UNIVERSITA' VITA-SALUTE SAN RAFFAELE

CORSO DI DOTTORATO DI RICERCA INTERNAZIONALE IN MEDICINA MOLECOLARE

CURRICULUM IN CLINICAL AND EXPERIMENTAL MEDICINE

Disentangling inflammatory drivers of fibrosis in IgG4-related disease

DoS: Prof. Lorenzo Dagna

Second Supervisor: Prof. Matthias J Löhr

Tesi di DOTTORATO di RICERCA di Marco Lanzillotta
Matr. 020161

Ciclo di dottorato XXXVII

SSD MED 09

Anno Accademico 2021/2022

Release of PhD thesis

Il sottoscritto, the undersigned Marco Lanzillotta

Matricola / registration number 020161

nato a/ born in Cuneo

il/on 04/11/1990

autore della tesi di Dottorato di ricerca dal titolo / author of the PhD Thesis titled

Disentangling inflammatory drivers of fibrosis in IgG4-related disease

NON AUTORIZZA la Consultazione della tesi per 12 mesi /DO NOT AUTHORIZE *the public release of the thesis for* 12 *months*

a partire dalla data di conseguimento del titolo e precisamente */ from the PhD thesis date, specifically*

Dal / *from* …1/04/2025… Al / *to* 31/3/2026

Poiché /*because*:

□ l'intera ricerca o parti di essa sono potenzialmente soggette a brevettabilità/ *The wholeproject or parts of it may be the subject of a patent application;*

X ci sono parti di tesi che sono già state sottoposte a un editore o sono in attesa dipubblicazione/ *Parts of the thesis have been or are being submitted to a publisher or arein the press;*

□la tesi è finanziata da enti esterni che vantano dei diritti su di esse e sulla loropubblicazione/ *the thesis project is financed by external bodies that have rights over it andits publication.*

E' fatto divieto di riprodurre, in tutto o in parte, quanto in essa contenuto / *reproduction of the thesis in whole or in part is forbidden*

Data /Date ....13/1/2025..……..... Firma /Signature …….....

DECLARATION

This thesis has been:

- composed by myself and has not been used in any previous application for a degree. Throughout the text I use both 'I' and 'We' interchangeably.

- has been written according to the editing guidelines approved by the University.

Permission to use images and other material covered by copyright has been sought and obtained. For the following image/s (Fig 3-4-5), it was not possible to obtain permission and is/are therefore included in thesis under the "fair use" exception (Italian legislative Decree no. 68/2003).

All the results presented here were obtained by myself, except for:

1) *Flow cytometry analysis (Results, chapter 3.1, figure 1) were performed in collaboration with Dr. Claudia Minici, Biology Department, Università vita salute San Raffaele.*
2) *General concepts related to IgG4-Related disease included in the Introduction have already been published as review on the British Medical Journal, the Lancet Rheumatology, and the UEG Journal in which the PhD candidate was the first author.*
3) *The author was granted approval for the inclusion of Figure 1,6,13,14 for the sake of PhD dissertation by the Journal Editors.*

All sources of information are acknowledged by means of reference.

ACKNOWLEDGMENTS

I would like to thank the colleagues from Prof Sitia's Lab and Mass Spectrometry facilities for insights and collaborations.

DEDICATION

I would like to thank Claudia for her expertise, patience, and guidance throughout these three years.

A Diana e Camilla, stelle polari del mio universo.

ABSTRACT

INTRODUCTION IgG4-related disease is an increasingly recognized fibro inflammatory disorder characterized by serum IgG4 elevation and fibrotic lesions in the affected organs. Organ fibrosis can lead to end organ damage or death if unrecognized and left untreated. B cell depletion reverses myofibroblast activation in affected tissues suggesting that B-lymphocytes and IgG4 antibodies might directly contribute to tissue fibrosis in this condition. Our aim was to investigate a potential IgG-mediated modulation of fibroblasts' phenotype and to identify a possible autoantigen targeted by IgG on fibroblasts.

