this systematic review have been only recently published demonstrating that awareness is increasing in pediatricians. One can postulate that the average age of patients is lower than suggested (7), and may be more frequent in the pediatric age group than these 25 published cases might suggest.

Symptoms and organ manifestation

The symptoms of IgG4-RD are variable and depend on the affected organs. It can be localized almost everywhere (Table 2). In adults, IgG4-RD mostly affects the orbit, the salivary tract, the pancreas and the lymph nodes, however, manifestations in almost every part of the human have been described (12). In this study we have demonstrated a similar distribution of disease localizations in children. As in adults, most pediatric patients had orbital or pancreatic localizations. Therefore, IgG4-RD in children apparently is the same entity as in adults. In cases of unexplained inflammatory conditions, especially when tumor-like

abnormalities are observed by physical examination or imaging studies in the preferential localization of the disease (pancreas, salivary glands, orbit, lymph nodes), one should rule out IgG4-RD. Furthermore, conditions previously called Mikulicz' s disease, sclerosing sialadenitis, inflammatory orbital pseudotumor or any pseudotumor, a subset of idiopathic retroperitoneal fibrosis and Riedel's thyroiditis are now mostly reclassified as IgG4-RD and should raise suspicion for IgG4-RD (13).

Table 2: Organ manifestations of IgG4-related disease

Pancreas Autoimmune pancreatitis type 1	**Lymph nodes** Ig4-related lymphadenopathy of several lymph nodes
Liver and bile duct IgG4-related sclerosing cholangitis IgG4-related cholecystitis IgG4-related hepatopathy	**Other abdominal manifestations** Inflammatory pseudotumor Retroperitoneal fibrosis Small bowel obstruction caused by peritoneal IgG4-RD IgG4-RD of stomach with chronic ulcer IgG4-related esophagitis
Kidneys Interstitial nephritis Glomerular lesions such as membranous nephropathy	**Skin manifestation** Erythematous, subcutaneous papules or nodules of IgG4 origin
Urological manifestation IgG4-related prostatitis Ureteral IgG4-RD Testicular inflammation as a manifestation of IgG4-RD	**Orbital and ophthalmic manifestation** Inflammatory pseudotumor of orbit Scleritis Retinopathy due to IgG4-RD with hypergammaglobulinemic hyperviscosity Trigeminal and orbital nerve compression Nasolacrimal duct obstruction
Pulmonary manifestation Interstitial lung disease/interstitial pneumonia Bronchial damage/asthma-like clinical presentation Plural manifestation of IgG4-disease Pulmonary arterial hypertension	**Cardiovascular manifestation** IgG4-related periaortitis IgG4-related aortitis Pericarditis IgG4-related coronary artery disease
Thyroid Riedel's thyroiditis Fibrosing Hashimoto thyroiditis	**Salivary and lacrimal gland** IgG4-RD Mikulicz's Küttner's tumor or IgG4-related submandibular gland disease
Nervous system Infundibular hypophysitis Hypertrophic pachymeningitis IgG4-related hypophysitis Intracerebral inflammatory pseudotumor Neuropathy	**Other manifestations** IgG4-related fibrosing mediastinitis IgG4-related myositis Multifocal fibrosclerosis Increased risk of malignancy: lung, colon and especially MALT lymphoma.

Diagnosis

The diagnosis of IgG4-RD can only be confirmed histologically, the gold standard, while clinical symptoms, serological and radiological findings could be supportive to establish the diagnosis. The typical histological abnormalities (Figure 3), according to the Boston consensus (6), are dense lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis. The ratio of IgG4/IgG positive plasma cells in tissues should be greater than 0.4 and the numbers of IgG4 positive plasma cells per high power field (HPF) should be greater than the numbers agreed in the consensus (6). IgG4 positive plasma cells in tissues could also be observed in several other conditions without meeting the histological diagnostic criteria for IgG4-RD. Therefore, alternative diagnosis such as xanthogranulomatous disease, granulomatosis with polyangiitis and sarcoidosis should be excluded before obtaining the diagnosis IgG4-RD (50). In current study, almost all cases were histologically proven, except a case of Riedel's thyroiditis, which is recently been recognized as a spectrum of IgG4-RD (40).

Figure 3. Histology of IgG4-RD

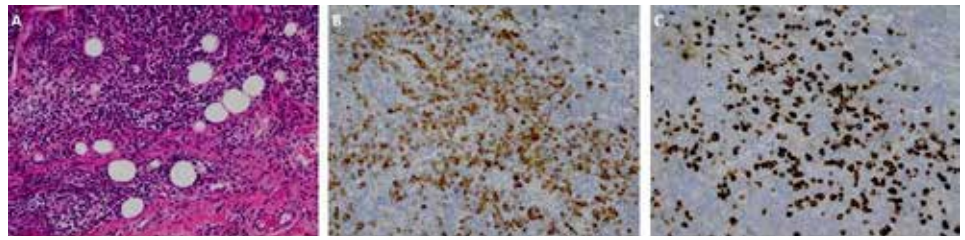


Figure 3. Histology of IgG4-RD. Histology of the orbital tissue of an adult patient from our hospital with IgG4-related orbital disease. **A** HE-staining demonstrating multiple lymphoid infiltrates and fibrosis. **B** Immunohistochemical staining for IgG showing diffuse scattered IgG (brown color). **C** Immunohistochemical staining for IgG4 revealing widely scattered IgG4 positive plasma cells (dark brown) with an average of 339 per HPF out of 2 HPF with a ratio of 0.67 to total IgG plasma cells in the tissue. HE = Hematoxylin and Eosin, HPF = High-power field.

Serum IgG4 is elevated in most of the cases of IgG4-RD, but about 30 to 50% of histologically confirmed cases have normal levels of serum IgG4, which can lead to falsely rejecting the diagnosis (31). A similar percentage of pediatric patients had elevated serum IgG4 levels (70%) to those reported in the adult population. In general, the specificity and positive predictive value of serum IgG4 are low, but if elevated can be useful in monitoring response to treatment (32). Inflammatory biomarkers such as erythrocyte sedimentation rate and C-reactive protein might be elevated, but normal levels of these biomarkers are frequently observed in IgG4-RD making them less specific as biomarkers (16). Moreover, recently, serological studies of IgG4 positive circulating plasmablasts have been shown

to be superior to serum IgG4 levels in IgG4-RD (17). So far, this technique has not been widely introduced for clinical applications.

Pathogenesis

The pathogenesis of IgG4-RD is unclear. Generally abundant serological T-helper cells 2 and regulatory T-cells are observed. These are most probably induced by an antigen triggering the immune system (7). Subsequently, interleukin (IL)-4,5,10,13 and transforming growth factor (TGF)-beta have been assumed to activate B-cells, hence producing IgG4 expressing B-cells and fibrosis (7). The role of IgG4 antibodies in the pathogenesis is unclear, but because of characteristics of these antibodies (19), they most probably act as anti-inflammatory antibodies as response to an unknown trigger (Figure 4).

Treatment

When untreated, IgG4-RD can cause irreversible organ damage hence early and aggressive treatment is indicated (51). Glucocorticoids are the first choice of the treatment for the adults, mostly effective at a prednisone dosage of 0.5 -1 mg/kg/day, adjusted according to aggressive disease (8). In the presented study prednisone appeared first choice therapy for pediatric IgG4-RD. There is no consensus on prednisone dosage in pediatrics, but in general prednisone 1 to 2mg/kg/day should be appropriate. Prednisone can thereafter be tapered according to individual response. Treatment with prednisone is often rapidly effective, but this treatment should be maintained for 2 to 4 weeks after initiation. In the presented study prednisone was generally effective first line therapy in 83% of the cases. However, only in 43% of the cases prednisone single therapy sufficed. The rest of the cases required maintenance (immunosuppressive) therapy. According to previous studies, especially on adults, about 25% of patients show relapse of the disease despite prednisone maintenance therapy making steroid sparing agents necessary (8). MMF, azathioprine and methotrexate were effective in about 50% of the cases in this study. The role of DMARDs in the treatment of IgG4-RD is not yet clear and management of this disease with these agents has not been outlined (8).

Figure 4. The IgG4-antibodies

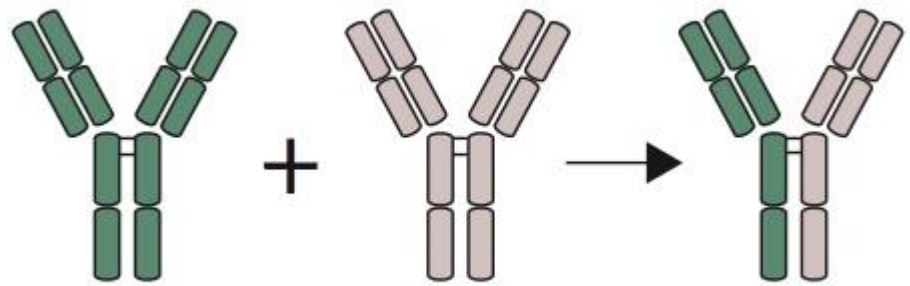


Figure 4. The IgG4-antibodies. Proposed mechanism of the formation of IgG4 antibodies by ''Fab-Arm" exchange. IgG4 antibodies continuously exchange half molecules with other antibodies making them bivalent reactive antibodies with two different antigen-binding fragments. These antibodies are also unable to activate the classical complement system and can bind to antigens. However, as a result of bivalent reactivity unable to form immune complexes. Because of these characteristics the IgG4 antibodies are most probably anti-inflammatory agents rather than pro-inflammatory. Fab = antigen binding fragment.

Recently, increasing evidence for the efficacy of rituximab treatment of IgG4-RD has been demonstrated (21). In this review four patients were treated with rituximab leading to significant clinical outcomes in all cases. We recommend rituximab as a strong alternative when a patient is refractory to therapy. Intravenous or subcutaneous immunoglobulin treatment has been successfully used in other inflammatory or immune mediated diseases, but this therapy has not yet been applied in IgG-RD (52).

Serum IgG4, when elevated, can be used in disease activity monitoring after initiating treatment, however, the role of serum IgG4 as disease activity marker has not yet fully been outlined (7). Studies should define the role of serum IgG4 as disease marker, same applies to circulating plasmablasts. Imaging studies, especially PET scan is useful in disease monitoring. Studies have shown the usefulness of FDG-PET/CT scan for diagnosis, staging and the degree of organ involvement and monitoring of therapy response, and this imaging method seems to detect more lesions than conventional methods like ultrasonography and CT (53).

CONCLUSION

In conclusion, IgG4-RD is a relatively new disease and generally unknown to pediatricians. The results of this study suggest that the average age of patients is lower than suggested in the literature. Early recognition and therapy are important to prevent serious and irreversible organ damage. Treatment with prednisone is the first choice for this disease, but maintenance therapy with DMARDs is often required. Rituximab may be a good alternative in therapy refractory disease. Further (epidemiological) studies should confirm these preliminary conclusions. Moreover, serological and histological studies and studies on treatment of children with IgG4-RD are needed in order to confirm the same results in children compared with previous studies performed in adults.

APPENDIX

Search terms used in the medical databases for the literature search in this systematic review on IgG4-RD in pediatrics.

Embase.com (251)

('immunoglobulin G4 related disease'/exp OR 'Mikulicz disease'/exp OR ((G4 OR igg4 OR 'igg 4' OR Mikulicz OR kuttner OR riedel*) NEAR/3 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*)):ab,ti) AND (child/exp OR newborn/exp OR adolescent/exp OR adolescence/exp OR 'child behavior'/de OR 'child parent relation'/de OR pediatrics/exp OR childhood/exp OR 'child nutrition'/de OR 'infant nutrition'/exp OR 'child welfare'/de OR 'child abuse'/de OR 'child advocacy'/de OR 'child development'/de OR 'child growth'/de OR 'child health'/de OR 'child health care'/exp OR 'child care'/exp OR 'childhood disease'/exp OR 'child death'/de OR 'child psychiatry'/de OR 'child psychology'/de OR 'pediatric ward'/de OR 'pediatric hospital'/de OR 'pediatric nursing'/exp OR 'pediatric anesthesia'/exp OR 'pediatric surgery'/exp OR (adolescen* OR infan* OR newborn* OR (new NEXT/1 born*) OR baby OR babies OR neonat* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR underag* OR (under NEXT/1 (age* OR aging)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool* OR picu OR nicu OR picus OR nicus):ab,ti)

Medline (224)

(Mikulicz' Disease/ OR ((G4 OR igg4 OR igg 4 OR Mikulicz OR kuttner OR riedel*) ADJ3 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*)).ab,ti.) AND (exp child/ OR exp infant/ OR adolescent/ OR exp pediatrics/ OR exp Child Health Services/ OR Hospitals, Pediatric/ OR (adolescen* OR infan* OR newborn* OR (new ADJ born*) OR baby OR babies OR neonat* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR underag* OR (under ADJ (age* OR aging)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool*).ab,ti.)

Cochrane (6)

(((G4 OR igg4 OR 'igg 4' OR Mikulicz OR kuttner OR riedel*) NEAR/3 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*)):ab,ti) AND ((adolescen* OR infan* OR newborn* OR (new NEXT/1 born*) OR baby OR babies OR neonat* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR underag* OR (under NEXT/1 (age* OR aging)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool* OR picu OR nicu OR picus OR nicus):ab,ti)

Web-of-science (126)

TS=((((G4 OR igg4 OR "igg 4" OR Mikulicz OR kuttner OR riedel*) NEAR/2 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*))) AND ((adolescen* OR infan* OR newborn* OR (new NEAR/1 born*) OR baby OR babies OR neonat* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR underag* OR (under NEAR/1 (age* OR aging)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool* OR picu OR nicu OR picus OR nicus)))

PubMed publisher (33)

(Mikulicz' Disease[mh] OR ((G4[tiab] OR igg4[tiab] OR "igg 4"[tiab] OR Mikulicz[tiab] OR kuttner[tiab] OR riedel*[tiab]) AND (related[tiab] OR associat*[tiab] OR autoimmun*[tiab] OR disease*[tiab] OR inflammat*[tiab] OR tumor*[tiab] OR thyroidit*[tiab]))) AND (child[mh] OR infant[mh] OR adolescent[mh] OR pediatrics[mh] OR Child Health Services[mh] OR Hospitals, Pediatric[mh] OR (adolescen*[tiab] OR infan*[tiab] OR newborn*[tiab] OR new born*[tiab] OR baby OR babies OR neonat*[tiab] OR child*[tiab] OR kid OR kids OR toddler*[tiab] OR teen*[tiab] OR boy*[tiab] OR girl*[tiab] OR minors OR underag*[tiab] OR under age*[tiab] OR under aging*[tiab] OR juvenil*[tiab] OR youth*[tiab] OR kindergar*[tiab] OR puber*[tiab] OR pubescen*[tiab] OR prepubescen*[tiab] OR prepubert*[tiab] OR pediatric*[tiab] OR paediatric*[tiab] OR school*[tiab] OR preschool*[tiab] OR highschool*[tiab])) AND (publisher[sb] OR inprocess [sb])

Google scholar (100)

"G4|igg4|Mikulicz|kuttner|riedel rd|related|associat|autoimmune|disease|inflammation|tumor|thyroiditis" child|children|adolescent|adolescents|adolescence|infant|infants|infancy

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Chapter 2

Novel organ involvements and complications of IgG4-related disease

Chapter 2.1 AA amyloidosis and IgG4-related disease

Based on:

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And

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ABSTRACT

IgG4-related disease (IgG4-RD) is a systemic fibroinflammatory condition. If untreated the disease can lead to fibrosis of the affected tissue. Timely diagnosis and treatment are therefore critical. Here we demonstrate a case of prolonged untreated IgG4-RD leading to secondary renal AA amyloidosis. The patient presented with mesenteric mass since more than 16 years. In the laboratory elevated levels serum IgG4, erythrocyte sedimentation rate (ESR) and CRP were observed. During the follow-up, patient developed kidney function impairment and nephrotic syndrome which appeared to be due to renal AA amyloidosis. Patient is thereafter treated with rituximab.

We then prospectively measured serum amyloid A (SAA), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) serum IgG4 and assessed the IgG4-Related Disease Responder Index (IgG4-RD RI). In total 28 patients with histologically proven IgG4-RD were included. In total 9 (32%) of these patients had elevated SAA, which significantly correlated with levels of CRP. No correlations were found between SAA and serum IgG4 and IgG4-RD RI.

In conclusion, patients with IgG4-RD may have increased CRP and SAA and may potentially develop AA amyloidosis if untreated.

INTRODUCTION

IgG4-RD is a systemic fibroinflammatory condition potentially affecting all parts of the human body (1). The diagnosis is based on the clinical presentation, elevated serum IgG4 and histology as the gold standard. The disease can lead to irreversible organ damage due to fibrosis (2). Therefore, prompt diagnosis and treatment are critical.

Serum IgG4 is increased in about 30% of the patients with IgG4-RD (3). Other inflammatory parameters such as ESR and CRP are less frequently elevated (4). Prolonged high levels of CRP, often associated with high levels of SAA, may lead to secondary AA amyloidosis (5). Here we demonstrate a case of a patient with IgG4-RD and secondary AA amyloidosis. Furthermore, we demonstrate the results of our study on SAA levels in a well-defined cohort of patients with IgG4-RD.

CASE PRESENTATION

A 53-year-old man with long-standing malaise and fatigue had a slow-growing mesenteric mass (5 cm in diameter) that had been present 16 years earlier on the basis of a review of previous imaging studies. In July 2015, the patient had received a diagnosis of mesenteric IgG4-related disease with involvement of surrounding lymph nodes, which had fulfilled international consensus criteria (Figure 1A and 1B). The only blood abnormalities at that time had been the levels of IgG4 (25 g/L), CRP (84 mg/L) and the ESR (119 mm per hour). After therapy with prednisone and azathioprine, both the size of the mass and the levels of inflammatory markers decreased rapidly. However, as the prednisone was being tapered, renal impairment with the nephrotic syndrome developed, as indicated by a creatinine level of 168 µmol/L), an albumin level of 22 mg/L per deciliter, and a ratio of urinary albumin (measured in milligrams per liter) to urinary creatinine (measured in grams per liter) of 10.3. Renal AA amyloidosis was diagnosed on kidney biopsy (Figure 1C and 1D). Retrospectively, the serum amyloid level from July 2015 had been elevated (350 mg/L). Treatment with intravenous methylprednisolone and rituximab led to slight decreases in the plasma creatinine level 150 µmol /L and in the ratio of urinary protein to creatinine (8.2) and a substantial decrease in the levels of both C-reactive protein (1.1 mg/L) and serum amyloid A (4 mg/L).

METHOD

To investigate the levels of SAA in IgG4-RD, we then prospectively measured the levels of inflammatory markers ESR, CRP and SAA in a well-defined cohort of patients with IgG4-RD. The patients were histologically diagnosed according to the Boston Criteria (6). Furthermore, the disease activity was assessed using the IgG4-RD RI in all patients at the time of measurement of these inflammatory markers (7).

RESULTS

In total, 28 patients with established SAA were included. In 9 patients (32%) SAA was elevated (normal range < 4mg/L, ranging from 5 to 350 mg/L) and correlated significantly with levels of CRP (correlation coefficient = 0.816, P = 0.001, Figure 2). No significant correlation was found between SAA and serum IgG4 (P = 0.642, Figure 2), however, a weak correlation between SAA and ESR was observed (correlation coefficient = 0.382, P = 0.0145, Figure 2). Furthermore, the IgG4-RD RI (7) was assessed at the moment of the measurement of these inflammatory parameters showing no correlation between IgG4-RD RI and SAA (P = 0.881, Figure 1).

Figure 1. Mesenteric- and Renal-Biopsy Samples from the Patient

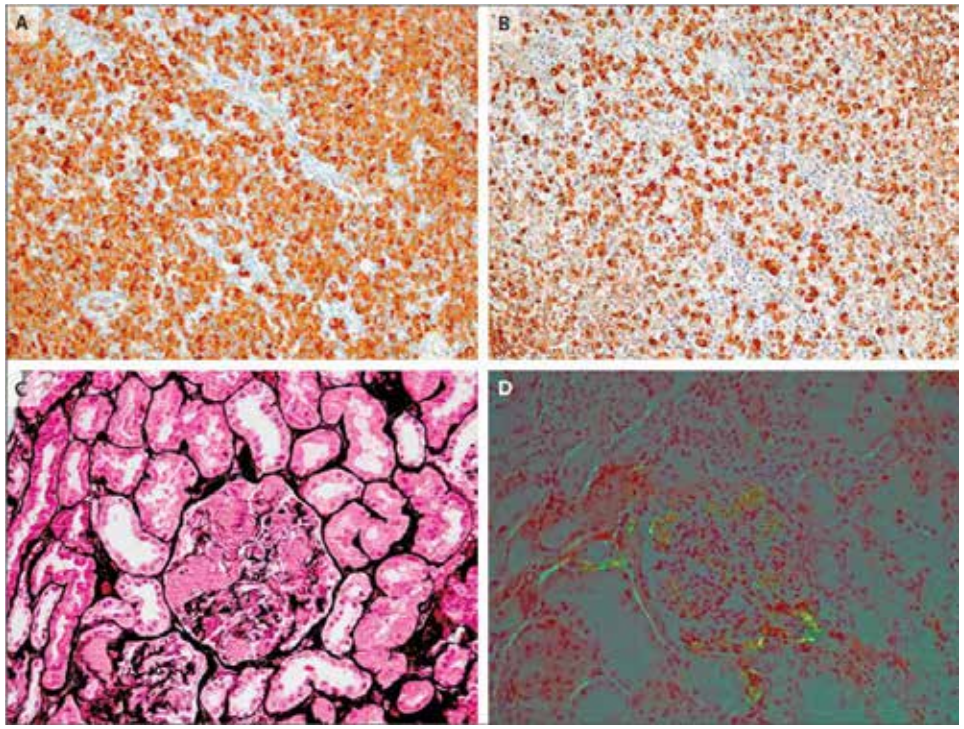

Figure 1. Mesenteric- and Renal-Biopsy Samples from the Patient. Shown are biopsy samples obtained from a 53−year−old man with renal AA amyloidosis that was apparently associated with IgG4−related disease. A sample that was obtained from a mesenteric mass underwent immunohistochemical staining for IgG (**Panel A**) and IgG4 (**Panel B**), with the latter showing diffuse, scattered IgG4−positive plasma cells. The average number of plasma cells that were positive for IgG4 (a mean of 421 cells per high−power field out of three analyses, with a ratio of IgG4−positive plasma cells to IgG−positive plasma cells of 0.53) fit the diagnosis of IgG4−related disease. The renal cortex shows expanded mesangial areas with a loss of argyrophilia and thickened capillary walls (**Panel C, silver staining**), and apple−green birefringence is visible in the mesangium and along capillaries under polarized light (**Panel D, Congo red staining**).

Figure 2. The levels of serum amyloid A

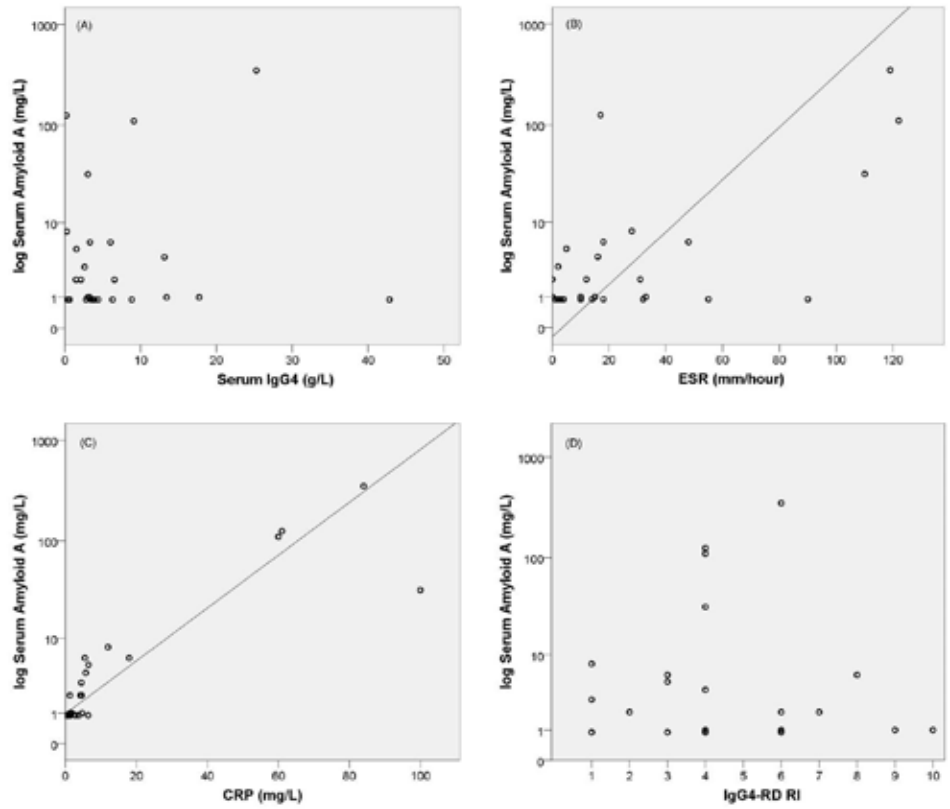

Figure 2. The levels of serum amyloid A. Scatter plotes of the association between SAA and serum IgG4 (A), ESR (B), CRP (C) and IgG4-RD RI (D).
ESR, Erythorcyte Sedimentation Rate; CRP, C-reactive protein; IgG4-RD RI: IgG4-Related Disease Responder Index; SAA, serum amyloid A.
*For significant correlations, a linear line was fitted to the scatterplot.

DISCUSSION

Here, we describe a patient with renal AA amyloidosis that was apparently associated with IgG4-RD. Furthermore, we demonstrate that in 32% of patients with IgG4-RD, SAA is elevated accompanied by elevated CRP.

IgG4-related disease is associated with previously unexplained clinical conditions that include idiopathic retroperitoneal fibrosis (8). It is considered to be a fibroinflammatory disease that is responsive to anti-inflammatory therapy, which may prevent fibrosis.

Various long-standing inflammatory conditions such as autoinflammatory and rheumatic disorders may lead to the deposition of AA amyloid (5). SAA and CRP are both acute-phase proteins that are often elevated during inflammation. The formation of SAA can be expected in IgG4-RD, with elevations in CRP in approximately 40% of patients (4). In current study the elevated levels of SAA were correlated with elevated levels of CRP, but not with serum IgG4. Furthermore, no correlation was found between SAA levels and scores on IgG4-RD RI.

AA amyloidosis is a serious clinical complication with increased risk of mortality, predominantly affecting the kidneys (9). AA amyloidosis is possibly a rare complication of IgG4-RD, because the majority of patients with IgG4-RD have normal levels of CRP (4) and because IgG4-RD is often treated after establishing the diagnosis leading to normalisation of CRP and SAA. In our patient, the disease appeared to have been dormant for at least 16 years, according to the deduced time that the abdominal mass had been present. However, we speculate that the patient's long-standing inflammatory state probably contributed to the development of amyloidosis.

In conclusion, patients with IgG4-RD may have elevated CRP with associated elevated SAA, which may lead to AA amyloidosis. We recommend standard measurement of CRP and SAA in patients with IgG4-RD. Elevated CRP and SAA should be part of treatment consideration and the treatment aim should be to decrease the levels of CRP and SAA to normal basal levels.

CHAPTER 2.2 IgG4-related disease as an emerging cause of scleritis

Based on:

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ABSTRACT

Purpose: Scleritis is a painful ocular inflammatory condition associated with a broad spectrum of disorders. Several infectious and inflammatory conditions have been associated with scleritis, however in about 50% of the cases the underlying cause remains unclear. IgG4-related disease (IgG4-RD) is being recognized as a systemic disease with orbital and ocular manifestations. In this study we evaluate the occurrence of IgGR4-RD in patients with idiopathic scleritis within a double tertiary referral centre cohort.

Methods: We performed a retrospective study. Medical records of patients with idiopathic scleritis diagnosed between April 1992 and July 2016 were reviewed for demographic and clinical characteristics. Patients with scleritis due to an identified associated systemic or infectious disease were excluded.

Results: Out of 38 patients with idiopathic scleritis, we obtained additional data on serum IgG4, histology and potential clues for IgG4-RD in 15 patients. We identified 2 definite cases of IgG4-RD and 3 cases of probable IgG4-RD.

Conclusion We emphasize the occurrence of scleritis as a manifestation of IgG4-RD. Awareness of IgG4-RD associated scleritis is important for adequate diagnosis and therapy.

INTRODUCTION

Scleritis, classified as anterior or posterior and subdivided as diffuse, nodular or necrotizing, is a painful, potentially vision threatening ocular inflammatory condition associated with a broad spectrum of disorders. Most frequently rheumatoid arthritis, granulomatosis with polyangiitis (GPA) and relapsing polychondritis, but also spondylarthropathy, systemic lupus erythematosus (SLE), sarcoidosis, Behçet disease and IgA nephropathy have been associated with scleritis. Infectious diseases such as syphilis, tuberculosis, aspergillus, herpes zoster and infection with human immunodeficiency virus (HIV) may also cause scleritis. Less frequent, the use of bisphosphonates and eye surgery are related to clinical features of scleritis. In about 50% of the cases, however, the underlying disease remains unclear (10, 11).

IgG4-RD is increasingly recognized as a systemic disease and several orbital manifestations of IgG4-RD, including lacrimal and soft tissue manifestations, have recently been described in different cohort studies (12, 13). However, the relationship between IgG4-RD and scleritis is seldom described (14-17). To evaluate the occurrence of IgG4-RD in patients with scleritis, we have analysed a well-defined cohort of scleritis patients.

MATERIALS AND METHODS

The Erasmus MC university medical centre Rotterdam and Rotterdam Eye hospital represent tertiary referral centres for patients with ophthalmic disorders including scleritis. Medical records of patients with idiopathic scleritis diagnosed between April 1992 until 04th of July 2016 were reviewed for demographic and clinical characteristics. The ophthalmologists and the internist-immunologists with scleritis in their area of interest were requested to provide identification numbers of the patients with idiopathic scleritis. Furthermore, using Diagnosis Treatment Combination (DTC), patients were tracked in the electronic patient records. Patients were registered according to clinical diagnosis in the DTC system. Patients with secondary scleritis due to an associated systemic or infectious disease were excluded. Retrospectively, serum IgG4, histology and potential clues for IgG4-RD were established if available. Other inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are unspecific disease markers for IgG4-RD and data on these markers were not used. The typical histological features of IgG4-RD are a

dense lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis. The ratio of IgG4/IgG positive plasma cells in tissues should be greater than 0.4 and the numbers of IgG4 positive plasma cells per high power field (HPF) should be greater than the numbers agreed (6). The diagnosis of IgG4-RD was set by established criteria for histology (2, 6).

To grade the value of the findings we classified the patients in 4 categories (Table 1). Histologically proven cases were classified as definite IgG4-RD (2). Idiopathic scleritis with elevated serum IgG4 levels was classified as probable IgG4-RD. A recently published meta-analysis suggested high sensitivity and specificity of serum IgG4 in the diagnosis of IgG4-RD making idiopathic scleritis with elevated serum IgG4 in our cohort probable IgG4-RD (18). When revised, histology samples strongly suspected for IgG4-RD were also classified as probable IgG4-RD. Cases where IgG4-RD could (most probably) be excluded, were classified as no IgG4-RD. Cases in which no statement could be made about possibility of IgG4-RD, because of lack of data, were classified as unknown.

Table 1. Criteria for establishing diagnosis IgG4-RD in patients within this study

Probability of IgG4-RD	Criteria	Numbers of patients
Patients with definite diagnosis	When the diagnosis IgG4-RD is histologically established (with or without elevated serum IgG4) and the patient was known with idiopathic scleritis.	2
Patients with probable diagnosis	Patients with idiopathic scleritis and elevated serum IgG4 levels, but not histologically proven (n=2). Histology suggestive for IgG4-RD, but insufficient according to criteria (n = 1);	3
Patients with (most probably) no IgG4-RD	Patients with idiopathic scleritis with no or insufficient clues for IgG4-RD	10
Patients with unknown diagnosis	The remaining cases of idiopathic scleritis: no data available to make a statement about possibility of IgG4-RD.	23

RESULTS

In total 38 patients with idiopathic scleritis were identified and presented. The mean age of the patients was 45 years with a slight female preponderance (63%). In 15 out of 38 cases sufficient data could be retrieved to adequately study the given classifications. Out of these patients, 2 definite cases of IgG4-RD and 3 cases of probable IgG4-RD were identified (Table 2).

One patient (patient 2) initially presented with idiopathic scleritis and was later diagnosed with histologically confirmed IgG4-related orbital disease (IgG4-ROD) and treated successfully. Another patient (patient 38) presented with recurrent scleritis after almost 20 years. However, histology of new onset unilateral orbital tumor as well revision of orbital tissue from 20 years ago confirmed IgG4-RD. This case has previously been published as a case report due to its interesting clinical course.(19) Two patients (patient 1 and 22) had elevated serum IgG4 levels. Patient 1 was refractory to prednisone and eventually required rituximab to achieve clinical remission. Patient 22 required besides prednisone also methotrexate maintenance therapy. Considering the idiopathic scleritis and elevated levels of serum IgG4, these two patients are classified as probable IgG4-RD. Additionally, histopathological revision of skin biopsy revealed suggestive IgG4-RD (IgG4/IgG ratio of 0.3) in one case (patient 9). According to the consensus criteria for IgG4-RD a definite diagnosis was not met in this patient.(6) Data on serum IgG4 was not available in this patient. The histology might also be obscured by concomitant use of immunosuppressive agents (6).

IgG4-RD could be ruled out in 10 cases based on clinical data. In the remaining 23 cases no statement could be made on the presence of IgG4-RD because of missing data.

Table 1: characteristics of patients with idiopathic scleritis and (suspected) IgG4-RD in our study compared with previously published case reports.

Characteristics of patients with idiopathic scleritis and (suspected) IgG4-RD in our study									
P	**Age**	**Sex**	**Medical history**	**Initial diagnosis**	**Serum IgG4 (g/l)**	**Histology**	**Imaging**	**Treatment**	**IgG4-RD classification**
1	62 y	M	Rectum carcinoma at the age of 51 for which resection and stoma with complete remission	Idiopathic scleritis	Elevated (1.84)	NA	PET imaging: normal*	Pred, later RTX	Probable IgG4-RD because of idiopathic scleritis and elevated serum IgG4
2	65 y	M	Unremarkable	Idiopathic scleritis	Normal (0.47)*	Orbital/lacrimal tissue: IgG4-RD according to Boston criteria	CT-orbit: lacrimal disease.	Pred, later Aza.	Definite IgG4-RD because of lacrimal histology confirming IgG4-RD
5	47 y	M	Dermatitis of unknown origin	Idiopathic posterior scleritis	NM	Skin biopsy revision from 1999: IgG4/IgG ratio 0.3 and average of 74 IgG4+ plasma cells/HPF	MRI orbit: normal	Pred, later MTX	Probable IgG4-RD, ** because of histology suggestive for IgG4-RD. Serum IgG4 not available
6	35 y	F	Unremarkable	Idiopathic scleritis	Elevated (1.47)	NA	MRI cerebrum: solitary thickening M. Rectus medial right	Pred and MTX	Probable IgG4-RD, because of idiopathic scleritis and elevated serum IgG4
15	39 y	F	Idiopathic orbital inflammation at the age of 19, complete remission with pred. Scleritis at the age of 20, Complete remission with pred	Idiopathic scleritis	NM	Periorbital tissue: IgG4-RD. Revision previous orbital tissue: also IgG4-RD.	NA	Pred and Aza	Definite IgG4-RD,*** because of periorbital histology confirming IgG4-RD
Characteristics of patients with scleritis and IgG4-RD in previously published case reports									
Case	**Age**	**Sex**	**Medical history**	**Diagnosis**	**Serum IgG4**	**Histology**	**Imaging**	**Treatment**	**Comments and differences with patients in our cohort**
(17) Paulus	66 y	M	NM	Scleritis and uveitis	Elevated (143 mg/dl)	Histopathology of conjunctival and inferior rectus muscle biopsies confirmed IgG4-RD	CT of the orbit: asymmetric thickening of right inferotemporal sclera	Pred and MTX	Very short history of scleritis and uveitis. In contrast to our cohort, diagnosis established by scleral biopsy
(15) Ohno	49 y	F	NM	Scleritis for several years	NM. Postoperative normal serum IgG4	Enucleation of the right eyeball: IgG4-RD confirming IgG4-RD	No other lesions on whole body CT, GS and MRI	Initially pred 10mg/day and cyclosporine 100mg/day. Later enucleation.	In contrast to our patients, diagnosis confirmed after enucleation. Prior, serum IgG4 not measured leaded to inadequate treatment
(16) Caso	17 y	M	NM	Idiopathic scleritis since 5 years	NM	right scleral biopsy confirming IgG4-RD	MRI: abdominal lymphadenopathy	RTX	Scleritis in a young patient due to IgG4-RD. In contrast to our cohort, diagnosis established by scleral biopsy
(14) Philippakis	63 y	F	Thyroidectomy	13 years history of recurrent scleritis	Elevated (135mg/dl)	Surgical scleral biopsy confirming IgG4-RD	MRI: sclera the only site of local inflammation. PET: small mediastinal lymphadenopathy	Pred and MTX	Adequate workup with serum IgG4 and PET, however, after 13 years of unexplained scleritis. In contrast to our cohort, diagnosis established by scleral biopsy
(20) Lee	79 y	F	breast cancer, for which lumpectomy and radiation, colon cancer, diabetes, hypertension	Nodular scleritis and later uveitis	NM	Episcleral/orbital biopsy confirming IgG4-RD	NM	Pred, infliximab and later enucleation	Scleritis and later uveitis. In contrast to our cohort, diagnosis established by eipscleral (and orbital) biopsy

P-Patient, Y-Years, F-Female, M-Male, Pred-Prednisone, Aza-Azathioprine, MTX-Methotrexate MMF-Mycophenolate, RTX-Rituximab, NM-Not measured, NA-Not applicable, CT-computed tomography, GS-gallium scintigraphy, MRI-magnetic resonance imaging.

* Diagnostic test performed during immunosuppressive therapy.

** Revision of skin biopsy from 1999 showing IgG4/IgG ratio of 0.3. According to the consensus criteria for IgG4-RD a definite diagnosis was not met in this patient.(6) Data on serum IgG4 was not available in this patient. However, the histology might be obscured.

*** This case has previously been published as a case report due to its interesting clinical course (19).

DISCUSSION

In this retrospective cohort study we identified IgG4-RD as a potential and important cause for scleritis in minimally 15% of patients. To our best knowledge, this study represents the largest cohort of idiopathic scleritis patients evaluated for IgG4-RD.

IgG4-RD has only recently been recognized as a systemic clinical entity (21). Several conditions such as Mikulicz disease, sclerosing sialadenitis, idiopathic orbital inflammation, a subset of idiopathic

retroperitoneal fibrosis and Riedel's thyroiditis are now reclassified under the umbrella of IgG4-RD (22). The first reports that IgG4-RD might be associated with scleritis arose in 2012 (17). Since then sporadic cases have been published (Table 1 provides an overview on previous published reports on scleritis and IgG4-RD). In this study we report the occurrence in a larger cohort of scleritis patients, which may potentially take an important place in the list of the causes of scleritis. There are some limitations in the presented study. Primarily the retrospective nature limited the access to potential additional histological or serological data. Additionally in the past the inability to screen patients systemically with sensitive and novel radiographic studies such as FDG-PET/CT scanning might cause underdiagnoses, since most patients were swiftly treated with immunosuppressive agents masking systemic symptoms of IgG4RD (23).

Furthermore, scleritis is not often histologically evaluated because of fear of triggering the disease, perforation, infection or scleral thinning at the site of biopsy (24). Patient 2 and 38 presented with scleritis followed later by periorbital/lacrimal manifestation enabling proper histological evaluation. In situations where biopsy is not possible, which is often the case in scleritis, elevated serum IgG4 levels could be supportive and indicative, due to high sensitivity and specificity, for further additional imaging with for example PET-scanning to establish tissue diagnosis (25). However, about 30% of histologically proven cases show normal levels of IgG4 potentially leading to misinterpretation and falsely rejection of the diagnosis (23). In our study, the diagnosis of idiopathic scleritis needing aggressive immunosuppressive therapy and one sample elevated serum IgG4 levels in two cases were sufficient to establish the diagnosis of probable IgG4-RD.

Inflammatory markers such as ESR and CRP are not specific for this disease and cannot be

used for establishing or ruling out the diagnosis (4). Detection of circulating IgG4 positive plasmablasts by flowcytometry was shown to be a superior alternative for measuring IgG4 concentrations in serum, however, this method has not widely been introduced for clinical application (3). FDG-PET/CT scan is useful for diagnosis, staging and the degree of organ involvement as well as for monitoring of disease activity. This imaging technique detects more lesions than conventional imaging methods like computed tomography (CT) (25). We recommend additional survey of serum IgG4 and PET imaging in cases of idiopathic scleritis. Possible abnormalities on PET could be potential sources for histological examination. Adequate diagnostics can prevent excessive diagnostics, delay in obtaining the diagnosis and subsequently treatment of the disease and can prevent systemic manifestation of IgG4-RD.

To diagnose IgG4-RD is relevant because potential irreversible organ damage due to fibrosis may occur. IgG4-RD requires systemic immunosuppressive treatment, whereas local (ophthalmic) disease might be sufficiently treated with applications (1, 26). When indicated, glucocorticoids are the first choice of treatment mostly successful at a dosage of 30-40 mg/day, usually adjusted to body weight and severity of the disease (27). Almost 25% of patients relapse after tapering prednisone and require steroid-sparing agents. A steroid-sparing agent is appropriate and indicated when the glucocorticoid dosage cannot be tapered because of persistently active disease (27). Conventional immunosuppressive agents such as mycophenolate mofetil, azathioprine and methotrexate have all been described, but management of this disease with these DMARDs has not been outlined (27). Improving evidence for rituximab in the treatment of IgG4-RD is emerging leading to clinical remissions in patients with IgG4-RD with different organ manifestations (28, 29). Efficacy of anti-TNF alpha in the treatment of IgG4-RD has only been published in case reports and evidence for its clinical application in studies is lacking (23, 30).

In conclusion, this study indicates that in patients with idiopathic scleritis potentially a substantial part of these cases might be related to IgG4-RD. This emphasizes the existence of IgG4-RD associated scleritis. In addition to the well-known causes, IgG4-RD can be added as a novel cause of scleritis. Improved awareness will lead to more effective and swift diagnosis and therapy, and may prevent irreversible organ damage.

Chapter 2.3 How to distinguish IgG4-related disease from granulomatosis with polyangiitis?

Based on:

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ABSTRACT

Introduction: IgG4-related disease (IgG4-RD) is a fibro-inflammatory disease that compromises almost every organ and mimics several immune mediated diseases. Indeed, previous unexplained conditions have now been reclassified as primarily IgG4-RD. Here, we describe three cases of IgG4-RD earlier mistaken as limited granulomatosis with polyangiitis (GPA).

Case presentations: Three cases presented with orbital mass, chronic nasal congestion, excessive rhinorrhea, formation of crusts, nasal polyps and development of a saddle nose. Anti-neutrophil cytoplasmic antibodies were negative and the histology was inconclusive. Because the clinical presentation suggested limited GPA patients were managed accordingly. Histopathological revision however, revealed IgG4-related eosinophilic angiocentric fibrosis consistent with IgG4-RD in all three cases.

Discussion: These three cases emphasize that orbital and nasal manifestation of IgG4-RD mimic GPA. The orbit, but also nose and sinus are often involved in IgG4-RD. The diagnosis of this recently introduced entity depends on the abundance of a set value of IgG4 presenting plasma cells in the histological sample. Proper diagnosis is important to initiate adequate treatment in order to prevent irreversible fibrosis and organ damage.

Conclusion: IgG4-RD is an emerging imitator that potentially can affect almost all body parts. Also in nasal disease it may mimic limited GPA. Awareness will alert the physician to differentiate from IgG4RD by evaluating the count of IgG4 presenting plasma cells in histological samples.

INTRODUCTION

IgG4-RD is a fibro-inflammatory disease that may affect almost all organs. This condition has only recently been recognized as a systemic clinical disease, manifesting as tumour-like infiltrations and characterized by IgG4 positive plasma cells (2, 21). The average age of affected patients with IgG4-RD at presentation is estimated to be older than 50 years, although IgG4-RD in children has been reported regularly (21). IgG4-RD mostly affects the orbit, the pancreas, the salivary and lacrimal glands and the lymph nodes. Several of these conditions are known as Mikulicz's disease, Küttner's tumor, sclerosing sialadenitis, idiopathic orbital inflammation, idiopathic scleritis, a subset of idiopathic retroperitoneal fibrosis and Riedel's thyroiditis. All are now reclassified under the umbrella of IgG4-RD (22, 29, 31). This disease may imitate many inflammatory, infectious and malignant disorders often leading to a delay in diagnosis. Irreversible organ damage due to fibrosis may occur when prompt and effective treatment is omitted (26). Nasal manifestation of IgG4-RD, mostly of the paranasal sinuses, has previously been described in case reports (32), but it can also manifest as a primary or secondary nasal disease such as chronic sinusitis and paranasal sinusitis with dacryoadenitis (33, 34). Since IgG4-RD localised in the nasal region remarkably resembles limited granulomatosis with polyangiitis (GPA), we hypothesize that in ANCA negative GPA, IgG4-RD might be an alternative diagnosis. We therefore re-evaluated three of such patients and could re-diagnose these in IgG4-RD with orbital and nasal manifestations. The histomorphological features matching IgG4-RD and absence of evident features of GPA were the reasons for immunohistochemical analysis in these cases leading to the diagnosis of IgG4-RD. Therefore, we stress the importance to be aware of IgG4-RD in ANCA negative ENT cases without a histologically proven vasculitis.

CASE PRESENTATIONS

Patient characteristics and main clinical features are presented in Table 1.

Case 1

A 53-year-old man with a periorbital tumor and solid swelling of the eyelid of unknown origin was treated with local glucocorticosteroid injections. The symptoms worsened and

he developed a solid swelling on the left side of the nose (Figure 1A+B). Further physical examination revealed no abnormalities. Computed tomography (CT) of the orbit demonstrated a homogeneous intraorbital tumor on the left side. Limited GPA was suspected without anti-neutrophil cytoplasmic antibodies (ANCA). However, serum IgG4 was elevated (1.45 g/l) and after re-evaluation histology of the orbit mass and internal nasal mucosa revealed lymphoplasmacytic infiltrates with eosinophils and angiocentric fibrosis compatible with IgG4-related eosinophilic angiocentric fibrosis (Figure 2) fitting the criteria of IgG4-RD. Treatment was started with prednisone 1mg/kg/day and methotrexate 20mg once a week (including folic acid) leading to a significant decrease in orbital and nasal inflammation.

Case 2

A 73-year-old man presented almost 18 years ago with recurrent nasal polyps and inflammation. Inspection of the nose revealed loss of cartilaginous support, leading to saddle deformity, and red congestive internal mucosa along with crusting. One year after presentation he developed an orbital tumour on the right side with significant painful exophthalmos. Histology demonstrated a lymphoplasmacytic infiltrate with eosinophils and angiocentric fibrosis. Diagnosed as limited ANCA negative GPA 1mg/kg/day prednisone, azathioprine and cotrimoxazole induced a clinical remission. However, after tapering the prednisone a relapse occurred and cyclophosphamide was initiated . Because of persistent pain, enucleation was performed followed by radiation eventually relieving the symptoms (Figure 1C+D). A revision of the orbital tissue was compatible with IgG4-related eosinophilic angiocentric fibrosis (Figure 2). He remained asymptomatic without treatment, but elevated serum IgG4 levels are recently observed almost 10 years after enucleation.