METHODS Patients fulfilling classification criteria for IgG4RD were included. Fibroblasts from IgG-RD patients were obtained via endoscopic ultrasound pancreatic biopsy and surgical biopsy of inflamed periorbital tissue**.** IgG1 and IgG4 from IgG4-RD patients and healthy controls were employed to stimulate fibroblasts. Soluble factors and collagen production were assessed by using ELISAs. Cell-based ELISA was used to assess the binding of IgG1 and IgG4 to fibroblasts. Quantitative RT-PCR were used to assess fibroblast modulation in the presence of IgG1 and IgG4. Immunoblotting was employed to detect the binding of IgG to pancreatic and orbital lysate. The anti-integrin monoclonal antibody volociximab was used as positive control.

RESULTS Ten IgG4-RD patients were included in this study. IgG1 and IgG4 from IgG-RD patients bind both autologous and heterologous fibroblasts. Stimulation of fibroblasts with IgG1 and IgG4 led to upregulation of laminin and PDGFRα genes and to higher collagen I concentration in the supernatant compared to not stimulated fibroblasts**.** IgG4 stimulation reduced fibroblasts proliferation compared to IgG1. IgG1 from patient IRD1 bound an antigen between 62 and 98 kDA on pancreatic lysate.

CONCLUSIONS IgG1 and IgG4 from IgG4-RD patients can modulate fibroblasts' phenotype toward a pro-fibrotic one. IgG4 appears to exert an anti-proliferative effect compared to IgG1. These features are mediated via binding of IgG1 or IgG4 on auto antigens located on fibroblast surface. Further mass spectrometry studies are required to identify the autoantigen targeted.

TABLE OF CONTENTS

Introduction 9
1.1 Introduction 9
1.2 Epidemiology 10
1.3 Clinical presentation and phenotypes of IgG4-RD 10
1.3.1 IgG4-RD Phenotypes 11
1.3.2 Proliferative and Fibrotic Phenotype 12
1.3.3 IgG4-related Head and Neck disease 15
1.3.4 IgG4-related lung disease 17
1.3.5 IgG4-related Pancreato-biliary disease 18
1.3.6 IgG4-related Kidney disease 20
1.3.7 IgG4-related Retroperitoneum involvement and aortitis 21
1.3.8 IgG4-related sclerosing mesenteritis 23
1.4 Diagnosis 23
1.4.1 ACR/EULAR classification criteria 25
1.4.2 Imaging features of IgG4-RD 26
1.4.4 Novel biomarkers for IgG4-RD diagnosis 31
1.6 Treatment of IgG4-RD 31
1.7 Novel disease activity biomarkers 37
1.8 Pathogenesis 38
1.8.1 Genetic Risk Factors 39
1.8.2 Bacterial Infection and Molecular Mimicry 39
1.8.3 Environmental risk factors 39
1.8.4 B cells 40
1.8.5 The role of IgG4 antibodies 41
1.8.6 Autoantibodies response in IgG4-RD 44
1.8.7 T cells 45
1.8.8 Possible pathogenetic model for fibrosis in IgG4-RD 47
Aim 51
Results 52

3.1 Clinical characteristic of the patients' cohort 52
3.2 IgG1 and IgG4 from IgG4-RD patients bind autologous fibroblasts 53
3.3 IgG1 and IgG4 from healthy controls and IgG4-RD patients bind heterologous fibroblasts 54
3.4 Gene expression modification after IgG1 and IgG4 stimulation 56
3.5 IgG1 and IgG4 increase collagen production from IgG4-RD pancreatic fibroblast 59
3.6 IgG1 from IgG4-RD patient bind an auto antigen on pancreatic lysate 61
3.7 Immunoprecipitation confirms Immunoblot analysis 62
3.8 IgG1 from patient IRD1 bind a surface antigen 63
Discussion 65
Methods 72
Patients 72
Pancreatic and orbital tissue acquisition 72
Isolation and Culture of Fibroblasts from Biopsy Samples 72
Immortalization of Fibroblasts via hTERT Transduction 74
Flow Cytometry 74
Isolation of IgG1 and IgG4 from Blood Using Affinity Resin 75
RNA Isolation, Reverse Transcription (RT), and PCR for Gene Expression Analysis in Fibroblasts Stimulated with IgG 76
Cell-based ELISA for detecting IgG Antibody Binding on Fibroblasts 77
Elisa sandwich for IL-6 and collagen I 77
Immunoblotting 78
Immunoprecipitation 79
Statistical analysis 79
References 80