Table 1. Characteristics of the patients

	Case 1	Case 2	Case 3
Gender	Male	Male	Female
Age	53 years	73 years	50 years
Medical history	-Chronic spontaneous urticarial disease -Unilateral orbital swelling without a definite diagnosis.	-Irradiated prostate carcinoma.	- 27 years ago diagnosed with Good-Pasture disease for which prednisone therapy.
Symptoms and duration of symptoms	Redness and swelling with orbital discomfort since 4 years. Thickening of the nose without other symptoms. No systemic symptoms suggestive for vasculitis were observed.	Recurrent nasal polyps with chronic nasal inflammation leading to multiple surgeries. Subsequent exophthalmos due to an orbital tumor leading to discomfort and pain. No systemic symptoms suggestive for vasculitis.	Unilateral left-sided orbital swelling. Recurrent excessive nasal mucus production, often curst formation and recurrent sinusitis. Allergies were excluded. No systemic symptoms suggestive for vasculitis.
Diagnosis	Orbital and nasal manifestations of eosinophilic angiocentric fibrosis including IgG4 plasma cell infiltration meeting the criteria for IgG4-RD.	Orbital and nasal manifestations of eosinophilic angiocentric fibrosis including IgG4 plasma cell infiltration meeting the criteria for IgG4-RD.	Orbital manifestations of eosinophilic angiocentric fibrosis including IgG4 plasma cell infiltration meeting the criteria for IgG4-RD.
Nose inspection	Lateral left side of the nose is swollen. Rhinoscopy: thickening of the mucosa without visible inflammation nor crusting.	Loss of cartilaginous leading to saddle deformity. Rhinoscopy: inflammation with severe crusting.	Normal.
Serum IgG4 pre-treatment (range normal values: 0.08-1.40 g/l)	1.45 g/l	3.30 g/l	0.36 g/l (current value after withdrawing prednisone, clinical remission).
ANCA	Negative	Negative	Negative
Other relevant findings	Normal CRP and ESR. Normal renal function.	Slightly elevated ESR (48 mm/hr.) and CRP (18 mg/l). Normal renal function.	Normal CRP and ESR. Normal renal function.
Imaging	-CT orbit: homogenous tumor of the orbit and the nose without compression of nervous optics and with bulbous shifting. - FDG-PET: increased activity in the left orbit and external nose.	-MRI orbit at presentation: orbital mass on the right side leading to exophthalmos. Nasal polyps and status after sinus surgery and septoplasty. -CT thorax and abdomen: no abnormalities.	-CT orbit: bilateral chronic dacryoadenitis, more prominent on the left side.
Histology	Surgical orbital biopsy: -Angiocentric lymphoplasmacytic infiltration with fibrosis, 90 IgG4 positive plasma cells per HPF and IgG4/IgG ratio of 0.45. No granulomas. Biopsy mucosa nose: -infiltration of lymphocytes, histiocytes, and plasma cells. 12 IgG4 positive plasma cells per HPF with an IgG4/IgG ratio of 0.33. No granulomas.	Surgical enucleation of the eye and orbital mass right: Angiocentric lymphoplasmacytic infiltration, fibrosis, 133 IgG4 positive plasma cells per HPF with an IgG4/IgG ratio of 0.7. No granulomas.	Lacrimal gland surgery: Angiocentric lymphoplasmacytic infiltration, fibrosis, 78 IgG4 positive plasma cells per HPF with IgG4/IgG ratio of 0.4. No granulomas.
Treatment	Prednisone 1mg/kg (currently tapering) and methotrexate 20mg weekly with folic acid.	Prednisone 1mg/kg and maintenance for 2 years, azathioprine 100mg daily for two years, cotrimoxazole 960mg twice a day for two years, 4 cycles of cyclophosphamide 750mg/m^2 and enucleation. Currently no treatment.	Previous treatment: Prednisone 1mg/kg and maintenance for almost 8 years with prednisone 5mg/day. Patient declined steroid sparing treatment. Current treatment: Prednisone recently withdrawn . In case of relapse rituximab will be considered.

Characteristics and the main clinical features of the 3 patients with IgG4-RD in the ENT region, previously diagnosed as limited GPA. CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, ANCA = anti-neutrophil cytoplasmic antibodies, HPF = high power field.

Figure 1. Clinical images of patient 1 and 2

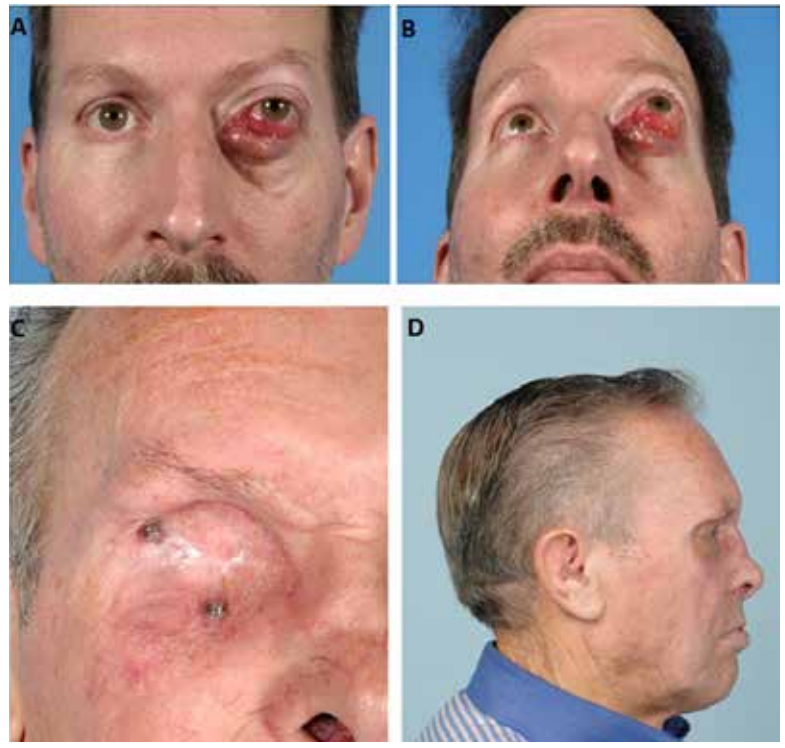

Figure 1. Clinical images of patient 1 and 2. A+B: Clinical images of patient 1 showing the orbital tumor and the swelling on the left side of the nose due to IgG4-RD. **C+D:** Clinical images of patient 2 demonstrating post-surgery status (after enucleation of the right eye). Currently, the patient uses an ocular prosthesis. The lateral image shows loss of cartilage support leading to saddle deformity of the nose. These images are used with written permission of the patients.

Case 3

A 50-year-old female was referred 9 years ago because of recurrent bilateral orbital swelling. Histology demonstrated a lymphoplasmacytic infiltrate with eosinophils without vasculitis or granuloma. Other complaints included recurrent excessive rhinorrhea, crust formation and sinusitis, although inspection of the nose revealed no abnormalities. ANCA antibodies were absent, but the symptoms were regarded as limited GPA. Corticosteroid treatment successfully achieved a complete remission and was slowly tapered and maintained at a low dose for almost 8 years. A revision of orbital tissue reclassified the lesion to be compatible with IgG4-related eosinophilic angiocentric fibrosis (Figure 2).

DISCUSSION

Herewith, we describe three cases of IgG4-RD initially suspected of limited GPA. The clinical course of these patients reflects the broad mimicking capacity of IgG4-RD that may obscure its real identity.

GPA is an inflammatory condition often involving the orbital and ENT regions. Classical histology includes granulomas, vasculitis, necrosis and fibrosis. However, especially in GPA limited to the ENT region, only about 16% of the cases present with classical histological features. Additionally ANCA's frequently lack in this situation and might lead to a presumed diagnosis of GPA (35, 36). In this light the presented patients with orbital and nasal symptoms were initially diagnosed as limited GPA. Patient 1 presented with thickening of the lateral part of the external nose and patient 2 presented with recurrent nasal polyps, crusting and saddle deformity of the nose (37). Patient 3 complained of recurrent excessive rhinorrhea, crusting and sinusitis but no abnormalities were seen on inspection of the nose. None of these patients had systemic symptoms, ANCA antibodies, radiological or histological signs of GPA. IgG4-RD is a rare disease preferentially manifesting in the orbit, salivary tract, pancreas and lymph nodes (29, 38). However, involvement of almost every part of the body has been described (39, 40). Infrequently nasal manifestation occurs, but such rare cases might easily be overlooked as in our patients (32-34). Recurrent unexplained nasal inflammation in presumed limited GPA with concomitant orbital inflammation and improved awareness of IgG4-RD has triggered us to re-evaluate the presented patients. Adequate and swift diagnosing of IgG4-RD is important because potential irreversible tissue damage due to fibrosis may occur and treatment modalities can be different. Furthermore, prolonged untreated inflammation due to IgG4-RD may lead also to AA amyloidosis, emphasizing timely recognition and treatment of the disease (41).

Diagnosis of IgG4-RD can be challenging and is based on the combination of clinical presentation and histology. The clinical presentation of IgG4-RD dependents on the organ involvement of this disease (2). A histological diagnosis requires demonstration of IgG4 presenting plasma cells in surgical specimens and a ratio of IgG4-positive to total IgG-positive plasma cells greater than 40% according to the Boston consensus criteria and remains the gold standard (6). The cases presented meet the both histomorphological features of eosinophilic angiocentric fibrosis previously proposed as a part of the spectrum of IgG4-RD (42, 43) and the immunohistochemical criteria for IgG4-RD(42, 43). Elevated serum IgG4 may be support a suspicion of IgG4-RD because of its relatively high specificity and sensitivity (18). However, up to 30% of the patients with histological proven cases may present with normal serum IgG4 (4). Inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) can be elevated in 40 to 50%, but are a-specific (4). Recently it was demonstrated that circulating IgG4 positive plasma blasts detected with flow cytometry might be a superior alternative to serum IgG4 (3). FDG PET/CT is a sensitive tool to determine the extent of the disease and may be used in monitoring of therapy response (44, 45).

Figure 2. Histology images of the 3 patients

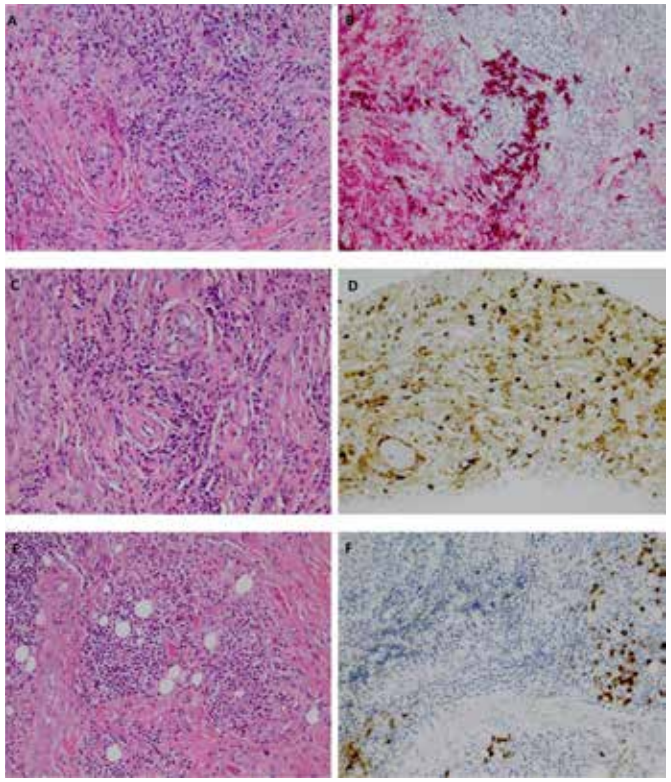

Figure 2. Histology images of the 3 patients. A: HE staining of orbital tissue in case 1 showing lymphoplasmacytic infiltration (and eosinophilic infiltration) with predominantly perivascular fibrosis without evidence for granulomatous inflammation. **B**: immunohistochemical staining for IgG4 demonstrating 90 IgG4 positive plasma cells per HPF with an IgG4/IgG ratio of 0.45. **C**: HE staining of orbital tissue in case 2 revealing lymphoplasmacytic infiltrations (and eosinophilic infiltration) and extended perivascular fibrosis. No evidence for granuloma. **D**: immunohistochemical staining for IgG4 showing 133 IgG4 positive plasma cells per HPF with an IgG4/IgG ratio of 0.7. **E**: HE staining of orbital tissue in case 3 showing extended perivascular fibrosis with lymphoplasmacytic (and eosinophilic) infiltration without signs of granulomatous inflammation. **F**: Staining for IgG4 demonstrating 78 IgG4 positive plasma cells per HPF with an IgG4/IgG ratio of 0.4.

Immunosuppressive treatment in IgG4RD is mandatory to prevent fibrosis and irreversible organ damage, and differs from the treatment of GPA for which maintenance therapy for localized disease includes cotrimoxazole, azathioprine or methotrexate (1, 26, 46). Except one case (32), evidence is lacking for intranasal steroid delivery in IgG4-related nasal disease, but could be considered in isolated nasal manifestation. The treatment strategies in IgG4-RD and GPA do not differ significantly, since both respond to glucocorticoids and

rituximab. However in limited GPA, the disease is often effectively treated with conventional immunosuppressants and cotrimoxazole (46). Conventional immunosuppressants such as mycophenolate-mofetil, azathioprine and methotrexate have all been described, but the evidence for their efficacy in IgG4-RD is poor and treatment may therefore require rituximab in an early stage (47).

Concluding, IgG4-RD is an emerging imitator, potentially mimicking GPA of the ENT region. We stress the importance of recognizing IgG4-RD, especially if orbital symptoms are present in supposed limited nasal GPA, because of the potential complications if treated inadequately and the presence of IgG4-RD in ANCA negative limited GPA must be studied in larger cohort of patients.

Chapter 2.4 The tarsal plate manifestation of IgG4-related disease

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ABSTRACT

Purpose

To describe a clinical case of bilateral biopsy-proven IgG4-related disease confined to the tarsal plate.

Method

Interventional case report.

Results

A 58-year-old woman presented with a tarsal swelling in the lateral part of the upper eyelids, with focal ulceration and mucus. Histology revealed fibrotic inflammation with increased IgG4-positive plasma cells fulfilling to the criteria of IgG4 related disease (IgG4-RD). Serum IgG4 levels were increased and pathological fluorodeoxyglucose (FDG) uptake at Positron Emission Tomography (P.E.T.)/ CT scanning was restricted to the upper eyelids. After treatment with oral and topical prednisone the tarsal lesions markedly regressed.

Conclusions

Periorbital IgG4-RD may be confined to the tarsal plate. Treatment with systemic and topical steroids may induce significant regression.

INTRODUCTION

IgG4-related disease (IgG4-RD) is an increasingly recognized fibro-inflammatory condition leading to tumor-like infiltrations of the affected tissues (48). It is characterized by a lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells, storiform fibrosis, obliterative phlebitis and often a mild to moderate tissue eosinophilia (49) . IgG4-RD predominantly affects the salivary glands, pancreas, orbital tissue and the retroperitoneal cavity, either singly or systematically, however, manifestations in almost all parts of body have been described (1, 50). In case of IgG4-related orbital disease patients mostly present with lacrimal complaints, peri-orbital masses or, less frequently, scleritis or uveitis (51, 52). Here we describe a rare case of bilateral tarsal IgG4-RD.

CASE PRESENTATION

A 58-year-old woman, with a history of rosacea, presented with painless bilateral swelling of the eyelids, mild hyperaemia of the conjunctiva and mucopurulent discharge since 3 months. She reported no improvement on topical antibiotics prescribed by her general practitioner. On clinical examination, tarsal swelling was observed, more apparent in the lateral part of the upper eyelid, right more than left, with focal ulceration and mucus (Figure 1A).

Figure 1

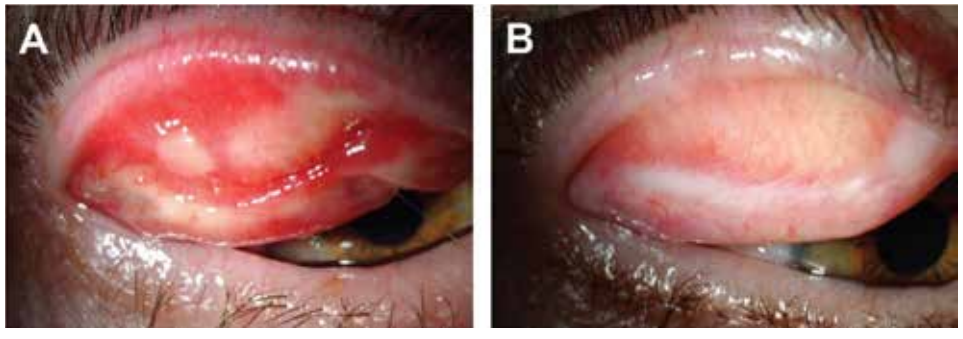

Figure 1. Photograph demonstrating tarsal IgG4-related disease before (A) and after treatment (B) 6 months of treatment with systemic and topical steroids

Histopathological examination from a tarsal biopsy demonstrated a storiform fibrosing inflammation with increased numbers of IgG4-positive plasma cells (>200 per high-power field, with IgG4/IgG ratio >0.80), fulfilling the criteria for IgG4-RD (Figure 2).

Figure 2

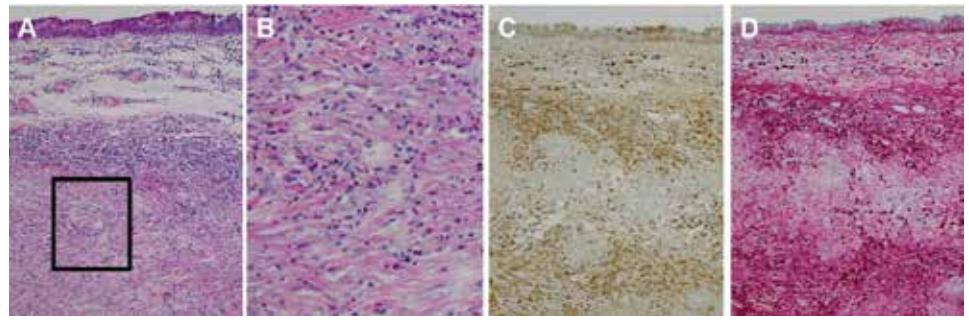

Figure 2. **A** Histopathology showing a lymphoplasmacellular infiltration with occasional eosinophilic granulocytes, atrophy of the tarsal glands and fibrosis visualized with haematoxylin and eosin stain. **B** Magnified view of (A), showing storiform fibrosis; the pattern is characterized by loosely arranged whorls of elongated spindled fibroblast-like cells, representing spokes of the wheel. **C** Increased numbers of IgG4-positive plasma cells ([200/HPF) visualized with IgG4 immunohistochemical staining in red. **D** Almost equal numbers of IgG-positive plasma cells (IgG4/IgG ratio 0,8) visualized with IgG immunohistochemical staining in brown. All images 9100 original magnification.

General blood testing showed no irregularities except for elevated serum IgG4 concentration at 439 mg/dL (normal < 135 mg/dL). FDG-PET/CT scans showed increased accumulation in the bilateral upper eyelids without signs of systemic manifestation of the disease.

For the management of the tarsal lesions oral prednisolone was administered at a 60 mg/day initial dose and was slowly tapered down within 6 months. In addition, topical steroids were prescribed. During the first 6 months after initiation therapy, there was marked regression leading to residual tarsal scarring (Figure 1B). No signs of recurrence, systemic involvement, or progression to lymphoma occurred after 10 months.

DISCUSSION

We here demonstrate a case of bilateral biopsy proven periorbital IgG4-RD confined to the tarsal plate successfully treated with local and systemic glucocorticoids.

IgG4-RD may primarily present in the orbit and periorbital tissue. IgG4-RD is increasingly recognized as part of the spectrum of orbital diseases (52). Recognition of IgG4-RD is important because the disease can present as a multisystem inflammatory disorder. Early recognition may avoid permanent organ dysfunction and disability (41) . The present case demonstrates the importance of pathologic confirmation. The differential diagnosis includes other causes of inflammation (infectious and auto-immune) and tarsal gland carcinoma (especially in unilateral disease).

To our knowledge, two cases of tarsal involvement have been reported previously (53, 54). The clinical presentation in these patients differed in that these lesions were more nodular, and in one case mimicking a chalazion. In these cases treatment was locally and included either excision or local injection of triamcinole acetonide. The ulceration in our case necessitated a combination of topical and systemic steroids yielding a complete regression.

We monitor the patient for signs of recurrence, systemic involvement, and potential progression to lymphoma.

In conclusion, IgG4-RD may manifest in the tarsal plate and may require systemic immunosuppressive treatment.

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Chapter 3

Novel biomarkers in diagnosis and disease monitoring of IgG4-related disease

Chapter 3.1 Expansion of blood IgG4+ B-cells, Th2 and Tregulatory cells in IgG4-related disease

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ABSTRACT

Background: IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition affecting various organs and has a diverse clinical presentation. Fibrosis and accumulation of IgG4+ plasma cells in tissue are hallmarks of the disease and IgG4-RD is associated with elevated IgG4 serum levels. However, disease pathogenesis is still unclear and these cellular and molecular parameters are neither sensitive nor specific for diagnosis of IgG4-RD.

Objective: We here sought to develop a flowcytometric gating strategy to reliably identify blood IgG4+ B-cells to study their cellular and molecular characteristics and investigate their contribution in disease pathogenesis.

Methods: Sixteen patients with histologically confirmed IgG4-RD, 11 patients with sarcoidosis and 30 healthy individuals were included for 11-color flowcytometric analysis of peripheral blood for IgG4-expressing B cells and T-helper (Th) subsets. In addition, detailed analysis of activation markers and chemokine receptors was performed on IgG4-expressing B cells and IgG4 transcripts were analysed for somatic hypermutation.

Results: Cellular and molecular analyses revealed increased numbers of blood IgG4+ memory B-cells in patients with IgG4-RD. These cells showed reduced expression of CD27 and CXCR5 and increased signs of antibody maturation. Furthermore, IgG4-RD patients, but not patients with sarcoidosis, had increased numbers of circulating plasma blasts and $CD21^{low}$ B-cells, as well as Th2 and regulatory T-cells, indicating of a common disease pathogenesis in IgG4-RD.

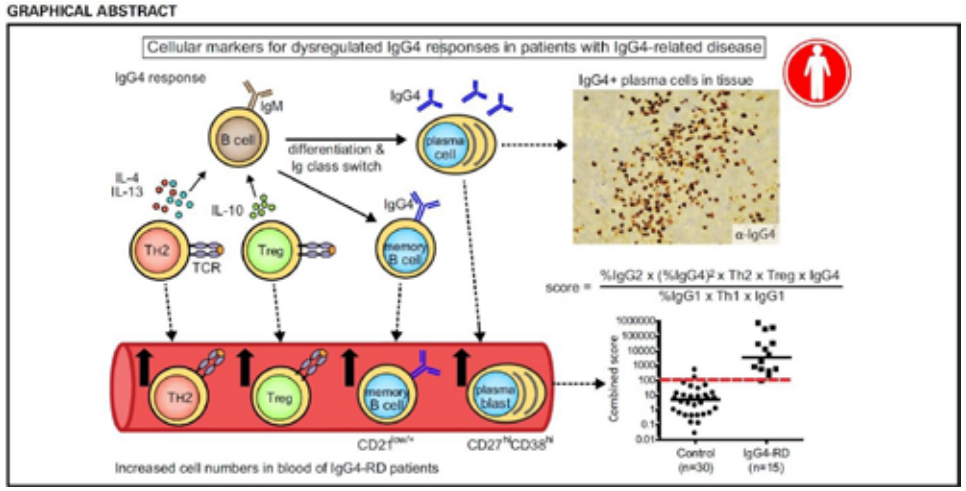
Conclusion: These results provide new insights into the dysregulated IgG4 response in patients with IgG4-RD. A specific “peripheral lymphocyte signature” observed in patients with IgG4-RD, could support diagnosis and treatment monitoring.

Key messages:

- IgG4+ memory B-cells can be reliably detected in human peripheral blood
- Patients with IgG4-RD have increased numbers of circulating IgG4+ memory B-cells. Molecular characteristics reveal that this is a result of an ongoing immune response in affected tissue

- The specific "peripheral lymphocyte signature" representing the combined changes in B- and T-cell subset numbers in patients with IgG4-RD might be exploited as a non-invasive marker for diagnosis and treatment monitoring.

GRAPHICAL ABSTRACT



INTRODUCTION

IgG4 related disease (IgG4-RD) is defined as a systemic fibro-inflammatory condition that can affect potentially any organ, but predominating the retroperitoneal space, thyroid, pancreas, salivary glands and orbital tissue (1, 2). The pathology is characterized by fibrosis and infiltration of IgG4-producing plasma cells of the affected organs (3, 4). As a result, a tumor-like swelling of the involved organ can occur, which causes organ dysfunction and, if left untreated, can lead to organ failure. Furthermore, AA amyloidosis can develop in IgG4-RD, emphasizing the need for prompt diagnosis and treatment of the disease (5). Previously, diseases with IgG4+ plasma cell infiltrates were defined predominantly by organ involvement, and only since 2012 these diverse manifestations have been recognized as one disease entity (3, 4).

The pathogenesis of IgG4-RD remains poorly understood. It is thought to be triggered by organ damage, resulting from e.g. a bacterial infection with molecular mimicry or from an underlying autoimmune process (1). In patients with pancreatic involvement, auto-antibodies directed against self-antigens have been observed (6, 7). Importantly, IgG4+ plasma cell infiltrates are not monoclonal (8), and serum IgG4 in patients is polyclonal with

reactivity to multiple antigens (9). The function of IgG4 antibodies remains elusive, because these display weak or negligible binding to both C1q and Fcγ receptors (10, 11). Furthermore, IgG4 molecules have the exclusive ability to exchange Fab-arms, thus creating monovalent bispecific antibodies that can prevent the formation of immune complexes (12). Therefore, IgG4 has a presumed immune dampening effect, however its role in the pathogenesis of IgG4-RD is controversial (13).

Immunoglobulin class switching of B-cells to IgG4 is regulated by T helper 2 (Th2) cytokines and by IL-10, produced by regulatory T-cells (14, 15). Substantial evidence indicates that indeed Th2 cells and regulatory T-cells (Tregs) are involved in the pathophysiology of IgG4-RD (16-18). Affected tissues express higher messenger RNA (mRNA) levels of the Th2 cytokines IL-4, IL-5 and IL-13 (16, 17). Furthermore, isolated circulating CD4+ T-cells from patients predominantly produce Th2 cytokines (18, 19). Similarly, IL-10 and tissue growth factor β (TGF-β) transcripts are increased in affected tissues (16), and patients with IgG4-related autoimmune pancreatitis have increased frequencies of circulating Tregs (20, 21). More recently, in affected tissue and blood of IgG4-RD patients clonal expansions of CD4 effector memory T(EM) cells have been identified, which potentially drive fibrosis and IgG4 production (22, 23).

Clinically, IgG4-RD manifestations can mimic those of many infectious, inflammatory and malignant disorders (24-30). Therefore, diagnosis of IgG4-RD can be challenging, often leading to a delay in start of proper treatment. The gold standard for diagnosis is histology (28, 31). Characteristic lymphoplasmacytic infiltrates, rich in IgG4 positive plasma cells, storiform fibrosis (cartwheel arrangement of fibroblasts) and obliterative phlebitis are seen in histological samples (28). An increase in serum IgG4 supports the diagnosis, but is found in only 50-70% of patients with histological proven IgG4-RD (32, 33). Recently, circulating plasma blasts have been postulated as a more reliable marker for the disease, irrelevant of IgG4 serum levels (34). However, the specificity of this marker is limited, because circulating plasma blasts are also increased in active infection, following vaccination and in other chronic diseases, such as systemic lupus erythematosus (SLE) (35-38).

Still the importance of B-cells in the pathophysiology of the disease is illustrated by recent observations demonstrating that B-cell depletion with rituximab is a promising therapy in IgG4-RD (39-42). As rituximab specifically binds CD20, it does not directly target

IgG4+ plasma cells, which do not express CD20. Thus, the therapeutic effect could lie in depletion of memory B cells that are chronically stimulated to generate IgG4+ plasma cells. Furthermore, other chronically activated B cells might be involved in the disease through antigen presentation and cytokine production, such as $CD21^{low}$ B cells that are found to be expanded in diverse chronic inflammatory diseases (43).

Despite previously reported expansions of IgG4+ plasma cells, CD4 Tem, Th2 cells and Treg cells in affected tissue, little is known about the pathogenesis of IgG4-RD. Therefore, we developed a new flow cytometric approach, which enabled us to study IgG4-expressing B cells and their pathogenic contribution to the disease. Our insights into IgG4-expressing B cells in combination with abnormalities in B and T-cell subsets reveal abnormal systemic immune regulation, which are valuable for the improvement of diagnosis and treatment of IgG4-RD.

METHODS

Patients.

Patients with IgG4-RD and patients with sarcoidosis were recruited following signed informed consent from the Immunology outpatient clinic at the Erasmus Medical Center Rotterdam and from the Rotterdam Eye Hospital, the Netherlands. All patients were >18 years and were diagnosed based on clinical, serological and histopathological findings. All IgG4-RD patients met the IgG4-RD diagnostic guidelines, including histological confirmation (28), and did not have a known history of an immunodeficiency or any auto-inflammatory disease other than IgG4-RD. All but two patients with sarcoidosis had tissue biopsy confirmed disease with typical presence of non-caseating granulomatous inflammation. In two patients the diagnosis was based on clinical presentation and supportive serological parameters (angiotensin converting enzyme and soluble interleukin-2 receptors) in combination with radiological imaging. Healthy controls were recruited from healthy individuals selected from department staff, and the control group was age- and gender-matched to the patient cohort. None of the healthy individuals showed signs of active inflammatory disease. This study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Erasmus MC (ethics approval numbers MEC-2014-476, MEC-2015-200 and MEC-2017-084).

Histopathology.

All patients with IgG4-RD were histologically diagnosed. The haematoxylin and eosin stainings were analyzed at the Department of Pathology of the Erasmus Medical Center Rotterdam by a trained pathologist with experience in diagnosing IgG4-RD. The deparaffinized formalin-fixed paraffin embedded sections of the tissue (4 mm thick) were stained using a BenchMark automated immunostainer (Ventana, Tucson, AZ, USA) with the Ultraview Universal diaminobenzidine detection kit (Ventana). Mouse anti-human IgG (clone A57H, 1:200, Dako, Carpinteria, CA, USA) and mouse anti-human IgG4 (clone HP6025, 1:600, Invitrogen Zymed, Camarillo, CA, USA) were used for immunohistochemical staining and were applied to the sections for 32 min. The amount of IgG4+ plasma cells per high-power field (0.28 mm^2) and the IgG4/IgG ratio were measured.

IgG serology.

IgG subclass serum levels were measured by immunonephelometry using a Siemens BN II nepholometer according to manufacturer guidelines. A possible prozone effect for IgG4 levels was excluded through dilution of serum samples until reliable values were obtained (44).

Flow cytometry of blood samples.

Patients and controls were included over a time period of three years. To ensure consistency in flowcytometry, standardized sample preparation, antibody staining and flowcytometer instrument settings were used (45). In short, absolute counts of CD3+ T-cells, CD19+ B-cells and CD16+/CD56+ NK-cells were obtained with a diagnostic lyse-no-wash protocol using commercial Trucount tubes (BD Biosciences, San Jose, Calif). For detailed 11-color flow cytometry, red blood cells were lysed with NH_4Cl prior to incubation of 1 million nucleated cells for 15 minutes at room temperature in a total volume of 100µL (antibodies listed in Supplementary Table 1). After preparation, cells were measured on a 4-laser LSRFortessa flow cytometer (BD Biosciences) using standardized settings. Data were analyzed with FACSDiva software V8.0 (BD Biosciences). Immunophenotypic definitions of lymphocyte and leukocyte subsets are listed in Supplementary Table 2.

Table 1. Characteristics of patients with IgG4-RD

patient	gender	age (yr)	organs affected	medication at inclusion	time until diagnosis	treatment started after inclusion	CRP (<10mg/L)	ESR (20mm/h)
1	F	60	orbita	n.i.m.	14 years	prednisone, methotrexate, cyclosporine, infliximab	**14**	**40**
2	F	67	orbita	n.i.m.	8 years	dexamethasone	1.1	1
3	M	63	orbita, lymph node, lung, prostate	prednisone	3 years	prednisone, rituximab, methotrexate	0.9	5
4	M	54	orbita	prednisone	10 years	prednisone	1.9	9
5	F	53	orbita	n.i.m.	3 years	unknown	1.7	n.d.
6	M	60	orbita, lymph node	t.n.	5 years	prednisone, methotrexate	0.7	16
7	M	44	orbita	t.n.	12 years	prednisone	0.4	1
8	F	59	skin	t.n.	5 months	hydroxochloroquine	6.0	11
9	M	18	lymph node, lung, cerebra	t.n.	1 month	dexamethasone, azathioprine	**50**	**107**
10	F	62	pancreas, lymph node, lung	t.n.	3 months	prednisone	6	2.4
11	M	74	lymph node, salivary gland, lung	t.n.	5 months	prednisone, azathioprine	0.8	**37**
12	M	53	mesenterium	t.n.	20 years	prednisone, azathioprine, rituximab	**84**	**119**
13	M	62	thyroid gland	t.n.	1 year	prednisone	1.9	6
14	M	32	serous membrane	t.n.	3 months	prednisone	**18.7**	8
15	M	79	lymph node, pancreas	t.n.	1 year	-	6.4	**90**
16	M	60	bile duct, lymph node	t.n.	2 weeks	prednisone, methotrexate	4	0.7

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; n.i.m., no immunomodulatory medication for at least 6 months prior to inclusion; t.n., treatment naïve; n.d., not determined

Molecular analysis of immunoglobulin heavy chain (IGH) gene rearrangements.

RNA was isolated from post-Ficoll mononuclear cells with a GenElute mammalian RNA kit (Sigma-Aldrich, St Louis, Mo) and reverse transcribed to cDNA with random primers (Invitrogen Life technologies). *IGHV* gene rearrangements were amplified in a semi-nested multiplex PCR approach using 4 different L-VH-family forward primers (46) in combination with *IGHG4*-specific (5'GGGCATGATGGGCAYGGGGGACCATA; first round) and *IGHG*-consensus (5'CACGCTGCTGAGGGAGTAG; second round) reverse primers. PCR products were cloned into a pGEMT easy vector (Promega, Madison WI), amplified by colony PCR, and sequenced on an ABI Prism 3130XL (Applied Biosystems, Foster city, CA). The sequences were analyzed with the IMGT database (www.imgt.org) and BASELINe program (selection.med.yale.edu/baseline) (47, 48).

Statistical analysis

Frequencies and absolute cell numbers were assumed a non-Gaussian distribution. All results are expressed as median values with interquartile range if applicable. Results were analyzed using the non-parametric Mann-Whitney *U* test. Linear regression was used to study the strength of association between cell-subsets with Spearman r to measure significance. All *P*-values are two-tailed and were considered statistically significant if values were lower than 0.05. Statistical analysis was performed using GraphPad Prism software, version 6 (GraphPad Software, La Jolla, CA). Principal component analysis (PCA) was performed with Infinicyt software (Cytognos, Salamanca, Spain). Specificity and sensitivity were calculated with SPPS software (IBM SPSS statistics 21.0, Armonk, NY)

RESULTS

Patient characteristics.

A total of 16 patients with IgG4-RD were included with a mean age of 56 years (range 18-79 yrs) and a male:female ratio of 2:1 (Table I). All patients were confirmed to have IgG4-RD based on the Boston consensus, with typical histopathologic characteristics and the presence of IgG4-producing plasma cells in affected tissue (Figure 1). Twelve out of 16 patients had increased serum IgG4 levels (mean 5.06g/L; range 0.27-25.25g/L). Four of these patients had an additional increase in serum IgG1 or IgG2 levels, and one pa-

tient with normal serum IgG4 had increased levels of IgG1 and IgG3 (see Supplementary Table 3). Six patients showed signs of active disease based on clinics and increased CRP (C-reactive protein) and/or ESR (erythrocyte sedimentation rate) (Table I).

The majority of IgG4-RD patients were treatment naive (no prior treatment), three patients had received immunomodulatory medication in the past (medication had been stopped for at least 6 months prior to inclusion) and only two patients received low dose prednisone (Table I). Most patients had normal counts of blood leukocytes and lymphocytes (see Supplementary Table 3). Increased lymphocytes resulted from high T-cell counts, often in combination with high B- and/or NK cell numbers. One of the two patients treated with prednisone had decreased numbers of B- and T-cells. In the analysis of the distribution of IgG subclasses within the total IgG memory B-cell compartment we analyzed material from 15 of the 16 patients.

Of the eleven patients with sarcoidosis, one patient used plaquenil. All other patients were therapy naive (see Supplementary Table 4). All patients had normal leukocyte counts, although some patients had slightly decreased or slightly increased T-cell or B-cell counts. One patient had an increased IgG4 serum level accompanied with an increased IgG1 serum level. One patient had a slightly decreased IgG4 serum level and from one patient IgG serum levels were not determined. Five patients showed signs of active disease based on CRP and/or ESR (see Supplementary Table 4).

Immunophenotypical analysis of IgG4-expressing B-cells.

The hallmark of IgG4-RD is the accumulation of IgG4-producing plasma cells in affected tissue. To study whether patients had systemic abnormalities in IgG4-expressing B cells, we developed a reliable flow cytometric gating strategy to distinguish cells expressing one of the four IgG subclasses (Figure 2A). Within total CD19+ B cells, CD27+CD38^{hi} plasma blasts were electronically gated and studied for expression of IgG1, 2, 3 and 4. However, surface Ig levels were too low to detect. Therefore, we next focused our attention on CD38^{dim}IgM-IgD- memory B cells. These contained presumed memory B cell subsets that expressed either IgG1, IgG2 or IgG3, and within the triple negative fraction, a sizeable IgG4-expressing subset could be identified (Figure 2A).

To confirm the activated nature and memory phenotype of all IgG+ B-cell subsets we studied the expression of B7 family members CD80 and CD86 and the TNF receptor superfamily member transmembrane activator and CAML interactor (TACI). All four IgG-subclass expressing B-cell subsets showed higher expression of the activation markers than naive B-cells (Figure 2B), fitting with an activated memory B-cell phenotype.

Figure 1. Histology images of patients with IgG4-RD

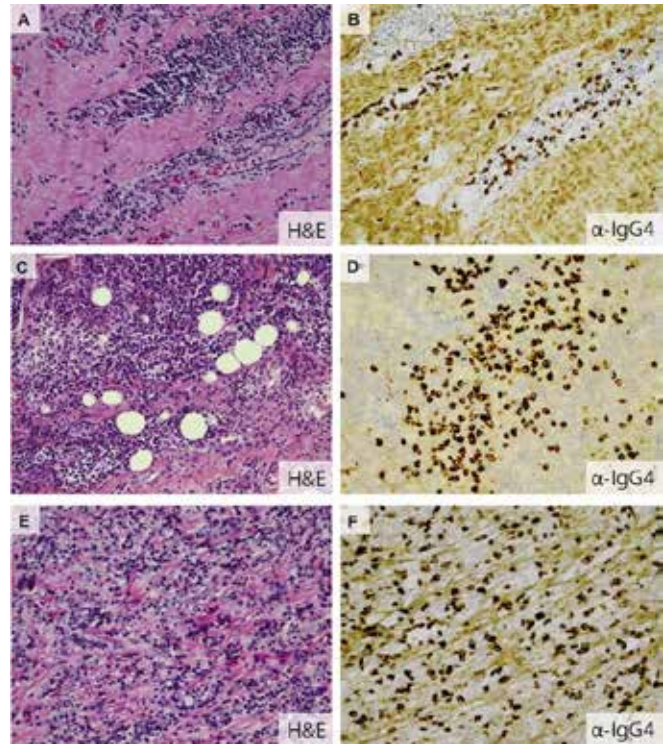


Figure 1. Histology images of patients with IgG4-RD. Representative hematoxylin and eosin (H&E) and IgG4 staining of tissue sections of affected organs in patients with IgG4-RD. **A:** Pericardial tissue of patient 15 demonstrating lymphoplasmacytoid fibrosis and obliterative phlebitis. **B:** IgG4 staining showing IgG4 positive plasma cells with a ratio of IgG4 positive/IgG positive cells > 0.4. **C:** Orbital tissue of patient 6 with lymphoid infiltration**. D:** IgG4 staining demonstrating IgG4 positive plasma cells (ratio IgG4/IgG positive cells > 0.4). **E:** Lung tissue of patient 10 with lymphoplasmacellular infiltrates and fibrosis. **F:** Staining with IgG4 confirming the diagnosis (IgG4/IgG positive plasma cells ratio > 0.4). All images are at 3200 magnification.

Figure 2. Identification and immunophenotyping of IgG subclass–expressing memory B cells

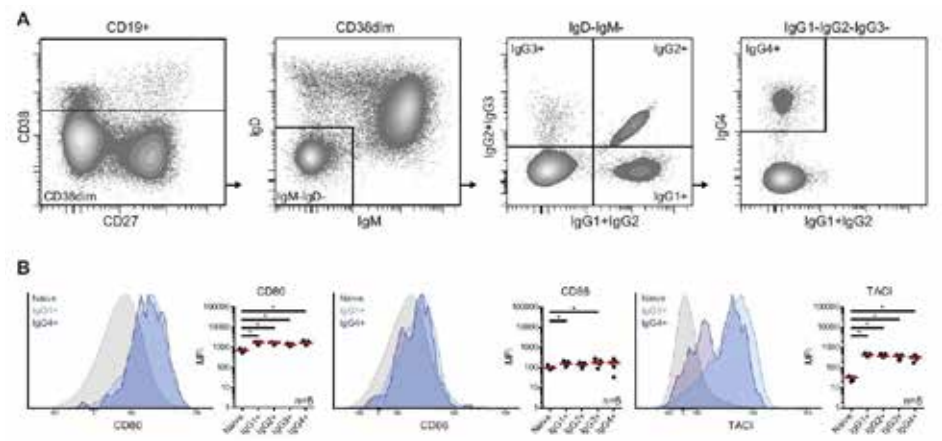


Figure 2. Identification and immunophenotyping of IgG subclass–expressing memory B cells.

A: Stepwise flow cytometric gating strategy to identify IgG1+, IgG2+, IgG3+ and IgG4+ memory B cells. **B**: Expression of activation and memory markers on B-cell subsets. Shaded gray histograms represent naive B cells, light blue histograms represent IgG+ memory B cells, and dark blue histograms represent IgG4+ memory B cells. Each dot represents 1 subject, and red lines indicate medians. Statistical analysis between the groups was performed with the Mann-Whitney U test. *P < .05.

Increased CD21^{low} B-cells and plasma blasts, but reduced IgM+IgD+ memory B cells in patients with IgG4-RD.

To study systemic abnormalities in IgG4-expressing B cells, we performed extensive immunophenotyping of B-cells in 16 patients with IgG4-RD and compared these with 30 age-matched healthy controls (Figure 3A). The median numbers of transitional and naive mature B cells were not different between patients and controls. Of the six major memory B cell subsets, only the IgM+IgD+ memory B cells were significantly affected in patients (reduced; *P*<0.05) (Fig 3B). In contrast, the numbers of CD21^{low} B-cells (CD19+CD38^{dim}CD21^{low}), and plasma blasts (CD19+CD38+CD27+) were significantly higher in patients with IgG4-RD (both *P*<0.05).

Patients with IgG4-RD have increased numbers of IgG4+ memory B-cells.

Despite the normal total numbers of IgG+ memory B-cells, patients with IgG4-RD had significantly higher numbers of IgG4+ memory B-cells than controls (*P*<0.01) (Figure 3C).

In addition, patients had reduced numbers of memory B cells expressing IgG1 (*P*<0.05), whereas IgG2 and IgG3 expressing B-cell numbers were similar to controls (see Supplementary Figure 1A). When analyzed as fraction of total IgG-expressing memory B cells, the decrease in IgG1 and increase in IgG4 in patients with IgG4-RD became more apparent (both *P*<0.0001). Furthermore, IgG4-RD patients showed a significant increase in the fraction of IgG memory B cells expressing IgG2 (*P*<0.01). To study if there was a direct relation with serum IgG4 levels, we performed linear regression analyses with the percentages and the absolute numbers of IgG4+ memory B-cells (see Supplementary Figure 1B). These did not reveal significant correlations, indicating that the memory B cells and serum IgG4 levels were not directly related.

Since both IgG4-expressing B cells and CD21^{low} B cells were significantly increased in IgG4-RD patients, we studied if these were related and investigated CD21^{low} B cells expressing IgG4 (see Supplementary Figure 1C). Indeed, patients carried significantly more CD21^{low}IgG4+ B cells (see Supplementary Figure 1D) and these numbers were directly correlated to the total number of CD21^{low} B cells (*P*<0.01) (see Supplementary Figure 1E).

Cellular and molecular analysis of IgG4-expressing B-cells in patients with IgG4-RD.

To analyze the nature of the IgG4-expressing B-cell expansion in patients with IgG4-RD, we first studied the expression of CD27 (Figure 4A). In healthy controls, the frequencies of cells that expressed CD27 were highest within the IgG2 and IgG4 subsets, followed by IgG1 and IgG3 (Figure 4B). Thus, CD27 positivity was higher in cells utilizing IgG subclasses encoded by the downstream genes within the *IGH* locus (Cγ2 and Cγ4) (Figure 4C). In patients with IgG4-RD, significantly fewer IgG4+ B-cells expressed CD27 than IgG4+ B-cells from healthy individuals.

To study whether the expanded IgG4-expressing B cells could be normally involved in immune responses, we analyzed the expression levels of cytokine receptors and chemokine receptors involved in germinal center responses. IL21 receptor (IL21R) and IL4R as well as C-C chemokine receptor type 7 (CCR7) expression levels were similar between IgG4+ memory B-cells from healthy individuals and from

Figure 3. Composition of the blood B-cell compartment in healthy subjects and patients with IgG4-RD

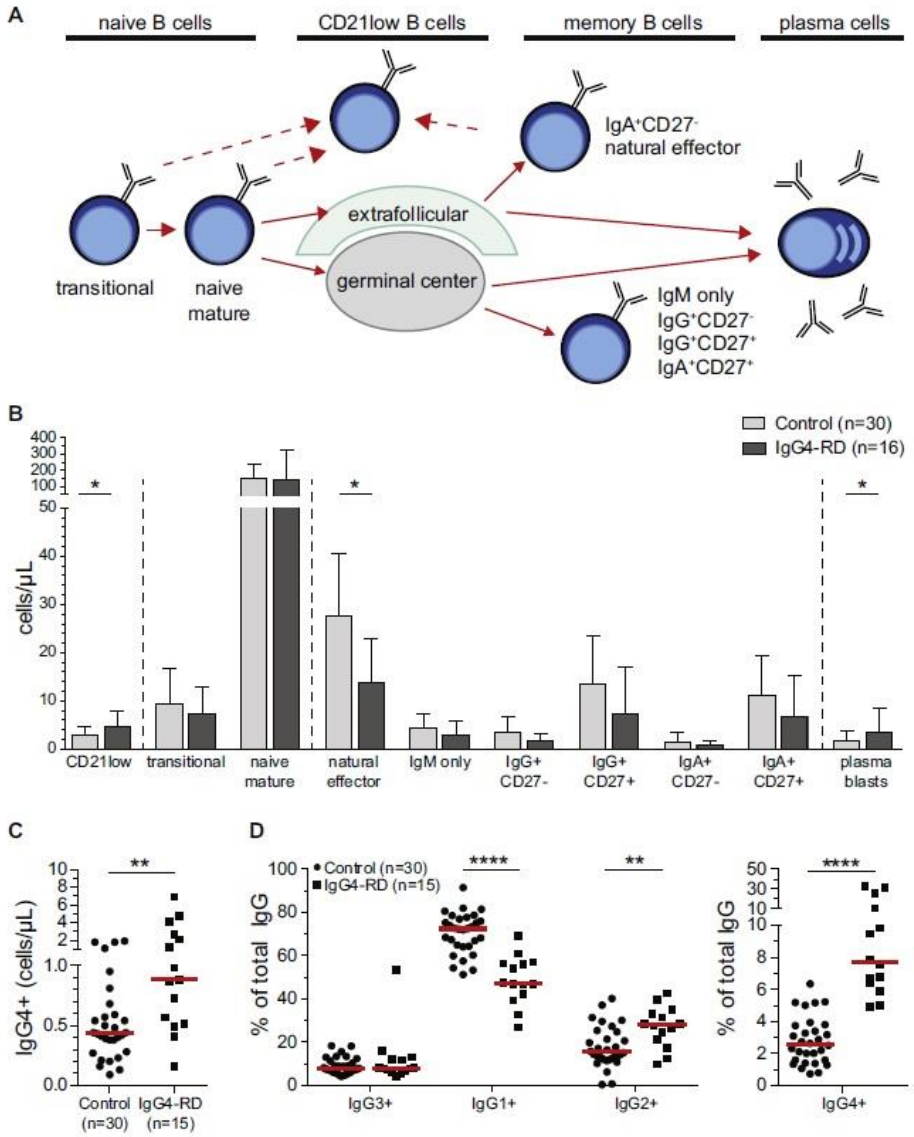


Figure 3. Composition of the blood B-cell compartment in healthy subjects and patients with IgG4-RD.
A: Model of peripheral B-cell maturation. **B**: Absolute numbers of naive and memory B-cell subsets and plasma cells. Columns indicate median values with interquartile ranges. **C**: Absolute numbers of IgG4+ memory B cells. **D**: Distribution of IgG subclass memory B cells within the total IgG memory B-cell compartment. Each dot represents 1 subject, and red lines indicate medians. Statistical analysis between groups was performed with the Mann-Whitney U test. *P < .05, **P < .01, and ****P < .0001.

Figure 4. Molecular characteristics of IgG4+ B cells from healthy subjects and patients with IgG4-RD

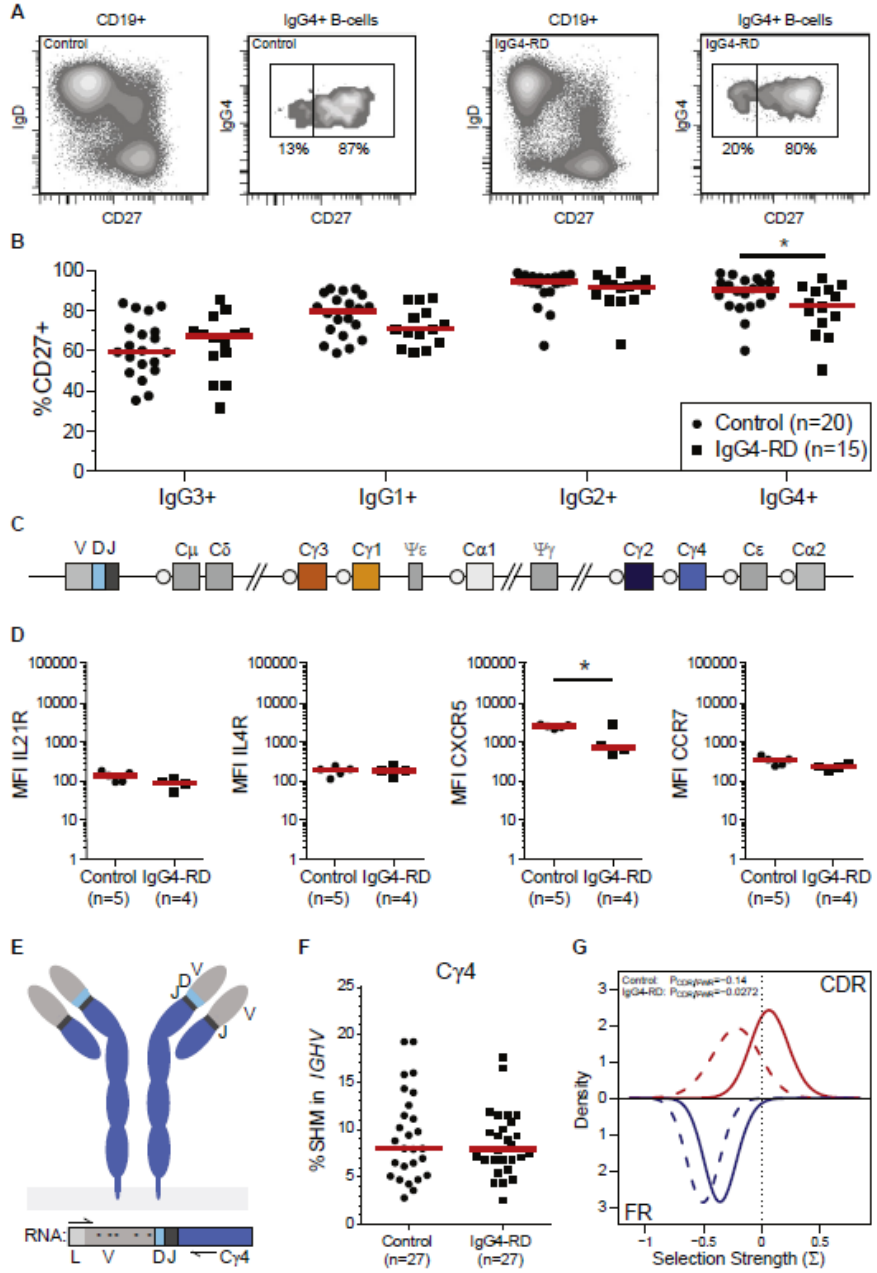


Figure 4. Molecular characteristics of IgG4+ B cells from healthy subjects and patients with IgG4-RD.
A: Representative flow cytometric plots of CD27 expression on total B cells and IgG4+ B cells from a healthy subject and a patient with IgG4-RD. **B**: Frequencies of IgG subclass memory B cells expressing CD27. **C**: Expression of cytokine and chemokine receptors on IgG4+ B cells. **D**: Schematic representation of the human IGH locus. MFI, Median fluorescence intensity. **E**: Representation of the membrane B-cell receptor with the variable domain of the immunoglobulin heavy chain (V, D, and J) and immunoglobulin light chain (V and J). Asterisks in the RNA represent mutations in the genes encoding the proteins of the B-cell receptor. L, Leader. **F**: Frequency of SHM in rearranged IGHV gene. **G**: Selection for replacement mutations in the CDR (red) and framework region (FR; blue) regions. Solid lines represent patients, and dotted lines represent healthy control subjects. A selection strength of more than 0 is indicative of positive selection. *P < .05.

patients with IgG4-RD (Figure 4D). In contrast, C-X-C receptor type 5 (CXCR5) expression was significantly lower on IgG4+ memory B-cells from patients.

To investigate the nature of the expanded IgG4+ memory B-cells in the pathogenesis of IgG4-RD, we studied somatic hypermutations (SHM) in the *IGHV* regions (Fig 4, *E*). Such mutations are molecular signs of B-cell responses and affinity maturation (49). In agreement with previous studies, SHM frequencies in IgG4 transcripts of healthy adults were higher than in the more proximal-encoded IgG1 and IgG2 (see Supplementary Figure 2A) (50, 51). The SHM levels in IgG4 transcripts of patients with IgG4-RD (7.89%) were similarly high as in healthy adults (7.98%; *P*=0.75) (Figure 4F). To study if the mutations were driven by selection for antigen binding, we analyzed the selection for replacement mutations in the complementarity determining regions (CDR) with the Bayesian estimation of Antigen-driven SELectIoN program BASELINe (48). IgG4 transcripts from healthy controls did not show more replacement mutations than expected by random chance in the CDR regions, i.e. absence of positive selection (Figure 4G). In contrast, CDR regions of IgG4-RD patients did show positive selection for replacement mutations. Both groups showed normal negative selection for replacement mutations in framework (FR) regions that compose the structure of the variable domain. Thus, IgG4+ B-cells in patients with IgG4-RD show increased selection for replacement mutations in CDR regions. Patients and controls displayed high diversity in sequence and length of the CDR3 region, which is encode by the junction of the V, D and J genes. Still, patients showed significantly shorter IGH-CDR3 regions in IgG4 transcripts than healthy individuals (see Supplementary Figure 2B). Furthermore, *IGHV* usage seemed to differ between groups with increased usage of IGHV5-51 in patients with IgG4-RD and reduced IGHV3-39 usage (see Supplementary Figure 2C), although this did not reach statistical significance (*P*=0.08).

Increased numbers of Th2 cells and Tregs in IgG4-RD.

The impaired expression levels of CXCR5 and absence of positive selection of SHM in IgG4+ B cells are suggestive of abnormal immune responses in patients with IgG4-RD. IgG4 responses are mediated by IL-4 and IL-13, produced by Th2 cells, and IL-10, produced by Tregs. Therefore, we performed additional studies into Th subsets in our patients (Figure 5A). Flowcytometrc immunophenotyping of their blood T cells demonstrated normal overall numbers of naive, central memory and effector memory CD4 T cells (see Supplementary Figure 3A). Still, patients with IgG4-RD had significantly more circulating Th2 cells (CD45RA-CCR6-CXCR3-CCR4+; *P*<0.0001) and Tregs (CD45RA-CD127-CD25+; *P*<0.001) than healthy individuals (Figure 5B). Th1 cells seemed slightly lower, but this was not significant and numbers of follicular helper T cells (Tfh) and Th17 were not different. Linear regression showed a direct correlation between the numbers of IgG4+ memory B-cells and Th2 cells (*P*<0.01), but not for Tregs (*P*=0.24; Fig 5, *C*). $CD21^{low}$IgG4+ B cell numbers were similarly correlated with Th2 cells (*P*<0.05) and not with Treg numbers (*P*=0.49; see Supplementary Figure 3A). IgG4 serum levels in patients did not correlate with Th2 nor Treg cell numbers (see Supplementary Figure 3B). In contrast to the changes in Th subsets, patients with IgG4-RD had no differences in numbers of CD4+CD45RA+CCR7- TemRA cells, CD4+CD45RA-CCR7- TemRO cells, nor CD27- TemRO cells (CD4 CTL) (see Supplementary Figure 4A and B). Finally, naive CD8 T-cell numbers were decreased in IgG4-RD patients, in absence of effector memory expansions (see Supplementary Figure 4C).

Skewing of Th subsets towards Th2 is also observed in allergies and frequently accompanied by eosinophilia (52). However, we did not find increased numbers of eosinophils ($SSC^{high}CD45+CD16^{dim}CD81+$) in patients with IgG4-RD (see Supplementary Figure 5), nor any differences in the other granulocyte subsets (neutrophils and basophils). In contrast, the numbers of plasmacytoid dendritic cells ($SSC^{low}CD45^{dim}HLADR+CD123+$), which are potent drivers of Th1 responses (53), were significantly lower in IgG4-RD patients. Thus, the increased numbers of IgG4-expressing B cells in IgG4-RD patients are accompanied by systemic reduction in Th1 immunity and increased Th2 and Treg cells.

IgG4+ memory B-cells and other lymphocyte subsets as a diagnostic tool for IgG4-RD.

Despite clinical indications and high serum IgG4 levels, diagnosis of IgG4-RD requires analysis of a tissue biopsy. Obtaining a biopsy can be difficult, is invasive, sample error sensitive and might not always be sufficiently discriminative from other immune-proliferative and auto-inflammatory diseases. A specific marker in blood could be a valuable means to overcome these diagnostic challenges. However, the differences in B-cell and T-cell subsets we identified do not provide a clear discrimination between patients and controls. Therefore, we explored if a combination of measurements could be used as a disease marker. First, we performed a principal component analysis (PCA) on the absolute cell numbers of all B and T-cell subsets from healthy individuals and patients with IgG4-RD and visualized this with the automated population separation (APS) tool of the Infinicyt program (54). In this analysis we also included a group of patients with treatment naive sarcoidosis, another fibro-inflammatory disease. IgG4-RD resembles many immune proliferative diseases, yet patients with sarcoidosis did not show typical expansions of IgG4+ memory B cells, Tregs and Th2 cells (see Supplementary Figure 6). Of the first 5 principle components, component 1 (PC1) separated patients with IgG4-RD from patients with sarcoidosis and from healthy controls. (Figure 6A). This analysis indicates the usefulness to combine multiple parameters for optimal discrimination between IgG4-RD patients and controls and potentially from other auto-inflammatory conditions with tissue fibrosis. Therefore, we continued to develop a score based on the B- and T-cell subsets that were most significantly different between IgG4-RD patients and both controls and patients with sarcoidosis (Figure 6B). Application of this scoring method to the 56 samples in our study resulted in 50 samples being correctly assigned as either non-IgG4-RD or IgG4-RD (Figure 6B). The calculated sensitivity for this score was 93.3% (95% confidence interval, 68.05% - 99.83%) and the calculated specificity was 87.8% (95% CI, 73.80% - 95.92%). Thus, IgG4-RD patients carry changes in their blood B- and T-cell subsets that together reflect a unique “lymphocyte signature”.

Figure 5. Blood TH subsets

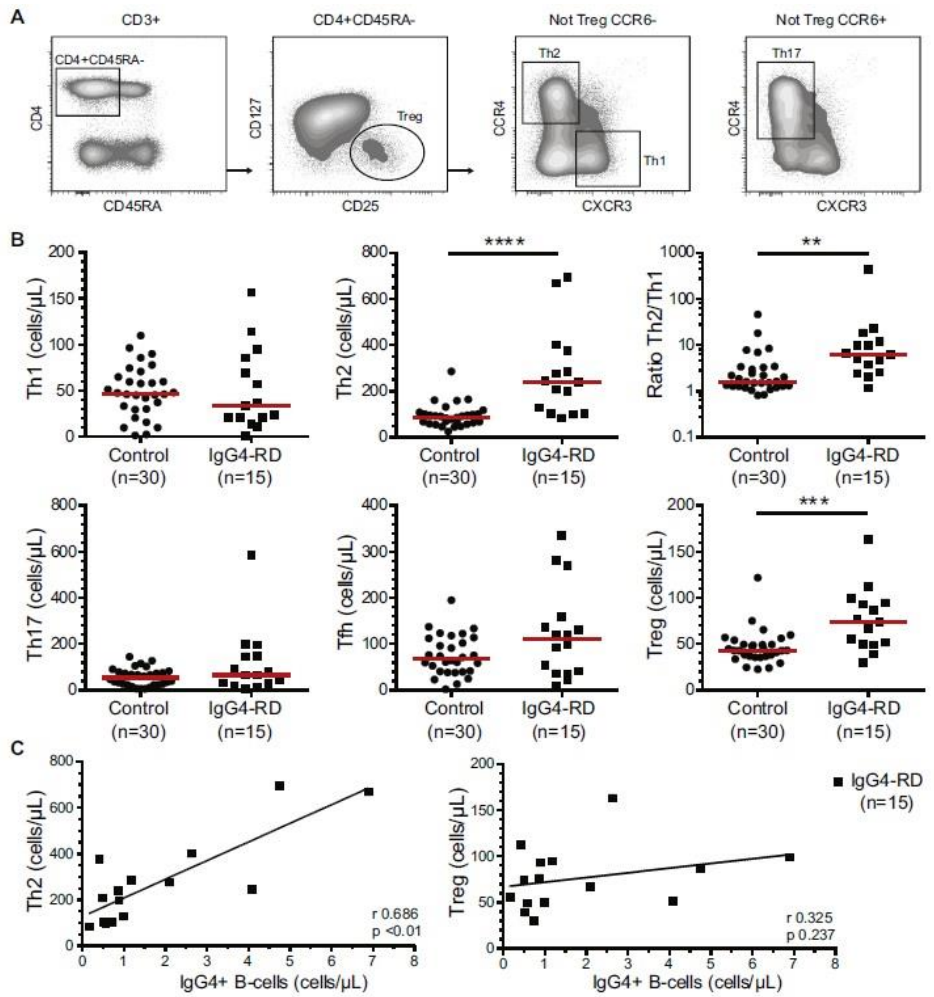


Figure 5. Blood TH subsets. A: Representative plots from flow cytometric analysis. **B**: Absolute numbers of TH1, TH2, follicular helper T (TFH), and Treg cells. **C**: Regression analysis of TH2 cells and IgG4+ B cells and of Treg cells and IgG4+ B cells. Each dot represents 1 subject, and red lines indicate medians. Statistical analysis between the groups was performed with the Mann-Whitney U test. **P < .01, ***P < .001, and ****P < .0001. Correlation was calculated with Spearman.

Figure 6. Multiparameter analysis and clustering of subjects based on blood lymphocyte subsets

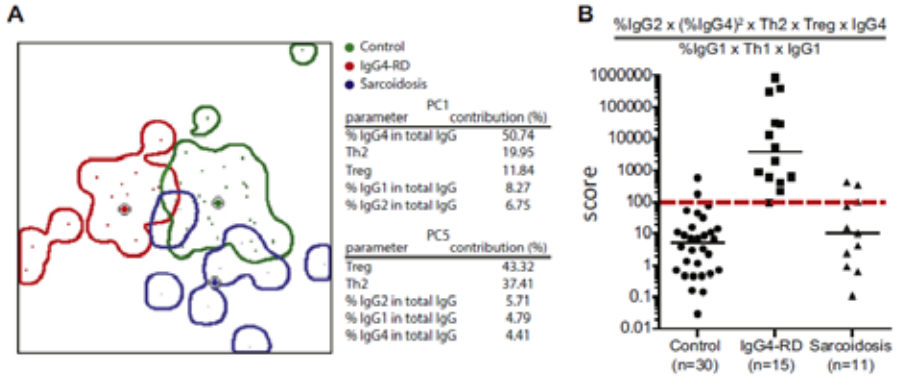


Figure 6. Multiparameter analysis and clustering of subjects based on blood lymphocyte subsets. A: PCA of peripheral blood lymphocyte subsets from healthy subjects (green), patients with sarcoidosis (blue), and patients with IgG4-RD (red). Each subject is represented by 1 dot, with indication of group median (large dot) together with 1 SD and 2 SD. After use of an automated population separator based on the absolute counts of various subsets, principal component (PC) 1 and PC5 were selected to visualize separation between healthy subjects, patients with sarcoidosis, and patients with IgG4-RD. The contribution of the top 5 components of each PC are listed. **B**: Scoring of blood samples based on a formula composed of the subsets that were significantly different between healthy subjects and patients with IgG4-RD. Percentages of IgG2+ and IgG4+ B cells within total IgG B cells are multiplied by the absolute numbers of TH2, Treg, and IgG4+ B cells and divided by the percentage of IgG1+ B cells within total IgG1 B cells multiplied by the absolute numbers of TH1 and IgG1+ B cells. Red dotted line indicates a score of 100 considered as a cutoff value.

DISCUSSION

We here demonstrate significant expansions of IgG4-expressing B cells in blood of patients with IgG4-RD. These changes were directly related to the previously described expansion of CD21^{low} B cells and are associated with expansions in plasma blasts, Th2 and Treg cells.

Increased numbers of circulating plasma blasts have been reported before in patients with IgG4-RD (8, 34). This is not a disease-specific effect as similar expansions have been observed in many chronic inflammatory diseases and in individuals with active infection or following vaccination (35-38). Thus, increased plasma blast numbers mostly reflect active humoral immune responses. Active inflammation is further supported by the increased numbers of CD21^{low} B cells in our patients. These cells have been reported to be increased in multiple states of inflammation (43, 55, 56) and are thought to result from

prolonged B-cell activity due to chronic activation by self-antigens (57). CD21 functions as a co-receptor for the BCR, and downregulation of its expression makes the cells anergic for chronic stimuli (58). We now demonstrated that the expansion of CD21^{low} B cells is in part due to the expansion of IgG4+ population, which directly links it to the disease. The nature of chronic B-cell stimulation in IgG4-RD remains unclear. It is likely that auto-antigens are involved, as plasma blasts from patients with IgG4-RD produce immunoglobulins that react against human cell lysates (8).

Insights into IgG4-expressing B cells in patients with IgG4-RD are limited, most likely due to the lack of proper reagents to specifically detect and isolate these cells. To overcome this, we developed a flow cytometric approach to stain for IgG4-expressing B cells using newly available reagents and found that frequencies and absolute numbers of IgG4+ memory B-cells were increased in patients with IgG4-RD. However, in contrast to previous observations, these numbers were not correlated to IgG4 serum levels (59). Absence of correlation is most likely due to the fact that these cells were immunophenotypically memory B-cells (60, 61) rather than IgG4-producing plasma cells. Previously we observed similar absence of correlation for IgE+ memory B-cell numbers and serum IgE levels in patients with atopic dermatitis (62).

On top of cell numbers, patients' IgG4+ memory B cells were phenotypically different from those of healthy controls. A lower frequency of cells expressed CD27, which is the conventional marker for memory B-cells (63, 64). Furthermore, expression levels of CXCR5 were lower in patients. These differences could originate from a different maturation pathway for IgG4+ memory B cells in patients. Generation of IgG+ memory B cells in humans is critically dependent on T-cell help (65). Yet, it is unclear if T-cell help is needed for sequential switching to IgG4 of memory B cells expressing other IgG subclasses. The reduced expression of CXCR5 on IgG4+ B-cells from patients could represent a reduced capacity for homing to B-cell zones in lymph nodes and would suggests that activation and differentiation of IgG4+ B-cells in patients occurs in peripheral tissue, rather than in lymphoid structures (66). Cytokines specifically inducing CSR to IgG4 have been found in IgG4-RD tissue biopsies (16). Moreover, expression of activation-induced cytidine deaminase (AID), the enzyme that triggers SHM and which is needed for CSR, was reported to be increased in tissue biopsies of IgG4-RD patients, conforming the ability of local CSR (15).

In addition to differences in IgG4+ memory B cells, we found systemic expansions of Th2 and Treg cells. Ig class switching to IgG4 is mediated by the cytokines IL4 and IL-10, which are predominantly produced by Th2 and Treg, respectively. This is referred to as "the modified T-helper 2 type response" (67), and has been reported in the context of the beneficial effects of immunotherapy in allergy, in which it favors CSR to IgG4 over IgE (68). Prolonged antigen exposure indeed leads to a serologic shift in the IgG4:IgG1 antibody ratio, as previously illustrated by natural immunization in bee keepers or during subcutaneous immunotherapy with grass pollen (69, 70). Previous observations in tissue and in blood have also linked the modified Th2 type response to IgG4-RD (16-18, 20, 69). Our data confirms such a response, with the observation of increased numbers of Th2 cells and Tregs in IgG4-RD and a positive correlation between Th2 cells and IgG4+ B-cells. In contrast to earlier observations we did not find an increase in Tfh cells (71). Yet, this finding supports the hypothesis that differentiation towards IgG4 in IgG4-RD takes place in peripheral tissue, rather than in follicles in lymphoid structures.

Despite significant differences in numbers of B- and T-cell subsets between patients and controls in the presented study, none of these were sufficiently discriminative to be used as biomarker. This included plasma blasts, which had recently been proposed as a biomarker for IgG4-RD (34). Importantly, the numbers of plasma blasts in our patients (median 3.36 cells/μL) were in the same range as previously reported (median 4.70 cells/μL) (34), whereas plasma blast numbers were extremely low in controls of that study (0.10 cells/μL) (34). As plasma blasts in our study (median 1.70 cells/μL) were in the same range as reported by others, it is questionable if this subset is a reliable biomarker (72). Therefore, we propose to derive a biomarker based on the specific "lymphocyte signature" in peripheral blood of patients with IgG4-RD. Combined analysis of various subsets has an advantage since it does not rely on one biomarker, rather it reflects the pathophysiology of the disease. Since IgG4-RD can affect various organs, the clinical presentation is diverse, yet PCA of the peripheral lymphocyte compartment illustrates that the underlying immune response is clearly distinct. This is confirmed by the fact that combined analysis with PCA not only distinguishes healthy from disease, but can also discriminate between different fibro-inflammatory conditions (i.e. sarcoidosis from IgG4-RD). Examples of scoring systems in medical practice are ample and systems medicine has been proven of value to study complex diseases and generate predictive models, although some caution is warranted (73, 74).

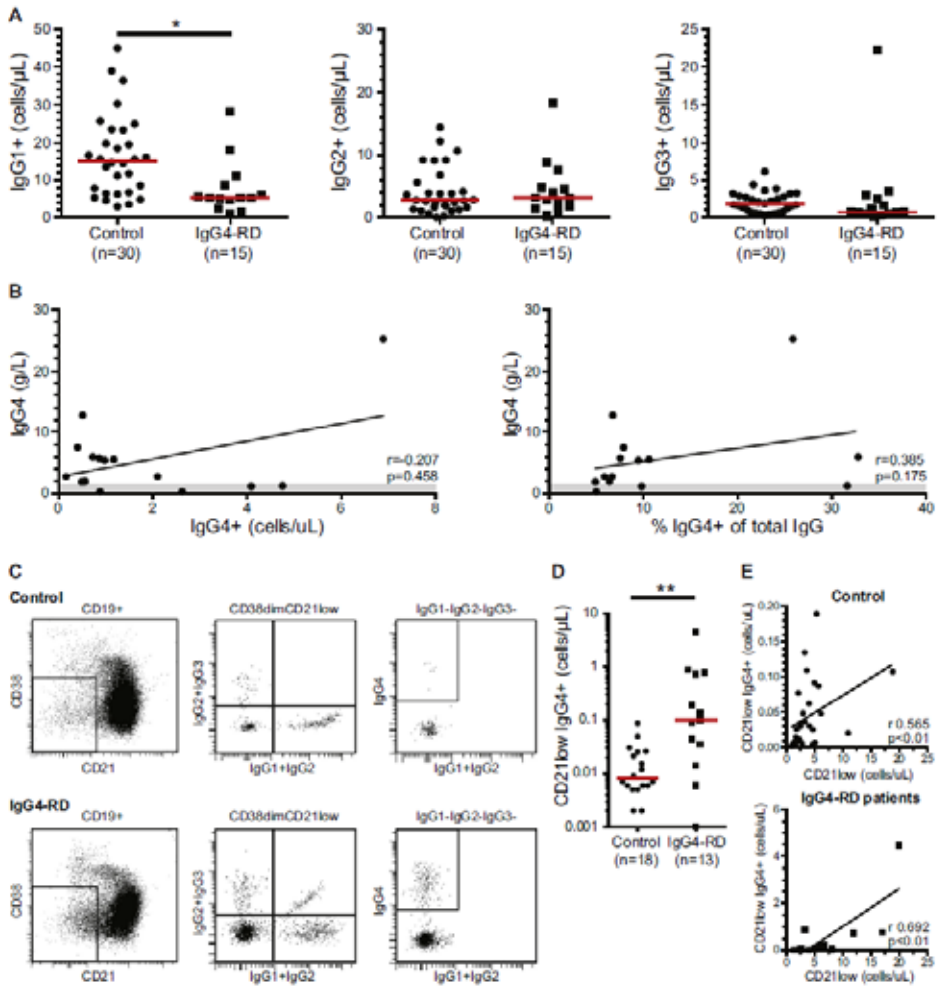
In conclusion, we here demonstrate that patients with IgG4-RD present with increased numbers of IgG4+ B-cells, $CD21^{low}$ B-cells and plasma blasts in blood. Molecular characteristics reveal that the latter expansion is a result of an ongoing immune response in affected tissue. Furthermore, this is characterized by increased numbers of Th2 cells and regulatory T-cells, known as the modified Th2 type response. This specific "peripheral lymphocyte signature" in patients with IgG4-RD indicates that patients suffering from IgG4-RD have a common disease pathogenesis and in the future, peripheral blood might be exploited as a non-invasive tool to employ for diagnosis and treatment monitoring in patients with IgG4-R

KEY MESSAGES

- IgG4 + memory B cells can be reliably detected in human peripheral blood.
- Patients with IgG4-RD have increased numbers of circulating IgG4+ memory B cells. Molecular characteristics reveal that this is a result of an ongoing immune response in affected tissue.
- The specific peripheral lymphocyte signature representing the combined changes in B- and T-cell subset numbers in patients with IgG4-RD can be exploited as a noninvasive marker for diagnosis and treatment monitoring.

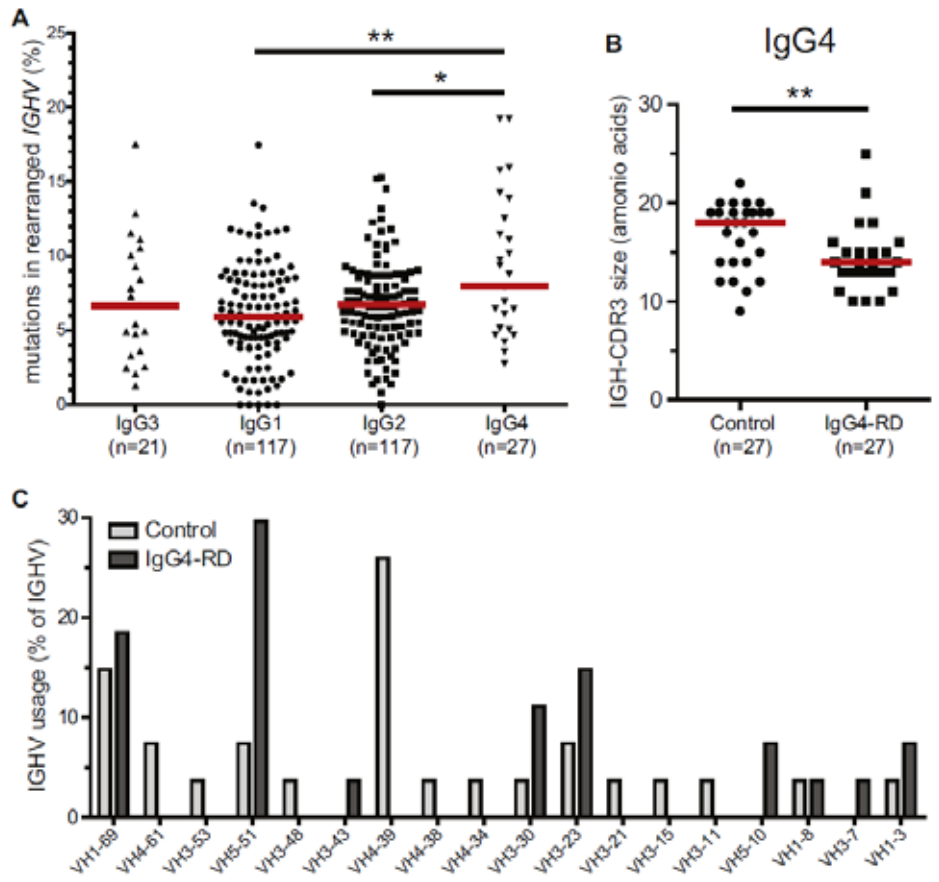
SUPPLEMENTARY FIGURES

Supplementary Figure 1



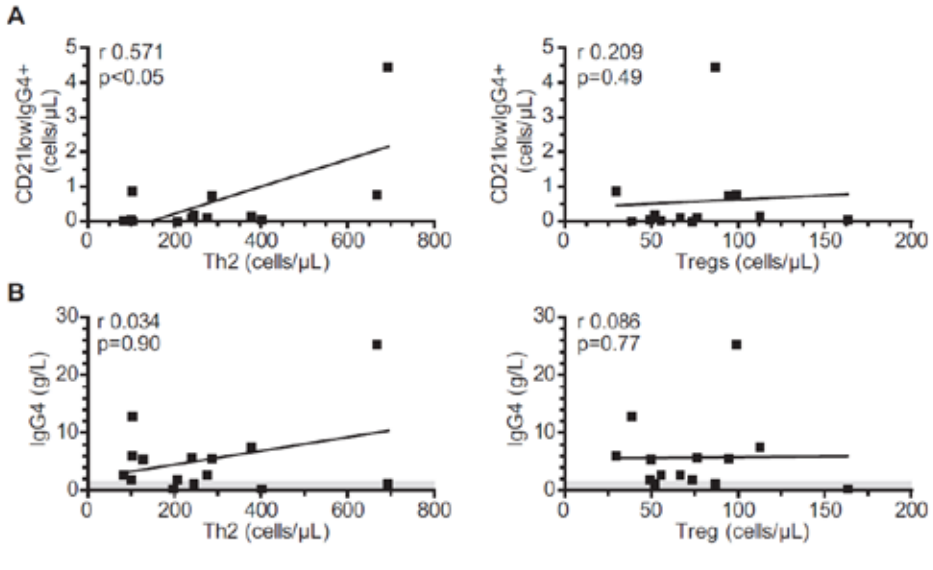
A: Absolute counts of IgG1+, IgG2+ and IgG3+ memory B-cells. Each dot represents one individual, red lines represent median values. Statistical analysis between the groups was performed with the Mann Whitney U test; *P<0.05. **B:** Correlation between IgG4 serum values and absolute numbers of IgG4+ memory B-cells and between percentages of IgG4+ B-cells within total IgG+ B-cells. Grey shaded area indicates the reference value for IgG4 serum values. Correlation was calculated with Spearman R. **C:** Representative plots of the flow cytometric analysis of IgG subclass expressing $CD21^{low}$ B-cells **D:** Absolute counts of $CD21^{low}$ IgG4+ B-cells. Each dot represents one individual, red lines represent median values. Statistical analysis between the groups was performed with the Mann Whitney U test; *P<0.05, **P<0.01. **E:** Correlation between $CD21^{low}$ B-cells and IgG4+ $CD21^{low}$ B-cells in healthy controls and in patients with IgG4-RD.

Supplementary Figure 2



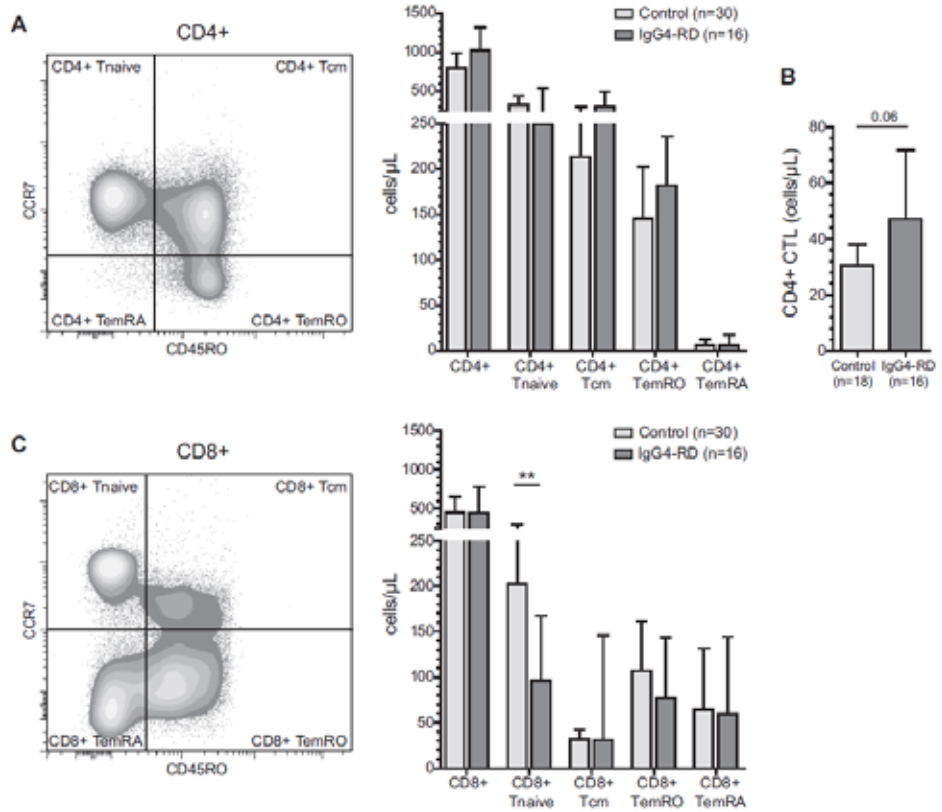
A: Frequencies of somatic hypermutations (SHM) in rearranged *IGHV* genes of IgG3+, IgG1+, IgG2+ and IgG4+ B-cells from healthy individuals. Each dot represents one sequence, red lines represent median values. Statistical analysis was performed with the Mann Whitney U test; *P<0.05, **P<0.01. **B:** IGH-CDR3 size of IgG4+ B-cells. Each dot represents one sequence, red lines represent median values. Statistical analysis was performed with the Mann Whitney U test; *P<0.05, **P<0.01. **C:** Frequencies of the *IGHV* gene usage in IgG4+ B-cells from healthy individuals and patients with IgG4-RD.

Supplementary Figure 3



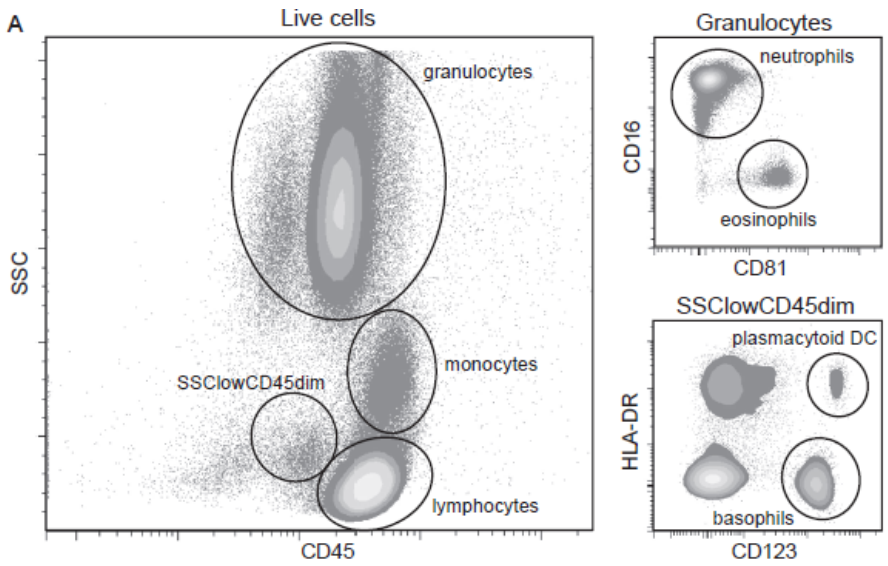
A: Correlation between CD21^{low} IgG4+ B-cells and Th2 cells and between CD21^{low} IgG4+ B-cells and Treg cells in patients with IgG4-RD. Correlation was calculated with Spearman R. **B**: Correlation between IgG4 serum values and absolute numbers of Th2 and of Treg cells. Grey shaded area indicates the reference value for IgG4 serum values. Correlation was calculated with Spearman R.

Supplementary Figure 4



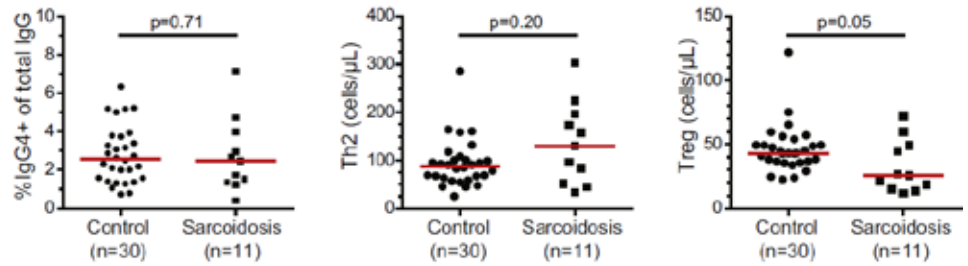
A: Representative plots of the flow cytometric analysis and absolute counts of CD4+ naive T-cells (CD4+ Tnaive), CD4+ central memory T-cells (CD4+ Tcm), CD4+ effector memory CD45RO+ T-cells (CD4+TemRO) and CD4+ effector memory CD45RO- T-cells (CD4+TemRA). **B:** Absolute numbers of CD4+CD45RO-CCR7-CD27- CTL cells **C:** Representative plots of the flow cytometric analysis and absolute counts of CD8+ Tnaive, CD8+ Tcm, CD8+TemRO and CD8+TemRA. Columns represent median values with interquartile range. Statistical analysis was performed with the Mann Whitney U test; **P<0.01.

Supplementary Figure 5



A: Representative plots of the flow cytometric analysis of granulocyte subsets and plasmacytoid dendritic cells (DC) **B**: Absolute counts of neutrophils, basophils, eosinophils and plasmacytoid DC. Columns represent median values with interquartile range. Statistical analysis was performed with the Mann Whitney U test, *P<0.05.

Supplementary Figure 6



Percentage of IgG4+ memory B-cells within total IgG memory B-cells and absolute counts of Th2 and regulatory T-cells in patients with sarcoidosis. Each dot represents one individual, red lines represent median values.

Supplementary Table 1. Antibodies used for flow cytometry

Antibody	Conjugate	Clone	Supplier
CD3	BV711	UCHT1	BD Biosciences, San Jose, CA
CD4	BV510	OKT4	BioLegend, San Diego, CA
CD8	APC-H7	SK1	BD Biosciences
CD16	PerCP-Cy5.5	3G8	BD Biosciences
CD19	PC7	J3-119	Beckman Coulter, Indianapolis, IN
CD19	BV785	SJ25C1	BioLegend
CD20	BV605	2H7	BioLegend
CD21	BV711	B-ly4	BD Biosciences
CD25	BV421	BC96	BioLegend
CD27	BV421	M-T271	BD Biosciences
CD38	APC-H7	HB7	BD Biosciences
CD45	OC515	GA90	Cytognos, Salamanca, Spain
CD45RA	BV605	HI100	BioLegend
CD45RO	FITC	UCHL1	Exbio, Vestec, Czech Republic
CD80	BV605	2D10	BioLegend
CD81	APC-H7	JS-81	BD Biosciences
CD86	PE-Cy7	IT2.2	BioLegend
CD123	PE-Cy7	6H6	eBioscience, San Diego, CA
CD124	PE-Cy7	G077F6	BioLegend
CD127	APC	A019D5	BioLegend
CD180	Biotin	RP/14	BioLegend
CD267	PE/Dazzle	1A1	BioLegend
CD360	PE-CF594	17A12	BD Biosciences
CCR4	PE-Cy7	L291H4	BioLegend
CCR6	PerCP-Cy5.5	G034E3	BioLegend
CCR7	PE	REA108	Miltenyi Biotec, Bergisch Gladbach, Germany
CXCR3	FITC	G025H7	BioLegend
CXCR5	APC	51505	R&D systems, Minneapolis, MN
HLADR	BV605	L243	BioLegend
IgA	FITC	IS11-8E10	Miltenyi Biotec
IgA	PE	IS11-8E10	Miltenyi Biotec
IgD	PerCP-Cy5.5	IA6-2	BioLegend
IgD	PE-CF594	IA6-2	BD Biosciences
IgE	FITC	Goat anti-human	Invitrogen, Waltham, MA
IgG	PE	IS11-3B2.2.3	Miltenyi Biotec
IgG1	PE	SAG1	Cytognos
IgG2	PE	SAG2	Cytognos
IgG2	FITC	SAG2	Cytognos
IgG3	FITC	SAG3	Cytognos
IgG4	APC	SAG4	Cytognos
IgM	BV510	MHM-88	BioLegend
Streptavidin	BV605	-	BioLegend

Supplementary Table 2. Immunophenotypic definitions of T- and B-cell subsets

$CD3^+$ T-cell subset	Immunophenotype
Total $CD8^+$ T-cell	$CD3^+CD8^+$
$CD8^+$ naive T-cell	$CD3^+CD8^+CCR7^+CD45RO^-$
$CD8^+$ central memory T-cell	$CD3^+CD8^+CCR7^+CD45RO^+$
$CD8^+$ effector memory RA T-cell	$CD3^+CD8^+CCR7^-CD45RO^-$
$CD8^+$ effector memory RO T-cell	$CD3^+CD8^+CCR7^-CD45RO^+$
Total $CD4^+$ T-cell	$CD3^+CD4^+$
$CD4^+$ naive T-cell	$CD3^+CD4^+CCR7^+CD45RO^-$
$CD4^+$ central memory T-cell	$CD3^+CD4^+CCR7^+CD45RO^+$
$CD4^+$ effector memory RA T-cell	$CD3^+CD4^+CCR7^-CD45RO^-$
$CD4^+$ effector memory RO T-cell	$CD3^+CD4^+CCR7^-CD45RO^+$
$CD4^+$ CTL cell	$CD3^+CD4^+CCR7^-CD45RO^+CD27^-$
T helper 1 cell	$CD3^+CD4^+CD45RA^-CCR6^-CCR4^-CXCR3^+$
T helper 2 cell	$CD3^+CD4^+CD45RA^-CCR6^-CCR4^+CXCR3^-$
T follicular helper cell	$CD3^+CD4^+CD45RA^-CXCR5^+$
Regulatory T-cell	$CD3^+CD4^+CD45RA^-CD127^-CD25^+$
$CD19^+$ B-cell subset	**Immunophenotype**
transitional B-cell	$CD19^+CD21^+CD38^{high}CD27^-$
naive mature B-cell	$CD19^+CD21^+CD38^{dim}IgD^+/IgM^+CD27^-$
natural effector B-cell	$CD19^+CD21^+CD38^{dim}IgD^+IgM^+CD27^+$
IgM^+ memory B-cell	$CD19^+CD21^+CD38^{dim}IgM^+CD27^+$
IgA^+CD27^- memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgA^+CD27^-$
IgA^+CD27^+ memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgA^+CD27^+$
IgG^+CD27^- memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgG^+CD27^-$
IgG^+CD27^+ memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgG^+CD27^+$
IgG1+ memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgG1^+$
IgG2+ memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgG2^+$
IgG3+ memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgG3^+$
IgG4+ memory B-cell	$CD19^+CD21^+CD38^{dim}IgD^-IgM^-IgG4^+$
plasma blast	$CD19^+CD38^{high}CD27^+$
$CD21^{low}$ B-cell	$CD19^+CD21^{low}CD38^{dim}$
$CD21^{low}IgG4^+$ B cell	$CD19^+CD21^{low}CD38^{dim}IgD^-IgM^-IgG4^+$

Supplementary Table 3. Basic immunological characteristics of patients with IgG4-RD

	serum immunoglobulins (g/L)				blood leukocytes and subsets (cells/μl)				
Patient	IgG1	IgG2	IgG3	IgG4	leukocytes	lymphocytes	T-cells	B-cell	NK-cells
1	8.61	**6.8**	0.4	**2.12**	6500	**3480**	**2640**	**620**	220
2	10.40	2.28	0.34	**2.73**	4500	2440	**1930**	400	*70*
3	5.24	2.36	0.38	**5.96**	7300	*790*	*610*	*60*	*90*
4	5.37	2.13	0.35	1.22	6200	1960	1640	200	120
5	4.59	3.16	0.62	0.27	**12100**	**4470**	**3500**	**490**	**450**
6	10.4	4.56	0.75	**7.50**	4800	1790	1480	110	180
7	6.49	2.49	0.32	**1.85**	7100	1650	1160	190	270
8	6.89	3.39	0.59	**1.93**	6500	2040	1510	270	220
9	**25.1**	5.12	0.96	**12.8**	5800	2360	1620	**570**	130
10	5.27	4.31	0.35	0.27	8800	**2970**	**2140**	**430**	370
11	10.4	2.93	0.32	**5.75**	7700	1280	1040	*60*	170
12	**11.4**	2.68	1.09	**25.25**	**10200**	**4100**	**3300**	280	**440**
13	6.85	4.66	0.23	**2.76**	5400	2280	1440	210	**530**
14	9.91	**7.15**	0,64	**5.40**	7600	1530	1130	110	260
15	**19.30**	2.27	**2.28**	1.13	7000	1710	1010	**470**	150
16	8.87	4.36	0.36	**5.55**	6800	1480	1150	*60*	160
normal range	4.9-11.4	1.5-6.4	0.2-1.1	0.08-1.4	3500-10000	1100-2500	700-1900	100-400	100-400

Subnormal values are depicted in italics, supranormal values in bold font

Supplementary Table 4. Clinical and immunological characteristics of patients with Sarcoidosis

patient	gender	age (yr)	organs affected	medication at inclusion	CRP (mg/L)	ESR (mm/h)	immunoglobulins (g/L)				Leukocytes (10^3 cells/μL)	T-cells (cells/μL)	B-cell (cells/μL)
							IgG1	IgG2	IgG3	IgG4			
1	F	43	lung, eye, skin	n.i.m.	2.6	3	n.d.	n.d.	n.d.	n.d.	5000	*430*	180
2	F	33	lymph node, eye	n.i.m	**12**	**22**	5.56	3.51	0.31	*0.05*	4300	*350*	*80*
3	M	67	ocular	n.i.m	1.0	5	5.90	2.15	0.27	0.36	8100	**2180**	200
4	M	49	lung, lymph node	n.i.m	3.9	**49**	**11.8**	6.38	0.79	**3.47**	6300	980	**450**
5	M	59	lymph node	n.i.m	0.7	**13**	9.0	4.74	0.30	1.22	8800	760	330
6	M	44	lymph node, eye	n.i.m	0.6	7	9.32	2.60	*0.13*	0.82	3600	930	140
7	F	66	lymph node, skin	n.i.m	2.4	12	6.81	3.21	0.35	0.17	7200	940	**570**
8	F	86	lymph node, eye	n.i.m	**27**	12	6.56	2.27	0.31	0.28	5500	830	*90*
9	M	51	eye, lymph node, kidney	n.i.m	**10**	12	7.12	3.88	0.47	0.23	4100	*430*	260
10	M	52	eye, lung	plaquenil	0.3	3	7.18	2.74	0.34	0.51	3700	*350*	*60*
11	F	58	lung, eye, skin, joint	n.i.m	n.d.	n.d.	7.78	2.66	*0.11*	0.55	5700	1220	380
normal range					<10mg/L	20mm/h	4.9-11.4	1.5-6.4	0.2-1.1	0.08-1.4	3500-10000	700-1900	100-400

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; n.i.m., no immunomodulatory medication, n.d., not determined; Subnormal values are depicted in italics, supranormal values in bold font.