ACRONYMS AND ABBREVIATIONS

IgG4: immunoglobulin G4**. IgE** immunoglobulin**. IgG4-RD:** IgG4-related disease**. AIP:** autoimmune pancreatitis**. TFH: T** follicular helper cells**. D** Day**. FAP:** fibroblast activation protein**. IL:** Interleukin**. PDGFR:** Platelet-derived grow factor receptor**. PDGF:** Platelet derived grow factor. **ACTA 2:** actin alpha 2. **GADPH**: Glyceraldehyde 3-phosphate dehydrogenase. **Ki-67**: Antigen Kiel 67. **MRI:** Magnetic resonance Imaging**. PET:** Positron emission tomography. **FDG**: [^{18}F]fluorodeoxyglucose. **CT**: Computer tomography. **EUS**: Endoscopic ultrasound. **ESR**: erythrocyte Sedimentation rate. **CRP**: C reactive protein. **ACR**: American college of Rheumatology. **EULAR**: European League against Rheumatism. **GPA**: granulomatosis with polyangiitis. **EGPA**: eosinophilic granulomatosis with polyangiitis. **CD**: cluster of differentiation. **RNA**: RiboNucleic Acid. **DNA**: Deoxyribonucleic acid. **cDNA**: complementary DNA. **HPF**: High power field. **DMARDs**: Disease Modifying Anti Rheumatic Drugs. **GCs**: glucocorticoids. **AZA**: azathioprine; **MMF**: mycophenolate mofetil; **MTX**: methotrexate; **LEF**: leflunomide; **RTX**: rituximab; **CYC**: cyclophosphamide. **ELF**: Enhanced Liver Fibrosis. **RANKL**: Receptor activator of nuclear factor kappa-B ligand. **CCL-18**: chemokine ligan 18. **BAFF**: B Cell Activating Factor. **APRIL**: a proliferation-inducing ligand. **HLA**: human leukocyte antigen. **CTL**: cytotoxic T cell. **TGF**: transforming growth factor. **FAB**: fragment antigen-binding **FC**: fragment crystallizable. **ADAMTS13**: a disintegrin and metalloproteinase with thrombospondin type 1 motif, 13. **GWAS**: genome-wide association study. **PD1**: programmed cell death-1. **BATF**: Basic Leucine Zipper ATF-Like Transcription Factor. **CD40L**: CD40 ligand. **ICOS**: inducible T cell costimulatory. **ICOSL**: ICOS ligand. **PDL1**: PD1 ligand. **SLOs**: secondary lymphoid organs. **TCR**: T cell receptor. **TLOs**: tertiary lymphoid organs. **FGF**: Fibroblast grow factor. **ECM**: extracellular matrix. **SLAMF7**: SLAM Family Member 7. **TCZ**: tocilizumab. **DAR**: daratumumab. **VOL**: Volociximab. **IRD**: IgG4-RD. **HC**: Healthy Controls. **PE**: phycoerythrin. **ELISA**: Enzyme linked Immunosorbent Assay. **HRP**: Horseradish Peroxidase. **SDSPAGE**: Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis. **PCR**: Polymerase Chain Reaction. **PBS**: Phosphate-buffered saline. **DMEM**: Dulbecco's Modified Eagle Medium. **EDTA**: Ethylenediaminetetraacetic acid. **PBMC**: peripheral blood mononuclear cell. **EPCAM**: Epithelial cell adhesion molecule. **FBS**: Fetal Bovine Serum. **Tris**: Tris(hydroxymethyl)aminomethane. **hTERT**: human

Telomerase reverse transcriptase. **dNTP**: deoxynucleotide triphosphates. **TMB**: tetramethylbenzidine. **SEM**: standard error of the mean. **PFA**: Paraformaldehyde. **FcγRs**: Fc gamma receptors.