Chapter 3.2 Soluble interleukin-2 receptor: a potential marker for monitoring disease activity in IgG4-related disease

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ABSTRACT

Background: IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition. T-cells play a crucial role in the pathogenesis and therefore serum interleukin-2 receptor (sIL-2R) may be a potential biomarker in this disease.

Method: We studied the levels of sIL-2R in 26 histologically proven IgG4-RD patients with available serum sIL-2R and compared to newly diagnosed and untreated sarcoidosis (n = 78), healthy individuals (n = 101) and with sIL-2R levels after treatment in IgG4-RD (n = 15). The disease activity was measured using the IgG4-related disease Responder Index (IgG4-RD RI).

Results: Median serum sIL-2R in IgG4-RD patients was 4667pg/ml compared to 1515 pg/ml in healthy individuals (P <0.001) and 6050 pg/ml in sarcoidosis (P = 0.004 compared to IgG4-RD). All IgG4-RD patients had elevated sIL-2R levels compared to the reference value of <2500 pg/ml in healthy controls and 85% elevated serum IgG4, however these did not correlate with each other. Both sIL-2R and IgG4 levels declined significantly after treatment (P = 0.001 and P = 0.01, respectively). The decrease in sIL-2R was significantly correlated with the decrease in disease activity assessed by IgG-RD RI (P = 0.044).

Conclusion: sIL-2R is elevated in IgG4-RD reflecting the inflammatory process with enhanced T-cell activation in the disease. Furthermore, sIL-2R might serve as a potential marker of response to treatment in IgG4-RD.

INTRODUCTION

IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition characterized by storiform fibrotic lesions and accumulation of IgG4 producing plasma cells in the affected tissues (30, 75, 76). It mimics infectious, chronic inflammatory and malignant disorders causing delay in diagnosis (2, 76). Yet, early diagnosis and treatment is important to avoid irreversible organ damage due to fibrosis or secondary amyloidosis in cases of longstanding high inflammatory conditions (5, 29). Histology remains the gold standard in the diagnosis of IgG4-RD (75). Serum IgG4 is used in the diagnosis of this disease. However, it is not a sensitive biomarker and may be normal in histology proven cases (75).

The pathogenesis of IgG4-RD is mostly unclear, but B cells, IgG4 positive plasma cells, IgG4 antibodies, as well as oligoclonal expansion of T-cells seem to play an important role in the immunopathophysiology (23, 77-79). T follicular helper-2 (Tfh2) cells are involved in driving the class switch to IgG4 (71). Cytokines, including interleukin (IL)-4 and transforming growth factor β, derived from T-helper 2 (Th2) cells and regulatory T cells may also contribute to the pathophysiology of IgG4-RD (19, 79-81). However, the exact role of Th2 cells and their specific cytokines in IgG4-RD is still a subject of debate (18, 82). Also CD4+ T-cells that display cytotoxic features are abundant in peripheral blood and diseased tissue sites of IgG4-RD patients and may contribute to the chronic inflammatory/fibrotic network via secretion of specific cytokines (23, 83).

Peripheral blood levels of soluble IL-2 receptor (sIL-2R) reflect the level of T-cell activation, and elevated serum levels correlate with disease activity in rheumatoid arthritis and sarcoidosis, diseases in which enhanced T-cell activity is centrally involved (84-86). Moreover, increasing sIL-2R levels may precede T-cell driven fibrotic responses (87). In light of the above, serum sIL-2R may also be elevated in IgG4-RD and represent a maker for disease activity.

Here we examined sIL-2R serum levels in a cohort of histologically diagnosed and therapy naïve patients with IgG4-RD in comparison to patients with sarcoidosis and healthy controls. Moreover sIL2R levels were related to clinical response upon treatment.

METHODS

Study population

The Erasmus MC University Medical Center represents a national referral centre for patients with IgG4-RD. Medical records of patients with IgG4-RD between 1999 and July 2017 were reviewed for clinical characteristics and the availability of serum sIL-2R measurements. Only patients with histologically confirmed IgG4-RD according to established Boston criteria for histology (28) and of whom serum sIL-2R were available were included. In total, serum sIL-2R was available from 26 patients with clinically active IgG4-RD. The serum sIL-2R levels from these patients were compared to those of patients with histologically proven sarcoidosis (n = 78, from the Erasmus MC University Medical Centre sarcoidosis database) and to healthy controls (n = 101, anonymous blood bank donors). Serum sIL-2R levels in untreated IgG4-RD patients were compared to sIL-2R levels after starting treatment (available in 15 patients). The disease activities at the time of measurement of serum sIL-2R levels before and after starting treatment were assessed using the IgG4-RD Responder Index (IgG4-RD RI) (88). This study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Erasmus MC (ethics approval numbers MEC-2014-476, MEC-2015-200 and MEC-2017-084).

Analysis of serum parameters

Serum IgG4 and sIL2R levels were measured by the standard laboratory diagnostic facility within Erasmus MC. IgG4 was measured by immunonephelometry using Siemens BN II nephelometer and serum sIL-2R levels were determined with ELISA (Diaclone, Besancon Cedex, France) according to manufacturer instructions in pg/mL. The reference range of sIL-2R is set at <2500 pg/mL within Erasmus Medical Center, based on the serum sIL-2R measurements in 101 healthy blood bank donors.

Statistical analysis

Characteristics of the patients with IgG4-RD, sarcoidosis and the healthy controls were described using descriptive statistics including the median and percentages. We tested for differences between the three groups of IgG4-RD, sarcoidosis, and healthy controls using a One-way ANOVA. To investigate the individual differences between the groups

(IgG4-RD versus healthy controls, sarcoidosis versus healthy controls and IgG4R versus sarcoidosis) we used independent sample t-tests. Because we used multiple t-tests, this could be classified as multiple testing. Therefore, we used a stricter P-value for those 3 specific tests ($P < 0.01$ is considered significant). For all other statistical test performed, we used the standard P value ($P < 0.05$ is considered significant). To investigate whether sIL-2R levels changed after treatment, a dependent samples t-test was performed. When calculating correlations, we used the Spearman's rank correlation coefficient. The statistical analyses were performed using IBM SPSS statistics 21.0.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

Table 1 demonstrates the characteristics of patients with IgG4-RD. Patients with IgG4-RD represent a heterogeneous group and 58% of the patients presented with involvement of more than one organ. Table 2 shows the basic characteristics of patients with IgG4-RD, sarcoidosis, healthy volunteers and the levels of sIL-2R. There was no difference between the mean age of IgG4-RD patients and sarcoidosis patients ($P = 0.311$). Significantly more patients with IgG4-RD were male (76.9%).

SIL-2R levels in the study population

The median level of serum sIL-2R in IgG4-RD was 4667 pg/ml, in sarcoidosis 6050 pg/ml and in the healthy population 1515 pg/ml. Serum sIL-2R levels in IgG4-RD were significantly higher compared to healthy controls ($P < 0.001$) and lower compared to sarcoidosis ($P = 0.004$) (Table 2, Figure 1).

SIL-2R levels after treatment in IgG4-RD

SIL-2R levels of 15 IgG4-RD patients were also available after treatment and decreased significantly from 5300 pg/ml (3695-6135) to 2864 pg/ml (2160-3653) ($P = 0.001$, Table 3). The duration of treatment varied per patient, but all patients of whom sIL-2R levels were available after treatment, showed significant clinical improvement on IgG-RD RI in response to treatment ($P = 0.01$, Table 3).

The correlation of serum sIL-2R with serum IgG4, C-reactive protein and IgG-RD Responder Index

In 19 patients with IgG4-RD, serum IgG4 levels were available before and after treatment and decreased significantly ($P = 0.001$) from 4.0 g/l (1.7-10.3) to 1.8g/l (1.3-3.3) and correlated with a clinical improvement of the disease. In all IgG4-RD patients sIL-2R was elevated, compared to 85% (22/26) for serum IgG4 (Table 1). No significant correlation was observed between the levels of serum sIL-2R and serum IgG4 before (Table 3, $P = 0.776$) and after (Table 3, $P = 0.450$) treatment. Elevated C-reactive protein (CRP) levels ($CRP \geq 10$ mg/l), were found in 27% (7/26) of patients before treatment. No significant correlation was observed between the levels of CRP and serum sIL-2R before (Table 3, $P = 0.279$) and after (Table 3, $P = 0.164$) treatment. In patients with IgG-RD, the decrease in sIL-2R significantly correlated with the decrease in disease activity assessed by IgG-RD RI ($P = 0.044$).

Table 1: characteristics of patients with IgG4-RD

P	Sex	Age	Disease manifestation of IgG4-RD	IgG4 before treatment	sIL-2R before treatment	Treatment/comments
1	M	53	Orbit and lymph nodes	17.7	3695	Rx: prednisone
2	M	36	Salivary gland	3.18	3441	Rx: initially prednisone and Aza, later RTX
3	M	42	Lymph nodes and kidney	3.0	12400	Rx: prednisone and RTX, now cellcept
4	M	60	Orbit and lymph nodes	5.22	3700	Rx: prednisone and MTX
5	F	46	Orbit	2.76	3400	Rx: now infliximab
6	M	61	Orbit, lymph node and prostate	13.48	5800	SIL-2R initially measured under low dose prednisone, but active disease. Now prednisone and MTX
7	M	57	Orbit, lymph node and prostate and pancreas	13.40	5151	Rx: prednisone
8	M	63	Orbit, pancreas, ENT and prostate	1.65	5700	Rx: prednisone
9	M	17	Lung, lymph nodes and brain	10.30	3900	Rx: prednisone and RTX
10	M	53	Mesenteric manifestation	25.25	5900	Rx: prednisone and RTX
11	M	32	Pericardial and pleural manifestation	5.40	3500	Rx: prednisone
12	M	39	Orbit	1.65	8135	Rx: prednisone and Aza
13	M	29	Lymph node (Kimura disease)	1.17	3800	Patient declined treatment
14	M	55	Hypophysis and ENT	1.57	3100	Rx: prednisone
15	M	48	Mesenteric manifestation	3.01	14027	Patient did not show up for follow up/treatment
16	F	52	Orbit	0.61	2859	Surgical resection periorbital mass, no systemic treatment
17	M	61	Biliary tract and ENT	5.55	5300	Rx: prednisone
18	M	79	Lymph node	42.8	9000	Only lymph node manifestation, no systemic treatment
19	F	39	Salivary gland	3.97	3374	Rx: prednisone
20	M	74	Orbit and lymph node	8.82	6135	Rx: dexamethasone
21	F	52	Orbit and lymph node	3.27	2639	Rx: prednisone
22	M	74	Orbit and ENT	3.30	7977	Status after ocular surgery, currently no systemic treatment
23	F	61	Orbit and ENT	0.36	4182	Rx: prednisone
24	M	65	Retroperitoneal fibrosis	3.44	7100	Rx: prednisone
25	M	53	Orbit and ENT	1.45	4105	Rx: prednisone, MTX and now RTX
26	F	59	Skin	1.18	10800	Rx: prednisone and plaquenil

Characteristics of patients with IgG4-RD, including serum IgG4 and serum sIL-2R before treatment.
P = patient, Rx = treatment, MTX = methotrexate, Aza = azathioprine, RTX = rituximab, NA = not applicable, NM = not measured. Normal range serum IgG4 is 0.08-1.40 g/l and normal range of serum sIL-2R is below 2500 pg/ml.

Table 2. The basic characteristics and SIL-2R levels in the study population

	IgG4-RD patients	Sarcoidosis patients	Healthy controls
Number	26	78	101
Median age (IQR)	53 years (41-61)	49 years (38-56)	not applicable
Males	20 (76.9%)	37 (47.7%)	not applicable
Females	6 (23.1%)	41 (52.6%)	not applicable
Median serum sIL-2R (IQR)	4667 pg/ml (3485-7319)	6050 pg/ml (4651-9475)	1515 pg/ml (1150-1880)

Figure 1. Levels of sIL-2R

Figure 1: Boxplots of serum sIL-2R (pg/ml) in newly untreated sarcoidosis and IgG4-RD, and sIL-2R levels after initiation of treatment in IgG4-RD and in healthy population.

* P = 0.004, ** P < 0.001, *** P = 0.001.

Table 3. The treatment response in IgG4-RD

P	IgG4 before treatment	IgG4 after treatment	sIL-2R before treatment	sIL-2R after treatment	CRP before treatment	CRP after treatment	IgG4-RD RI before treatment	IgG4-RD RI after treatment
1	17.7	10.10	3695	3443	1.8	1.6	9	3
2	3.18	3.32	3441	2864	1.0	2.5	4	2
4	5.44	0.46	3700	1600	0.5	0.9	6	1
6	13.48	6.70	5800	2900	0.3	0.3	10	4
7	13.40	3.27	5151	3653	4.7	1.4	10	3
8	1.65	0.28	5700	2800	1.0	0.6	6	1
9	10.30	4.99	3900	1800	50.0	4.5	7	3
10	25.25	4.58	5900	4300	80.0	35.0	6	2
11	5.40	2.79	3500	819	4.8	0.4	5	1
12	1.65	1.54	8135	3700	10.0	7.5	6	1
14	1.57	1.33	3100	2160	5.2	10.0	6	3
17	5.55	1.84	5300	2200	0.3	0.3	6	1
20	8.82	1.28	6135	2222	2.8	38	4	0
24	3.44	1.35	7100	3616	31	6.8	6	1
26	1.29	1.76	10800	5000	8.0	7.6	4	3

Treatment response in IgG4-RD with levels of serum IgG4, CRP and sIL-2R before and after treatment. The disease activity has been measured using the IgG4-RD RI.
Normal range serum IgG4 is 0.08-1.40 g/l, normal range of serum sIL-2R is below 2500 pg/ml and normal range of CRP is below 10 mg/l.
CRP = C-reactive protein, IgG4-RD RI = IgG4-Related Disease Responder Index.

DISCUSSION

Here we demonstrate that sIL-2R levels are elevated in all patients with active and untreated IgG4-RD. Furthermore, the decrease in sIL-2R levels after treatment significantly correlates with clinical improvement in a small cohort of patients with IgG4-RD.

These observations in patients with clinically active IgG4-RD suggest that sIL-2R reflects the inflammatory process in these patients. SIL-2R is secreted by activated T-cells and elevated levels are used as marker for T-cell activity in other inflammatory diseases (89). Although the pathophysiological mechanism of IgG4-RD is not yet fully elucidated, T-cell activation is currently considered an important contributor (23, 77, 79). Our observation of declining sIL-2R serum levels upon clinical improvement after immunosuppressive treatment supports the pathological role of (excessive) T-cell activity in IgG4-RD and further indicates that serum sIL-2R levels reflect IgG4-RD disease activity. Although the posttreatment measurement intervals of sIL-2R differed in patients, it will probably not have influenced the results, because the decrease in sIL-2R correlated with clinical improvement of the disease activity obtained by IgG4-RD RI.

Theoretically, serum IgG4 levels might reflect disease activity in IgG4-RD. However, in general serum IgG4 is normal in ~30% of IgG4-RD patients (30). Indeed 15% of our patients with IgG4-RD had normal serum IgG4 levels, whilst sIL-2R was elevated in all patients. Moreover, there was no direct correlation between serum IgG4 and sIL-2R and between CRP and sIL-2R levels in the cohort studied.

Sarcoidosis often presents with clinical presentations similar as IgG4-RD and is associated with elevated serum sIL-2R (this study and others) and if untreated fibrosis may develop (84, 85, 90). Consequently sarcoidosis represents an appropriate disease control for comparison of serum sIL-2R levels with IgG4-RD. The levels of sIL-2R were significantly lower in IgG4-RD compared to sarcoidosis, yet significantly higher than that observed in the healthy population. This may be indicative of a more vigorous T-cell component, or burden of activated T cells, in sarcoidosis. Because sIL-2R is a non-disease specific T-cell activation marker, it might not suite as a specific diagnostic tool in IgG4-RD (86). However,

as all IgG4-RD patients displayed elevated serum sIL-2R, compared to 85% and 27% for serum IgG4 and CRP, its negative predictive value can be considered high and thus may be helpful in diagnostic evaluation of IgG4-RD. Moreover, sIL-2R could be useful in monitoring disease activity, disease dynamics and early detection of a recurrence.
This study is limited by its retrospective character and the relatively small population. Therefore larger studies are required to obtain the sensitivity and specificity of sIL-2R in IgG4-RD.

In conclusion, we demonstrate that serum sIL-2R is elevated in IgG4-RD. Furthermore, serum sIL-2R may have potential as a tool for monitoring disease activity/treatment response in IgG4-RD. *The value of serum sIL-2R for this application needs further confirmation in prospective and larger studies, also in comparison to diseases with mimicking capacity of IgG4-RD such as granulomatosis with polyangiitis.*

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Chapter 4

Insights in the pathogenesis of IgG4-related disease

Chapter 4.1 Local and systemic signs of chronic B-cell responses in IgG4-related disease

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ABSTRACT

Background: IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition. B cells and T-cells play a crucial role in the pathogenesis. In addition to previously described T-cells, recent reports underline the important role of Tfh2 cells in IgG4-RD and the presence of B-cell follicles in the affected tissues.

Method: We studied the affected tissues of 19 patients with IgG4-RD for presence of B-cell follicles. Detailed flowcytometry was performed to study the numbers of follicular helper-T (fh) cells in 18 patients, 30 controls and 11 sarcoidosis patients, as well as Tfh1 and Tfh2 subsets in 8 patients, 20 healthy individuals and 11 sarcoidosis patients. Furthermore, we studied the levels of somatic hypermutation (SHM) in IgG transcripts blood B cells.

Results: Seventeen out of 19 patients with IgG4-RD appeared to have B-cell follicles in the affected tissues. No increase in the absolute numbers of blood Tfh nor Tfh1 cells in IgG4-RD was observed. However, significantly more Tfh2 cells were present in both IgG4-RD and in sarcoidosis than in controls. SHM levels were similar between IgG4-RD patients and controls for all 4 IgG subclass transcripts, whereas patients with sarcoidosis had significantly more SHM in IgG1 and IgG2.

Conclusion: The affected tissues of patients with IgG4-RD contain B-cell follicles and active responses are reflected by increased blood Tfh2 cell numbers. Still, circulating B cells do not show molecular signs of enhanced responses. Together with our previously identified immunophenotype with reduced CD21 and CD27 expression on IgG4+ B cells, this might reflect a means to dampen B-cell responses through anergy.

INTRODUCTION

IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition potentially affecting all parts of the human body. The disease is characterized by storiform fibrotic lesions and accumulation of IgG4 producing plasma cells in the affected tissues (1-3). IgG4-RD may mimic infectious, chronic inflammatory and malignant disorders often leading to delay in diagnosis and treatment (3, 4). Timely diagnosis and treatment are important to avoid fibrosis or secondary amyloidosis in cases of longstanding high inflammatory conditions (5, 6). Histology of affected tissue is still the gold standard in the diagnosis of IgG4-RD (2). Serum IgG4 is used in the diagnosis of this disease, however, it is not a sensitive biomarker and may be normal in histology proven cases (2). Recently, a "lymphocyte signature" was developed using combined scores of IgG4+ B cells, T-helper (Th)2 cells and regulatory T-cells (Tregs), which potentially could be used in the diagnosis of IgG4-RD as non-invasive test (7).

The pathogenesis of IgG4-RD is mostly unclear. Several studies have demonstrated that B cells, IgG4 positive plasma cells, IgG4 antibodies, as well as T-cells play an important role in the immunopathophysiology of IgG4-RD (7-10). Various cytokines including interleukin (IL)-4 and transforming growth factor (TGF-)β, derived from T-helper 2 (Th2) cells and regulatory T cells may contribute to the disease pathophysiology (7, 11-13). The exact role of Th2 cells and their specific cytokines in IgG4-RD is however still a subject of debate (14, 15). Furthermore, CD4+ T-cells displaying cytotoxic features have been shown to be abundantly present in peripheral blood and diseased tissue sites of IgG4-RD patients and may possibly contribute to the chronic inflammatory/fibrotic network of the disease by secreting specific cytokines (10, 16).

We recently demonstrated expansion of IgG4+ B cells, Th-2 cells and Tregs in IgG4-RD (7). The importance of follicular T-helper cells (Tfh) subsetting is a relative new finding in IgG4-RD (8). T follicular helper-2 (Tfh2) cells, but not the total Tfh cells, play possibly a role in the pathogenesis of IgG4-RD. Tfh2 cells are involved in driving the Ig class switching of B cells to IgG4 by producing IL-4 (17). The aim of this study was to investigate the levels of Tfh2 in the peripheral blood of patients with IgG4-RD and to investigate the affected tissues of patients for presence of B-cell follicles. Furthermore, we aimed to study extensively the levels of somatic hypermutation (SHM) in IgG4 + B-cells, which were previously suggested to be increased in IgG4-RD (18).

METHODS

Patients.

Patients with IgG4-RD and patients with sarcoidosis were recruited following signed informed consent from the Immunology outpatient clinic at the Erasmus Medical Center Rotterdam and from the Rotterdam Eye Hospital, the Netherlands. All patients were >18 years and were diagnosed based on clinical, serological and histopathological findings. All IgG4-RD patients met the IgG4-RD diagnostic guidelines, including histological confirmation (19), and did not have a known history of an immunodeficiency or any auto-inflammatory disease other than IgG4-RD. All but two patients with sarcoidosis had tissue biopsy confirmed disease with typical presence of non-caseating granulomatous inflammation. In two patients the diagnosis was based on clinical presentation and supportive serological parameters (angiotensin converting enzyme and soluble interleukin-2 receptors) in combination with radiological imaging. Healthy controls were recruited from healthy individuals selected from department staff, and the control group was age- and gender-matched to the patient cohort. None of the healthy individuals showed signs of active inflammatory disease. This study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Erasmus MC (ethics approval numbers MEC-2014-476, MEC-2015-200 and MEC-2017-084).

Histopathology

All patients with IgG4-RD were histologically diagnosed. The amount of IgG4+ plasma cells per high-power field (0.28 mm^2) and the IgG4/IgG ratio were measured and all patients met the Boston criteria for IgG4-RD (19). The haematoxylin and eosin stainings were analyzed at the Department of Pathology of the Erasmus Medical Center Rotterdam by a trained pathologist with experience in diagnosing IgG4-RD. The deparaffinized formalin-fixed paraffin embedded sections of the tissue (4 mm thick) were stained using a BenchMark automated immunostainer (Ventana, Tucson, AZ, USA) with the Ultraview

Universal diaminobenzidine detection kit (Ventana). Mouse anti-human IgG (clone A57H, 1:200, Dako, Carpinteria, CA, USA) and mouse anti-human IgG4 (clone HP6025, 1:600, Invitrogen Zymed, Camarillo, CA, USA) were used for immunohistochemical staining and were applied to the sections for 32 min.. Stainings were performed with CD3 (2GV6, ready to use), CD79A (Sp18, ready to use), Bcl-2 (Sp66, ready to use, all from Ventana), Bcl-6 (G11GE/A8, 1:10) and PD-1 (Nat105, 1:50, both from Cell Marque, Rocklin, CA, USA).

Molecular analysis of IgG transcripts

RNA was isolated from post-Ficoll mononuclear cells of IgG4-RD patients 5, 7, 8, 10, 11 and 12with a GenElute mammalian RNA kit (Sigma-Aldrich, St Louis, Mo) and reverse transcribed to cDNA with random primers (Invitrogen Life technologies). Rearranged IgG transcripts were amplified in a multiplex PCR approach using 4 different L-VH-family forward primers in combination with an *IGHG*-consensus (5'CACGCTGCTGAGGGAGTAG) reverse primer.(20) PCR products were cloned into a pGEMT easy vector (Promega, Madison WI), amplified by colony PCR, and sequenced by Micromon facility of Monash University on an Applied Biosystems 3730s DNA Analyzer (Thermo Fisher). Obtained sequences were analyzed using the IMGT database (http://www.imgt.org/IMGT_vquest/vquest) to assign the *IGHV*, *IGHD* and *IGHJ* genes and alleles, and to identify SHM. Of each unique clone, the position and frequency of mutations were determined within the *IGHV* gene (CDR1-FR3). SHM were determined as variations on the best matched V-gene and represented as the percentage of mutations of the total sequenced V-gene nucleotides. The IgG receptor subclasses were determined using the *IGH* reference sequence (NG_001019). All results of patients with IgG4-RD were compared with previously generated data sets of controls and of patients with sarcoidosis (20, 21).

Flow cytometry

Stored PBMC from controls, IgG4-RD patients and patients with sarcoidosis were thawed and prepared for detailed flowcytometric analysis of Th and Tfh subsets. Two million PBMC were incubated for 15min at room temperature in a total volume of 100µl with the following antibodies: CD4-BV510(RPA-T4), CD25-BV421(BC96), CD45RA-BV605(HI100), CD127-APC(A019D5), CCR4-PE-Cy7(L291H4), CCR6-PerCP-Cy5.5(G034E3; all from Biolegend, San Diego, CA), CD3-BV711(UCHT1), CD8-APC-H7(SK1), CCR7-PE-CF594(150503), CXCR3-PE(1C6/CXCR3), CXCR5-BB515(RF8B2; all from BD Biosciences, San Jose, CA). After preparation, cells were measured on a 4-laser LSRFortessa flow cytometer (BD Biosciences) using standardized settings. Data were analyzed with FACSDiva software V8.0 (BD Biosciences). Tfh cells were defined as $CD3^+CD4^+CD45RA^-CXCR5^+$, and within this subset the Tfh2 cells were defined as being $CCR6^-CXCR3^-CCR4^+$.

Statistical analysis

Somatic hypermutation frequencies and both frequencies and absolute cell numbers were assumed a non-Gaussian distribution. Results were analyzed using the non-parametric Mann-Whitney *U* test. IgG subclass distributions were analyzed with the $\chi 2$ test. All *P*-values are two-tailed and were considered statistically significant if values were lower than 0.05. Statistical analysis was performed using GraphPad Prism software, version 6 (GraphPad Software, La Jolla, CA).

RESULTS

Patients

A total of 19 patients with IgG4-RD were included with a mean age of 60 years (range 18-79 years) and a male/female ratio of 3/1 (Table I). All patients were confirmed to have IgG4-RD based on the Boston consensus, with typical histopathologic characteristics and the presence of IgG4-producing plasma cells in affected tissue (19). Fifteen out of 19 patients had increased serum IgG4 levels (range 0.27-25.25g/L). The majority of IgG4-RD patients were treatment naive (no prior treatment), three patients had received immunomodulatory medication in the past (medication had been stopped for at least 6 months prior to inclusion) and only two patients received low dose prednisone (Table I). Sixteen out of 19 patients in this study were previously included in the study on IgG4+ B cells in IgG4-RD (7). Of the eleven patients with sarcoidosis, one patient used plaquenil. All other patients were therapy naïve, as defined in the study of Heeringa et al (7).

Table 1. Histological findings in patients with IgG4-RD

patient	gender	age (yr)	organs affected	IgG4+ PC/HPF (ratio IgG4/IgG)	B-cell follicles	medication at inclusion	time until diagnosis	serum IgG4 (g/L)
1	F	60	orbita	149 (> 0.4)	+	n.i.m.	14 years	**2.12**
2	F	67	orbita	>50 (>0.4)	+	n.i.m.	8 years	**2.73**
3	M	63	orbita, lymph node, lung, prostate	>100 (>0.4)	+	prednisone	3 years	**5.96**
4	M	54	orbita	156 (0.90)	+	prednisone	10 years	1.22
5	F	53	orbita	>64 (0.4)	+	n.i.m.	3 years	0.27
6	M	60	orbita, lymph node	411 (0.67)	+	t.n.	5 years	**7.50**
7	M	44	orbita	110 (0.4)	+	t.n.	12 years	**1.85**
8	F	59	skin	>135 (0.95)	-	t.n.	5 months	**1.93**
9	M	18	lung, lymph node, cerebra	150 (>0.4)	+	t.n.	1 month	**12.8**
10	F	62	pancreas, lymph node, lung	131 (0.72)	+	t.n.	3 months	0.27
11	M	74	lymph node, salivary gland, lung	138 (0.7)	+	t.n.	5 months	**5.75**
12	M	53	mesenterium	458 (0.5)	+	t.n.	20 years	**25.25**
13	M	62	thyroid gland	330 (0.8)	+	t.n.	1 year	**2.76**
14	M	32	serous membrane	136 (0.70)	+	t.n.	3 months	**5.40**
15	M	79	lymph node, pancreas	1223 (0.9)	+	t.n.	1 year	1.13
16	M	60	bile duct, lymph node	205 (0.63)	n.d.	t.n.	2 weeks	**5.55**
17	M	48	mesenterium	120 (0.56)	+	t.n.	5 months	**3.01**
18	M	60	orbit, ENT	207 (0.85)	+	t.n.	6 years	**15.02**
19	M	74	pancreas, orbit, lymph node	210 (0.8)	+	t.n.	3 years	**8.82**

PC, plasma cells; HPF, high power field; n.i.m., no immunomodulatory medication for at least 6 months prior to inclusion; t.n., treatment naive; n.d., not determined; ENT, ear-nose-throat. IgG4 serum levels above normal range are shown in bold font. Histology analysis was performed on the affected organ listed first.

B-cell follicles in affected tissues

The accumulation of IgG4-expressing B cells and plasma cells is thought to result from enhanced T-cell dependent germinal center responses in lymphoid and non-lymphoid tissue of affected patients. Indeed, 17 out of 19 patients (89%) had B-cell follicles in affected tissues. The affected tissue of one patient did not show B-cell follicles, while the analysis could not be performed for one other patient (Table 1). As illustrated in Figure 1A, the follicles contained CD79A positive B cells with low Bcl-2 expression as well as CD3+ T cells that expressed Bcl-6 and PD-1, which are typical for Tfh cells.

Figure 1. B-cell follicles and circulating follicular helper T-cell subsets in patients with IgG4-RD

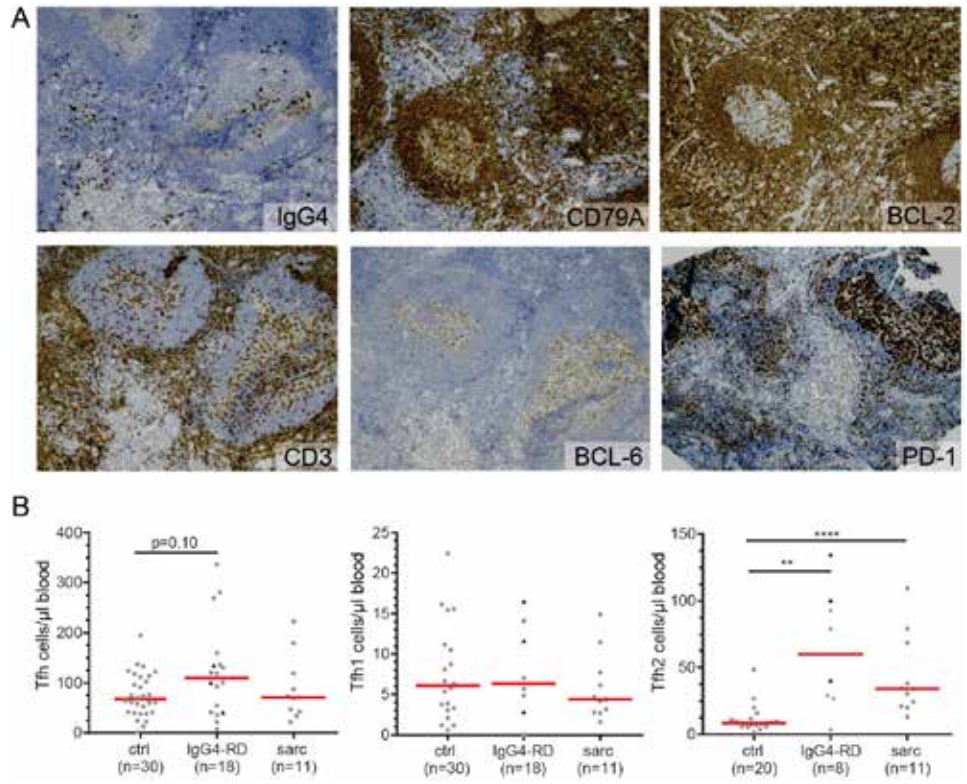

Figure 1. B-cell follicles and circulating follicular helper T-cell subsets in patients with IgG4-RD. A: Immunohistochemical analysis of orbital tissue from patient 7 reveals the presence of IgG4+ plasma cells and CD79A-positive Bcl2-negative B-cell follicles containing Bcl-6 positive, PD-1+ B follicular helper T(fh) cells. **B:** Numbers of Tfh, Tfh1 and Tfh2 cells in blood of controls, patients with IgG4-RD and patients with sarcoidosis. ** p <0.01, *** p <0.001, **** p <0.0001.

Increased numbers of follicular T-helper 2 cells

As recently suggested, Tfh2 cells possibly play a role in the pathogenesis of IgG4-RD (7). The observed B-cell follicles in the affected tissues of patients with IgG4-RD support this. We analysed the blood Tfh subsets in patients with IgG4-RD and compared that with healthy controls and with patients with sarcoidosis. The absolute numbers of Tfh cells were not significantly increased compared to healthy individuals (P = 0.10) and patients

with sarcoidosis, nor were Tfh1 cells (Figure 1B). The numbers of Tfh2 cells in IgG4-RD were, however, significantly higher than in controls ($p < 0.01$) (Figure 1B). The numbers of Tfh2 cells were also significantly elevated in sarcoidosis ($p < 0.0001$) compared to healthy controls.

Molecular signs of antibody maturation

We previously showed that IgG4+ memory B cells have a more anergic phenotype with significant more of these cells lacking CD21 and CD27 expression, and reduced expression levels of CXCR5, a chemokine receptor important for homing to B-cell follicles (7). Moreover, transcripts of these IgG4+ B cells did not show enhanced accumulation of somatic SHM. We here performed additional SHM analysis on transcripts amplified with an IgG consensus primer. This revealed no increased SHM in the other IgG subclasses either, which is in contrast to patients with sarcoidosis (Figure 2) (21). Still, the frequency of IgG2 transcripts within the data set generated with the IgG consensus primer was significantly increased (Figure 2), fitting with our flowcytometry data (7) and repeated immune responses in patients with IgG4-RD (20).

Figure 2. Somatic hypermutation analysis in IgG transcripts

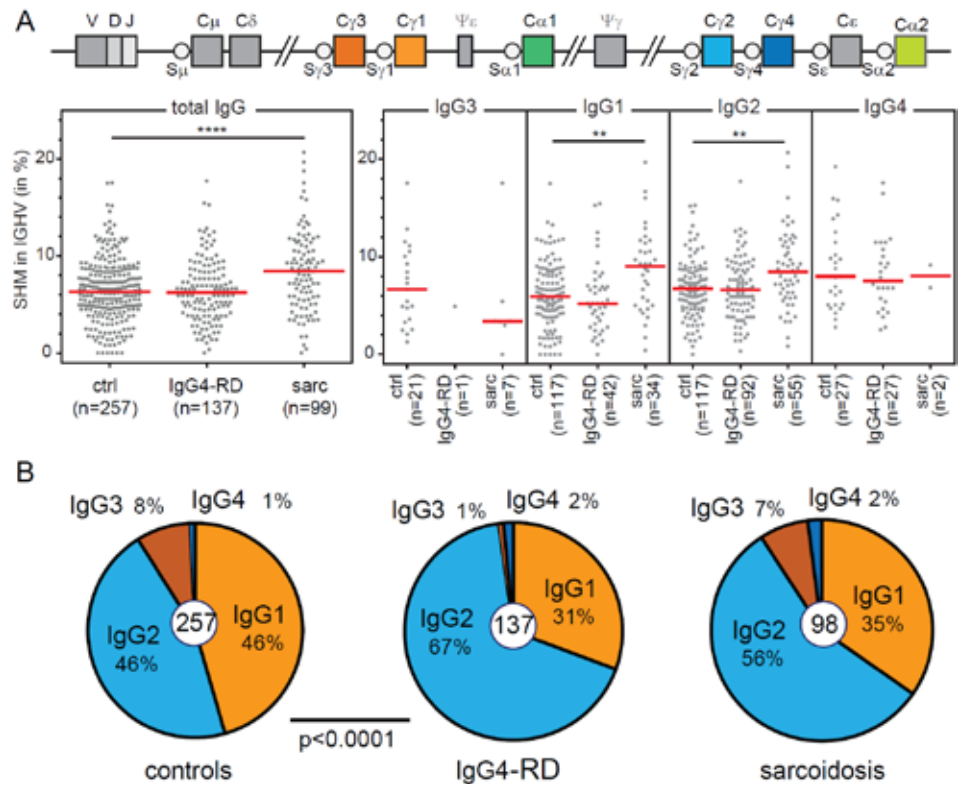

Figure 2. Somatic hypermutation analysis in IgG transcripts. **A:** Somatic hypermutations (SHM) in IgG transcripts and IgG subclasses of adult controls, [1] patients with IgG4-RD and patients with sarcoidosis[7]. Statistics: Mann-Whitney U test, ** $p < 0.01$, **** $p < 0.0001$. **B:** The relative composition of IgG subclasses in transcripts from IgG4-RD patients generated with a consensus IgG primer as compared to previously generated data from controls and from patients with sarcoidosis (20, 21). Statistics, $\chi 2$ test.

DISCUSSION

We here demonstrate the presence of B-cell follicles in the affected tissues of patients with IgG4-RD and show significantly increased levels of Tfh2 in patients with IgG4-RD compared to healthy controls. Moreover, we show that circulating B cells do not have enhanced accumulation of SHM in IgG transcripts.

The gold standard for diagnosis of IgG4-RD is histology. The affected tissues of IgG4-RD contains lymphoplasmacytic infiltration, fibrosis and often phlebitis and eosinophils (19).

The affected tissues further frequently reveals B-cell follicles consisting of CD-20 positive B-cells surrounded by CD19+ cells (18). In these lymphoid follicles IgG4-class switching take place under influence of Tfh and interleukin(IL)-4 (8). Indeed, in our study these B-cell follicles were observed in the tissues of almost all patients with IgG4-RD. Additional immunohistochemical analysis in patient 7 also revealed expression of BCL-6 (regulator of Tfh cells) and PD-1 (negative regulator of activated T-cells). Looking in the peripheral blood by flowcytometry, the absolute numbers of circulating Tfh cells in patients with IgG4-RD were not significantly increased, which was in agreement with previous study (22). The numbers of Tfh2 were, despite the lower amount of included patients for this analysis, significantly increased in patients with IgG4-RD compared to healthy controls. However, circulating Tfh2 cell numbers were also significantly increased in patients with sarcoidosis, and are therefore less specific for IgG4-RD as circulating IgG4+ B cells, Treg cells and Th2 cells (7). The role of increased levels of blood Tfh2 cells in sarcoidosis is unclear. B-cell follicles are usually not seen in the affected tissues of sarcoidosis (21). Still, the elevated levels of Tfh2 in IgG4-RD, despite the possible role of these cells in the pathogenesis, are therefore less specific diagnostic marker for IgG4-RD. This was in contrast with circulating levels IgG4+ B cells, Treg cells and Th2 cells (7).

Sarcoidosis is an immune mediated disease often with similar clinical presentation as IgG4-RD. Patients with sarcoidosis may also develop fibrosis if untreated (23-25). In diagnostic workup of IgG4-RD, sarcoidosis should be excluded and vice versa. Because of the elevated levels of Tfh2 cells in sarcoidosis, these cells are not disease specific for IgG4-RD. Larger cohort studies comparing the circulating levels of Tfh2 in IgG4-RD, sarcoidosis and other disease mimickers of IgG4-RD such as granulomatosis with polyangiitis should give more insight in the circulating levels of Tfh2.

We previously reported that IgG4+ memory B cells in patients with IgG4-RD were phenotypically different from controls due to lower frequency of expressed CD27 cells, which acts is the conventional marker for memory B-cells (7). We also found lower expression levels of the chemokine receptor CXC chemokine receptor 5 (CXCR5) in IgG4+ B cells, which is required for cells to migrate into germinal centres (8). Upregulation of CXCR5+Tfh cells has however previously been described (8). It may be that circulating IgG+ B cells are

less easy to activate and migrate to follicles possibly explaining the reduced expression of CXCR5 in these cells. Otherwise, these differences could originate from a different maturation pathway for IgG4+ memory B cells in patients. Moreover, transcripts of these IgG4+ B cells did not show enhanced accumulation of SHM. Additional SHM analysis on transcripts amplified with an IgG consensus primer revealed no increased SHM in the other IgG subclasses either, which is in contrast to patients with sarcoidosis (21). Still, the frequency of IgG2 transcripts was significantly increased, fitting with our flowcytometry data (7) and repeated immune responses (20). This contrasted a previous study (18), where increased SHM were found in the FR3 and CDR3 regions of IgG4 transcripts. It should be noted that FR3 and CDR3 form less than 1/3rd of the variable domain and that mutations in the CDR3 are notoriously difficult to define, because this includes the junctional regions between *IGHV*, *IGHD* and *IGHJ* genes with random nucleotide insertions and deletions from the V(D)J recombination process. Our analysis involved the strongly recommended approach for SHM analysis, which included the whole *IGHV* gene (>280 nt) including FR1, FR2 and FR3, and the CDR1 and CDR2.(26) Thus, the patients in our original IgG4-RD cohort do show signs of chronic B-cell responses in affected tissue, whereas their circulating B cells have increased anergic properties.

In conclusion, our studies indicate that despite local and systemic signs of chronic immune responses in patients with IgG4-RD, the circulating B cells do not show phenotypic nor molecular signs of chronic activation. Hence, this could reflect a mechanism by which the immune system attempts to modulate the reactiveness of the involved B cells, which could provide new avenues for studies into pathogenesis and treatment of patients with IgG4-RD.

Chapter 4.2 A metadherin gene variant is associated with IgG4-related disease in two unrelated families

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Submitted

ABSTRACT

IgG4-related disease (IgG4-RD) is a fibroinflammatory disease. We describe two unrelated IgG4-RD patients with an identical deleterious exon 1 metadherin (*MTDH*) gene variant. In other IgG4-RD patients, we detected another *MTDH* variant in exon 1. MTDH is associated with inflammatory responses acting through several pathways, including the PI3K-AKT and NF-κB pathways that can be involved in processes associated with organ fibrosis. We observed increased PI3K-AKT and NF-κB signaling in PBMCs of both index patients and their fathers who are carrying the same variant. Our findings suggest that *MTDH* variants may be involved in the immunopathogenesis of IgG4-RD.

INTRODUCTION

IgG4-related disease (IgG4-RD) is a fibroinflammatory disease of one or multiple organs that generally manifests in adults (7). The gold standard of diagnosing IgG4-RD is histopathology revealing fibrotic lesions combined with excessive IgG4-positive plasma cell infiltrations. Early diagnosis and treatment are important to avoid irreversible fibrotic organ damage and secondary amyloid A (AA) amyloidosis. The treatment of IgG4-RD is often challenging: glucocorticoids and rituximab are effective, but the relapse rate is high, often resulting in patients requiring longstanding immunosuppressive treatment. The underlying immunopathogenesis of IgG4-RD remains unclear; however, alterations in circulating IgG4+ memory B cells, plasmablasts, CD21+ low B cells, T helper 2 (Th2) cells and regulatory T cells (Tregs) and the involvement of follicular T helper (Tfh) cells have been described (7). Recently, combined analysis of IgG subclass-positive B cells and T helper cell subsets in blood revealed a specific ‘‘lymphocyte signature” for IgG4-RD (7). This peripheral blood lymphocyte signature is a potential noninvasive diagnostic approach to distinguish patients with IgG4-RD from healthy individuals and from those with other fibroinflammatory diseases (7).

Genetic involvement has been suggested to underlie IgG4-RD because of familial aggregation in monozygotic twins and a sporadic case of a possible genetic association in a patient with IgG4-RD (27, 28). Identification of a genetic association may lead to further understanding of the underlying pathogenesis and the discovery of potential therapeutic targets for this disease. Here, we present identical missense variants in the metadherin (*MTDH*) gene in two unrelated young patients with IgG4-RD. In both cases, over-activation of *MTDH*-related inflammatory pathways in peripheral blood mononuclear cells (PBMCs) was observed.

CASE PRESENTATIONS

Patient 1: A 17-year-old male with an unremarkable medical and family history presented with an epileptic seizure. He had persistent cough and occasional hemoptysis for four months. The only laboratory abnormalities were an increased erythrocyte sedimentation rate (ESR) of 107 mm/hr, a C-reactive protein (CRP) level of 50 mg/l and increased serum IgG4 levels of 12.9 g/l (reference range 12-18 years; 0.035-2.3 g/l). Magnetic resonance imaging (MRI) and computed tomography (CT) scans revealed a cystic lesion in the left frontal lobe and a large space-occupying lesion in the left hemithorax with multiple central calcifications (Figure 1A, B). Open lung and left frontotemporal biopsy samples demonstrated a sclerotic inflammatory process characteristic of IgG4-RD (Figure 1C-F). Treatment with dexamethasone 10 mg/day and azathioprine 2 mg/kg/day was initiated; however, because of persistent pulmonary disease activity on positron-emission tomography (PET) and increased levels of inflammatory markers (CRP, ESR and serum AA) while tapering the dexamethasone dose, rituximab was administered and achieved a successful response.

Patient 2: A previously healthy 32-year-old male with an unremarkable family history was diagnosed with cardiac tamponade and pleural effusion (Figure 1G). The only laboratory abnormalities were elevated CRP (121 mg/l) and IgG4 levels (5.40 g/l; reference range > 18 years; 0.08-1.4 g/l). The pericardiectomy histological samples confirmed IgG4-RD (Figure 1H+I). Prednisone treatment 0.5 mg/kg/day resulted in a long-lasting complete resolution of the pericardial and pleural effusion and resulted in a stable serum IgG4 level of 2.79 g/l. This case was previously described in a case series on IgG4-RD (1).

Figure 1

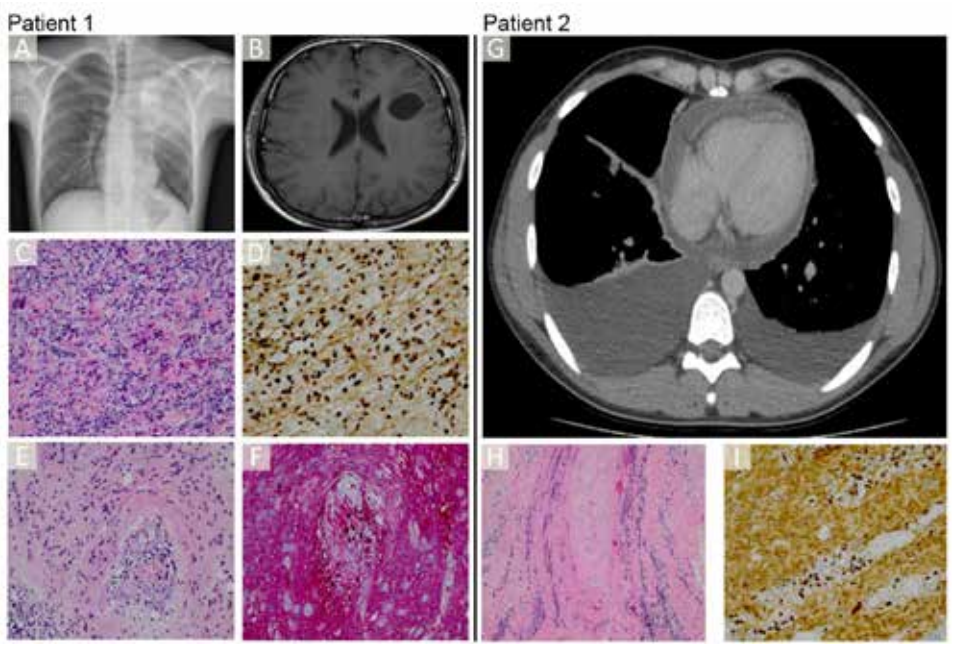

Figure 1: **Imaging and histology specimens of patients 1 and 2**. **A**: Plain film of the thorax of patient 1 showing a large space-occupying lesion of the left hemithorax, with multiple branched calcifications. **B**: MRI of the cerebrum of patient 1 demonstrates a cystic space-occupying lesion of the left temporal lobe of the brain. **C**: Biopsy of the pulmonary upper lobe of patient 1. HE staining revealed multiple lymphoid infiltrates and fibrosis. **D**: Immunohistochemical IgG4 staining revealed widely scattered IgG4-positive plasma cells (brown color) with a ratio of > 0.4 to the total number of IgG-positive plasma cells according to the diagnostic criteria of IgG4-RD. **E**: HE staining of the cerebral lesion in patient 1, showing chronic fibrotic meningoencephalitis with plasma cell invasion. F: Immunohistochemical staining demonstrated IgG4-positive plasma cells (red color) with a ratio of > 0.4 to the total IgG plasma cells/HPF. G: CT scan of the thorax of patient 2 demonstrated bilateral pleural effusion and pericardial effusion. H: HE staining of the pericardium biopsy showed lymphocyte infiltration and fibrosis. I: IgG4 Immunohistochemical staining showed IgG4-positive plasma cells (brown color) with a ratio of 0.7 to the total number of IgG plasma cells. Figure H is at x100 magnification, and panels C, D, E, F and I are at x200 magnification.
HE = Hematoxylin and eosin, HPF = High-power field.

METHODS

Genomic analysis

Whole exome sequencing (WES) was performed on the two patients and their asymptomatic mothers after informed consent. Variants were annotated with ANNOVAR software and filtered with TIBCO Spotfire version 7.5.1. Functional effects of gene variants were evaluated using SIFT, PolyPhen-2, MutationTaster, VarElect and CADD PHRED algorithms. Confirmation using Sanger sequencing of the coding regions of *MTDH* was performed for the two index patients, the parents of both patients and 18 additional IgG4-RD patients who were diagnosed according to the international consensus (7). PCR was performed with the TaqGold amplification system (Applied Biosystems, Foster City, CA, USA), followed by direct Sanger sequencing. Primer sequences are available upon request. Analyses were performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Erasmus MC (MEC-2017-279).

Immunohistochemical analysis for phosphorylated AKT (pAKT)

The levels of pAKT Ser473 and pAKT Thr308 were assessed in lesion biopsies of the two index patients. The two index patients and 18 other patients with IgG4-RD were histologically diagnosed according to the international consensus on IgG4-RD. Additionally, we assessed the levels of pAKT Ser473 and pAKT Thr308 in the two index patients. Immunohistochemistry was performed with an automated staining system (Ventana BenchMark ULTRA, Ventana Medical Systems Inc., Tucson, AZ, USA) using the alkaline phosphatase method for all antibodies and a brown or red chromogen. Following deparaffinization and a 60-minute heat-induced antigen retrieval of the tissue sections, they were incubated with primary antibody targeting p-AKT Ser473 (sc-135651; 1:25, Santa Cruz Biotechnology; Dallas, TX, USA), p-AKT Thr308 (sc-135650, 1:50, Santa Cruz Biotechnology), beta-catenin and TGF-β receptor for 1 hour at 36°C. A subsequent amplification step was performed by incubating the sections with hematoxylin II counterstain for 8 minutes and followed by bluing reagent for 8 minutes according to the manufacturer's instructions (Ventana). Using the immunoreactive scoring (IRS) method, the samples were subsequently scored as described by Remmele and Stegner (29). Representative pictures of staining were taken using an Olympus DP25 camera and acquired by Olympus CellSens Entry 1.9 software. A detailed description of histological analyses is available upon request.

PI3K-AKT and NF-κB pathway analysis

AKT phospho flow analysis in response to anti-IgM and anti-CD19 stimulation was performed to determine PI3k-AKT-mTOR pathway activity as described previously (30). In order to study NF-κB pathway activation, IL-8 synthesis by peripheral blood mononuclear cells (PBMC) was measured following stimulation with TNF-a and LPS.

Phospho-flow

Phospho-flow analysis was performed as previously described by Wentink et al. (30). Frozen PBMCs were thawed, allowed to rest for 30 minutes in medium without FCS, and stained with anti-CD20-BV421 (2H7, Biolegend) and anti-CD14-PE (MoP9, BD Biosciences, San Jose, CA, USA) prior to stimulation with anti-IgM (Southern Biotech, Birmingham, AL, USA) and anti-CD19 (J3-119, Beckman Coulter). PMA was used as a positive control. Cells were fixed in Cytofix (BD Biosciences) buffer according to the manufacturer's protocol. After fixation, cells were permeabilized with Perm Buffer III (BD Biosciences) according to the manufacturer's protocol. Cells were then stained for 30 minutes at room temperature with the following labeled antibodies: AKT-Alexa488 (55/PKBa/AKT), pAKT-Alexa488 (M89-61) (both BD Biosciences), CD3-BV711 (UCHT-1, BD Biosciences) and CD56-BV510 (HCD56, Biolegend). Next, labeled cells were analyzed on an LSRII flow cytometer (BD Biosciences). Different lymphocytic and monocytic subsets were analyzed for AKT and pAKT expression using Infinicyt software. The pAKT/AKT ratio was calculated using the mean fluorescent intensities.

Peripheral blood mononuclear cell stimulation

Cryopreserved PBMCs were thawed, washed and subsequently suspended in RPMI (Gibco, Paisley, UK) supplemented with 10% FCS (Bio Whittaker) and penicillin and streptomycin (100 IU/ml; BioWhittaker, Verviers, Belgium). Cells were seeded in 96-well plates at 0.5 x 10^6 cells per well and stimulated with 100 ng/ml tumor necrosis factor alpha (TNF-α; R&D Systems Inc., Minneapolis, MN, USA) or 10 ng/ml lipopolysaccharide (LPS; Escherichia coli 0111:B4; Sigma Aldrich, Zwijndrecht, Netherlands) for 24 hours at 37°C in a humidified 5% CO_2 incubator. Culture supernatants were collected, and IL-8 concentrations were determined by ELISA according to the manufacturer's guidelines (Cytoset; Invitrogen, Waltham, MA, USA).

IgG4-positive B cells and lymphocyte signature

Circulating IgG4-positive B cells, T helper subsets and peripheral blood lymphocyte signatures in the two index patients and their fathers were assessed as previously described (7).

Peripheral blood flowcytometry

IgG4-positive B cells, T helper cell subsets and peripheral lymphocyte signatures were determined in the two index patients and their fathers were assessed as previously described (7). To ensure consistency with the flow cytometry analysis, standardized sample preparation, antibody staining and flow cytometer instrument protocols were used (31). In short, absolute counts of CD3+ T cells, CD19+ B cells and CD16+/CD56+ NK cells were obtained with a diagnostic lyse/no-wash protocol using commercial Trucount tubes (BD Biosciences). For detailed 11-color flow cytometry, red blood cells were lysed with NH_4Cl before approximately 1 million nucleated cells were stained for 15 minutes at room temperature in a total volume of 100 µL (antibodies listed in repository table E1). After staining, cells were measured on a 4-laser LSRFortessa flow cytometer (BD Biosciences) using standardized settings, and the data were analyzed with FACSDiva software V8.0 (BD Biosciences).

RESULTS

Genetic analysis

On both the IgG4-RD patients and their mothers WES was performed to identify possible causal genetic variants or predispositions for IgG4-RD. These two patients were specifically selected based on their relatively young age at disease onset and their extraordinarily severe clinical manifestations. WES identified the same heterozygous variant in exon 1 of the *MTDH* gene (*MTDH*: rs140652237; NM_178812 c.160G>A: p.V54M) in both index patients, which has a low frequency in Europeans and the Dutch population (Table 1) (32). For in silico gene variant filtering and analysis, SIFT, PolyPhen-2, Mutation Taster and CADD PHRED scores were used (33). The results and details are summarized in Table 1.

Both patients are Dutch Caucasian and were confirmed to be unrelated by 'Vcf tools relatedness' as described by Yang et al. (34). The mothers of both patients lacked the exon 1 variant. Sanger sequencing confirmed this variant in the patients, and further analysis revealed that both fathers were also heterozygous for this *MTDH* variant, indicating coinheritance (Figure 2A and 2B).

To determine the occurrence of these and possible other *MTDH* variants, Sanger sequencing of *MTDH* was performed in 18 unrelated IgG4-RD patients (Table 2), none of whom carried the exon 1 variant (rs140652237). However, another heterozygous variant in exon 1 (rs17854373; *MTDH*: NM_178812.3:c.232G>T, p.A78S) was found in 17% (MAF 0.083) of the IgG4-RD patient cohort (Tables 1 and 2).

Additionally, a silent variant (*MTDH*: rs2331652; NM_178812:exon9:c.1353G>A: p.K451K) was found in exon 9 in both index patients, their parents (Figure 2) and 4 other IgG4-RD control patients (Tables 1 and 2). This variant has a very high frequency in the European population, does not represent a splice site variant and does not lead to amino acid changes; therefore, it is not considered as potentially damaging as the exon 1 variant.

The protein structure of MTDH

The *MTDH* gene consists of 12 coding exons and is located at 8q22 (35). It encodes a highly basic 582 aa protein with a molecular mass of approximately 64 kDa. The protein contains several distinct functional regions as described in the Pfam database: an N-terminal transmembrane domain (aa51-72), putative nuclear localization domains (aa79-91, aa432-451 and aa561-580) and various protein interaction sites (35). The transmembrane domain (TMD) (aa 1–71) is required for MTDH-induced NF-κB activation, although the NF-κB p65 subunit interacts with aa 101–205 (36). Of further interest is that aa position 54 is located in a SUMO-interaction motif region (aa 50-56). It is a pleiotropic protein that potentially localizes to different cellular compartments, including the cell membrane, cytoplasm, endoplasmic reticulum, nucleus and nucleoli. The I-TASSER server permits the prediction of protein structure and function (37) and was used to estimate the three-dimensional structure of the mutant protein based on the aa sequence. The predicted structure with the highest confidence score that illustrates the mutations is presented in Figure 2C. The variances were located and mutated using Swiss-PdbViewer software (38). The replacement of the valine residue with a methionine residue is likely to result in the formation of an additional hydrogen bond between this methionine and the arginine at position 33 (Figure 2D), leading to a reduction in the hydrophobic protein surface area (Figure 2E).

Figure 2

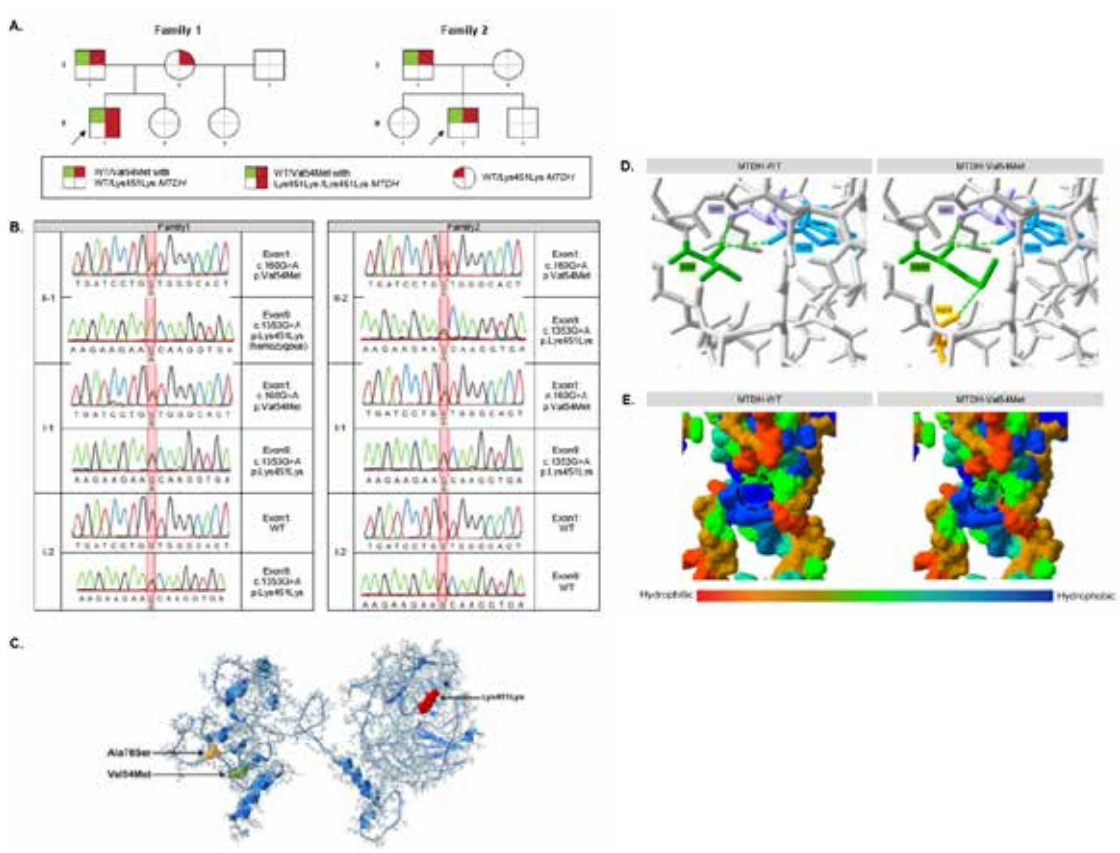

Figure 2: A: Family pedigree of both probands (arrows), colored symbols indicate the gene variants. The green symbol represents Val54Met and the red symbol represents Lys451Lys variants. **B:** Sanger sequencing chromatogram of each proband and his parents. **C:** Three-dimensional structure of the MTDH protein with the mutated amino acids highlighted (Val54Met in green, Lys451Lys in red, and Ala78Ser in yellow). **D and E:** Schematic of the three-dimensional structure of the MTDH protein illustrating the difference between the wild-type (WT) and Val54Met mutant protein. An additional hydrogen bond was presumed upon the substitution of valine with methionine, changing the predicted molecular surfaces of WT MTDH and the Val54Met mutant MTDH. The surfaces were colored according to the hydrophobicity grade.

Table 1. Gene variant analysis.

MTDH variant	SIFT prediction	PolyPhen-2	MutationTaster	CADD score	Frequency
Exon 1 (rs140652237)*	0.09 (tolerated)	0.815 (possibly damaging)	Polymorphism	= 23.0 (CADD PHRED-like score > 15 is suggested threshold for the deleteriousness	ExAC: ALL: 0.0033 European (Non-Finnish): 0.0050 Dutch population: 0.002***
Exon 1 (rs17854373)	0.17 (tolerated)	0.995 (possibly damaging)	Disease causing	The CADD PHRED score was 25.7 indicating this variant as potentially pathogenic	ExAC: ALL: 0.0543 European (Non-Finnish): 0.07026 Dutch population: 0.0543
Exon 9 (rs2331652)**	-	-	-	-	ExAC: ALL: 0.098 European (Non-Finnish): 0.02514 Dutch population: 0.01 Other population ExAC (e.g., Latino): 0.3656

*Variant found in both index patients and their fathers.

**This variant does not lead to amino acid changes and therefore is not considered potentially damaging.
*** According to the Genome of the Netherlands (Go.NL).

Table 2. MTDH gene variants in 18 patients with IgG4-RD

Patient	Gender	Age	Ethnicity	Organs affected	MTDH variants
1	M	46	Dutch	Orbit	None
2	M	38	Dutch	Salivary gland	None
3	M	74	Dutch	Salivary gland and lymph node	None
4	F	60	Dutch	Skin	None
5	M	65	Dutch	Orbit, lymph nodes, ENT, prostate	None
6	F	64	Dutch	Pancreas, lung	None
7	M	61	Chinese	Orbit and lymph nodes	Nose
8	M	53	Dutch	Orbit and nose	**Exon 1: rs17854373 heterozygous**
9	M	61	Dutch	Cholangitis and ENT	None; sequencing last 15 nucleotides of exon 10 not possible
10	M	55	Dutch	Orbit, lymph nodes	None; sequencing last 40 base pare of exon 1 not possible
11	M	54	Dutch	Mesenterium	None; sequencing last 120 base pare of exon 1 not possible
12	M	74	Indonesian	Orbit, lymph node, gall bladder	**Exon 9: c.1353G>A:p.K451K**
13	M	80	Indonesian	Lymph node, pancreas	**Exon 9: c.1353G>A:p.K451K**
14	M	60	Dutch	Orbit, ENT	**Exon 1: rs17854373 heterozygous**
15	M	74	Dutch	Orbit, ENT	**-Exon 1: rs17854373 heterozygous** **-Exon 9: c.1353G>A:p.K451K** - sequencing last 15 nucleotides of exon 10 not possible
16	M	43	Hungarian?	Lymph node, kidney	None; sequencing last 15 nucleotides of exon 10 not possible
17	F	68	Curacao	Orbit, lymph node	**Exon 9: c.1353G>A:p.K451K**
18	M	64	Dutch	Orbit, lymph node, pancreas, prostate	None

Functional analysis

MTDH acts through several cellular pathways, including the AKT-PI3k and NF-κB pathways (35). MTDH overexpression activates PI3K/AKT signaling, thereby modulating numerous downstream processes, including cellular proliferation, epithelial-mesenchymal transition (EMT), neovascularization, apoptosis, and survival (32). Therefore, activation of both the AKT-PI3K and NF-κB pathways was explored.

High levels of cytoplasmic pAKT were observed in the plasma cells present in the examined tissues of both index patients. This phosphorylation appeared to be more prominent at Ser473 than at Thr308 (data not shown) (Figure 3A, B, D). Thus, pAKT (Ser473) levels were subsequently determined in PBMCs by flow cytometry and compared to the levels in PBMCs from healthy controls (HCs). Clearly, elevated pAKT/AKT ratios were observed in unstimulated B cells, T cells and monocytes from the patients, while the NK cells displayed only a marginally increased pAKT/AKT ratio (Figure 3D). However, total AKT levels declined in patients compared to the control subjects, which may result from the permissiveness of AKT to become degraded upon phosphorylation (39). This elevated pAKT/AKT ratio may indicate increased basal activity of AKT in both patients with *MTDH* variants, which could correspond with a gain-of-function (GOF) mutation in MTDH. However, we cannot rule out that disease activity itself may also be a causative factor for increased AKT phosphorylation in isolated mononuclear cells.

Additionally, the fathers of both patients with the *MTDH* exon 1 variant displayed an increased pAKT/AKT ratio in unstimulated PBMCs (Figure 3E). NK cells and monocytes from the father of patient 1 showed increased AKT activation. Two of three IgG4-RD patients without any of the described *MTDH* variants had increased AKT phosphorylation in B cells, T cells and NK cells, while the third patient exhibited a pAKT/AKT ratio similar to that of the controls. Taken together, these findings support a possible link between the identified *MTDH* exon 1 variant and increased AKT activation. In addition, the data indicate that increased AKT activation is not a prerequisite for IgG4-RD, but we postulate that it might increase either the risk of developing IgG4-RD or the severity of this disease. The patients with the c.232G>T, p.A78S variant in exon 1 were not included due to lack of availability of the blood specimens were not available.

Figure 3

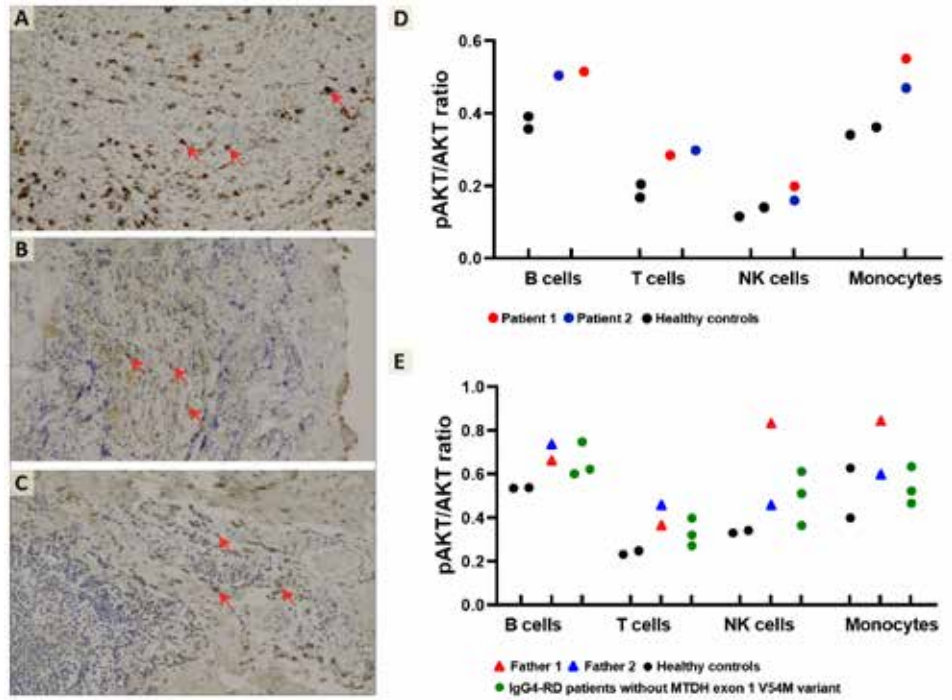

Figure 3. **AKT phosphorylation in tissue and blood cells**. **A+B**: pAKT Ser473 staining (brown, red arrows) in the lung and brain tissue of patient 1, showing high expression of AKT. **C**: pAKT Ser473 expression in pericardial tissue of patient 2. **D:** The pAKT/AKT ratio in the two IgG4-RD patients with the Val54Met *MTDH* variant and in 2 healthy controls. **E:** The pAKT/AKT ratio in the fathers of the IgG4-RD patients with the *MTDH* exon 1 variant, in IgG4-RD patients without the *MTDH* exon 1 variant and in healthy controls. Panels D and E represent independently performed experiments.

IL-8 production is strongly regulated by NF-κB signaling (40). Therefore, the capacity of PMBCs to produce IL-8 was examined for two patients with the Val54Met *MTDH* variant, in both fathers with the Val54Met *MTDH* variant, in 3 patients with IgG4-RD without any *MTDH* variants and in 2 HCs (Figure 4). Unstimulated PBMCs from both patients with the *MTDH* exon 1 variant produced significantly higher amounts of IL-8 than did PBMCs from the HCs ($P < 0.01$). Despite the high basal levels of IL-8 production in the patients with the *MTDH* exon 1 variant, further increases in IL-8 production upon TNF-α or LPS stimulation were observed. Upon TNF-α stimulation, the IL-8 levels produced by the IgG4-RD patients with the *MTDH* variants were also higher than those produced by the HCs, the IgG4-RD

patients without *MTDH* variants and the fathers of the IgG4-RD patients with the *MTDH* exon 1 variant. Upon LPS (10 ng/ml) stimulation, the IL-8 levels in patients with *MTDH* variants (and their respective fathers) were not significantly higher than those in the HCs. It should be noted that, in contrast to the pAKT analysis, this analysis used PBMCs from patient 1 while he was undergoing treatment with low-dose glucocorticosteroids, which may have influenced the outcomes.

Figure 4

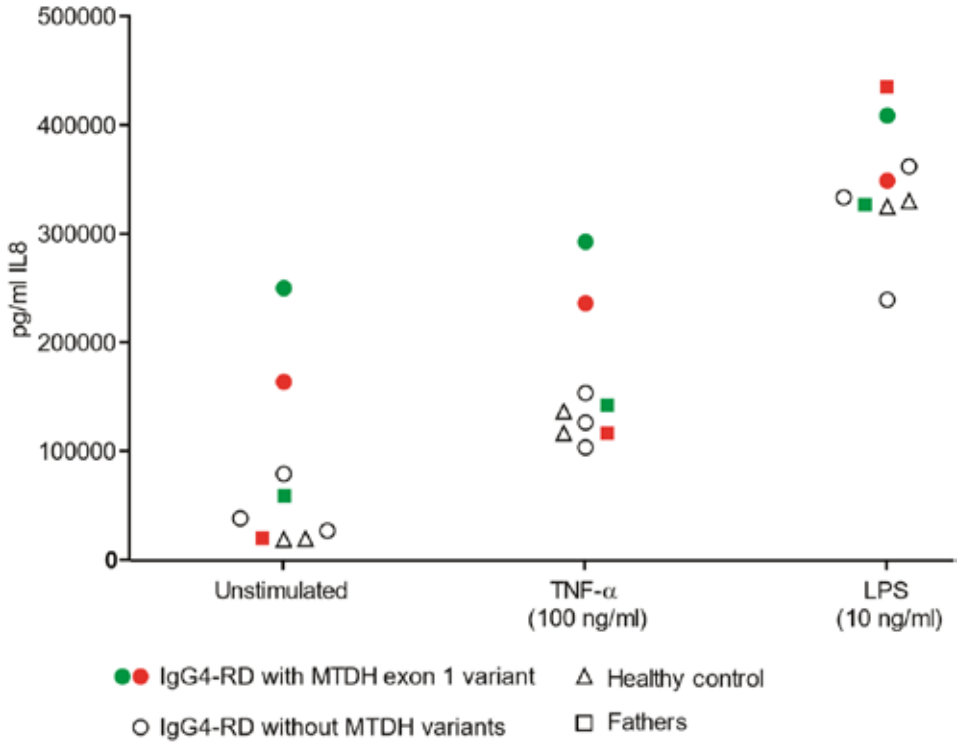

Figure 4. **IL-8 production by PBMCs following stimulation with TNF-α and LPS.** Included are the two IgG4-RD patients with the *MTDH* Val54Met variant (patient 1: red circle, patient 2: green circle), their fathers (red and green squares), patients with IgG4-RD with wild-type *MTDH* variant, and healthy controls. PBMCs were cultured in the absence (left column) or presence of TNF-α 100 ng/ml (middle column) or LPS 10 ng/ml (right column). NB. PBMCs were obtained after starting immunosuppressive medication in patient 1, IgG4-RD patients with wild-type *MTDH* variants were therapy naive.

IgG4-positive B cells

Recently, we studied different lymphocyte subsets, including IgG2- and IgG4-positive B cells, T helper 2 (Th2) cells and regulatory T cells (Tregs), in a cohort of patients with IgG4-RD. IgG4-positive B cells were expanded in IgG4-RD patients. A ''lymphocyte signature'' based on different B and T cell subsets appeared to distinguish patients with IgG4-RD from those with other fibroinflammatory diseases, such as sarcoidosis (7). Both described patients were included in the previous study and had a positive IgG4-RD signature. In the presented study, we analyzed the IgG4-RD lymphocyte signature in both fathers who were carrying the *MTDH* exon 1 variant. The father of patient 1 showed a positive score of 250, while the father of patient 2 had a negative score (< 100). The absolute numbers of IgG4-positive B cells and the percentages of IgG2- and IgG4-positive B cells within the total IgG-positive B cell pool in the father of patient 1 were in the upper 25% of those from HCs (Figure 5A-D). In contrast, the numbers and percentages of IgG2- and IgG4-positive B cells were not altered in the father of patient 2.

Figure 5

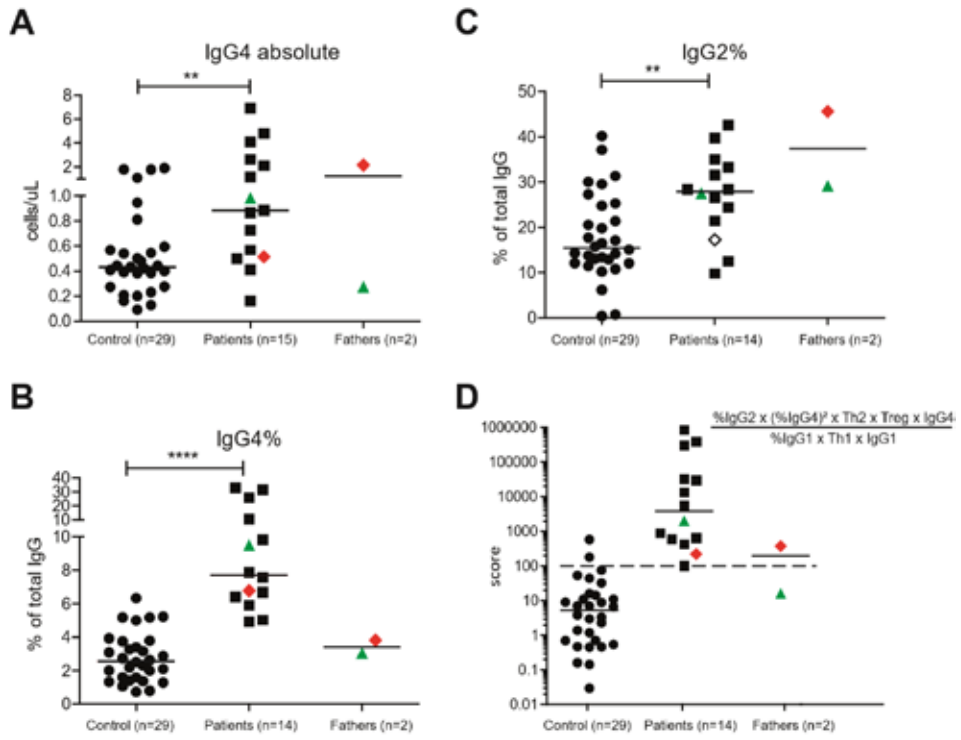

Figure 5: **A:** The absolute numbers of IgG4-positive B cells**, B:** the percentage of IgG4-positive B cells compared to the total number of IgG-positive B cells and **C:** the percentage of IgG2-positive B cells compared to the total number of IgG-positive B cells in patients with IgG4-RD, the fathers of the two index patients with the *MTDH* exon 1 variant and healthy controls. **D:** Scoring of blood samples based on a formula composed of the different cell types indicated significant differences between healthy individuals and patients with IgG4-RD. The scores were also determined in both fathers. (The percentage of IgG2+ B cells and IgG4+ B cells within the total IgG B cell population were multiplied by the absolute numbers of Th2 cells, Tregs and IgG4+ B cells and divided by the percentage of IgG1+ B cells within total IgG+ B cells multiplied by the absolute numbers of Th1 and IgG1+ B cells. A score of 100 (red dotted lines) is considered the cut-off value.)
Rhombus (red) indicates patient 1 (and father 1); triangle (green) indicates patient 2 (and father 2).

DISCUSSION

Here, we identified a rare inherited specific variant in exon 1 (c.160G>A: p.V54M) of the *MTDH* gene in two unrelated families that is most likely related to IgG4-RD. In three other patients with IgG4-RD, we found another, more common, variant in exon 1 (c.232G>T, p.A78S) of *MTDH*. This last variant is directly adjacent to the putative nuclear localization signal (aa 79–91); however, this variant was not further explored in this study.

The *MTDH* gene is recognized as an oncogene and is involved in various biological processes (both canonical and pathological), including cell proliferation and survival, angiogenesis and EMT. It activates critical signaling pathways, particularly the PI3K/AKT, NF-κB, Wnt/β-catenin and the mitogen-activated protein kinase pathways (41). EMT, PI3K/AKT and NF-κB are also associated with fibrosis and inflammatory effects that are characteristic for IgG4-RD. Indeed, we found an elevated pAKT/AKT ratio in PBMCs from both index patients as well as their healthy fathers who carry the same variant in exon 1. The NF-κB protein complex is a prototypical proinflammatory signaling pathway that is strongly associated with chronic inflammatory diseases (42). The MTDH protein translocates to the nucleus and upregulates NF-κB in TNF-α-treated cells to facilitate binding of the NF-κB-CBP (cyclic AMP response element-binding protein) complex on the IL-8 gene promoter in proinflammatory responses (43). MTDH induces IL-8 expression, which depends on the 71 aa on the N-terminus of MTDH. Accordingly, we observed a significantly higher baseline of activated IL-8 production by PBMCs from both index patients compared with HCs and IgG4-RD patients without MTDH variants, indicating enhanced NF-κB activity (36).

IgG4-RD presents characteristic histopathologic features: excess IgG4+ plasma cell infiltrates, fibrosis and obliterative vasculitis. MTDH is highly expressed in normal B lymphoid cells, malignant lymphomas and myeloma plasma cells (44). It is unknown whether MTDH has a direct role in IgG class switching, particularly in IgG4-producing plasma cells. B cell-ablative therapy with rituximab was successful in patient 1 and has been reported to be an effective treatment in refractory IgG4-RD in a case series (45). Although the pathophysiology of IgG4-RD is still mostly unclear, the therapeutic efficacy of rituximab in IgG4-RD suggest that B lymphoid cells might be key components in the immune-pathogenesis. T lymphocytes are also involved, as follicular Th2 cells are thought to direct class switching into IgG4-producing B cells in IgG4-RD. The abundant EMT/fibrosis component

suggests an interaction between IgG4+ plasma cells and fibroblasts. Both B lymphoid cells and fibroblasts express MTDH (43). As part of an IgG4-triggered response, IgG4-producing plasma cells may produce significant amounts of TGF-β that stimulate EMT leading to fibrosis. EMT is a process wherein epithelial cells dedifferentiate into myofibroblasts and acquire mesenchymal features (e.g., αSMA, collagen and fibronectin expression) that promote migratory capacity, invasiveness and traction forces (46). Essentially, EMT represents a normal physiological tissue response to injury to promote wound closure and tissue repair. EMT is normally tightly controlled by various (key transcription) factors that typically repress the expression of epithelial proteins while simultaneously enhancing the expression of mesenchymal proteins (46). However, in fibrotic situations, EMT programs are not attenuated, resulting in persistent myofibroblast formation and excessive accumulation of extracellular matrix proteins such as collagen (47). TGF-β and Wnt/β-catenin signaling pathways are considered to represent major signaling pathways in fibrosis and are highly potent inducers of EMT. MTDH is involved in both of these signaling pathways and participates in TGF-β-induced EMT. Additionally, MTDH is a downstream effector of the TGF-β receptor. The PI3K-AKT and NF-κB pathways are also considered important downstream pathways of MTDH and have also been associated with EMT (48). Moreover, MTDH (and NF-κB signaling) regulate the epigenetic activation of TWIST1 expression, which is an important transcription factor in pathological EMT/fibrosis (49). Since fibrosis is the hallmark of IgG4-RD, variations in the *MTDH* gene could plausibly be involved in the immune-pathogenesis. Skin biopsy for the derivation of fibroblast cultures and to assess the response to TGF-β might have been valuable tests in the two index patients described in this study; however, skin biopsies in these patients were not performed.

The fathers of the index patients are carriers of the *MTDH* variants but do not present any signs of IgG4-RD; therefore, we hypothesize that this variant contributes to disease in specific circumstances. We postulate an initial trigger (environmental, infection, tissue damage) generates a pathogenic IgG4 response in facilitated by the mutation, leading to an uncontrolled fibroinflammatory state manifesting as IgG4-RD. Notably, father 1 demonstrated high levels of pAKT, enhanced NF-κB activation, elevated levels of IgG4-positive B cells and a high probability score without any signs of disease despite carrying

the same *MTDH* variant. These data suggest that the exon 1 variant of *MTDH* might be linked to an elevated score, although not with complete penetrance. The above-mentioned hypotheses warrant further in-depth genetic and biochemical research to elucidate the exact pathophysiological mechanisms and to identify disease targets in IgG4-RD. In conclusion, we present a genetic variant in exon 1 of the *MTDH* gene and its pathophysiological consequences in two unrelated patients with IgG4-RD. The prevalence of *MTDH* variants in IgG4-RD and their exact role in the pathophysiology of IgG4-RD are subject to further exploration.

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Chapter 5

Treatment modalities for IgG4-related disease

Chapter 5.1 Infliximab for IgG4-related orbital disease

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ABSTRACT

IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory condition with unclear pathophysiology. It may occur as a single organ disorder, but multi-organ presentation is common and can mimic several conditions. The preferred therapy consists of steroids, but definite maintenance strategy remains unclear. We describe a case of a 61-year-old woman, initially diagnosed with idiopathic orbital inflammation (IOI) refractory to multiple immunosuppressive agents. The disease was complicated with epilepsy, vision loss and trismus. Treatment with various immunosuppressive agents was unsuccessful. Eventually the patient was effectively treated with infliximab. This is the second case of IgG4-RD treated with a TNF-blocker documented in literature and the first description to demonstrate its superiority over steroid sparing agents. Though speculative, TNF-blockers might exert their effect in IgG4-RD by interfering with the possible overexpressed TNF alpha due to fibrosis in this disease. Treatment with infliximab appears a good alternative for refractory IgG4-RD. However, further studies are required to define the value of infliximab in IgG4-RD.

INTRODUCTION

IgG4-RD is a fibro-inflammatory condition, which can affect almost all parts of the human body (1) and characterized by a tumor-like appearance with infiltration of polyclonal IgG4-positive plasma cells, mostly with storiform fibrosis and elevated serum IgG4 concentration (2) . It can affect a single organ, but the condition may also occur in multiple organs simultaneously. IgG4-RD mimics many infectious, inflammatory and malignant disorders. It is important that physicians are aware of this disease in order to avoid delay in diagnosis and the adjusted treatment (3). IgG4-RD can cause significant morbidity and even lead to irreversible organ damage. Intensifying immunosuppressive treatment might therefore be necessary, especially when vital organs are at risk (4). We describe a patient with IgG4-related orbital disease (IgG4-ROD) successfully treated with infliximab, a chimeric monoclonal TNF-alpha inhibitor.

CASE REPORT

A 61-year-old female was previously diagnosed with an idiopathic orbital inflammation (IOI) located in the right pterygopalatine fossa and orbit. The first clinical presentations were epilepsy with secondary generalization, partial external ophthalmoplegia and binocular diplopia due to limited ocular motility of the right eye in all directions. The pupils were isocore and the pupillary reflexes were normal. Our patient had a mild ptosis of the right upper eyelid.

An MRI of the brain showed a diffuse space-occupying lesion localized in the masticator space, in particular in the inferior orbital fissure, the orbital and the pterygopalatine fossa / infratemporal fossa with also meningeal enhancement of the right temporal lobe and secondary edema of the right temporal lobe (Figure 1). These abnormalities were most likely the focus of epilepsy. The ophthalmoplegia was most probably the result of orbital mass leading to limitation of ocular movements, but a pupil-sparing oculomotor nerve palsy could not be excluded. Cerebrospinal fluid showed no abnormalities. Routine laboratory tests revealed normal blood count, normal liver and kidney biochemistry values and increased erythrocyte sedimentation rate (ESR of 104 mm/h). Immunological examination showed negative antinuclear antibodies (ANA) and especially negative Anti-neutrophil cytoplasmic antibodies (ANCA). At that time of diagnosis, there was an increased total

immunoglobulin G (IgG) level (18.5 g/l) and increased serum IgG4-level (2.76 g/l), while levels of other subclasses of IgG were within normal ranges. Serum protein electrophoresis did not show any abnormalities. Microbiological analyses on tuberculosis, human immunodeficiency virus (HIV), hepatitis C, hepatitis B, Epstein-Barr virus, toxoplasma, Lues and Borrelia were negative. A computerized tomography (CT) scan of the lumbar vertebrae as well as a CT scan of the thorax and abdomen did not show abnormalities.

Figure 1. MRI images of the orbits

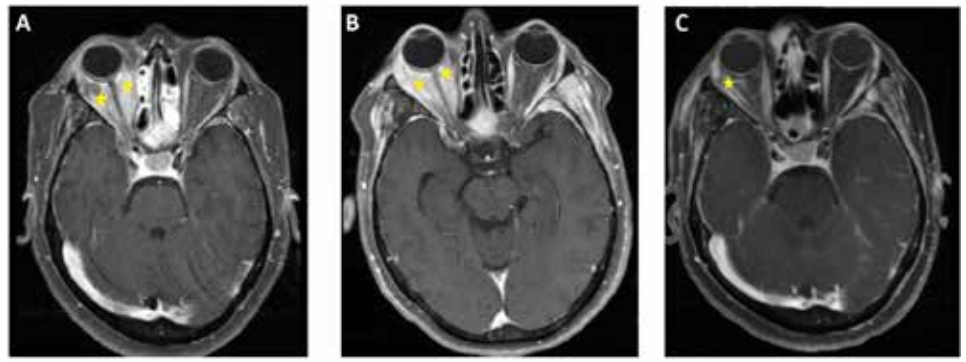

Figure 1. MRI images of the orbits. SE T1 weighted MR axial images of the orbits (TR 480 ms., TE 10 ms.) with fat saturation post-gadolinium. **A:** March 2006: Note the extensive contrast enhancement of the redundant tissue in the orbital fat on the right side, both intra- and extraconally on the medial and lateral side. The stretched non-enhancing hypointense optic nerve is seen against an enhanced background. This imaging was essentially unchanged in the past 5 years since the beginning of the disease. Meanwhile, she was treated with steroids, methotrexate and cyclosporine. **B)** One year later (2007), despite treatment with methylprednisolone and cyclophosphamide, no significant improvement occurred. **C)** Three years later (2010), shortly after initiating treatment with Infliximab, a striking decrease in the enhancement is visible. The intraorbital fat has mostly regained its fat signal.
SE = Spin Echo, MR = Magnetic Resonance, TR = Repetition Time.

Biopsy of the pterygopalatine fossa lesion revealed the histopathology of a chronic inflammation and fibrosis (Figure 2A). Additional staining techniques regarding of Grocott, Gram and Periodic Acid Schiff (PAS) were negative for micro-organisms. Immunohistochemical analysis showed polytypic plasma cells. Later, re-evaluation with novel IgG4 staining techniques, revealed an average of 149 IgG4 positive plasma cells per high-power field (HPF) out of 3 HPF with a ratio >0.4 to total IgG plasma cells in the tumorous tissue (Figure 2). The initial diagnosis of IOI was then revised into orbital IgG4-RD. The patient started with

steroids (prednisone 60 mg once daily). Consequently, she developed steroid induced diabetes mellitus, for which treatment with oral anti hyperglycaemic agents was started. Thereafter several types of immunosuppressive medications respectively, methotrexate, cyclosporine and cyclophosphamide were given. All these treatments had temporary or insufficient results or unacceptable side effects. As a result of direct compression of the optic nerve by the lesion, the patient also developed loss of vision of the right eye. At that time the visual acuity of the right eye was light perception positive and visual acuity of the left eye was 1.0. There was global restriction of the motility of the right eye. In addition, a trismus developed, leading to an inability to open her mouth more than 4 mm. *Coronoidectomy* was performed with moderate and temporary effect. Radiotherapy was rejected by the patient.

Figure 2. Histology of the pterygopalatine fossa

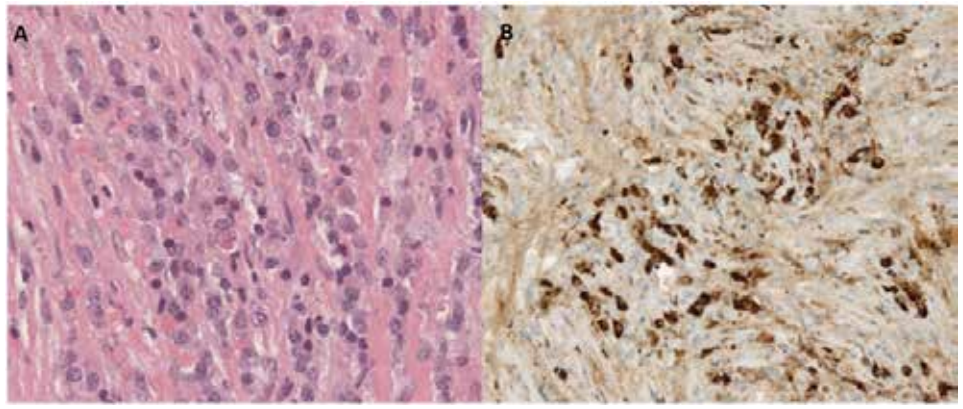

Figure 2. Histology of the pterygopalatine fossa. A: HE-staining showing chronic fibrosing inflammatory cells, including lymphocytes, plasma cells, and macrophages. **B:** Immunohistochemical staining for IgG4 (brown color) showing widely scattered IgG4 positive plasma cells. Both figures are at x400 magnification.
HE = Hematoxylin and Eosin.

Because of the refractory disease and the severe complications, experimental treatment with Infliximab in a dose of 200 mg (=3mg/kg) every 8 weeks, resulted in significant improvement of the inflammation and clinical, biochemical and radiological improvements were observed. The trismus recovered and the mouth could be opened properly. The ocular movements improved markedly and were now only limited in the horizontal directions.

The visual acuity of the right eye showed slight improvement from positive light perception to 1/300. Furthermore, no epileptic seizures occurred since infliximab was initiated accompanied by decrease of serum IgG4 and ESR (to 1.35 g/l, 35 mm/h, respectively) and significant improvement of the lesions on the MRI of the brain (Figure 1). To date, our patient is continuously treated with infliximab for at least 5 years with favourable and stable clinical outcomes. She has experienced no side effects of this treatment.

DISCUSSION

We present a case of IgG4-ROD successfully treated with a TNF-alpha blocker. This patient was initially diagnosed with IOI with serious complications over a course of 10 years. In retrospect, this patient fulfills the diagnostic criteria of IgG4-related orbital disease. A clinical constellation of findings including orbital pseudotumor, elevated serum IgG4, histological presence of fibrosis and IgG4 positive plasma cells infiltration fit in the presentation of IgG4-RD according to the Boston Consensus criteria for IgG4-RD (5).

IgG4-RD can primarily manifest in the orbit, giving the clinical appearance of a pseudotumor (6). Recently IgG4-RD is more often recognized as part of the spectrum of orbital diseases (7), and may give both local and systemic inflammation (8). It can cause significant morbidity and even lead to organ damage and therefore may require intensive immunosuppressive therapy as demonstrated in this therapy refractory patient. Intensive treatment in therapy refractory patients is a well-recognized clinical problem in IgG4-RD, especially when vital organs are at risk (4). Glucocorticoids are the first choice of treatment for most types of IgG4-RD and are mostly effective at a prednisone dose of 0.6-1 mg/kg/day for a single organ manifestation (3, 9). In most cases this treatment turns out to be rapidly effective, allowing the dose of prednisone to be reduced by 10% every two weeks according to clinical responses. About 61% of patients with IgG4-RD achieve complete remission after one year of treatment with corticosteroids (3). However, relapse rates hereafter are high. In case of relapse, about 50% of patients suffer from manifestation of IgG4 disease in other organ systems and may require second-line maintenance treatment (10). Management of further immunosuppressive therapy has not been outlined (11). Conventional steroid-sparing agents like mycophenolate mofetil, azathioprine or methotrexate have all been used in treatment of IgG4-RD, but no case series or clinical

trials supporting their efficacy and long-term outcomes exist (12). Although limited studies available, radiotherapy has been suggested to be effective in steroid refractory IgG4-ROD (13). Rituximab, a monoclonal antibody directed against the B lymphocytic-specific antigen CD20, might target the subset of plasma cells that can produce IgG4 antibodies in cases of IgG4-RD. This therapy, although limited studies are available, has induced substantial clinical remission in patients with IgG4-RD (3, 14, 15).

Infliximab is a chimeric monoclonal antibody against tumor necrosis factor alpha and successfully used for several immune mediated inflammatory conditions including rheumatoid arthritis, inflammatory bowel disease, sarcoidosis and uveitis (16). There are also cases published of successful treatment of IOI with infliximab (17). So far there has been only one published case report of a steroid refractory patient with ocular adnexal IgG4-RD and uveitis treated successfully with infliximab (18). Our patient appeared refractory for several steroid-sparing agents. Infliximab was initiated as a rationale to treat inflammatory symptoms in view of therapeutic efficacy of TNF alpha blockers in IOI (19).

The pathogenesis of IgG4-related disease is unclear and there are no studies about the role of TNF alpha in this disease. Although speculative, it is conceivable that TNF alpha plays a role in the pathogenesis of IgG4-RD leading to positive effect of infliximab. T-cells are involved in the pathogenesis of IgG4-RD. Although controversial, studies have demonstrated increased numbers of T-helper cells 2 and regulatory T-cells in IgG4-RD most probably resulting from a certain antigen triggering the immune system. Production of different cytokines such as interleukin (IL)-4, 5,10,13 and transforming growth factor (TGF)-beta leads to co-activations of B-cells, production of IgG4 expressing B-cells and fibrosis (3), whereby a role of T helper cell-1 mediated cytokines such as TNF alpha cannot be ruled out entirely. Despite the unclear pathogenesis, fibrosis is the hallmark of IgG4-RD. Several studies have demonstrated the relationship between TNF alpha and fibrosis, such as in pulmonary fibrosis, kidney fibrosis, cardiac fibrosis and skin fibrosis (20-23). Given the fibrosis in IgG4-related disease, it is quite possible that there is in some way excessive production of TNF alpha which could be a target for therapy.

This case report suggests that infliximab can constitute a superior alternative to conventional steroid sparing agents in the treatment of refractory IgG4-RD disease. Studies have to elucidate the role of TNF alpha in IgG4-RD and more case series or prospective studies are required in order to define the effect of infliximab in IgG4-related disease.

CONCLUSION

We report a case of retrospective established therapy refractory IgG4-related orbital disease successfully treated with infliximab.