LIST OF FIGURES AND TABLES

Table 1. Available therapeutic strategies for inducing and maintaining remission of IgG4 related disease (IgG4-RD). Adapted from Lanzillotta et al, 2020.

Table 2. Established, emerging, and novel potential biological therapies for IgG4 related disease. Adapted from Lanzillotta et al, 2020.

Table 3. Clinical and laboratory characteristics of enrolled patients. *Abbreviations: F: female; M: male; IRD: IgG4-related disease; IgG4RD-RI: IgG4-RD Responder Index. Yrs: years; FBs: fibroblasts; I: immortalized; D: discarded.*

Figure 1. Differences and similarities among IgG4 related disease (IgG4-RD) phenotypes in clinical and serological features, outcomes, and therapeutic approaches. *IgG4-RD has a strong male predominance. Compared with white patients, Asian patients are at higher risk of developing IgG4-RD in the head and neck region. Patients with head and neck involvement also seem to more often have atopic manifestations. Patients with Mikulicz's/ systemic disease have more organs involved, higher values of IgG4-RD responder index (RI), and serum IgE concentrations. Patients with retroperitoneal and head and neck involvement seem more prone to have a fibrotic outcome than do those with other IgG4-RD phenotypes.*

Figure 2. Clinical, radiological, laboratory, and pathological features of fibrotic (left) and proliferative (right) phenotype of IgG4-RD.

Figure 3. Head and Neck disease in IgG4-RD. *A) Left submandibular gland involvement. (B) Bilateral swelling of sublingual glands. (C) Lacrimal gland involvement leading to orbital pseudotumor. (D) IgG4-related dacryoadenitis. (E) Left-sided infra-orbital swelling due to soft tissue mass infiltrating the pterygopalatine fossa. (F) Synchronous head and neck IgG4-RD involving lacrimal glands (red circle), frontal nerves (red arrows), infraorbital nerves (red asterisk), and optic nerve sheath (arrowhead). Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol.*

Figure 4. Lung involvement in IgG4-RD. *(G) Bronchovascular subtype, with thickening of interlobular septa mainly in peripheral zones; (H) IgG4 related ground-glass opacity; and (I,J) a solid nodular subtype. (K) circumferential aortic wall thickening (aortitis). (L) Coronary aneurysms due to IgG4-related coronary arteritis. Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol.*

Figure 5. Pancreato-biliary manifestations of IgG4-related disease. *(A–C) Diffuse pancreatic swelling during (A) late arterial phase, (B) portal venous phase, and (C) late phase of contrast administration. Hyperattenuation of pancreatic parenchyma with hypoattenuating pancreatic rim (halo sign) indicated by the yellow arrow (A–C). The red arrow (A) indicates a dilated pancreatic duct, the blue arrow (B) indicates concentric wall thickening of the common bile duct with a plastic stent within the duct lumen. (D) Pancreatic mass (asterisk) and adjacent atrophy (red arrow) due to autoimmune pancreatitis. Iso-hyperintensity of the pancreatic mass during the portal venous phase on CT scan (D) and low apparent diffusion coefficient (E) help to differentiate mass-forming autoimmune pancreatitis from pancreatic adenocarcinoma. (F) Capsule-like rim (halo sign) is typically hypointense in T2 weighted images (dashed yellow arrow). (G) Temporal wasting associated with PEI. (H) Intrahepatic biliary stenosis (red arrows) connected by segments of less stenotic or normal caliber biliary ducts (skip lesions). Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol.*