Chapter 5.2 The treatment outcomes in IgG4-related orbital disease: a systematic review of the literature

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ABSTRACT

Purpose: IgG4-related disease (IgG4-RD) is an immune-mediated systemic fibro-inflammatory disease. Treatment of IgG4-related orbital disease (IgG4-ROD) is often indicated to relieve the symptoms and to prevent complications. For IgG4-ROD no international formal treatment guidelines are available and the optimal treatment strategy is uncertain. In this systematic review, we describe the efficacy of conventional and biologic disease modifying antirheumatic drugs (DMARDs) in IgG4-ROD.

Methods: A systematic search of Embase, Medline, Web-of-Science, PubMed publisher, Cochrane and Google Scholar was performed for treatment outcomes in IgG4-ROD. Relevant articles on treatment of IgG4-ROD were retrieved to last date of inclusion January 3rd 2018. The following inclusion criteria were used: articles in English or English translation, studies evaluating the use of DMARDs (conventional and biologic) in the treatment of IgG4-ROD. Meta-analysis and review articles were excluded. A final selection after full-text evaluation was made by independent reviewers, based on treatment of IgG4-ROD with DMARDs and the availability of treatment outcomes.

Results: With this systematic review, we identified 35 studies and case reports/series on IgG4-ROD, describing 95 patients, treated with conventional and/or biologic DMARDs. The success of conventional DMARDs varies between 36-75% in patients with IgG4-ROD, while rituximab is successful in the majority (93%) of the patients.

Conclusion: Based on this systematic review, rituximab is the most effective DMARD in IgG4-ROD, while the efficacy of conventional DMARDs is limited. We propose early initiation of rituximab in case of refractory and organ- or life-threatening disease.

Keywords: IgG4-related disease, orbit, disease modifying antirheumatic drugs, rituximab

INTRODUCTION

IgG4-related disease (IgG4-RD) is an immune-mediated systemic fibro-inflammatory condition, that may mimic a variety of disorders. IgG4-RD is characterized by tumor-like lesions, lymphoplasmacytic infiltrate enriched with IgG4-positive plasma cells, storiform fibrosis and obliterative phlebitis.(5, 24) Serum IgG4 is often elevated, however, histologically proven cases may have normal serum IgG4 concentrations (25). Histology remains therefore the gold standard (25-27).

IgG4-RD has been reported in virtually every organ, but orbital structures are frequently involved (8, 28-30). IgG4-related orbital disease (IgG4-ROD) is most frequently reported in the lacrimal gland, but also in the orbital soft tissue, extraocular muscles, eyelids, sclera, optical and trigeminal nerves and orbital bones (31, 32). In contrast to other localizations, obliterative phlebitis is uncommon in orbital lesions (5, 33). Treatment of IgG4-ROD is often indicated to relieve the symptoms and to prevent complications.(34-36) Although an international consensus guidance statement on the treatment of IgG4-RD is suggested (24) there are no international formal treatment guidelines. Different treatment strategies have been proposed, including glucocorticoids and steroid-sparing immunosuppressive agents (24, 37-39). The use of steroid-sparing agents like conventional and biologic disease modifying antirheumatic drugs (DMARDs) in the treatment of IgG4-RD varies across countries due to different practice styles and/or the lack of access to certain steroid-sparing agents (24). The proposed treatment strategies are mostly based on case series and experience with the treatment of autoimmune pancreatitis (24, 40, 41). Less is known about the treatment of IgG4-ROD and it may therefore be challenging to develop guidelines for the management of this disorder. In this systematic review, we describe the reported efficacy of conventional and biologic disease modifying antirheumatic drugs in IgG4-ROD.

METHODS

A systematic search of the literature was conducted to provide an overview of all studies, case reports and case series regarding treatment of IgG4-ROD with DMARDs. The study was performed and reported in accordance with the PRISMA statement for systematic reviews.

Data source

Relevant articles on treatment of IgG4-ROD were retrieved from Embase.com, Medline (Ovid), Web-of-Science, and the Cochrane Library to last date of inclusion January 3th 2018. Possible additional references were obtained from PubMed (the subset as supplied by publisher, containing references not yet indexed in Medline) and Google Scholar (the most relevant citations). Filters for date or language were not used in the search strategy (see appendix 1 for the full search strategies for all databases).

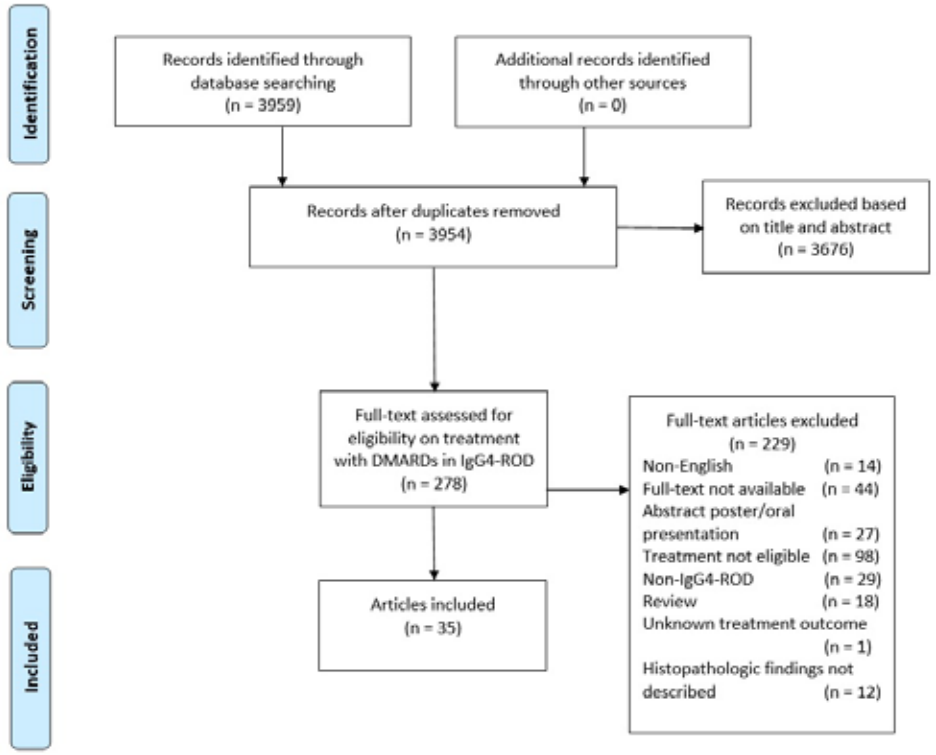
Study selection

After extracting duplicates, all titles and abstracts under this search strategy were screened (42). The following inclusion criteria were used: articles in English or English translation, studies evaluating the use of DMARDs (conventional and biologic) in the treatment of IgG4-ROD. Meta-analysis and review articles were excluded. A final selection after full-text evaluation was made by independent reviewers (SD and AFK), based on treatment of IgG4-ROD with conventional and biologic DMARDs and the availability of treatment outcomes. A consensus on the inclusion of each article was reached. The articles' methodologic quality was graded as level 4 according to the levels of evidence described by OCEBM Levels of Evidence Working Group "The Oxford 2011 Levels of Evidence". Overall response to therapy was defined as no response, partial or complete response (28). No response was defined as no improvement or worsening of the disease. Partial response was defined as improvement of the disease, but not complete remission or maintenance therapy was required. Good response was defined as complete remission or stable without treatment.

RESULTS

A total of 3959 articles were identified by the search (Figure 1). Of these, 279 articles on the treatment of IgG4-ROD were assessed for eligibility and 35 articles were included (see appendix 2 for an overview of the outcomes of included articles).

Figure 1. Search strategies and selection of the articles.



Patients

With this systematic review, we identified 35 studies and case reports/series on IgG4-ROD, describing 95 patients, treated with conventional and/or biologic DMARDs. The median age of the patients was 49 years, ranging from 5 to 83 years of age. Of these patients, 41 (43%) were female. The mean serum IgG4 concentration was 563 mg/dL (n=40, range 13-3820, normal values: 8-140). The mean follow-up was 36 months (n=66, 5-360).

Orbital manifestations

The included patients presented with different orbital manifestations of IgG4-RD. Most cases reported involvement of the lacrimal glands (66%), soft tissue of the orbit (32%) and extraocular muscles (29%).

Systemic organ manifestations

The reported cases included a broad spectrum of systemic organ manifestation in IgG4-ROD in 65 (68%) of the patients. Involvement of salivary glands was most frequently reported in 41 patients (43%), followed by lymph node manifestations in 26 patients (27%). Pancreas involvement was present in 19 (20%) of patients with IgG4-ROD.

Diagnosis

In all patients characteristic histology was found. However, 7 studies did not describe the IgG4/IgG ratio (18, 43-48) and 6 did not describe exact plasma cell counts (14, 49-53). These studies were included, due to characteristic histology.

Therapy and outcome

Glucocorticoids were the first line of treatment in 79 out of the 95 patients. In 11 patients rituximab monotherapy was used as initial treatment and in another patient rituximab and methotrexate combination was used as primary treatment (Table 1).

The doses of the glucocorticoids were not mentioned in all cases. However, when specified the induction doses of prednisone was usually between 0.5-1mg/kg/day and of methylprednisolone 1g intravenously. The overall treatment outcome did not differ between studies that mentioned the glucocorticoid dosage and studies that did not. Almost all patients (89%) responded to primary glucocorticoid treatment. Relapse whilst tapering glucocorticoids was described in 12 (14%) out of a total 83 patients with glucocorticoid therapy. In 30 (36%) patients relapse occurred when glucocorticoids were discontinued. In 2 (2%) patients no distinction was made between tapering or cessation. In 25 (30%) patients a relapse after tapering glucocorticoids was not described in the study. Complete and lasting remission after cessation of glucocorticoids was described in 3 patients (4%) patients. One (1%) patient had no reported relapse after tapering of glucocorticoids.

In 1 (1%) patient with no reported relapse, no distinction was made between tapering and cessation of the glucocorticoids. The maximum time of follow-up was almost seven years. However, the most reported time of follow-up was between 2-3 years. Side effects from glucocorticoids, including obesity, herpes zoster, diabetes mellitus, glucocorticoid-induced avascular necrosis of the humeral heads, were reported in 4 (5%) of the patients.

Of the conventional DMARDs, methotrexate was used in 27% of the cases, followed by azathioprine in 15%. Mycophenolate mofetil was used in 11% of the cases. Other DMARDs included cyclosporine (3%) and mizoribine (1%). Rituximab was used in 59% of the cases, infliximab in 5%. Abatacept and adalimumab were both used once. Tacrolimus and indomethacin were both prescribed to 1 patient. In 17% of the patients cyclophosphamide was prescribed, 1 patient received tamoxifen, 1 fludarabine and 1 R-CHOP (rituximab/cyclophosphamide/doxorubicin/vincristine/prednisolone). See appendix 2 for doses of therapy. Unfortunately, exact treatment regimens were not always described in the included articles.

Table 1. Summary of treatment outcomes in IgG4-related orbital disease.

Therapy (n)	Good response (%)	Treatment failure (%)	Comment
Methotrexate (n=26)	12 (46%)(28, 44, 50, 54-59)	14 (54%)(14, 29, 60-63)	In 2 (6%) patients a switch in therapy was required due to side effects.(14)
Azathioprine (n=14)	5 (36%)(51, 54, 59, 64)	9 (64%)(15, 28, 29, 46, 65)	In 5 (36%) patients treatment was discontinued due to side effects. (15, 28, 46)
Mycophenolate mofetil (n=10)	7 (70%)(15, 28, 43, 45, 54)	3 (30%)(28, 60, 61)	One (10%) patient experienced toxicity.(28) One (10%) patient required mycophenolate mofetil only as maintenance after treatment with rituximab.(15)
Mizoribine (n=1)	NA	1 (100%)(49)	NA
Infliximab (n=5)	4 (80%)(18, 47, 63, 66)	1 (20%)(62)	Two (40%) patients required additional surgery.(47, 66)
Abatacept (n=1)	1 (100%)(49)	NA	NA
Adalimumab (n=1)	1 (100%)(67)	NA	Maintenance with adalimumab required.
Rituximab (n=57)	53 (93%)(14, 15, 28, 29, 48, 49, 52, 53, 58, 60-62, 65, 68-71)	4 (7%)(29, 49, 72)	Five (9%) patients relapsed after treatment.(29, 48, 49, 53) In 11 (19%) patients maintenance therapy was required after initial treatment with rituximab.(15, 28, 48, 53)
Glucocorticoids (n=83)	75 (90%)(14, 15, 28, 29, 43-51, 53-55, 57-65, 67-71)	8 (10%)(18, 29, 52, 66, 72)	Relapse on tapering glucocorticoids was described in 12 (14%) patients with glucocorticoids as induction therapy.(28, 49, 50, 58, 64) In 30 (36%) patients relapse occurred when glucocorticoids were discontinued.(14, 15, 28, 29, 43, 45-47, 59, 60, 62, 65, 67-71) Side effects from glucocorticoids, were reported in 4 (5%) of the patients.(14, 60, 63)
Cyclophosphamide (n=16)	12 (75%)(54, 59)	4 (25%)(18, 52, 62, 63)	NA
Cyclosporine A (n=3)	NA	3 (100%)(29, 49, 63)	NA
Tacrolimus (n=1)	NA	1 (100%)(44)	NA
Indomethacin (n=1)	NA	1 (100%)(62)	NA
Fludarabine (n=1)	1 (100%)(68)	NA	Fludarabine was used in combination with rituximab.
R-CHOP (n=1)	NA	NA	R-CHOP was used in one patient with unknown outcome.(53)

Of the 26 cases treated with methotrexate, 12 (46%) demonstrated a good initial response. In the remaining cases, methotrexate was insufficiently effective or treatment was stopped due to side effects. A switch in treatment was required in 14 patients. Two patients experienced side effects from the methotrexate. Therefore, a switch to rituximab therapy was made. A total of 14 patients received azathioprine, showing a good response in 5 (36%) patients. In 9 (64%) patients, azathioprine was inefficient. One (7%) patient did not respond to this treatment at all. In 2 (14%) patients the treatment was discontinued due to intolerable side effects. Of 10 patients treated with mycophenolate mofetil, 7 (70%) patients had a good response. One patient required mycophenolate mofetil only as maintenance after treatment with rituximab. In 3 (30%) patients, mycophenolate mofetil was insufficient, these patients required a switch to rituximab. Mizoribine was unsuccessfully used in 1 patient.

Rituximab was the most prescribed treatment modality after glucocorticoids and was used in 57 (60%) of the 95 patients described in this study. In patients treated with rituximab, a good response was reported in 53 (93%) of the patients. One (2%) patient developed rituximab resistance and 3 (5%) patients had progressive disease under rituximab therapy. In 5 (9%) patients relapse after rituximab treatment was described. In 11 patients maintenance therapy with glucocorticoids (5 patients), mycophenolate mofetil (2 patients) and rituximab (1 patient) was required after induction therapy with rituximab. Of 2 patients the maintenance therapy was unknown. The dose of rituximab used was not always described. When specified, the dose was usually 1 g for two doses, two weeks apart. When specified, the mean follow-up time was 29 months (3-112 months). Infliximab was used as treatment in 5 patients, showing a good clinical response in 4 (80%) patients. One patient required infliximab maintenance therapy and the second patient relapsed after infliximab was ceased, 2 patients required additional surgery and 1 patient showed no response to infliximab treatment. Abatacept and adalimumab were both used once. Both patients responded well to these therapies.

Cyclosporine A was used in 3 patients, tacrolimus and indomethacin in 1 patient; all patients with insufficient results. Fludarabine was used in combination with rituximab in one patient, with good clinical result. R-CHOP was used in one patient, with an unknown result. Cyclophosphamide was used in 16 patients. In 12 (75%) patients, improvement

after treatment was reported. One (6%) patient required a switch to rituximab and in 2 (13%) patients cyclophosphamide treatment showed treatment failure.

Radiotherapy (usually between 20-30 Gy in 10-15 fractions) was used as additional therapy in 6 patients for recurrent disease. Of these patients, 4 (67%) showed a good response, 1 (17%) patient had no response and the response in another (17%) patient was not described (15, 44, 48, 53, 71, 72).

In a total of 6 patients, surgery was performed for different reasons. Two patients required additional orbital surgery after systemic therapy for mechanical problems (44, 69). Two patients underwent surgery before systemic therapy to resect IgG4-RD lesions and both patients required systemic treatment. Two patients underwent additional surgery after systemic treatment (29, 45, 66).

In 1 patient treatment with glucocorticoids, cyclophosphamide, indomethacin, methotrexate and infliximab were not sufficient. The patient developed a painful blind eye that led to enucleation. Histopathology of the globe showed features suggestive of IgG4-related scleritis and panuveitis (62).

In this review, 7 children were included. The median age was 7 years, ranging from 5 to 14 years of age. All patients were treated with glucocorticoids, 6 patients relapsed after tapering and 1 patient had a partial response. All patients required additional therapy. Two children received mycophenolate mofetil, one rituximab, one methotrexate, one infliximab and one adalimumab (43, 45, 47, 51, 56, 67, 70).

DISCUSSION

In this systematic review of the literature, we describe the efficacy of systemic treatment in IgG4-ROD.

Glucocorticoids

Currently, the initial treatment approach for active IgG4-RD is systemic glucocorticoids (24). Glucocorticoids are broad-spectrum, strong anti-inflammatory and immunosuppressive agents that act on immune cells including T cells, B cells, dendritic cells, macrophages, B cells, cytokines and endothelial cells (73). Glucocorticoids were the primary treatment in 88% of the patients with ocular involvement. Prednisone was given in an initial dose of 0.5-1 mg/kg/day and tapered within months. The initial response rates of patients with IgG4-ROD on glucocorticoids is slightly lower than reported response rates of patients with IgG4-RD of the pancreas or sclerosing cholangitis and the percentage non-responders is higher (74). Patients with systemic involvement might have a higher relapse rate on glucocorticoid monotherapy (75). Patients with IgG4-ROD involving the sclera or optic nerve might show insufficient response to glucocorticoids and require a switch to rituximab and/or radiation.(13, 76, 77) A relapse rate of 50% is seen in patients tapering or stopping glucocorticoids. Relapses usually are recorded when the prednisolone were tapered under 10 mg/day. However, one patient relapsed with a dose of 45 mg oral prednisolone per day.(64) Insufficient duration of initial glucocorticoid treatment is associated with relapse (78). Recurrent lesions are mostly seen in the lacrimal gland (78). Involvement of the lacrimal gland is a risk factor for relapse. The incidence of relapse in the first 6 months was 26% (79). Glucocorticoids are effective as induction therapy, but the high recurrence rates and unwanted side effects indicate the need for steroid-sparing therapy in IgG4-ROD (74, 80). Side effects of glucocorticoid treatment were reported in 5% of patients. This percentage is much lower than reported in other studies (74, 81). Glucocorticoid side effects might be underreported in the included studies of this review. All children treated with glucocorticoids required additional treatment. There is no consensus on prednisone dosage in pediatrics, but it seems effective as a first line therapy. However, in almost half of the pediatric patients glucocorticoid monotherapy is not sufficient (82).

Methotrexate

Methotrexate is a folate antagonist that affects T cells and macrophage recruitment and function (83). It has anti-inflammatory properties and is the most frequently reported DMARD in the treatment of IgG4-ROD in this study. Previous studies show good effect of methotrexate on systemic IgG4-RD (56, 84). Methotrexate can be initiated as a steroid-sparing agent. However, almost 50% of the patients required a switched to rituximab because of an insufficient response.

Azathioprine and mizoribine

Azathioprine is an inhibitor of purine synthesis and the second most frequently reported DMARD in the treatment of IgG4-ROD (85). Azathioprine has been widely used in the treatment of IgG4-related pancreatitis (37, 86). Of the patients with IgG4-ROD treated with azathioprine, 36% respond well. However, the majority of patients required a therapy switch. In patients with active IgG4-related pancreatitis, the transition from glucocorticoids to azathioprine monotherapy is likely to fail (37). It is possible that, in some patients, the transition from glucocorticoids to azathioprine has been made during active disease and therefore failed. Treatment-limiting side effects are reported in 10-30% of patients treated with azathioprine (37, 87). This is in the same range as in the presented review. Azathioprine is effective in a minority of patients with IgG4-ROD, with a high percentage of patients reporting side effects. The efficacy of mizoribine, another purine antagonist, cannot be established at this point in time as only 1 case has been reported.

Mycophenolate mofetil

Mycophenolate mofetil inhibits the purine synthesis. It has anti-inflammatory and anti-fibrotic characteristics (88, 89). The last is an interesting effect in this fibroinflammatory disorder. Adding mycophenolate mofetil to glucocorticoid maintenance therapy might help maintain initial response and prevent relapse in systemic IgG4-RD (90). Mycophenolate mofetil for treatment of IgG4-ROD has only been described in some case reports being successful in 70%. Although only small numbers of patients have been treated with mycophenolate mofetil, this agent might be a potential conventional DMARD.

Infliximab and adalimumab

Monoclonal antibody against tumor necrosis factor alpha (anti-TNFα), such as infliximab or adalimumab are known to be effective in various immune-mediated inflammatory conditions. It causes programmed cell death of T lymphocytes, binds TNFα and might have antifibrotic properties (23, 91). Data on the efficacy of anti-TNFα in IgG4-ROD are limited (18, 63, 67). In this study, treatment with infliximab has been reported in 5 patients, of whom 4 demonstrate a good response. Treatment with anti-TNFα might be effective in patients with IgG4-ROD. However, due to limited evidence, the treatment with anti-TNFα should be carefully considered.

Rituximab

Rituximab is a monoclonal therapeutic antibody against CD20 and is the most reported biologic DMARD in the treatment of IgG4-RD.(74) B cell-ablative therapy with rituximab is successfully used in various autoimmune conditions (92). It appears to be an effective induction and maintenance therapy, even without concomitant glucocorticoid use in IgG4-RD (80, 93). Rituximab is effective in part because it depletes B lymphocytes, preventing plasma cells from producing IgG4. It is also hypothesized that B lymphocytes have a role in presenting antigen to T lymphocytes and therefore play a role in re-activating the disease (94). Rituximab is initiated as a second or third line therapy and induces clinical response in a majority (93%) of the patients. When specified, the dose was usually 1 g for two doses, two weeks apart (Table 2). In 19% of the patients, maintenance therapy was required. In 9% of the patients, recurrent disease was reported. Of these patients, the dose was either unknown or deviant from 1 g for 2 doses, two weeks apart. These relapses may be due to suboptimal treatment schedules of rituximab in IgG4-ROD.

Compared to other treatments modalities, rituximab is an efficient therapy for patients with refractive IgG4-ROD. Further prospective studies on the treatment of IgG4-ROD with rituximab are required.

Table 2. Rituximab doses in the treatment of patients with IgG4-related orbital disease.

Reference	Rituximab dosage
(58)	Two infusions of 1000 mg, two weeks apart; re-treated after six months.
(49)	Unknown
(62)	Unknown
(68)	Two infusions of 1000 mg, two weeks apart; re-treated after glucocorticoid therapy with 500 mg monthly for four months.
(69)	Two infusions of 1000 mg, two weeks apart; re-treated after five months with two series every six months.
(60)	Two infusions of 1000 mg, two weeks apart.
(70)	Two infusions of 1000 mg, two weeks apart.
(52)	Unknown
(72)	Unknown
(28)	Unknown
(29)	Two infusions of 1000 mg, two weeks apart with a median of two cycles (range 1-5).
(48)	Unknown
(53)	Eight cycles of 375 mg/m^2 monthly.
(61)	Two infusions of 1000 mg, two weeks apart with a median of 2 cycles (range 1-3).
(15)	Patient 1: 375 mg/m^2, four infusions at weekly intervals, followed by the same dose at 3, 4 and then 6-monthly intervals. Patient 2: 375 mg/m^2, four infusions at weekly intervals, followed by the same dose at 3 and 6-monthly intervals. Patient 3: 375 mg/m^2, four infusions at weekly intervals, followed by the same dose at 2-monthly intervals. Patient 4: 500 mg one infusion. Patient 5: 100 mg, two infusions two weeks apart.
(14)	Two infusions of 1000 mg, four weeks apart.
(65)	100 mg/week for four weeks.
(71)	Four infusions of 1000 mg/week.

Other treatment modalities

The alkylating agent cyclophosphamide is used as a cytostatic agent as well as an immunosuppressant in life- or organ-threatening autoimmune disease (95, 96). In IgG4-RD a combination of glucocorticoids and cyclophosphamide showed lower relapse rates.(96) Cyclophosphamide is reported in 16 patients. It must be noted that the follow-up time was short, with a mean follow-up of 6.3 month. Therefore, the relapse rate might be underestimated. Alternative agents such as indomethacin, cyclosporine A and tacrolimus have not been successful. Abatacept, a cytotoxic T lymphocyte-associated antigen 4 fusion protein that acts as a negative regulator of CD28-mediated T cell activation,(97) has exerted a response in the only patient treated with it. Because limited numbers of patients are described with those alternative agents, no substantial conclusions can be made.

Radiotherapy

Radiotherapy was used as additional therapy in 6 patients. Radiotherapy was used for recurrent disease or residual masses. In 4 patients a good response to radiotherapy was reported. Three of these patients had orbital radiotherapy, one patient received radiotherapy on a residual mass on the cheek. Therefore, radiotherapy might be considered in patients with localized refractory IgG4-ROD or patients with compressive optic neuropathy with a dose of 20-30 Gy in 10-15 fractions. The efficacy of orbital radiotherapy cannot yet be established due to the limited patient data.

Surgery

A total of 6 patients underwent surgery. Of these patients, 4 underwent additional surgery after systemic treatment and 2 underwent surgery before systemic treatment to resect IgG4-related lesions. One patient underwent primary surgical debulking after biopsy and of one patient the reason for surgery was not described. Both patients with surgery before systemic treatment required additional therapy and additional surgery was performed to excise remaining lesions. The value of surgical debulking requires future investigation, before conclusions on its efficacy can be drawn.

CONCLUSIVE REMARKS

In this systematic review of the literature, the reported outcomes of systemic treatment of patients with IgG4-ROD were evaluated. This study emphasizes the good response to glucocorticoids in general. However, the results of conventional DMARDs are rather disappointing. The high relapse rate indicates the need for maintenance therapy in IgG4-ROD patients. Rituximab is a promising treatment modality for induction or maintenance therapy and indicates the involvement of the B cell system in the pathophysiology. Rituximab needs to be studied in larger controlled trials.

This study has several limitations. Most of the included articles were 'successful' case reports or case series. This introduces the likelihood of reporting bias. Limited conclusions can be drawn due to the lack of large and randomized studies. Furthermore, the recently proposed IgG4-RD responder index (IgG4-RD RI) (98, 99) for assessing disease activity was only used in a minority of the cases (61, 100). Therefore, a number of included studies did not have standardized treatment outcomes. These limitations are most likely a result of the rarity of IgG4-ROD.

RECOMMENDATIONS FOR THE TREATMENT OF IGG4-RELATED DISEASE

The treatment of IgG4-ROD is often challenging. Studies included in the current consensus guidance show that treatment with conventional DMARDs in IgG4-RD is a second line treatment option. In the presented study with IgG4-ROD patients, we would rather favor the biologic DMARD rituximab as a secondary treatment strategy. Larger prospective studies are required to understand the role of conventional DMARDs and rituximab in IgG4-ROD. Based on our experience and on the presented data, we therefore suggest the following treatment strategy in IgG4-ROD:

- Glucocorticoids are the preferred first line systemic treatment of IgG4-ROD. The initial glucocorticoid dose is prednisone 0.5-1mg/kg/day.
- In severe refractory or vision-threatening disease, rituximab of 2 x 1 gram with 2 weeks in between combined with glucocorticoids tapered in 3 months is started. When indicated, a high dose of glucocorticoids pulse therapy (1 gram 1-3 days intravenously) can be used as induction strategy. Rituximab initiation will be followed every 6 months for the duration of 2 years. When refractory, cyclophosphamide can be initiated.
- In less severe cases, mycophenolate mofetil or infliximab can be considered as maintenance or steroid-sparing therapy.
- Consider radiotherapy or surgery in localized refractory disease in patients with compressive optic neuropathy or proptosis. The dose of radiotherapy is usually ranged between 20-30 Gy in 10-15 fractions.

APPENDIX 1. Search terms used in the medical databases for this systematic literature search in IgG4-ROD.

Embase.com

('immunoglobulin G4 related disease'/exp OR 'Mikulicz disease'/exp OR ((G4 OR igg4 OR 'igg 4' OR Mikulicz OR kuttner OR riedel*) NEAR/3 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*)):ab,ti) AND (therapy/exp OR therapy:lnk OR 'drug administration'/exp OR 'treatment outcome'/exp OR (therap* OR treat* OR agent* OR drug* OR corticosteroid* OR pharmac* OR prednisone OR rituximab OR Glucocorticoid* OR steroid* OR mycophenolat* OR azathioprine* OR thalidomide* OR dmard* OR administ* OR colchicin*):ab,ti)

Medline (OvidSP)

APPENDIX 2. Outcomes of included articles on DMARDs in IgG4-related orbital disease.

(Mikulicz' Disease/ OR ((G4 OR igg4 OR igg 4 OR Mikulicz OR kuttner OR riedel*) ADJ3 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*)).ab,ti.) AND (exp therapeutics/ OR therapy.fs. OR drug therapy.fs. OR exp treatment outcome/ OR (therap* OR treat* OR agent* OR drug* OR corticosteroid* OR pharmac* OR prednisone OR rituximab OR Glucocorticoid* OR steroid* OR mycophenolat* OR azathioprine* OR thalidomide* OR dmard* OR administ* OR colchicin*).ab,ti.)

Cochrane

(((G4 OR igg4 OR 'igg 4' OR Mikulicz OR kuttner OR riedel*) NEAR/3 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*)):ab,ti) AND ((therap* OR treat* OR agent* OR drug* OR corticosteroid* OR pharmac* OR prednisone OR rituximab OR Glucocorticoid* OR steroid* OR mycophenolat* OR azathioprine* OR thalidomide* OR dmard* OR administ* OR colchicin*):ab,ti)

Web-of-science

TS=((((G4 OR igg4 OR "igg 4" OR Mikulicz OR kuttner OR riedel*) NEAR/3 (rd OR related OR associat* OR autoimmun* OR disease* OR inflammat* OR tumor* OR thyroidit*)))

AND ((therap* OR treat* OR agent* OR drug* OR corticosteroid* OR pharmac* OR prednisone OR rituximab OR Glucocorticoid* OR steroid* OR mycophenolat* OR azathioprine* OR thalidomide* OR dmard* OR administ* OR colchicin*)))

Google scholar

"G4|igg4|Mikulicz|kuttner|riedel

rd|related|associat|autoimmune|disease|inflammation|tumor|thyroiditis"

therapy|treatment|agent|drugs|corticosteroids|prednisone|rituximab|Glucocorticoids|steroids|mycophenolate|azathioprine|thalidomide|dmards|colchicine

APPENDIX 2. Outcomes of included articles on DMARDs in IgG4-related disease

Reference	Age	Sex	IgG4 related orbital manifestation	Non-orbital organ manifestation	Serum IgG4 (mg/dl)	Therapy	Overall response	Follow-up (months)	Comment
(43)	14	F	Orbit H+	None	NM	Pred MMF	Partial	Unknown	Relapse under pred. MMF maintenance was required.
(58)	40	M	LG, orbit, EOM H+	Pancreas	662	Pred MTX 15mg/w RTX 1g/2w	Good	132	Orbitotomy was performed. Relapse after pred and MTX were discontinued. Pred and RTX gave complete remission.
(64)	38	F	LG, eyelid, sclera H+	None	NM	Pred 80mg/d NSAID eye drops 3dd Aza 125mg/d	Good	14	Relapse after tapering pred. Aza was added, with good response.
(49)	65	F	LG H+	SG, pancreas	NM	Pred 40mg/d CsA, mizo RTX, abatacept 500mg/4w	Good	8	Relapse after tapering pred. Insufficient efficacy with CsA and mizo. After six cycles of RTX, the patient was RTX resistant without anti-RTX antibodies. Abatacept was started, with good response.
(44)	71	F	EOM, LG, ON, IO H+	Sinus	202	Pred, MTX	Good	47	Excellent response to treatment.
	29	M	EOM, LG, ON, IO H+	Sinus	NM	Pred, tacrolimus, R/T 20-24 Gy, surgery	Good	33	Relapse after tapering pred. Received R/T and OD was performed.
(66)	40	M	Orbit, ON H+	None	NM	Pred, surgery, MP, IFM	Good	38	Failed response on oral steroids. Resection of lesion. Marked improvement on iv steroids and infliximab.
(45)	5	F	EOM H+	Sinus	152	Surgery, pred 1mg/kg/d MMF 600mg/m^2	Partial	Unknown	Surgical debulking. Weaned of pred, but required MMF as maintenance due to intermittent relapses.
(62)	79	F	Sclera, uvetitis H+	None	NM	Pred, cyclo, indomethacin, MTX, IFM	No	Unknown	Relapse after pred, cyclo and indomethacin. MTX and IFM had no response, leading to enucleation.
	76	M	LG H+	None	NM	Pred, RTX	Good	24	Relapse after tapering pred. No recurrence 2y after RTX.
(57)	66	M	Sclera, conjuncti-va, EOM H+	None	142	Pred 1mg/kg/d MTX 15 mg/w	Good	24	Previous subconjunctival injection of triamcinolone. No treatment after 2 months. Stable for 2y.
(54)	29	M	MD H+	LN, lung	Elevated	Pred, cyclo, MTX	Good	Mean 6.3 (2-13)	Pred was administered in all patients, with a dosage of 0.5-0.8mg/kg/d for 1 month, then tapered to £7.5 mg. The clinical condition improved after treatment. One patient received cyclo and MTX for refractory disease.
	41	F		Pancreas		Pred, cyclo			
	52	F		None		Pred, MTX			
	32	M	MD, orbit H+	None		Pred, cyclo			
	65	F	MD H+	Pancreas, BT, mediastinum		Pred, aza			
	46	F		Sinus		Pred, MTX			
	40	M		LN, pharynx		Pred, MTX			
	56	M		Brain		Pred, cyclo			
	51	M		Pancreas		Pred, MMF			
	57	M		Mediastinum, prostate		Pred, cyclo			
	73	M		RPF, TIN, LN		Pred, MMF			
	31	F		LN		Pred, cyclo			
	49	M		Pancreas, BT		Pred, cyclo			
	59	F		LN		Pred, cyclo			
	24	F		LN		Pred, cyclo			
	57	M		LN, prostate		Pred, cyclo			
	44	M		LN		Pred, cyclo			
(46)	69	M	LG, orbit H+	Skin	45.2*	Pred 80mg/d, aza 200mg/d, tamoxifen 20mg/d	Partial	Unknown	Aza was discontinued due to side effects. After pred was stopped, the patient had flares of skin manifestation. After tamoxifen was stopped, the patient relapsed.

(50)	63	F	Sclera H+	None	135	Pred 1mg/kg/d, MTX 20mg/w	Partial	12	Relapse after tapering pred, MTX was started. Stable on pred 3mg/d.
(55)	20	F	Sclera H+	None	97	Pred, MTX	Good	Unknown	After tapering, no relapse of disease.
(68)	33	M	LG H+	SG, LN, sinus	2690*	Pred 1mg/kg/d, DXM 40mg/d, RTX 1g iv 2 doses, fludarabine 40mg/m^2	Good	12	Relapse after tapering pred. DXM was complicated by pneumonia and admission to the ICU. Good clinical response on 3 cycles RTX/fludarabine
(69)	51	F	LG, eyelids H+	None	202	Pred 0.6mg/kg/d, RTX 1g iv 2 doses, surgery	Good	36	Relapse after tapering pred. Fibrosis of eyelid required surgery. 1y stable after treatment.
(51)	7	M	EOM H+	None	109	Pred 1mg/kg/d, aza 2mg/kg/d	Good	Unknown	Relapse after tapering pred. Improvement after therapy.
(60)	56	M	EOM, LG H+	None	670	Pred 80mg/d, MTX 25mg/w, MMF, RTX 1g iv 2 doses	Good	360	Not able to taper pred. Glucocorticoid-induced herpes zoster and necrosis of humeral heads. Relapse after 2y on pred and MTX. Refractory to MTX and MMF. RTX good response and pred was discontinued.
(59)	58	F	EOM, sclera, ON H+	Meckel's cave	143	Pred 50mg/d, aza 50mg/bid, MTX 2.5mg/w, cyclo	Partial	7	Radiological worsening post treatment with pred, aza, MTX. Stable on cyclo.
(70)	12	F	Orbit H+	Sinus	Normal	MP 1g iv, pred 40mg/d, RTX 1g iv	Good	12	Relapse on tapering pred. No recurrence 1y after RTX.
(56)	10	M	LG, EOM H+	None	Normal	MP 3.5mg/kg/d, pred 1mg/kg/d, MTX 15mg/m^2	Good	14	Only partial response on pred, complete response on pred and MTX.
(63)	61	F	Orbit H+	Meninges, masticator space	276	Pred 60mg/d, MTX, CsA, cyclo, IFM 200mg/8w	Partial	60	Steroid-induced DM. MTX, CsA, cylco without effect. Good response on IFM, maintenance IFM is required.
(18)	40	M	Sclera, LG, uveitis H+	None	127	MP, pred, cyclo, IFM 5mg/kg/2w	Partial	13	Progressive disease under pred and cyclo. Good response on IFM. Relapse after stop IFM.
(52)	61	M	LG H+	Sinus, lung, RPF, kidney, mastoid	Normal	MP, cyclo 800mg, RTX	Good	12	Progression under MP and cyclo. Regression on RTX.
(72)	67	M	LG, orbit H+	Pancreas	149	Pred 0.6mg/kg/d, RTX, R/T 20 Gy	No	23	Non-responder. Bilateral vision loss due to compressive optic neuropathy.

(28)	58	F	LG, eyelid H+	RPF, pancreas, SG, BT, LN	30	Pred, aza	Partial	115	Relapse after tapering pred. Pred maintenance required.
	68	M	EOM, orbit, ON, orbital bone H+	None	103	Pred, RTX	Partial	29	Relapse after tapering pred. Pred maintenance required.
	61	M	Orbit H+	SG	32	Pred, RTX	Partial	23	Relapse after tapering pred. Pred maintenance required.
	53	M	LG H+	Pancreas, SG, TIN, LN	13	Pred, RTX	Partial	47	Relapse after tapering pred. Pred maintenance required.
	61	F	Orbit, EOM, V1 and V2 nerves H+	None	730	Pred, aza, RTX	Partial	112	Relapse after tapering pred. Pred maintenance required.
	35	M	LG H+	TIN, prostate, testis, lung, pancreas, LN	1850	Pred, aza, RTX	Good	40	Relapse after tapering pred. After aza and RTX stable without treatment.
	22	M	LG, EOM, eyelid H+	Sinus, pancreas, SG, TIN, LN, lung	300	Pred, MTX	Partial	52	Relapse after tapering pred. Pred maintenance required.
	38	F	Orbit, EOM H+	None	104	Pred, RTX	Good	19	No relapse, stable without treatment.
	42	M	Orbit H+	None	NM	Pred, RTX	Partial	51	Relapse after tapering pred. Pred maintenance required.
	65	M	Orbit, EOM, orbital bone H+	Sinus	NM	Pred, aza, RTX, MMF	Partial	32	Relapse after tapering pred. MMF maintenance required.
	70	F	LG, eyelid H+	SG, LN, preverterbral, RPF	500	Pred, aza, MMF	Partial	60	Relapse after tapering pred. Pred and MMF maintenance required.
	54	M	LG, orbit, EOM, V2 nerve H+	SG, LN	1225	Pred, aza, MMF, RTX	Good	50	Relapse after tapering pred. Stable without treatment.

(29)	45	M	EOM, orbit H+	SG	NM	Pred, MTX, RTX	Good	36	Persistent disease on pred and MTX. Complete response on RTX.
	59	F	LG, EOM, V1 nerve H+	SG		Pred, MTX, RTX	Good	36	Unable to taper pred and MTX. Complete response on RTX.
	72	F	LG H+	SG, skin		Pred, RTX	Partial	24	Pred no significant effect. Improvement on RTX.
	29	M	LG, EOM, V2 nerve H+	None		MTX, pred, RTX	Partial	36	Relapse on MTX. Relapse without pred. Improvement on RTX.
	24	F	Orbital bone H+	Skull bone, meninges, sinus		Pred, aza, MTX, RTX	Partial	12	Relapse when pred was tapered. No clear response on MTX or aza. Improvement on RTX.
	60	F	Orbit H+	None		Surgery, pred, RTX	No	24	No response on pred. Worsening of disease under RTX.
	63	M	LG, orbit, EOM H+	Pancreas, pericardium		RTX	Partial	12	Improvement with treatment.
	42	M	Palpebra H+	None		Pred, RTX	Partial	Unknown	Relapse after tapering pred. Improvement on RTX.
	52	F	NLD H+	Pancreas, RPF, SG		MTX, surgery, RTX	Partial	Unknown	Recurrence after treatment.
	57	M	LG H+	SG, pancreas		MTX, CsA, pred, RTX	Good	Unknown	Relapse when off pred. Unable to taper MTX. Complete response on RTX.
	38	F	LG H+	Thyroid, lung, BT		RTX	No	36	No change in outcome on RTX.
	21	M	Sclera H+	LN, SG		RTX	Good	36	Complete response on RTX.
	55	M	Orbit H+	None		Pred, RTX	Partial	12	Disease worsened on pred. Improvement on RTX.
	54	M	NLD H+	Pancreas, RPF, LN, lung, palate		RTX	Good	12	Complete response on RTX.
	37	M	LG H+	Pancreas, prostate		Pred, RTX	Partial	12	Relapse after tapering pred. Improvement on RTX.
	58	M	LG H+	SG, LN, skin		RTX	Partial	Unknown	Improvement on RTX.
	79	M	LG H+	Lung, pancreas, LN, kidney, RPF		Pred, RTX	Good	Unknown	Relapse after tapering pred. Complete response on RTX.
(47)	13	F	LG, eyelid H+	Pelvis, kidney	NM	Pred 30mg/d, IFM	Partial	Unknown	Relapse after tapering pred. Abdominal masses improved on IFM. The LG mass was excised.
(48)	39	F	LG, EOM H+	None	NM	RTX	Good	38	One relapse after RTX. Stable without disease.
	40	M	LG, EOM H+	LN	NM	Pred, RTX, R/T	Good	25	No relapse after treatment.
	37	M	LG, orbit, ON H+	Pancreas, LN, cavernous sinus	NM	RTX	Good	96	Stable disease.
	70	M	EOM, IO H+	BT, cavernous sinus	563	RTX	Partial	54	Maintenance therapy required.
	30	M	LG H+	Liver, LN	167	RTX	Partial	38	Recurrence after RTX.
	52	F	LG H+	SG	47.7	RTX	Partial	46	Maintenance therapy required.
	53	F	LG, orbit H+	None	NM	RTX	Good	5	No relapse after treatment.

(53)	54	F	LG H+	SG	1262	Pred 0.6mg/kg/d, RTX, R-CHOP, R/T	Partial	30	Previously treated with R-CHOP, R/T, RTX. Good response, but recurrence after treatment. Stable on maintenance pred.
(61)	55	M	Orbit H+	SG	1560	Pred, MTX, RTX	Good	12	IgG4-RD RI 5 to 0 points after RTX.
	72	F	LG H+	Skin	1140	Pred, RTX	Good	6	IgG4-RD RI 6 to 1 point after RTX.
	56	M	LG H+	None	670	Pred, MTX, MMF, RTX	Good	6	IgG4-RD RI 3 to 1 point after RTX.
	40	F	LG H+	Thyroid, LN, lung	70.8	Pred, tamoxifen, RTX	Partial	6	IgG4-RD RI 6 to 3 points after RTX.
	57	F	Orbit H+	SG	58	Pred, MTX, RTX	Good	6	IgG4-RD RI 2 to 0 points after RTX.
(15)	46	F	Orbit, EOM H+	None	NM	Pred 1mg/kg/d, RTX 600mg iv	Partial	24	Triamcinolone injection subconjunctival before pred. Relapse after tapering pred. RTX maintenance required.
	49	M	Orbit, LG, EOM H+	None	NM	Pred 1mg/kg/d, RTX 800mg iv	Good	57	Relapse after tapering pred. Subconjunctival injection triamcinolone with moderate improvement. Complete response after RTX.
	64	M	Orbit, LG, EOM H+	SG, LN	NM	Pred 1 mg/kg/d, RTX 700mg iv, R/T	Good	38	Relapse after tapering pred. Complete remission after RTX. Residual mass on cheek treated with R/T.
	83	M	EOM, LG H+	SG	271	Pred 1mg/kg/d, RTX 500mg iv	Good	7	Relapse after tapering pred. Good response after RTX, no relapse.
	48	F	Orbit, LG H+	None	152	Pred 50mg/d, aza 100mg/d, RTX 1000mg iv, MMF	Partial	30	Relapse after tapering pred. Aza side effects. Clinical remission after RTX, MMF maintenance therapy required.

(14)	54	F	Orbit H+	SG	401	Pred 15mg/d, MTX 17.5mg/w, RTX	Good	20	Side effects of pred and MTX. Relapse after tapering. After RTX stable disease.
	53	M	Orbit, EOM H+	Cranial nerve, sinus, LN, SG	1560	Pred 60mg/d, MTX 15-20mg/w, RTX		12	Side effects of MTX and pred. RTX good response, pred stop.
(65)	57	M	Orbit H+	Lung, pituitary, SG, sinus, paravertebral, gall bladder, intestine	83.9	MP 36mg/d, aza 100mg/d, RTX 100mg/w	Good	6	Relapse after tapering MP. Complete remission after RTX.
(71)	74	M	LG, EOM H+	Skin, LN	3820	MP 1g iv, Pred 100mg/d, R/T 20 Gy, RTX 1g iv	Good	23	Relapse after MP. Start R/T and RTX. Stable disease after treatment.
(67)	9	F	LG H+	None	NM	Pred 40mg/d, adalimumab 40mg/2w	Partial	12	Relapse after tapering pred. Improvement with adalimumab, but maintenance adalimumab required.

*On treatmentAza: Azathioprine, BT: Biliary tract, CsA: Cyclosporine A, Cyclo: Cyclophosphamide, DM: Diabetes mellitus, DMARD: disease modifying antirheumatic drug, DXM: Dexamethasone, EOM: Extra ocular muscle, F: Female, H+: Histology performed, H-: Histology not described/performed, ICU: Intensive care unit, IFM: Infliximab, IgG4-RD, RI: IgG4-related disease responder index, IgG4-ROD: IgG4-related orbital disease, IO: Infraorbital nerve, LG: Lacrimal gland, LN: Lymph node, M: Male, MD: Mikulicz's disease (defined as bilateral involvement of lacrimal and salivary glands), MMF: Mycophenolate mofetil, MP: Methylprednisolone, Mizo: Mizoribine, MTX: Methotrexate, NA: Not applicable, NLD: Nasolacrimal duct, NM: Not measured, NSAID: Nonsteroidal anti-inflammatory drugs, OD: Orbital decompression, ON: Optic nerve, Pred: Prednisone, R-CHOP: Rituximab/cyclophosphamide/doxorubicin/vincristine/prednisolone, RPF: Retroperitoneal fibrosis, RTX: Rituximab, R/T: Radiotherapy, SG: Salivary gland, TIN: Tubulointerstitial nephritis, Y: Year

No response was defined as no improvement or worsening of the disease. Partial response was defined as improvement of the disease, but not complete remission or maintenance therapy was required. Good response was defined as complete remission or stable without treatment.

Chapter 5.3 The treatment outcomes in IgG4-related disease

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ABSTRACT

Introduction: IgG4-related disease (IgG4-RD) is an emerging systemic inflammatory disease involving nearly all organs eventually leading to fibrosis. Prompt and adequate treatment to prevent irreversible organ damage is therefore pivotal. To evaluate the treatment outcomes, we studied a well-defined cohort of patients with IgG4-RD.

Method: 32 patients with histologically confirmed IgG4-RD diagnosed between 1999 and April 2017 were included and reviewed for demographic and clinical characteristics. The response to treatment with glucocorticoids, disease modifying antirheumatic drugs, rituximab and other therapeutic interventions were evaluated.

Results: Glucocorticoids as well as rituximab appeared successful therapeutic drugs leading to clinical remission (complete or partial remission) in all patients. Recurrences however, were frequently (62% versus 100%, respectively) seen. Diseases modifying antirheumatic drugs (DMARD's), including azathioprine, methotrexate and mycophenolate mofetil were effective in less than half of the cases. A minority of patients was treated with alternative treatments including hydroxychloroquine, thalidomide and infliximab which all appeared effective. Surgical intervention and radiotherapy in local disease seemed to induce clinical remission and were associated with low recurrence rates.

Conclusion: Glucocorticoids and rituximab induce substantial responses as well as primary surgical intervention and radiotherapy, while the efficacy of DMARD's is limited. Based on the few data available, hydroxychloroquine, infliximab and thalidomide may be promising treatment options for second or third line strategies.

INTRODUCTION

IgG4 related disease (IgG4-RD) is a systemic fibro-inflammatory disease potentially affecting all parts of the human body (101). Eventually fibrosis may lead to irreversible organ damage and even secondary amyloidosis may occur (34). Therefore early recognition and swift initiation of adequate therapy remain critical (102-104).

The pathogenesis of IgG4-RD is still unclear and the trigger causing the inflammation seen in IgG4-RD is unknown, but recently evolving knowledge is leading to a better understanding of the disease. The elevated IgG4 levels and the good response of IgG4-RD patients to treatment with rituximab (B-cell depletion treatment), suggest a role of the humoral immune system in the pathogenesis of IgG4-RD (105, 106). IgG4 positive B-cells have been studied in IgG4-RD demonstrating increased numbers of blood IgG4 positive B-cells in patients compared to controls (107, 108). In addition, oligoclonally circulating total plasmablasts, are increased and appear to play a role in IgG4-RD and the number of plasmablasts decreased after B-cell depletion (105, 109, 110). These plasmablasts show extensive somatic hypermutation (SHM) in the rearranged variable regions, which indicates a T-cell depended response (110, 111). Different T-cell subsets have been studied in IgG4-RD and have shown that T-cells are also important in the pathogenesis of IgG4-RD (105, 108, 112). T follicular helper-2 (Tfh2) cells are possibly involved in driving the class switch to IgG4 (113). Different cytokines, including interleukin (IL)-4 derived from T-helper 2 (Th2) cells may also contribute to the pathophysiology of IgG4-RD (108, 114-116), but the role of Th2 cells in IgG4-RD remains unclear (117, 118). Recently, CD4+ T-cells displaying cytotoxic features appeared to be abundantly present in peripheral blood and diseased tissue sites of patients with IgG4-RD and possibly also contribute to the pathogenesis of the disease (112, 119).

Glucocorticoids are the first choice of treatment. The relapse rate after tapering glucocorticoids is high, hence steroid-sparing maintenance therapy is often required (36). Several disease modifying anti-rheumatic drugs (DMARD's) such as methotrexate and azathioprine have been used as steroid-sparing treatment of IgG4-RD, but studies confirming their efficacy are lacking (11). Most of the reports on the use of steroid-sparing treatment with DMARD's are case-based reports (3). Emerging data from case series reveal promising results for rituximab in the treatment of patients with IgG4-RD (101, 120-122). The aim of the current study is to evaluate the different treatment outcomes in a well-defined cohort of patients with IgG4-RD.

MATERIALS AND METHODS

The Erasmus University Medical Center represents a tertiary referral centre for patients with IgG4-RD. All patients diagnosed with IgG4-RD are treated and monitored prospectively, however, patients may also be diagnosed with IgG4-RD retrospectively. Medical records of patients with IgG4-RD between 1999 and April 2017 were reviewed for demographic and clinical characteristics. Only patients with histologically confirmed IgG4-RD according to established Boston criteria for histology were included (123). The efficacy of all therapies including glucocorticoids, DMARD's (mycophenolate mofetil, methotrexate and azathioprine), hydroxychloroquine, cyclophosphamide, rituximab, thalidomide, infliximab, surgery and radiotherapy were evaluated. The disease activity in patients was evaluated using the IgG4-Related Disease Responder Index (IgG4-RD RI), a monitoring tool for disease activity in IgG4-RD using the clinical, laboratory and radiological outcomes (98). IgG4-RD RI is designed for the physicians to easily score the extent of the disease activity (124). A score of 0 signifies the absence of active disease in an organ, a score of 1 indicates improvement of the disease activity within an organ, a score of 2 indicates that the disease within an organ has remained unchanged, a score of 3 indicated the presence of new or recurrent disease activity and a score of 4 refers to disease that has worsened despite treatment (124). The levels of serum IgG4 are scored as same manner and scored according to normal, improved, persistent or new/recurrent/worsened despite treatment (124).

Active disease was defined by an IgG4-RD RI score of ≥ 3 (96, 125). Improvement in the disease activity and complete response are defined as decline of ≥ 2 points compared to the baseline score or IgG4-RD RI of < 3 and decline ≥2 after treatment, respectively. Partial response and disease relapse were defined as decline of ≥ 2 points in IgG4-RD RI, but still ≥ 3 and worsening of clinical, radiological and serological (serum IgG4) findings, respectively.

This study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Erasmus MC (ethics approval numbers MEC-2017-1169).

RESULTS

In total 32 patients with IgG4-related disease were included with a mean age of 57 years, ranging from 17 to 77 years, of which 72% were male. The main outcomes of this study are presented in Table 1. An overview of the different treatment modalities in these 32 patients is presented in Table 2A and the different treatment outcomes (response, failure and relapse) for each treatment strategy are demonstrated in Table 2B.

The patients represented a heterogeneous group of IgG4-RD with different organ manifestations. Most of the patients had ocular (53%) manifestation of the disease. The medical history was unremarkable in most of the cases, though 5 patients had asthma. All the cases were histologically confirmed using the Boston consensus criteria for histology (123).

Almost all patients (29/32= 91%) were treated with glucocorticoids (mostly prednisone 0.5-1 mg/kg/day) leading to clinical response in all. In the remaining 3 patients, initial treatment with methotrexate followed by hydroxychloroquine in 1 patient and surgery in 2 patients led to complete response. In 27 (27 out 29) patients glucocorticoids were initiated initially and in 2 (2 out 29) patients glucocorticoids were started after relapse or failure of the initial glucocorticoid sparing regime (patients 4 and 6). In 10 (10 out 27) patients glucocorticoids were initiated as monotherapy and in the remaining patients these were combined (Table 2A).

Complete response was observed in the 72% of the patients receiving glucocorticoids, versus a partial response in 28%. Glucocorticoids were usually continued for a period of 4 to 6 weeks and thereafter tapered slowly and withdrawn in a period of 3 months to 1 year. Only in 2 patients a short course of prednisone was started after initial surgical excision of the affected tissue. Despite a good clinical response initially, a flare of the disease occurred when the glucocorticoids were tapered or discontinued in 62% of the patients necessitating additional (steroid-sparing) treatment. Methotrexate was initiated in 7 patients usually in a dose of 15 mg per week, being effective in a minority (= 29%, 1 complete response and 1 partial response). In the 5 other patients methotrexate failed to suppress the disease activity. In 1 of these 5 patients, methotrexate also caused gastro-intestinal toxicity.

In total 12 patients were treated with azathioprine, with only one complete response (8%), concerning a patient with pancreas and lymph node involvement in which azathioprine was successfully continued as maintenance treatment after prednisone induction

therapy. In 6/12 patients (50%) azathioprine was terminated because of toxicity (liver, gastro-intestinal, muscle symptoms) before disease activity could be assessed. In the remaining 5/12 patients azathioprine proved to be insufficient to suppress the disease activity and led to discontinuation of this treatment (treatment duration > 3 months).

Mycophenolate mofetil was initiated as maintenance treatment after steroid induction in 3 patients. Two patients showed treatment failure. One patient, with renal and lymph-node involvement of IgG4-RD refractory to prednisone tapering, azathioprine and rituximab showed a partial response with low dose prednisone and mycophenolate mofetil.

In 4 patients hydroxychloroquine was used as a maintenance therapy showing complete response in 2 patients (50%), after initial therapy with methotrexate and prednisone respectively. In one (25%) patient initial therapy with hydroxychloroquine was ineffective and replaced by prednisone resulting in clinical remission. In another patient hydroxychloroquine was withdrawn because of gastro-intestinal side effects.

Cyclophosphamide was used in 3 patients with organ function threatening disease after induction treatment with prednisone, which caused complete remission of the disease in only 1 (33%) patient, however, the remission was temporary and relapse occurred thereafter.

Among other agents cyclosporine A was used once without clinical effect. Thalidomide was initiated in 1 case of therapy refractory salivary disease leading to complete remission of the disease. However, the patient experienced neurological side effects and is using thalidomide now on demand.

Table 1. Patient characteristics and treatment outcomes , treatment failures, side effects and current treatment of patients

Patient characteristics											
Patient	**Age**	**Sex**	**Medical history**	**Diagnosis**	**Elevated serum IgG4**	**Initial treatment (IgG4-RD RI at presentation)***	**Therapy failure**	**Therapy side effects**	**Therapy response**	**Relapse after therapy**	**Current status / treatment**
1	60y	F	-	IgG4-related skin disease	Yes	Methotrexate (4)	Methotrexate	Methotrexate (liver toxicity)	Hydroxychloroquine (CR)	NA	Hydroxychloroquine
2	55y	M	Asthma	IgG4-related orbital, lymph node and skin disease	Yes	Prednisone (8)	-	-	Prednisone (PR)	Prednisone	Prednisone. Rituximab is being considered.
3	38y	M	-	IgG4-related salivary disease	Yes	Prednisone and azathioprine (4)	Mycophenolate mofetil, azathioprine	Thalidomide (neuropathy)	Prednisone (CR), rituximab (PR), thalidomide (CR)	Prednisone, rituximab	Thalidomide on demand due to neurological side effects
4	45y	M	-	IgG4-related orbital disease.	Yes	Hydroxychloroquine (4)	Hydroxychloroquine	-	Prednisone (CR)	NA	No treatment
5	65y	M	-	IgG4-related orbital disease and scleritis.	No	Prednisone and azathioprine (4)	-	Azathioprine (liver toxicity)	Prednisone (CR)	NA	No treatment
6	43y	M	2006: cervical follicular B-cell lymphoma: later revised to IgG4-RD	IgG4-related lymph node and renal disease	Yes	Radiotherapy (3)	-	-	Radiotherapy (CR), prednisone (PR), rituximab (CR), mycophenolate mofetil (PR)	Radiotherapy, prednisone, rituximab	Mycophenolate mofetil and low dose prednisone
7	68y	F	Carpal tunnel syndrome	IgG4-related orbital disease	Yes	Prednisone and Mycophenolate mofetil (6)	Mycophenolate mofetil	-	Prednisone (CR), rituximab (CR)	Prednisone tapering, rituximab	Dexamethasone tapering
8	77y	M	Asthma, hypertension	IgG4-related skin and lymph node disease	Yes	Prednisone and azathioprine (8)	-	Azathioprine (liver toxicity)	Prednisone (CR), rituximab (CR)	Prednisone, rituximab	Rituximab every 6 months
9	61y	M	-	IgG4-related orbital and lymph-node disease	Yes	Prednisone and methotrexate (6)	Methotrexate	-	Prednisone (CR)	Prednisone	Rituximab is being initiated
10	63y	F	-	IgG4-related orbital disease with epilepsy, vision loss and trismus	Yes	Prednisone (4)	Methotrexate, cyclosporine A, cyclophosphamide	Prednisone (diabetes mellitus)	Prednisone (CR), infliximab (CR)	Prednisone	Infliximab
11	64y	M	Diabetes mellitus type 2 and asthma.	IgG4-related orbital, lymph-node, pancreas and prostate disease.	Yes	Prednisone and methotrexate (10)	-	-	Prednisone (PR), methotrexate (PR)	NA	Methotrexate
12	60y	M	Diabetes mellitus type 2 and asthma.	IgG4-related orbital, lymph node, pancreas and prostate disease	Yes	Prednisone and azathioprine (10)	-	Azathioprine (gastro-intestinal toxicity)	Prednisone (CR)	Prednisone	Prednisone. Rituximab is being considered
13	64y	M	Hypothyroidism	IgG4-related orbital, lymph node, thyroid, prostate and pancreas disease	Yes	Prednisone (10)	-	-	Prednisone (CR)	Prednisone	Prednisone. Rituximab is being considered.
14	17y	M	-	IgG4-related lung, lymph node and cerebral disease	Yes	Dexamethasone and azathioprine (8)	Azathioprine	-	Dexamethasone (PR), rituximab (PR)	Dexamethasone, rituximab	Rituximab every 6 months
15	63y	F	Hypothyroidism and COPD Gold 1	IgG4-related pancreatitis, lung, lymph node and salivary gland disease	No	Partial pancreas resection followed by prednisone (8)	-	-	Surgery (CR), prednisone short course (CR)	NA	No treatment
16	54y	M	Mesenteric mass for 16 years	IgG4-related mesenteric disease	Yes	Prednisone and azathioprine (4)	Azathioprine	-	Prednisone (PR), rituximab (PR)	Prednisone, rituximab	Methylprednisolone monthly
17	63y	M	Optic neuritis	IgG4-related thyroid disease	Yes	Hemithyroidectomy followed by prednisone (6)	-	-	Surgery (CR), prednisone short course (CR)	NA	No treatment
18	59y	M	-	IgG4-related lung disease and ocular disease	No	Surgical resection lung lesion followed by prednisone and methotrexate (4)	-	-	Surgery (PR), prednisone (CR), methotrexate (CR)	NA	No treatment anymore
19	33y	M	-	IgG4-related pericarditis and pleural disease	Yes	Pericardiectomy followed by prednisone (8)	-	-	Surgery (PR), prednisone (CR)	NA	No treatment

20	42y	M	-	IgG4-related orbital and salivary disease	Yes	Prednisone (6)	Azathioprine	-	Prednisone (CR), hydroxy-chloroquine (CR)	Prednisone tapering	Hydroxychlo-roquine
21	42y	F	-	IgG4-related periorbit-al disease and scleritis	No	Prednisone and azathioprine (4)	-	Azathioprine (muscle pain)	Prednisone (CR)	NA	No treatment
22	70y	M	Myocardial infarction	IgG4-related orbital, nose and salivary gland disease	Yes	Prednisone (8)	-	-	Prednisone (CR)	NA	No treatment
23	60y	M	COPD Gold 1	IgG4-related cholan-gitis, lymph node and nasal disease	Yes	Prednisone (8)	Methotrexate	Azathioprine (gastro-intesti-nal toxicity) Hydroxy-chloroquine (gastro-intesti-nal toxicity)	Prednisone (CR),	Prednisone	Prednisone. Rituximab being consid-ered
24	65y	M	-	IgG4-related orbital, lymph node and pancreas disease	Yes	Prednisone (8)	-	-	Prednisone (PR)	Prednisone	Prednisone. Additional treatment is being consid-ered
25	74y	F	Asthma, acute rheumatic fever	IgG4-related lung diseases	Yes	Surgical resec-tion lung lesion (4)	-	-	Surgery (CR)	NA	No treatment after surgery
26	65y	M	Benign prostate hypertrophy	IgG4-related retroperi-toneal fibrosis	Yes	Prednisone (6)	-	Azathioprine (gastro-intesti-nal toxicity)	Prednisone (CR)	Prednisone	No treatment
27	53y	M	Chronic sponta-neous urticaria	IgG4-related orbital and nasal disease	Yes	Prednisone and methotrexate (6)	Methotrexate	-	Prednisone (CR)	Prednisone	Rituximab is being initiated
28	73y	M	Prostate carci-noma for which radiotherapy	IgG4-related orbital and nasal disease	No	Prednisone and azathioprine (6)	Azathioprine, cyclophospha-mide	-	Prednisone (PR), surgery (enucleation = CR)	Prednisone tapering	No treatment after surgery
29	50y	F	-	IgG4-related orbital disease	No	Prednisone (4)	-	-	Prednisone (CR)	NA	No treatment
30	54y	F	-	IgG4-related orbital disease	No	Prednisone (2)			Prednisone (PR), cyclo-phosphamide (CR) Radiotherapy (CR)	Prednisone, cyclophospha-mide	No treatment after radio-therapy
31	71y	M	-	IgG4-related pancre-atitis and lymph node disease	Yes	Prednisone and azathioprine (8)	-	-	Prednisone (CR), azathio-prine (CR)	NA	Azathioprine
32	61y	F	-	IgG4-related lung disease presenting as recurrent tumors.	No	Surgical resec-tion lung lesions (6)	-	-	Surgery (CR)	NA	No treatment after surgery

CR = complete response; PR = partial response; IgG4-RD RI = IgG4-Related Disease Responder Index

*IgG4-RD RI at baseline. The activity scores during the follow-up are not shown, but are available upon request.

Table 2. An overview of the different treatment modalities

Initial therapy	N = 32	Additional therapy	Maintenance therapy
Glucocorticoids	10	Glucocorticoids (n = 7) Glucocorticoids + cyclophosphamide + Radiotherapy (n = 1)	6 (60%): Glucocorticoids (n = 4) Hydroxychloroquine (n = 1) Infliximab (n = 1)
Glucocorticoids + Methotrexate	3	Glucocorticoids (n = 2)	3 (100%): Glucocorticoids (n = 2) Methotrexate (n = 1)
Glucocorticoids + Azathioprine	9	Glucocorticoids (n = 1) Glucocorticoids + surgery (n = 1) Glucocorticoids + rituximab (n = 4)	5 (67%): Azathioprine (n = 1) Glucocorticoids (n = 2) Rituximab (n = 1) Thalidomide on demand (n = 1)
Glucocorticoids + Mycophenolate mofetil	1	Glucocorticoids + Rituximab	1(100%): Glucocorticoids
Methotrexate	1	Hydroxychloroquine	1 (100%): Hydroxychloroquine
Hydroxychloroquine	1	Glucocorticoids	0
Surgery	2	0	0
Surgery + glucocorticoids	3	0	0
Surgery + glucocorticoids + methotrexate	1	0	0
Radiotherapy	1	Glucocorticoids, rituximab, mycophenolate mofetil	1 (100%): Mycophenolate mofetil

Therapy	N = 32, including 76 treatment episodes	Complete response	Partial response	Therapy failure	Relapse after initial response	Side effects
Table 2B. ***Response to different treatment modalities in patients with IgG4-related disease***						
Glucocorticoids	29	21 (72%)	8 (28%)	0	18 (62%)	1 (3%)
Methotrexate	7	1 (14%)	1 (14%)	5 (71%)	0	1 (14%)**
Azathioprine	12	1 (8%)	0	5 (42%)	0	6 (50%)
Mycophenolate mofetil	3	0	1 (33%)*	2 (67%)	0	0
Hydroxychloroquine	4	2 (50%)	0	1 (25%)	0	1 (25%)
Cyclophosphamide	3	1 (33%)	0	2 (67%)	1 (33%)	0
Thalidomide	1	1 (100%)	0	0	NA	1 (100%)
Cyclosporine A	1	0	0	1 (100%)	NA	NA
Infliximab	1	1 (100%)	0	0	0	0
Rituximab	6	3 (50%)	3 (50%)	0	6 (100%	0
Surgery	7	5 (71%)	2 (29%)	0	0	0
Radiotherapy	2	2 (100%)	0	0	1 (50%)	0

NA = not applicable (because treatment was discontinued)*In combination with low dose prednisone
**A patient with therapy failure and gastro-intestinal side effects

Infliximab was used once in a patient with orbital disease refractory to prednisone tapering, methotrexate, cyclosporine A and cyclophosphamide. Infliximab has induced a complete remission and the disease has been in remission since more than 5 years (126). Rituximab was initiated, usually as third line treatment, in 6 patients as maintenance therapy (50% complete response versus 50% partial response). The clinical remission after single dose of 2 gram observed in these patients was however temporarily and relapse of the disease occurred in all these patients within 6 months to 2 years after initiating rituximab. Two patients with relapse after treatment with rituximab are currently receiving rituximab every 6 months with complete remission of the disease.

Primary surgical intervention in 7 patients included hemithyroidectomy, resection of pulmonal masses, pericardiectomy and partial pancreas resection led to durable complete responses (median = 36 months). In 2 cases with solitary pulmonary involvement, resection of the lung lesions was performed and there was no further indication for systemic immunosuppressive therapy. In 1 case with orbital and nasal involvement, enucleation of the eye was eventually performed because of persistent symptoms of pain after treatment failure to prednisone tapering, azathioprine and cyclophosphamide. The enucleation led to clinical remission and pain symptoms resolved. Diagnosis of IgG4-RD was established retrospectively after enucleation (127). In two other patients short course of prednisone after surgery was sufficient to achieve complete remission of the disease. Furthermore, radiotherapy was used in 2 patients for lymph node involvement and therapy refractory IgG4-related orbital disease respectively, causing complete remission in both cases. The patient with lymphadenopathy developed a recurrence of the lymphadenopathy with new onset renal involvement of the disease a couple of years later for which the patient started with systemic immunosuppressive therapy. The other patient is in clinical remission 5 years after radiotherapy.

DISCUSSION

With this study we describe the treatment outcomes in 32 IgG4-RD patients with various organ manifestations. The observations in the presented study emphasize that the treatment of IgG4-RD can be challenging and is often tailor made.

The current study represents a heterogeneous cohort of patients with various organ manifestations. As in most studies IgG4-RD occurs mostly in men and occurs in middle-aged patients (36). However, in a previously published systemic review we demonstrate that IgG4-RD can also affect children of all ages (82).

Glucocorticoids

Glucocorticoids are commonly initiated as immunosuppressive induction therapy. Various types of cells of the immune system, including B and T-cells and macrophages, can be influenced by glucocorticoids (128). Glucocorticoids are considered as the mainstay treatment of IgG4-RD and usually effective in a dose of 0.5-1.0mg/kg/day, depending on severity or organ threatening disease despite the absence of randomized clinical trials (11, 101, 129). In the presented study a high response rate is followed by swift recurrences after tapering prednisone in figures corresponding previous reports (11). The high response rates warrant the use of glucocorticoids as first line treatment despites the frequent recurrences. Such high recurrence rates emphasize the need of alternative steroid-sparing therapy which has been advocated in many case series (11). The results in the current study alternative treatments are discussed below.

Azathioprine

Azathioprine is frequently used in immune mediated diseases (130). Among DMARD's, the use of azathioprine has been most frequently reported in patients with IgG4-RD, especially in IgG4-related pancreatitis (125). According to the consensus guidance, azathioprine, methotrexate or mycophenolate mofetil may be initiated as prednisone sparing therapy in IgG4-RD (11). A recent study of 18 patients showed therapeutic efficacy for azathioprine in preventing relapse of IgG4-related pancreatitis (131). Furthermore, case reports have suggested azathioprine to be effective in several manifestations such as IgG4-related cholangitis, IgG4-related kidney disease and hypophysitis (132-134). In the presented study however, azathioprine appeared effective in only a minority (8%). The only patient with a clinical response on azathioprine had inflammatory pancreatic disease and enlarged lymph nodes. The remaining 10 patients had other disease manifestations without pancreatic involvement. Therefore azathioprine may serve as second

line therapy for this subgroup of patients with pancreatic localizations. Remarkably, the continuation of azathioprine was restricted because of toxicity in a substantial number of patients. This toxicity is not known to appear in such high percentages. It remains of interest whether this is a disease specific complication. The efficacy of azathioprine in IgG4-RD remains therefore unclear.

Methotrexate

Except for two small studies suggesting methotrexate as a good steroid-sparing drug (100, 125), its application in IgG4-RD has only been described in limited case reports (55). Methotrexate affects the function of memory T-cells that produce pro-inflammatory and pro-fibrotic cytokines and is therefore a drug of interest in the treatment of IgG4-RD (135). In the current study however, methotrexate does not seem to be very beneficial, despite its low toxicity.

Mycophenolate mofetil

Mycophenolate mofetil selectively inhibits cytotoxic T-lymphocytes and is being used as an anti-inflammatory agent. Furthermore, mycophenolate mofetil is related with anti-fibrotic effects possibly due to inhibition of the transforming growth factor beta pathway (88) and can therefore be a potential interesting drug in the treatment of IgG4-RD. Just like the other DMARD's, mycophenolate mofetil has not been studied in large cohorts, but reported in case reports (89, 136-138). In the current study, mycophenolate mofetil caused partial remission in combination with low-dose prednisone in only 1 of 3 patients without toxicity. The use of mycophenolate mofetil therefore may be evaluated in larger cohorts.

Hydroxychloroquine

Hydroxychloroquine is initially designed as an antimalarial drug, but because of the accompanying antirheumatic effects broadly used in rheumatic and autoimmune disease. The exact role of hydroxychloroquine has not been identified, but it is believed that it has anti-inflammatory and possibly also anti-fibrotic effects (139-141). In current study hydroxychloroquine was initiated because of its positive outcomes in other inflammatory diseases like sarcoidosis (142). There are no published reports on

hydroxychloroquine in IgG4-RD, but this is often used in treatment of other immune mediated diseases. In our study, hydroxychloroquine was used in 4 cases as maintenance therapy and showed complete response in 2 (50%) patients. Gastro-intestinal complaints led to withdrawal of hydroxychloroquine in 1 (25%) patient. Generally, the drug is not associated with severe adverse effects except for retinal toxicity at higher doses (143). The efficacy of hydroxychloroquine needs to be evaluated in larger cohorts, but in cases of less severe IgG4-RD, it may be a potential drug to initiate.

Cyclophosphamide

Cyclophosphamide has been regarded ineffective in IgG4-RD (144). However, in a recent study additional effect of cyclophosphamide yielded a lower relapse rate when combined with glucocorticoids compared to monotherapy with glucocorticoids (96). In the present study 3 patients with organ threatening disease were treated with cyclophosphamide and glucocorticoids, because of relapse or failure to DMARD's. Patients treated with cyclophosphamide were those diagnosed with IgG4-RD retrospectively. Only one patient achieved a temporarily complete remission. The efficacy of cyclophosphamide in IgG4-RD should be therefore restricted if no other therapy is available in organ threatening disease.

Rituximab

The B-cell ablative chimeric monoclonal antibody rituximab is an emerging effective treatment strategy for IgG4-RD (101), however, large randomized controlled studies have not yet been performed. Despite the good clinical response, the relapse rate after treatment with rituximab is high (122). In the current study, clinical remission occurred in all 6 patients (50% complete response versus 50% partial response) treated with rituximab. Rituximab was initiated after failure to DMARD's, or relapse after prednisone tapering. However, after single dose treatment with rituximab, the disease recurred in all those patients 6 months to 2 years after initiating this treatment. One of the patients with severe IgG4-related skin disease is currently treated with rituximab 1000mg every 6 months. Also another patient with systemic disease and recurrence after rituximab is also being treated with rituximab every 6 months. This treatment strategy is already used in other

diseases such as granulomatosis with polyangiitis (145). As suggested previously (122), rituximab maintenance treatment is a potential effective strategy in patients with relapses. This needs to be investigated in future studies.

Other therapeutic modalities

Cyclosporine A is a calcineurin inhibitor in which a decrease in interleukin-2 production and, therefore, T-cell proliferation is established. Carbajal et al report on a case of IgG4 related disease with cardiac involvement which was refractory on mycophenolate mofetil, azathioprine, methotrexate, or cyclophosphamide, but in the end could be treated with prednisone in combination with cyclosporine (146). Cyclosporine A was used once in our study without any clinical effect.

Thalidomide has previously been shown to be effective in two patients with IgG4-related skin disease (147). Because of therapy refractory salivary disease due to IgG4-RD, one patient in our study was successfully treated with thalidomide. Its mechanism of action is not completely understood but suppression of TNF-α production is considered a possibility (148). However, neurological side effects of thalidomide could lead to discontinuation of this treatment. Infliximab, a chimeric monoclonal antibody against TNF-α, has only been reported in case report. In our study, one patient was successfully treated with infliximab after treatment failure to methotrexate, cyclosporine A and cyclophosphamide (149). Fibrosis, an hallmark of IgG4-RD, has been associated with overexpression of TNF-α and might be a target for infliximab and thalidomide in the treatment of IgG4-RD (149).

Surgery

IgG4-RD mostly presents as tumor-like infiltrations and therefore can be treated by surgery if possible (36). Surgical treatment of IgG4-RD often leads to regression of the disease (150-152). Patients primarily treated with surgical intervention in our study revealed a recurrence free disease period after long-term follow-up. These small figures suggest a favorable outcome after surgery and surgical incision/resection of the disease should be considered if possible.

Radiotherapy

Radiotherapy has successfully been reported in IgG4-RD (153, 154) and caused complete remission in one patient with therapy refractory orbital disease and another patient with IgG4-related lymphadenopathy in the current study. After a couple of years, the patient with lymphadenopathy developed a recurrence, but the patient with orbital disease is in remission since 5 years. In this case, the disease was previously refractory to prednisone and cyclophosphamide and eventually responded very well to radiotherapy. Radiotherapy can be considered as a possible treatment strategy for localized and symptomatic therapy in refractory IgG4-RD.

CONCLUSION REMARKS

In this observational study of 32 patients with IgG4-RD, we demonstrated the different treatment outcomes. The rarity of the disease and its many different manifestations at the time of diagnosis create a challenge to assess the optimal treatment. This study has some limitations. First of all, all these data concern observational data. The choice of treatment was based on recommendations of the existing literature and the experience of the prescribing immunologist. Furthermore, patients in this cohort presented with IgG4-RD with different organs involved. The treatment outcome could be different, based on the type of organ involved. However the cohort described in this study is too small to perform any sub-analyses. Ideally the treatment would be investigated in a randomized controlled trial. This is a challenge because of the rarity of the disease.

Nevertheless, this study shows that glucocorticoids and rituximab induce substantial responses as well as primary surgical intervention and radiotherapy. The efficacy of DMARD's is limited. Alternative strategies with hydroxychloroquine, thalidomide and infliximab are promising. More data are needed to confirm these observations, so eventually evidence based treatment guidelines can be developed to improve the treatment of patients with IgG4-RD disease.

RECOMMENDATIONS FOR THE TREATMENT OF IGG4-RELATED DISEASE

The treatment of IgG4-RD is often challenging. Previously small reports have emphasized the efficacy of DMARDs in IgG4-RD, which we unfortunately did not observe. The current consensus guidance however recommends treatment with DMARDs as a second line treatment options in IgG4-RD patients. Larger prospective studies are required to understand the role of DMARDs in IgG4-RD. With current knowledge we recommend the following treatment strategy in IgG4-RD:

- Use the IgG4-RD RI to obtain the disease activity and to monitor the disease activity;
- Check whether there is a treatment indication taking into account that IgG4-RD almost always requires treatment because of the possible secondary complications;
- In case it is possible (for example in a patient with single organ manifestation of the disease), surgery is preferred because of the favorable disease course with possible less relapses. Consider short course of glucocorticoids after surgery;
- Glucocorticoids (usually prednisone 0.5-1.0mg/kg/day or equivalent) are preferred as first line therapy of IgG4-RD. The initial dosage of glucocorticoids should be maintained for 2-4 weeks. Thereafter, glucocorticoids can be tapered slowly. It is recommended to continue glucocorticoids 3-6 months;
- In case of relapse after tapering glucocorticoids, consider DMARDs as second line treatment. In case of severe disease activity, consider rituximab as second line treatment;
- In case of liver and pancreas manifestation of the disease, azathioprine should be considered. In case of other organ manifestations, consider methotrexate, mycophenolate mofetil or hydroxychloroquine in case the (vital) organs are not threatened. The patients should be monitored frequently to assess the disease activity and the exclude the possible side effects of the DMARDs;
- Consider radiotherapy if organs are threatened by the mass effect of tumor/IgG4-RD;
- Rituximab should be started as third line treatment of IgG4-RD, or earlier in the treatment course when vital organs are affected. Consider rituximab maintenance therapy after induction of 2 gram of rituximab. Evaluate after couple of years of treatment if the maintenance therapy can be quitted;
- Other treatment options are cyclophosphamide. Consider this treatment in cases of therapy refractory cases. Rituximab is clearly preferred for cyclophosphamide. Also thalidomide can be considered in patient with therapy failure.

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Chapter 6

Summary

IgG4-related disease (IgG4-RD) is a relative new systemic fibro-inflammatory disease. The studies and reports presented in this thesis were aimed to obtain more insights in the clinical presentations, complications, diagnostics, pathogenesis and treatment of IgG4-related disease.

In Chapter 1, we described 3 different cases of IgG4-RD and provide an overview of IgG4-RD. The three cases are patients with; 1) orbital IgG4-RD with systemic manifestations as demonstrated with PET-CT scan, 2) a patient with IgG4-related mesenteric disease and 3) a patient with pericardial and pleural manifestations of IgG4-RD. Subsequently, this Chapter deepens on epidemiology, pathogenesis, diagnosis and treatment of IgG4-RD using the best current knowledge available. With a systematic review we also describe the manifestations of IgG4-RD in children.

We present in Chapter 2, novel clinical manifestations and describe organ damage caused by IgG4-RD. At first we present a patient with secondary renal AA amyloidosis due to prolonged untreated IgG4-RD. Furthermore, in another study, investigating C-reactive protein (CRP) and serum amyloid A (SAA) levels we demonstrated that about 32% of patients with IgG4-RD have increased SAA levels, which potentially may lead to secondary AA amyloidosis. We also show in a retrospective study on idiopathic scleritis that IgG4-RD is associated with scleritis. In 38 patients with idiopathic scleritis, we obtained data of serum IgG4 levels and histology and identified 2 definite cases of IgG4-RD and 3 cases of probable IgG4-RD. Finally, we studied 3 cases of previously diagnosed ANCA negative limited granulomatosis with polyangiitis (GPA) and could change the diagnosis in to IgG4-RD, we further included a very rare case of IgG4-RD of the tarsal plate.

In Chapter 3 we introduce novel techniques and parameters in the diagnostics of IgG4-RD. We demonstrate that serum interleukin (IL)-2 receptor is elevated in IgG4-RD and that these levels correlate with the disease activity. Furthermore, in a cohort of patients with IgG4-RD we studied the different subsets of lymphocytes including IgG4 positive B-cells, T-helper 2 (Th2) cells and regulatory T-cells (Tregs). IgG4 positive B-cells were demonstrated in patients and controls and we observed expanded IgG4 producing B-cells in IgG4-RD patients. A ''lymphocyte signature'' based on different subsets of B and T cells appears to distinguish patients with IgG4-RD from healthy controls and patients with sarcoidosis.

To investigate the pathogenesis of IgG4-RD, we showed in Chapter 4 (and 3.2) that IgG4 positive memory B-cells were phenotypically different from controls due to lower frequency of expressed CD27 (marker for memory B-cells) and lower expression of chemokine receptor CXC chemokine receptor 5 (CXCR5). We also demonstrated that IgG4 positive B-cells do not show enhanced somatic hypermutation (SHM). This suggest that IgG4 positive B-cells modulate the reactiveness of the involved B-cells. In this Chapter we also show that follicular T-helper 2 cells (Tfh2) are increased in patients with IgG4-RD and a survey of the histology specimens demonstrated B-cell follicles, suggesting a possible role of Tfh2 in the pathogenesis of IgG4-RD. However, the levels of Tfh2 were also increased in sarcoidosis, while in sarcoidosis the levels of IgG4 positive B-cells, Th2 cells and Tregs were not significantly enhanced. With the current data we described the role of humoral immune system and emphasized the role of (different) types of T-cells. Off course, future studies need to provide more insights in the immunological responses in IgG4-RD. Furthermore, we described the same variant in the MTDH gene in two unrelated families with IgG4-RD. The MTDH gene is involved in different pathways and has been described in different types of cancer and inflammatory processes. The mutation in this gene is supposed to be involved in the fibro-inflammatory reaction observed in these patients.

Finally, in Chapter 5 we evaluated the treatment outcomes of IgG4-RD. We described a case of a therapy refractory IgG4-related orbital disease who responded well on infliximab therapy. In a systematic review of literature we studied the treatment outcomes in patients with IgG4-related orbital disease and we described 33 patients with IgG4-RD that responded well on glucocorticoid and/or rituximab therapy, however, relapse rate remained high. Rituximab maintenance treatment may be more effective, as we showed in two therapy refractory patients. This indicated also the pathogenetic role of IgG4-B cells. We also described that failure rate of DMARD's is more than 50%, but drugs as hydroxychloroquine, thalidomide and infliximab (anti-TNF alpha) are potential effective drugs and these need further clinical exploration. Besides, primary surgical intervention as well as radiotherapy cause long-term recurrence free disease period, indicating the importance of the local environment in this disease entity.

Chapter 7

Overzicht IgG4-gerelateerde ziekte in het Nederlands

Chapter 7 Overzicht IgG4-gerelateerde ziekte in het Nederlands

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SAMENVATTING

IgG4-gerelateerde ziekte (afgekort als IgG4-RD = IgG4-related disease) is een systemische fibro-inflammatoire ziekte met manifestaties in bijna alle organen en derhalve een breed palet aan klinische presentaties. De ziekte wordt gekenmerkt door fibrosering en infiltratie van IgG4-positieve plasmacellen in de aangedane organen waarvan precieze pathogenese vooralsnog onbekend is. Alhoewel het serum IgG4 behulpzaam kan zijn in de diagnostiek van IgG4-RD, is histologie vooralsnog de gouden standaard om tot een diagnose te komen. Kennis van IgG4-RD is belangrijk om vertraging in de diagnostiek of misdiagnose te voorkomen. Onbehandelde IgG4-RD kan leiden tot fibrosering van de betrokken organen of zelfs secundaire AA amyloïdose. De eerstelijns behandeling bestaat in het algemeen uit corticosteroïden. Helaas is er vaak reactivatie van ziekte na het afbouwen of stoppen van corticosteroïden, waardoor steroidsparende behandeling noodzakelijk is. Conventionele immuunsuppressieve middelen zoals methotrexaat of azathioprine kunnen worden gebruikt in de behandeling, maar helaas werken deze niet altijd afdoende. Er is toenemend bewijs voor de effectiviteit van rituximab in patiënten die refractair zijn voor conventionele middelen of met ernstige presentatie bij aanvang.

INLEIDING

IgG4-RD is een systemische fibro-inflammatoire aandoening met betrokkenheid van bijna alle organen (1, 2). De ziekte kenmerkt zich door tumorachtige afwijkingen in de aangedane organen, waarbij het serum IgG4 vaak verhoogd is en er histologisch fibrose en infiltratie van IgG4 positieve plasmacellen wordt waargenomen (3). IgG4-RD is voor het eerst beschreven in 2001 in Japan. Hamano en zijn collega's vonden bij toeval verhoogde waardes van het serum IgG4 bij patiënten met auto-immuun scleroserende pancreatitis en concludeerden dat het serum IgG4 waarschijnlijk belangrijk is om auto-immuun pancreatitis te onderscheiden van andere ziektes van de pancreas en galwegen (4). Twee jaar later ontdekte Kamisawa dat deze ziekte zich niet beperkte tot alleen de pancreas: hij beschreef patiënten met multipele orgaanbetrokkenheid (5). Vanaf dat moment wordt IgG4-RD gezien als een systeemziekte en zijn er inmiddels beschrijvingen van manifestaties in bijna alle organen gepubliceerd (6).

ORGAANMANIFESTATIES

IgG4-RD kan zich manifesteren in één orgaan, maar het kan ook voorkomen in twee of meer organen tegelijk (7, 8). Het vaakst aangedaan zijn de lymfeklieren en de exocriene organen, zoals de traanklieren/periorbitale weefsels, speekselklieren en de pancreas (zie Tabel 1). Verschillende onbegrepen aandoeningen waarvan initieel gedacht werd dat het aparte ziektebeelden waren, zijn inmiddels toegevoegd aan het spectrum van IgG4-gerelateerde ziekte. Voorbeelden hiervan zijn onder andere de ziekte van Mikulicz, scleroserende sialadenitis, scleroserende dacryoadenitis, periorbitale xanthogranulomen, eosinophilic angiocentric fibrosis, Küttner's tumor, Riedelse thryreoïditis, en voor een deel ook idiopathische retroperitoneale fibrose en idiopathische scleritis (7-9). IgG4-RD kan bovendien diverse infectieuze, inflammatoire en maligne aandoeningen imiteren wat vaak leidt tot vertraging in de diagnose en behandeling, met mogelijke potentiele irreversibele orgaanschade (6).

KLINISCHE PRESENTATIES

De exacte gegevens over de prevalentie en incidentie van deze ziekte zijn helaas nog niet bekend. IgG4-RD komt vaker voor bij mannen dan bij vrouwen, en kan zich op elke leeftijd

voordoen. In een recente systematische review naar IgG4-RD bij kinderen, zijn er 25 gepubliceerde casussen gevonden van IgG4-RD bij kinderen, waarbij de jongste patiënt 22 maanden oud was (2). Dit geeft aan dat IgG4-RD ook bij kinderen kan voorkomen. De klinische presentatie van IgG4-gerelateerde ziekte is erg divers en uiteraard afhankelijk van de orgaanmanifestatie(s) (Tabel 1). Een patiënt met een orbitale manifestatie presenteert zich met andere symptomen dan een patiënt met pulmonale klachten. Doorgaans hebben patiënten voornamelijk lokaal last van orgaandysfunctie door fibrose en ontsteking en door de ruimte-innemende werking van de IgG4-RD laesies. Zo kunnen er bijvoorbeeld retro-oculaire pijn, exoftalmus en siccaklachten ontstaan als er periorbitale lokalisatie is, of hydronefrose door retroperitoneale lokalisatie (Figuur 1). Constitutionele symptomen zoals koorts, gewichtsverlies of vermoeidheid worden niet vaak gezien.

Tabel 1. Een overzicht van verschillende orgaanmanifestaties en de klinische uitingen van IgG4-gerelateerde ziekte

Orgaan	Kliniek	Orgaan	Kliniek
Pancreas	Auto-immuun pancreatitis	**Huid**	IgG4-gerelateerde ziekte zich doorgaans uitend als erythemateuze, subcutane papels of nodulaire afwijkingen
Lever en galwegen	-Cholangitis -Cholecystitis -Leverziekte	**Lymfeklieren**	-Inflammatoire lymfadenopathie, dit kan unilokaal of diffuus zijn. -Ontsteking submandibulaire klieren: voorheen Küttner's tumor
Retroperitoneum	Retroperitoneale fibrose: voorheen ziekte van Ormond	**Ogen/orbita**	-Scleritis -Uveitis -Dacryoadenitis -Pseudotumor orbitae
Urogenitaal	-Prostatitis -Testiculaire inflammatie -Tubulo-interstitiële nefritis -Membraneuze glomerulonefritis	**Speekselklieren**	Scleroserende sialadenitis: voorheen Mikulicz syndroom
Abdominale manifestaties	-Mesenteritis -Colon obstructie bij IgG4-RD -Oesofagitis	**Schildklier**	Sclerosende thryreoïditis: voorheen Riedel's thryreoïditis
Longen	-IgG4-gerelateerde longziekte: zich meestal uitend in infiltratieve noduli. -Interstitiële longziekte -Pleuritis -Pulmonale arteriële hypertensie	**Zenuwstelsel**	-Cerebrale pseudotumor -Hypertrofische pachymeningitis -Hypofysitis -Polyneuropathie
Cardiovasculair	-(Peri-)aortitis -Pericarditis	**KNO**	-Chronische sinusitis -Scleroserende larynx stenose

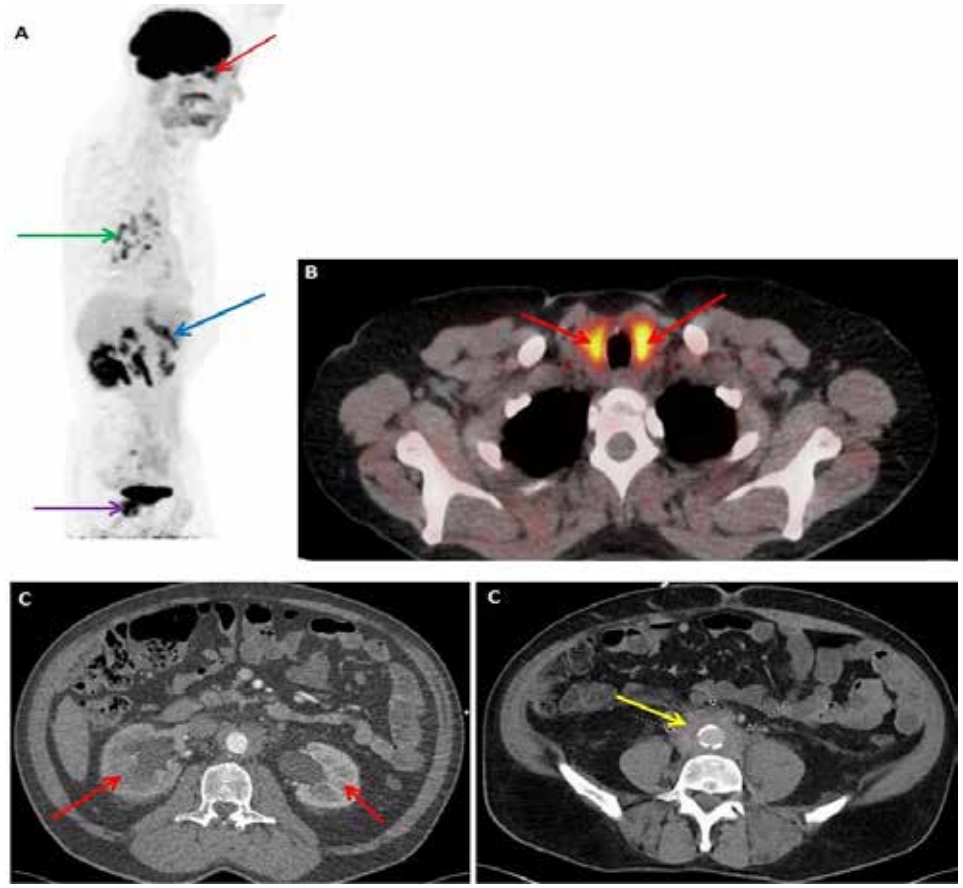
Veel voormalig idiopathische syndromen blijken nu op IgG4-gerelateerde ziekte te berusten.
NB. voor de meeste bovenstaande klinische symptomen zijn uiteraard nog meer oorzaken dan IgG4-ziekte.

PATHOGENESE

Recente studies geven nieuwe inzichten in het ziekteproces van IgG4-RD, maar de exacte pathogenese blijft nog onduidelijk. Het is met name niet bekend welke factor(en) ervoor zorgt dat het immuunsysteem actief wordt op een manier die uiteindelijk leidt tot het fenotype van IgG4-RD. Het principe van *molecular mimicry* na een infectie, bijvoorbeeld Helicobacter pylori, werd gesuggereerd, maar het bewijs hiervoor blijft uit. Een recente studie in een cohort van meer dan 50 patiënten met IgG4-RD liet geen verhoogde prevalentie zien voor helicobacter infectie (10). De IgG4 antilichamen blijken polyklonaal te zijn en er zijn geen specifieke auto-antilichamen gevonden, hetgeen een auto-immuunziekte onwaarschijnlijk maakt. Er zijn vooralsnog geen monogenetische afwijkingen bekend voor IgG4-RD. Echter, een recente beschrijving van IgG4-RD in een eeneiige tweeling suggereert dat een genetische predispositie mogelijk een rol speelt in de pathogenese van de ziekte (11). Een genetisch onderzoek in deze tweeling is echter niet gedaan. Daarnaast hebben wij twee patiënten beschreven met IgG4-RD met een variant in het *MTDH* gen, waarbij die variant ook bij de vaders van die patiënten voorkomen. MTDH is vooral beschreven in de oncologie en is betrokkenheid in verschillende processen zoals NF-κB en PI3K-AKT. De prevalentie van deze variant in de IgG4-RD en de exacte rol van deze variant in deze ziekte moeten nog worden uitgezocht.

Wat we wel weten, is dat zowel de B-cellen als de T-cellen een belangrijke rol spelen in de pathofysiologie van de ziekte. De IgG4-antilichamen worden geproduceerd door IgG4 positieve plasmacellen die voortkomen uit B-cellen. Hier volgt uit dat B-cellen een belangrijke rol hebben in de pathogenese van IgG4-gerelateerde ziekte (3, 12). De aanwezigheid van IgG4 positieve B-cellen in relatie tot andere subklasse producerende B-cellen is echter nog niet goed bestudeerd. In een recente studie hebben we verhoogde expressie van IgG4 positieve B-cellen in IgG-RD kunnen waarnemen vergeleken met gezonde populatie en vergeleken met sarcoïdose (13). Daartegenover vonden we verlaagde expressie van IgG1 positieve B-cellen. Langdurige blootstelling aan antigen leidt tot een serologische shift van IgG4:IgG1 ratio, zoals dit eerder ook beschreven is bij graspollen en wespen immunotherapie (14). De parallel hieraan suggereert dat ook bij IgG4-RD er sprake is van langdurige activatie van het immuunsysteem, bijvoorbeeld door een pathogeen of allergeen. Deze hypothese zal de komende jaren verder onderzocht moeten worden.

Figuur 1. Beeldvormende onderzoeken bij patiënten met IgG4-gerelateerde ziekte



Figuur 1. Beeldvormende onderzoeken bij patiënten met IgG4-gerelateerde ziekte. **A:** een 63 jarige man met presentatie vanwege orbitale IgG4-RD. Op PET scan systeemmanifestatie van de ziekte met orbitale (rode pijl), mediastinale/hiliaire lymfeklier (groene pijl), pancreas (blauwe pijl) en prostaatbetrokkenheid (paarse pijl). **B:** een 52 jarige vrouw met presentatie vanwege orbitale IgG4-RD. Op PET scan ook betrokkenheid van de schildklier/Riedelse thryreoïditis (rode pijlen). **C:** een 65 jarige man met acute nierinsufficiëntie vanwege hydronefrose beiderzijds (rode pijlen) door IgG4-gerelateerde retroperitoneale fibrose/peri-aortale infiltratie (gele pijl) met obstructie van de urinewegen.

Verder zijn er meerdere studies verschenen die bewijs leveren voor de aansturende rol van verschillende T-cel subsets, waaronder T-helper 2, regulatoire T-cellen, folliculaire T-helper cellen, maar ook de zogenaamde CD4+ cytotoxische T-cellen (15, 16). Het zal nog duidelijk moeten worden welke van deze T-helper subtypes het belangrijkste zijn in de pathogenese.

Er bestaat vooralsnog het meeste bewijs voor betrokkenheid van T-helper 2 cellen: T-helper 2 cellen, en ook regulatoire T-cellen, komen verhoogd tot expressie in het perifere bloed van patiënten. Daarnaast is er verhoogde expressie van de cytokines die door deze T-cellen worden geproduceerd aangetoond, zowel in het perifere bloed als in de aangedane weefsels (17, 18). Cytokines, waaronder interleukine (IL)-4 en IL-10 geproduceerd door respectievelijk Th2- en regulatoire T-cellen, zorgen voor een klasse switch van B-cellen naar IgG4 producerende B-cellen. Hier is eveneens een parallel te vinden met allergeen immunotherapie, aangezien deze Th2 respons ook is beschreven in de context van het gunstig effect van een dergelijke immunotherapie, waarbij de klasse switch richting IgG4 ten koste van IgE verloopt (19). Transforming growth factor β (TGF-β) en IL-10 hebben naast een regulatoire immunologische werking, een sterk pro-fibrotisch effect (20).

Vergeleken met de andere IgG subklassen zijn IgG4-antilichamen bijzonder qua vorm en functie. Zij fungeren waarschijnlijk vooral als anti-inflammatoire antistoffen. Deze antilichamen hebben namelijk twee verschillende antigenbindende fragmenten vanwege een zwakke disulfide binding tussen de twee Fc-fragmenten, waardoor er steeds willekeurige uitwisseling plaatsvindt van halve IgG4-antilichamen (Figuur 2). Deze antilichamen zijn dus bivalent. Ze kunnen wel antigenen binden, maar geen immuuncomplexen vormen (2). Daarnaast kan het Fc-fragment van IgG4 geen complement activeren. De IgG4 antilichamen binden niet aan activerende Fc-receptoren, maar waarschijnlijk wel aan remmende FcγRIIb receptoren, zoals dit onder andere gebleken is voor mestcellen (21). Gebaseerd op deze kenmerken zou het mogelijk zijn dat de IgG4-antilichamen geen direct pathogenetische rol spelen in het ziekteproces, maar meer als epifenomeen gezien worden omdat het afweersysteem continu aan het werk is om een chronische ontsteking te onderdrukken.

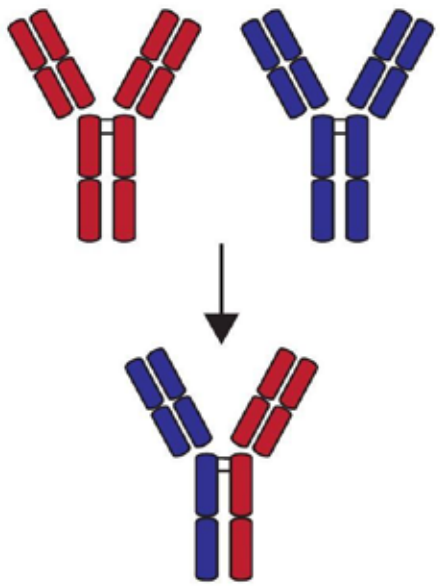
DIAGNOSTIEK

De diagnostiek van IgG4-gerelateerde ziekte kan lastig zijn. De klinische presentaties van patiënten met IgG4-RD overlappen immers sterk met klinische presentaties van andere aandoeningen, zoals sarcoïdose of een maligniteit. De inflammatoire markers zoals bezinking en C-reactieve proteïne (CRP) zijn in de meerderheid van de gevallen laag. Bepaling van het serum IgG4 kan wel behulpzaam zijn in de diagnostiek van IgG4-RD. Het serum

IgG4 is echter in ongeveer 30% van de histologisch bewezen gevallen binnen de normaalwaarden, wat kan leiden tot misdiagnose (6). Dit maakt het serum IgG4 niet eenduidig te interpreteren. Een verhoogd serum IgG4 kan hooguit de diagnose meer waarschijnlijk maken of richtinggevend zijn voor verder onderzoek. Wel kan het goed als marker voor ziekte activiteit worden gebruikt, indien het voor start van de behandeling verhoogd is (6). In een studie is aangetoond dat circulerende plasmablasten mogelijk een betere marker zijn in de diagnostiek en monitoring van ziekteactiviteit dan het serum IgG4 (22). Deze methode is echter nog niet overal bruikbaar voor klinische toepassing en de rol van deze plasmablasten moet in meer studies worden aangetoond. We hebben in een studie aangetoond dat combinatie van verschillende B cel subsets en T cel subsets een goede methode is om patiënten met IgG4-RD te onderscheiden van gezonde controles en van patiënten met sarcoïdose (13). Deze methode dient in meer studies te worden onderzocht en biedt potentie om als biomarker gebruikt te worden. We hebben ook aangetoond dat oplosbare serum interleukine-2 receptor (sIL-2R) in alle patiënten met een actieve IgG4-RD verhoogd is (23). SIL-2R levert geen diagnose, maar geeft de indicatie van actieve T-cellen en kan wel gebruikt worden om ziekte activiteit te monitoren.

Histologisch onderzoek blijft daarom vooralsnog de gouden standaard. De typische histomorfologische kenmerken van IgG4-RD zijn lymfoplasmocytaire infiltratie, fibrose, vaak flebitis en soms ook infiltratie van eosinofielen (Figuur 3). Immunohistochemisch kunnen IgG4 positieve plasmacellen worden gevisualiseerd. In de internationale consensus is voor elk orgaan het minimale aantal vereiste IgG4 positieve plasmacellen per high-power field (HPF) afgesproken (24). Daarnaast moet de ratio IgG4 positieve plasmacellen/totaal IgG positieve plasmacellen hoger zijn dan 0.4 (24). Dit is belangrijk, omdat IgG4 positieve plasmacellen, in lagere ratio's, ook gezien kunnen worden in de weefsels van patiënten met bijvoorbeeld granulomatose met polyangiitis (GPA) en de ziekte van Castlemann (25). In ongeveer 30% van patiënten met IgG4-RD is er sprake van perifere eosinofilie welke gepaard kan gaan met eosinofilie in de biopten van aangedaan weefsel (26). IgG4-RD dient dan ook in de differentiaal diagnose te staan van het hypereosinofieel syndroom.

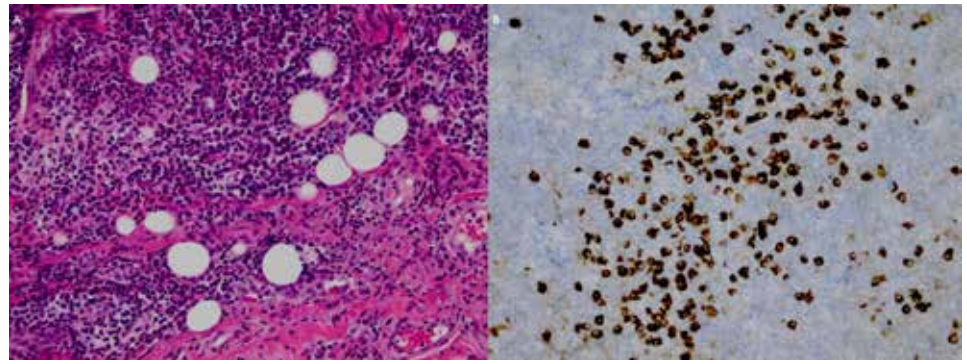
Figuur 2. Schematische weergave van IgG4-antilichamen



Figuur 2. Schematische weergave van IgG4-antilichamen. Een IgG4-antilichaam wisselt continue een half molecuul met een ander IgG4-antilichaam. De IgG4-antilichamen zijn daarom bivalent met twee verschillende antigenbindende fragmenten.

Een 18F-FDG-PET scan is een goede methode om de ziekte te stadiëren of om op zoek te gaan naar een geschikte plek voor een biopsie. Vaak worden met behulp van een PET scan meer IgG4-RD lokalisaties gevonden dan met conventionele beeldvormende technieken, zoals een CT scan (6).

Figuur 3. Histologische plaatjes van een patiënt met IgG4-gerelateerde orbitale ziekte



Figuur 3. Histologische plaatjes van een patiënt met IgG4-gerelateerde orbitale ziekte. A: traanklierbiopt waarin lymfocyteninfiltratie en fibrose te zien is. **B**: met immunohistochemisch onderzoek worden IgG4 positieve plasmacellen (bruin) aangetoond met gemiddeld 340 (N<100) positieve plasmacellen per high-power field en met een ratio van IgG4/IgG positieve plasmacellen van 0.67.

BEHANDELINDICATIES EN BEHANDELMOGELIJKHEDEN

Naast het feit dat de symptomen van IgG4-RD vaak belemmerend zijn, kan IgG4-RD irreversibele orgaanschade veroorzaken door fibrosering (1, 2). Hierdoor is er bijna altijd een behandelindicatie. Afhankelijk van de betrokken organen, bijvoorbeeld bij solitaire lymfeklierlokalisatie, kan er soms een afwachtend beleid worden afgesproken. Behalve fibrosering kan langdurig onbehandelde IgG4-RD ook leiden tot secundaire amyloïd A amyloïdose (AA amyloïdose) (27). Deze complicatie treedt waarschijnlijk op alleen bij langdurig inflammatoire tekenen in het bloed, zoals verhoogde CRP en verhoogde serum amyloïd A waardes.

Indien er een behandelindicatie is, is de eerstelijns therapie vaak glucocorticoïden, meestal in een dosisequivalent van 0.5mg/kg mg prednison per dag, of hoger op basis de ernst van de ziekte (28). In het algemeen wordt aangeraden om de initiële dosis voor 2-4 weken te continueren en daarna geleidelijk af te bouwen op geleide van de klinische symptomen. Helaas krijgt ruim 25% van patiënten een terugval tijdens het afbouwen of na het stoppen van prednison (28). In het geval van terugval is behandeling met conventionele prednison-sparende therapie aangewezen. Diverse middelen zoals methotrexaat, azathioprine maar ook mycofenolaten zijn beschreven als behandeling van IgG4-RD (1).

Echter, echte substantiële bewijs voor de effectiviteit van deze middelen ontbreekt. We adviseren desondanks om patiënten te behandelen met een van deze middelen indien de prednison niet kan worden afgebouwd tot <10 mg per dag. In het geval van refractaire ziekte ondanks deze conventionele prednison-sparende middelen, is er indicatie voor behandeling met rituximab. Hoewel rituximab in IgG4-RD ook niet in grote (gerandomiseerde) studies is onderzocht, blijken patiënten met verschillende orgaanmanifestaties klinisch erg goed te reageren op deze behandeling (29, 30). De lange termijneffecten van conventionele immuunsuppressieve middelen en rituximab in IgG4-RD zijn nog onbekend en moeten in toekomstige prospectieve studies worden onderzocht.

CONCLUSIES

IgG4-RD is een zeldzame en een nieuwe klinische entiteit met vele gezichten en manifestaties in vrijwel het hele lichaam. Vroege erkenning en behandeling zijn essentieel om irreversibele orgaanschade te voorkomen. De diagnose is gebaseerd op histologisch en immunohistochemisch onderzoek, waarbij bepaling van het serum IgG4 ondersteunend kan zijn. Glucocorticoïden zijn de eerste keus van de behandeling, maar vanwege frequente reactivatie bij het afbouwen is er vaak behoefte aan onderhoudstherapie. In het geval van refractaire ziekte kunnen conventionele immuunsuppressieve middelen evenals rituximab worden gebruikt bij de behandeling van IgG4-RD, waarbij rituximab klinisch het meest effectief lijkt te zijn.

AANBEVELINGEN VOOR DE PRAKTIJK

1. IgG4-gerelateerde ziekte is een systeemziekte: manifestaties in bijna alle organen zijn beschreven;
2. Het serum IgG4 kan behulpzaam zijn in de diagnostiek, maar histologisch onderzoek blijft de gouden standaard. Kennis van de typische histologische kenmerken is belangrijk om misdiagnose te voorkomen;
3. IgG4-RD kan, indien onbehandeld, leiden tot fibrose en AA amyloïdose met irreversibele orgaanschade. Tijdige diagnose en behandeling blijven daarom essentieel;

4. Corticosteroïden vormen de hoeksteen van de behandeling, er kan bijvoorbeeld gestart worden met prednison (of een andere glucocorticoïd) in een dosering van 0.5mg/kg per dag. Continueer deze dosering voor 2-4 weken of op basis van respons en daarna geleidelijk afbouwen. In het geval van een recidief: overweeg prednison sparende therapie met een conventioneel immuunsuppressief middel of rituximab.
5. De ziekte kan gemonitord worden aan de hand van de klinische symptomen, het serum IgG4, de bezinking en het CRP (indien verhoogd), eventueel aangevuld met serum amyloïd A en beeldvorming (PET scan).

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Chapter 8

Discussion and future directions

The clinical manifestations and complications of IgG4-related disease

IgG4-related disease is an emerging systemic disease with a heterogeneous clinical presentation and significant complications. We described various patients with disease localizations in all parts of the human body, including the usual sites such as the orbita, lymph nodes, salivary glands, aorta and pancreas, but also rarely affected sites such as lungs, sclerae, kidneys, prostate and brain (Chapter 1 and 2) (1). The relative recent discovery of IgG4-RD as a disease entity provided the opportunity to re-diagnose previous clinical unsolved issues. As a consequence of this, the numbers of case reports and case series about IgG4-RD are growing rapidly. These emerging novel findings increase the knowledge of the clinical presentation of the disease.

IgG4-RD mimics various (non)infectious diseases and malignancies requiring a careful workup which is especially based on histopathological examination; increasing awareness is warranted (2). We and others demonstrated that patients with inflammatory lesions of unknown origin, such as inflammatory orbital masses or nasopharyngeal vasculitis may actually suffer from IgG4-RD (Chapter2). In detail, we have identified that IgG4-RD may also explain a significant proportion of idiopathic scleritis, as depicted in Chapter 2.2. Scleritis is painful ocular inflammation with a variety of underlying causes (3, 4). Patients with scleritis usually undergo extensive diagnostics, but still in about 50% of the cases no underlying cause can be identified. Based on these observations, we recommend to exclude IgG4-RD in patients with idiopathic scleritis. Additional analysis with serum IgG4, PET-CT imaging and biopsy should be considered. However, scleral biopsies are only very rarely performed because of the potential severe complications, which implies a need for the additional diagnostic tools. Furthermore, granulomatosis with polyangiitis (GPA), an autoimmune disease with serious complications, may present similarly as IgG4-RD. ANCA negative limited GPA in the ENT region regularly occurs, in these cases IgG4-RD may be an alternative diagnosis, as described in Chapter 2.3. In limited GPA, ANCA antibodies are often negative and the histology does not deliver a classic feature of granulomas and vasculitis (5, 6). In these cases, IgG4-RD should be excluded. Distinguishing GPA from IgG4-RD is necessary because of the different complications and treatment considerations in both diseases. Conventional immunosuppressive drugs are often sufficient for limited GPA, while IgG4-RD does not respond well to these drugs and rituximab therapy

may be initiated earlier (2). Larger studies on so-called ANCA negative limited GPA should be performed in order to determine how often IgG4-RD is an explanation in these cases. The clinical description of the cases and case series predict that the disease spectrum of IgG4-RD will increase in the near future and therefore the diagnostic and therapeutic strategies will evolve subsequently.

It is generally recognized that IgG4-RD can lead to irreversible organ damage due to destructive fibrosis (7). Additionally, elevated C-reactive protein (CRP) and serum amyloid A (SAA) may indicate secondary amyloidosis, another tissue destructive complication (Chapter 2.1.). Careful monitoring of CRP and serum amyloid therefore, should be part of the routine workup and follow-up. However, the values of CRP in patients with IgG4-RD seem to vary globally. In a study of the Japanese population, CRP levels appeared to be lower compared to patients in western countries (8). Larger international studies on CRP, SAA and other potential new inflammatory markers (to be found by for example with proteomics or large cytokine arrays) are required to optimize diagnosis and monitoring of therapy in IgG4-RD.

Novel diagnostic strategies in IgG4-related disease

IgG4-RD may present with a typical clinical pattern of orbital, salivary gland, lymph node or pancreas manifestations, however a large part of patients may present differently. Furthermore, IgG4-RD mimics various other clinical disorders such as malignancy, infectious diseases and inflammatory diseases like sarcoidosis. Knowledge and awareness are critical to avoid misdiagnosis (1). Serum IgG4 is not a reliable diagnostic marker since it may be normal in histologically proven cases. However, when elevated and IgG4-RD is diagnosed, serum IgG4 can be used for monitoring disease activity (1). Erythrocyte sedimentation rate (ESR) and CRP are not always elevated and do not differentiate between IgG4-RD and other inflammatory or infectious diseases. Imaging, especially PET-CT scan is a useful tool to study and evaluate the systemic involvement and activity. A FDG-PET-CT scan often shows more inflammation sites as compared to conventional CT scans (9). However, a PET-CT scan cannot differentiate between IgG4-RD and other diseases, therefore, histology remains the gold standard. Histological features of lymphoplasmacytic infiltration, storiform fibrosis and often phlebitis and eosinophilic infiltrations are typical

for the disease. Staining on IgG4 positive plasma cells confirms the diagnosis further using the current Boston Consensus criteria for histology of IgG4-RD (10).

Additional, non-invasive, diagnostic tools are thus needed, since histology cannot always be obtained easily, because of the risk of complications. Up to now a few inflammatory parameters have been studied. We demonstrate in Chapter 3.1 that serum soluble interleukin-2 receptor (sIL-2R) is elevated in all patients with active IgG4-RD. Furthermore, the levels of sIL-2R declined significantly after treatment and correlated with disease activity. Thus, sIL-2R might serve as a potential additional diagnostic and monitoring marker. However, sIL-2R is also not specific. Other disorders with enhanced T-cell activity demonstrate increased levels of sIL-2R as well, including sarcoidosis and viral infections (11-13). SIL-2R may therefore be used in monitoring disease activity, but increased sIL-2R is insufficient to confirm the diagnosis of IgG4-RD. However, since all patients with active disease demonstrated increased sIL-2R, in contrast to serum IgG4, the specificity of sIL-2R may be high.

Novel detection strategies might evolve from in depth analyses of B-and T-cell repertoires in patients with IgG4-RD. Recently, circulating plasmablasts were demonstrated to be enhanced in IgG4-RD and appeared to be more specific for the disease than serum IgG4 (14). These circulating plasmablasts are however not specific for IgG4-RD and may also be increased in other diseases. We demonstrate that IgG4 positive B-cells, the precursors of circulating plasmablasts, are significantly enhanced in patients with IgG4-RD as compared to healthy controls and patients with sarcoidosis (Chapter 3.2). Also, the levels of T-helper 2 (Th2) cells and regulatory T-cells (Tregs) are increased in these patients. The combination of these B and T-cell subsets appears to be a helpful diagnostic tool to distinguish patients from healthy controls and patients with sarcoidosis. This ‘’specific lymphocyte signature” may potentially be used as a new noninvasive diagnostic tool in IgG4-RD. Further studies with larger amount of patients are required to investigate its value to evaluate the monitoring capacity of this ‘’specific lymphocyte signature”.

The pathogenesis of IgG4-related disease

Despite the increasing knowledge on IgG4-RD, its underlying pathogenesis is still unclear. It is assumed that an unknown exposure/antigen triggers the immune system leading to the phenotype of IgG4-RD (15). The pattern do not fit with a classical autoimmune disease, the male/female ratio is in favor of male patients and laboratory studies have failed to demonstrate specific auto-antibodies and/or auto-reactive T cells in IgG4-RD. Furthermore, infectious disease with molecular mimicry was hypothesized to play a role, but evidence supporting this hypothesis is poor (16). Despite the unknown underlying trigger, the type of immune response becomes more and more understood. The humoral immune response seems to play a crucial role in the disease, which is primarily based on increased IgG4 positive B-cells numbers, circulating plasmablasts and serum IgG4 levels. The IgG4 positive memory B-cells were phenotypically different from controls due to lower frequency of CD27 expression (conventional marker for memory B-cells) and lower expression of chemokine receptor CXC chemokine receptor 5 (CXCR5) (Chapters 3.2 and 4.1). Moreover, no enhanced SHM in the transcripts of these IgG4+ B were observed. This finding disagrees with a previous study (14), where increased SHM is found in the FR3 and CDR3 regions of IgG4 transcripts. However, FR3 and CDR3 form less than 1/3rd of the variable domain and mutations in the CDR3 are notoriously difficult to define, since this includes the junctional regions between *IGHV*, *IGHD* and *IGHJ* genes with random nucleotide insertions and deletions from the V(D)J recombination process. The results of Mattoo *et al.* might be influenced by a different methodological approach where our analysis involved the strongly recommended approach for SHM analysis, which included the whole *IGHV* gene (>280 nt) including FR1, FR2 and FR3, and the CDR1 and CDR2 (17). We concluded that patients in our original IgG4-RD cohort showed signs of chronic B-cell responses in affected tissues, whereas their circulating B cells have increased anergic properties. This could possibly reflect a mechanism by which the immune system attempts to modulate the reactiveness of the involved B-cells, which could provide new avenues for studies into pathogenesis and treatment of patients with IgG4-RD. The role, phenotype and differentiation of IgG4 positive B-cells and their interactions with other immune cells, fibroblasts and ‘’organ homing’’ needs more in depth studies.

Furthermore, T-cells are also of importance in IgG4-RD (1, 18-20). Previously, various cytokines including interleukin (IL)-4 and transforming growth factor (TGF-)β, derived from Th2-cells and Tregs, were thought to contribute to the disease pathophysiology (1, 21-23). Indeed, as demonstrated in Chapter 3.2, we observed increased levels of these Th2-cells and Tregs in patients with IgG4-RD. The exact role of Th2 cells and their specific cytokines in IgG4-RD is however still a subject of debate, but they may play a role in class-switch. Recent studies have demonstrated that follicular T-helper 2 cells (Tfh2) are possibly key players in IgG4-RD, in particular for IgG4 class-switching, plasmablast and plasma cell differentiation, and germinal center formation (24, 25). Indeed, in our study the levels of Tfh2-cells were significantly higher in patients as compared to healthy controls (Chapter 4.1). However, the observed absolute count of total circulating Tfh cells, instead of looking specifically in to Tfh2-cell subset, appears within normal limits, comparable with previous studies, but the Tfh2 subset seems to be more abundant as compared to healthy controls (26).he levels of circulating Tfh2 cells were also significantly increased in sarcoidosis in our studies, indicating that these cells are not specifically involved in IgG4-RD. The possible roles of different involved T-cell subsets need further exploration in future studies. Nevertheless, despite a possible role of Tfh2 cells in the pathogenesis of IgG4-RD, circulating Tfh2 cells are not a specific diagnostic marker for IgG4-RD which is in contrast to the increased circulating levels IgG4+ B cells, Treg cells and Th2 cells as presented in Chapter 3.1.

More recently, CD4+ T-cells displaying cytotoxic features were abundantly present in peripheral blood and in affected tissue sites of IgG4-RD patients. This may possibly contribute to the chronic inflammatory/fibrotic network of the disease by secreting specific cytokines (20, 27).

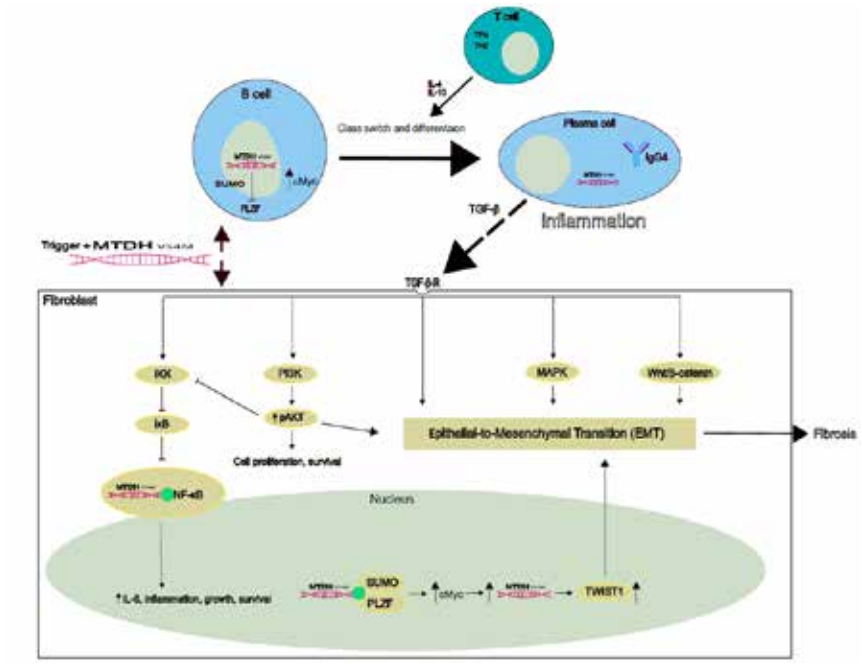
Familial aggregation of IgG4-RD has been reported in monozygotic twins (28), however monogenetic defects have not been published yet. We described an identical variant in exon 1 of the metadherin (*MTDH*) gene with activation of *MTDH* related pathways in two unrelated families (Chapter 4.2). MTDH is involved in many processes, including; cell proliferation, survival, migration, tissue invasion, apoptosis, angiogenesis and metastasis. In these processes diverse activated signalling pathways are involved including PI3K/AKT, NF-κB, Wnt/β-catenin and mitogen-activated protein kinase (MAPK) (29, 30). We

postulate that defects in MTDH result in activation of different inflammatory pathways, possibly under influence of environmental and local organ conditions leading to the phenotype of IgG4-RD. In our cases, the fathers and sons share a rare variant in *MTDH* gene. The father of one patient also demonstrated both elevated phosphorylated AKT levels and increased IgG4-positive B-cell counts without having the disease. The likelihood of familial IgG4-RD seems strong, however, it is unknown whether the fathers will develop the disease ever or not. This may depend on exposure to the appropriate trigger. Interestingly, in three out 18 other IgG4-RD patients another variant was found in exon 1 of *MTDH* which is more prevalent than expected in this small case series. The contribution of the MTDH, both gene and protein function, needs more intensive studies in laboratory setting as well as in larger IgG4-RD patient cohorts. We speculate that other variants may be involved in the above mentioned *MTDH* related pathways, this warrants genetic research in cohorts of IgG4-RD.

We include a hypothetical model representing an alternative pathophysiological model for IgG4-RD (Figure 1). In this model we focused on the possible role of *MTDH* in the pathogenesis. IgG4 has a limited effector function and has immunomodulatory/suppressive effects on the immune system. IgG4 exhibits a broad repertoire and has additionally the distinct property to undergo a process what is called Fab-arm exchange. These characteristics expend the functional properties of IgG4 in inflammation where it probably has a regulatory action. Elevated levels of IgG4 are associated with immune tolerance under conditions of chronic specific antigen/allergen exposure, such as tolerance to bee venom in beekeepers (31) or reduced allergic symptoms after allergen-specific immunotherapy (32, 33). Moreover, IgG4 levels correlates well with Tregs and negatively with cytotoxic T lymphocytes (34), supporting its involvement of immune tolerance in cancer. We hypothesize that individuals with IgG4-RD overreact to specific (local) triggers such organ damage or infection. B-lymphocytes class-switch to IgG4 presenting B cells, regulated by Tfh2 cells. IgG4 B-lymphocytes differentiate into IgG4 plasma cells that produce TGF-_, this cytokine is a strong activator of fibroblasts, resulting in a fibro-plasmacellular inflammatory response. In case of the described families, MTDH may act on several levels, including IgG4-class switching. Small Ubiquitin-like Modifier (or SUMO) pro-

teins interact with the SUMO-interaction motif region (aa 50-56) of MTDH. So, SUMO potentially interacts with the a54 variant in MTDH. Through Promyelocytic Leukaemia Zinc Finger (PLZF)-SUMO-MTDH regulation of cMyc occurs, which is involved in class switching. MTDH acts at several levels in fibroblasts. MTDH is a downstream intermediate in the TGF-β receptor pathway, activates further the PI3k-AKT pathway, NF-κB pathway and also stimulates of β-catenin expression, which is also known as a strong fibroblast activator. MTDH activates (environmental) transcription factor "Twist Family bHLH Transcription Factor 1" (TWIST-1), which is known to be involved in epithelial-mesenchymal transition, this factor correlates well with MTDH expression. The interesting interaction between IgG4 producing B cells and fibroblasts is centrally placed in this hypothesis. Identification of genetic variants and involved pathways may lead to further understanding of the underlying pathogenesis and may lead to better targeted treatment strategies. The successful treatment of IgG4-RD with immunosuppressive drugs and specifically rituximab implies an important role for B lymphoid cells in IgG4-RD pathogenesis.

Figure 1. An hypothesized overview of the pathophysiology of IgG4-RD with a possible role of *MTDH*



Treatment of IgG4-related disease

As previously mentioned, prompt treatment of IgG4-RD is essential to avoid irreversible organ damage. Glucocorticoids are effective in IgG4-RD and are still the mainstay of treatment. However, recurrence rate after tapering/withdrawing glucocorticoids is high (up to 50%) and glucocorticoids have a significant side-effect spectrum. Treatment with rituximab was also reported as an effective therapy, however, no large randomized studies are yet performed with rituximab in IgG4-RD. The disease modifying antirheumatic drugs (DMARD's) are usually not effective, as demonstrated in Chapter 5.2. This study showed a good response to glucocorticoids and rituximab, but the therapeutic efficacy of DMARD's was disappointing. Other treatment modalities such as hydroxychloroquine, thalidomide and infliximab are promising, but also here, studies are required to evaluate the efficacy of these treatment options. Besides drug therapies, primary surgical intervention and radiotherapy also seemed to cause a favorable clinical course of the disease. This suggest a pathophysiological role for the local organ environment in maintaining disease activity. The treatment outcomes of IgG4-RD requires large and prospective studies to evaluate the efficacy of the different treatment regimes. These studies are necessary in order to compose appropriate guidelines for physicians who are involved in the treatment of IgG4-RD.

Conclusion

IgG4-RD is an intriguing systemic disease with an organ-destructive character, in which more research efforts including immune cell-fibroblast interactions, genetics and homing studies are necessary to increase our understanding of the pathophysiology. Detailed registration of clinical presentations and complications will undoubtedly broaden the current spectrum of IgG4-RD. The diagnostics of IgG4-RD needs to be optimized, detection of IgG4 positive B lymphocytes may be a new additional tool in the diagnostic process. Furthermore, more insights in the pathogenesis, in particular genetics and detailed immunological examination will result in better understanding and adjusted (personalized) treatment options for patients with IgG4-RD.

Nevertheless, the current work delivers new insights in the clinical presentations and complications, demonstrates potential novel disease markers and deepens in to the pathogenesis and shows treatment outcomes of IgG4-RD, this offers a good clinical basis for future studies.

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Chapter 9

PhD portfolio

Curriculum vitae

Acknowledgements/Dankwoord

PhD PORTFOLIO

Summary of PhD training and teaching

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Erasmus MC, department of Internal Medicine, section Clinical Immunology

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PhD period: 2015-2019

Promotor: Prof. dr. P.M. van Hagen

Supervisor: Dr. J.A.M. van Laar

ACTIVITIES	YEAR	WORKLOAD (ECTS)
Courses		
Course “werken met endnote”	2015	0.1
Course “systematisch literatuuronderzoek met Pubmed”	2015	0.2
Course “D.O.O. Evidence Based Medicine”	2016	0.3
Course “D.O.O. Medical Ethics”	2016	0.1
Course “Annual advanced course immunology” (Molmed)	2016	3.0
Course ” Cursus medische immunologie Erasmus MC”	2016	5.0
BROK (Basiscursus Regelgeving Klinisch Onderzoek)	2017	1.0
Course “Workshop on Photoshop and Illustrator CS6 for PhD-students and other researchers” (Molmed)	2017	0.3
Course “Introduction in GraphPad Prism Version 6” (Molmed)	2017	0.3
Course “Basic introduction course on SPSS” (Molmed)	2017	1.0
Course “Groninger Basis cursus immunologie” UMCG Groningen	2019	0.3
Presentations and (inter)national conferences		
Speed date on IgG4+ B-cells in IgG4-related disease. EWIMID conference Amsterdam	2015	1.0
Attending and poster presentation (2016) on IgG4-related disease at Science Days Erasmus Internal Medicine, Antwerp.	2016, 2017	3.0
Annual conference Nederlandse Internisten Vereniging 2015, 2016 and 2017. Presentation “A patient with unusual cerebral manifestation of IgG4-related disease” at annual meeting in 2016.	2015, 2016, 2017	3.0
Symposium “7e Symposium Medische Immunologie”	2016	1.0
Conference: “5de Nationale Congres Klinische Immunologie en Allergologie”	2016	1.0

Symposium: “International SSc Symposium: update and practice in systemic sclerosis”	2016	2.0
Symposium: ”Dutch symposium on C1 inhibitor deficiency and other forms of angio-edema”	2016	0.1
Symposium: “Symposium Klinische Immunologie 9 februari 2017: Nieuwe ontwikkelingen in diagnostiek en behandeling van immuungemedieerde aandoeningen”. Presentation: a case of IgG4-related disease.	2016	0.5
Symposium: “NVVA (Nederlandse Vereniging voor Allergologie) dagen”	2017	0.1
Expert Meeting: “Annual Werkgroep Immuundeficiëntie (WID) meeting”. Presentation: Clinics and progress in diagnoses of IgG4 associated diseases.	2017	2.0
Regionale Nierbiopsie Avond Rotterdam Presentation: biopsy proven renal AA amyloidosis in IgG4-related disease	2017	0.3
Conference: “European Association of Allergy and Clinical Immunology (EAACI)”. Succeeded in Annual EAACI examination	2017	5.0
Presentation “An update on IgG4-related disease’’at annual conference Nederlandse Internisten Vereniging (NIV).	2018	0.3
Poster presentation “The treatment outcomes in IgG4-related disease” at the European Congress Immunology (ECI) 2018.	2018	0.3
Peer-reviewing for journals		
Netherlands Journal of Medicine (3 manuscripts)	2016, 2017, 2018	0.3
Rheumatology (1 manuscript together with dr. van Laar)	2016	0.1
American Journal of Case-reports (2 manuscripts)	2016, 2017	0.2
Clinical interventions in aging (1 manuscript)	2017	0.1
Breast cancer: targets and therapy (1 manuscript)	2017	0.1
SAGE open medical case reports (1 manuscript)	2017	0.1
Teaching		
Course “Teach the Teacher”	2016	2.0
Lessons: ’’Klinisch Redeneren Onderwijs” for medical students	2017	1.0
Lessons: “Casusonderwijs immunologie” for medical students	2017	0.2
Coaching of medical students: Lauren Westenberg: a bachelor medical student who I have been coaching since 2015. She is a coauthor in two articles on IgG4-related disease and joined first author in a systematic review about the relationship between allergies and cancer (in preparation)	2015, 2016, 2017	3.0
Total ECTS		**38.3**

CURRICULUM VITAE

Werkervaringen

2018-heden

Internist-Immunoloog en Allergoloog in het Groene Hart ziekenhuid Gouda.

Deelname aan de commissies infectiepreventie en geneesmiddelen commissie.

2015-heden

01-01-2015: Promotieonderzoek in het Erasmus naar IgG4-gerelateerde ziekte. Promotoren: professor van Hagen en dr. Van Laar. Research school: Molmed. Verwachte promotiedatum: eind 2019.

April 2019-heden

Lid hoofdredactieraad Nederlands Tijdschrift voor Allergie, Astma en Klinische Immunologie (NTvAAKI).

Mei 2017-Januari 2018

Internist-Immunoloog en Allergoloog in het Erasmus MC Rotterdam.

2010-2017

01-12-2010: AIOS interne geneeskunde in het Erasmus MC Rotterdam.

01-01-2015: Fellow klinische immunologie en allergologie. Einddatum: 01-06-2017.

2006-2007

Werkzaam bij het studententeam afdeling A2 te Erasmus MC-Daniel den Hoed.

2007-2008

Vrijwilligerswerk bij Mediceventsupport: *het leveren van eerste hulp tijdens diverse evenementen*.

Opleidingen

2003-2010

01-09-2003: Geneeskunde aan het Erasmus MC Rotterdam.

01-08-2004: propedeuse. Mei 2008: doctoraal. November 2010: artsexamen.

1998-2003

VWO, Stevin College te Den Haag. Diploma: behaald in 2003.

Bestuur en organisatie

2019-heden

Commissielid van de commissie infectiepreventie en geneesmiddelencommissie in het Groene Hart ziekenhuis Gouda.

2008-2012

Oprichter en teamleider **Student-Tolk**: het inzetten van geneeskunde studenten als tolk in het Erasmus MC-Sophia. Prijzen: 3e plaats communicatieprijs Medisch Contact 2012. Publicatie: Medisch contact 16 december 2011 nr. 50.

2008-2010

Oprichter en organisator **Masterclass ECG** voor geneeskunde studenten van het Erasmus MC Rotterdam. Docent en coördinator: dr. J.W. Deckers, cardioloog en dr. A.P.J. Klootwijk, cardioloog en onderwijsdecaan Erasmus MC.

2005-2009

Oprichter en voorzitter Aria Afghan Students Association. Aria is toegelaten tot Kaseur (koepelorgaan diverse studentenverengingen EUR): www.ariastudents.nl.

2004-2005

Actief lid jaarvertegenwoordiging (JVT-2) bij de medische faculteit vereniging Rotterdam (MFVR).

Nevenactiviteiten/relevante cursussen

- Verzorgen van het onderwijs klinisch redeneren aan geneeskunde studenten;
- Cursus algemene beginselen van het tolken in een ziekenhuis;
- Cursus behandeling van patiënten met HIV/aids;
- Cursus omgaan met lichamelijk onverklaarbare klachten (SOLK);
- Cursus ABCDE methodiek;
- Cursus DESG (Diabetes Mellitus);
- Cursus begeleiden van coassistenten (teach the teacher);
- Masterclass nagelriem capillairmicroscopie;
- BROK cursus afgerond;
- Internationale symposium over systemische sclerose 2016;
- Symposium medische immunologie 2016;
- Dutch symposium on C1 inhibitor deficiency and other forms of angio-edema 2016;
- Deelname aan alle verplichte regionale en landelijke onderwijsmomenten voor AIOS interne geneeskunde.
- Deelname EAACI congres 2016 en 2017;
- Deelname Groninger basis cursus immunologie 2019.

Publicaties en presentaties

Publicaties

1. **A.F. Karim** , J.M.L. Stouthard en M.B.L. Leys. Portale hypertensie ten gevolge van extramedullaire hematopoëise. **Ned Tijdschr Hematol** 2013;10:190-3);
2. Alidjan FM, Karim F et al. A Patient with Autoimmune Pancreatitis Type 1 with Previously Known Lymphadenopathy, Both in the Context of IgG4-related Disease. The American Journal of cse reports. 2015 Nov 5;16:790-3;
3. **Karim A. F.** et al. Infliximab for IgG4-Related Orbital Disease. OPRS. 2016 Jan 18;
4. **Karim A. F** et al. IgG4-related disease: a systematic review of this unrecognized disease in pediatrics. Pediatric rheumatology online. 2016 Mar 25;14(1);
5. **Karim A. F.** et al. An inflammatory condition with different faces: immunoglobulin G4-related disease**.** The Netherlands Journal of Medicine**.** 2016 Mar;74(3):110-5;
6. **Karim A. F.** et al. IgG4-RD as an emerging cause of scleritis. Acta Ophthalmologica. Feb 2017;

7. **Karim F** et al. AA amyloidosis and IgG4-RD. New England Journal of Medicine. Feb 2017;
8. Heeringa JJ and **Karim A. F** et al. Expansions of blood IgG4+ memory B-cells, Th2 and regulatory T cells in IgG4-related disease: implications for diagnosis and therapy monitoring. Journal of Allergy and Clinical Immunology**.** Mei 2018**;**
9. **Karim A.F.** et al. The call for considering follicular helper T cells in IgG4-related disease: reply. Journal of Allergy and Clinical Immunology. Mei 2018;
10. **Karim A.F**. et al. Implications of elevated C-reactive protein and serum amyloid A levels in IgG4-related disease: comment on the article by Perugino et al. Arthritis Rheumatol. Oct 2017;
11. **Karim A.F.** et al. To distinguish IgG4-related diseases from granulomatosis with polyangiitis. **Rheumatology.** Dec 2017;
12. **Karim A.F.** et al. IgG4-gerelateerde ziekte: stand van zaken. Nederlands Tijdschrift voor Allergie, Astma en Klinische Immunologie (NTvAAKI). Feb 2018;
13. **Karim A.F**. et al. Soluble Interleukin-2 Receptor: A Potential Marker for Monitoring Disease Activity in IgG4-Related Disease. Mediators of Inflammation. March 2018;
14. Hermans M.A.W, **Karim A. F.** et al. Nonsteroidal anti-inflammatory drug hypersensitivity: not always an allergy. Neth J M. Jan 2018;
15. **Karim A. F**. et al. The tarrsal plate manifestation of IgG4-related disease. Int Ophthalmology. March 2018;
16. **Karim A.F**. et al. The treatment outcomes in IgG4-related disease. Neth J M. Aug 2018;
17. Sanne Detiger and **Karim A.F.** et al. The histological absence of IgG positive plasma cells in juvenile xanthogranuloma: commens on systemisch juvenile xanthogranuloma: a case report and brief review. Clin Exp Dermatology. Sep 2018;
18. **Karim A.F.** et al. The treatment outcomes in IgG4-related orbital disease: a systematic review. Acta Ophthalmologica. Feb 2019;
19. **Karim A.F**. et al. The association between allergic diseases and cancer: a systematic review of the literature. Neth J M. Feb 2019.
20. **Karim A**.F. et al. A metadherin gene variant is associated with IgG4-related disease in two unrelated families. Submitted.

Presentaties

- EWIMID congres Amsterdam 2015: speeddate over de resultaten onderzoek naar IgG4-positieve B-cellen;
- Wetenschapsdagen Erasmus MC 2016: poster presentatie over resultaten B-cel studie IgG4-gerelateerde ziekte;
- Presentatie IgG4-gerelateerd ziekte op de Nederlandse internisten dagen;
- Presentatie IgG4-gerelateerde ziekte op de immunologie avond 2017;
- Poster presentatie IgG4-gerelateerd ziekte op de ECI congres 2018.

Talen

- Nederlands: vloeiend
- Dari/Farsi: moedertaal/vloeiend
- Urdu: vloeiend
- Engels: goed

Hobby's

- Cricket.
- Muziek: harmonium.

DANKWOORD

Promotieonderzoek is een proces van samenwerking tussen meerdere personen en disciplines waarbij vanuit verschillende kanten ideeën, suggesties en hulp komen die uiteindelijk leiden tot een proefschrift. Dit proefschrift is tot stand gekomen dankzij de steun en bijdrage van velen. Zowel op het werk als thuis en in mijn sociale omgeving heb ik mogen genieten van mensen die mij gedurende mijn promotietraject enorm hebben gesteund. Hierbij wil ik allen van harte bedanken.

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De leden van de leescommissie: prof. dr. R.P. Peeters, prof. dr. F.J.T. Staal en prof. dr. M. R. van Dijk dank ik voor het kritisch lezen en beoordelen van mijn proefschrift. Tevens wil ik de overige leden van de commissie bedanken voor de bereidheid zitting te nemen in de commissie.

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We zijn een van de eersten in de wereld die een genafwijking hebben ontdekt in twee families met IgG4-gerelateerde ziekte. Er moet nog veel gebeuren, maar dit eerste begin zou niet mogelijk zijn geweest zonder inzet van prof. Peter van der Spek en Sigrid Swagemakers. Ik dank jullie van harte. Tevens wil ik Pauline van Schouwenburg, Marjolein Wentink, Kornvalee Meesilpavikkai, Sita Virakul bedanken voor ieder hun bijdrage aan dit

mooie resultaat. Pauline, je was enorm enthousiast en tevens kritisch over de bevindingen. Mede dankzij jouw werk hebben we uiteindelijk mooie resultaten kunnen behalen. Dank je wel en veel succes in je verdere carrière.

Beste Wichor Bramer, ik ben onder de indruk van je kennis over het uitvoeren van systematisch literatuuronderzoek. Als een biomedical information specialist ben je een onmisbaar persoon in het Erasmus MC. Dank voor je steun.

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Beste Judy, wat heb ik veel steun gehad van je! Voor allerlei vragen en problemen kon ik bij je binnenlopen en je was altijd bereid om mij te helpen. Super veel dank.

Zonder goede collega's om je heen is goed werk verrichten onmogelijk. Ik heb het geluk dat ik zowel in het Erasmus MC als in het Groene Hart ziekenhuis te maken heb met uitstekende collega's. Mijn dank is groot voor de collegialiteit, steun en waardering die ik van jullie ontvang.

Onderzoek naar IgG4-gerelateerde ziekte is onmogelijk zonder patiënten. Ik heb enorm veel waardering voor patiënten die meedoen aan wetenschappelijk onderzoek. Alle patiënten die hebben meegedaan aan de studies in dit proefschrift wil ik van harte bedanken.

Het verhaal eindigt hier niet, maar brengt mij helemaal terug naar het begin. Vanaf mijn geboorte tot de dag van vandaag hebben mijn ouders mij altijd gesteund. Lieve papa en mama, jullie hebben veel meegemaakt. De goede tijden van ooit, werden ineens de slechte tijden van oorlog en ellende. De offers die jullie hebben gebracht om ervoor te zorgen dat wij een veilig onderdak kregen, konden studeren en een goed leven opbouwden, zijn niet met woorden te beschrijven. Het is waarschijnlijk verschrikkelijk geweest voor een advocaat, mijn papa, om in een klap zijn huis, land en zijn baan kwijt te raken

en te leven als een vluchteling. Hetzelfde geldt voor mijn mama die goed werk leverde als docente. Maar ik weet dat jullie enorm dankbaar zijn voor de manier waarop jullie ontvangen zijn in Nederland en ik hoop dat ik enigszins jullie trots heb kunnen maken. Bedankt voor jullie onvoorwaardelijke steun en liefde. Faiq, Sijaar en Jaid: we zijn meer vrienden dan broers, ik ben trots op jullie.

Daarnaast ben ik gezegend met geweldige schoonouders en schoonfamilie. Mijn schoonouders staan onvoorwaardelijk klaar voor me en hierbij wil ik ze hartelijk bedanken. Met mijn twee schoonbroers en een lieve schoonzus ben ik drie vrienden rijker!

Mijn vrienden: we spreken elkaar tegenwoordig vaker in een app-groep dan tijdens een feestje, maar jullie zijn geweldig.

Lieve Nahid, een betere partner dan jij kan ik me niet voorstellen. Jouw onvoorwaardelijke steun, liefde en inlevingsvermogen hebben me de kracht gegeven om steeds verder te gaan met mijn promotieonderzoek. Je maakt me op alle fronten gelukkig. We hebben samen de meest schattige kinderen (Fridaus, Farhan en Salman) van de hele wereld (een dikke kus voor jullie drie). Ik hoop dat we nog vele jaren met geluk en gezondheid samen met onze kinderen, ouders, vrienden en familie bij elkaar kunnen zijn.

Wais Jan, je hebt ons te vroeg verlaten. Wat was jij een geweldig persoon en een schat van mens. Rust in vrede.