Figure 6. Renal involvement in IgG4-RD. *(A) Diffuse bilateral kidney swelling. (B,C) Multiple, hyperintense parenchymal lesions (red arrows in B) and substantial reduction of apparent diffusion coefficient value in diffusion weighted image (dashed arrows in C). (D–F) Full disappearance of all lesions and normalization of renal size after 1 month of glucocorticoid treatment. Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol*

Figure 7. Fibrotic lesions in IgG4-RD. *Retroperitoneal fibrosis: soft tissue circumferential to the abdominal aorta (dashed circle) and the right ureter causing right kidney hydronephrosis and functional exclusion (arrow). Right kidney does not accumulate or excrete iodinated contrast media on CT scan. (B) Hypertrophic pachymeningitis: thickening of the dura mater (arrowheads) showing uptake of the contrast media on MRI. (C, D) IgG4-related thyroid disease in a patient before and after treatment. Before treatment, the thyroid gland has a hard, woody feel (brackets; C). The patient was treated with rituximab with only minor dimensional improvement after 4 months (brackets; D). (E) Fibrosing mediastinitis: soft tissue encasing branches of the thoracic aorta on CT scan (asterisk). (F) Sclerosing mesenteritis: hypodense thickening of the mesentery (asterisk).*

Figure 8. Radiological findings of fibrotic IgG4-related disease. *(A) Paravertebral soft tissue showing T1-weighted isointense (i; arrows) and T2-weighted low-signal intensity (ii; arrows) suggesting fibrosis. Gadolinium enhancement (iii; dashed arrows) suggesting a state of active rather than end-stage fibrosis. (Bi) CT scan showing mesenteric nodularity (asterisks). (Bii) Residual fibrosis remains (asterisk) after glucocorticoid therapy. (C) Riedel's thyroiditis (i; dashed circle) showing patchy [18F]-FDG accumulation with areas of intense uptake (ii; dashed arrow, active inflammation) and areas of physiological uptake (arrow, unaffected thyroid tissue or areas of end-stage fibrosis). (Di, ii) Atrophic pancreas after glucocorticoid therapy showing absent [18F]-FDG uptake reflecting fibrotic evolution (arrowhead). (Ei–iii, Fi–iii) [18F]-FDG avid soft tissue surrounding the infrarenal aorta (dashed frame). (Ei–iii) Axial and coronal CT scan shows extensive soft tissue anterolateral to the aorta (dashed circle and frame). (Fi–iii) Follow-up CT after glucocorticoid therapy shows minimal [18F]-FDG-PET avidity (dashed frame) and residual retroperitoneal fibrosis (dashed circle and frame). [18F]-FDG=[18F] fluorodeoxyglucose.*

Figure 9. Pathological features of IgG4-RD. *A. Pancreatic ducts are not affected by the fibroinflammatory infiltrate in IgG4-related autoimmune pancreatitis (arrowhead). B-C. Areas of storiform fibrosis in IgG4-related autoimmune pancreatitis (asterisks). D. Obliterative phlebitis: an obliterated vein surrounded by an inflammatory nodule (arrowhead), next to an intact artery (arrow). E-F. Immunohistochemistry for IgG (E) and IgG4 (F) on sequential sections shows an IgG4/IgG ratio > 40%.*

Figure 10. IgG4-RD treatment algorithm. *Abbreviations. AZA: azathioprine; MMF: mycophenolate mofetil; MTX: methotrexate; LEF: leflunomide; RTX: rituximab; CYC: cyclophosphamide.* Risk factors :**Previous relapses; very high serum IgG4 at baseline; very high IgE at baseline; multiorgan involvement; proximal biliary tree involvement **Damage is not reverted by immunosuppressive treatment.*

Figure 11. IgG4 Fab – Arm exchange. *IgG4 forms disulfide bonds within the heavy chains in its hinge region because, in contrast to the other IgG subclasses, the disulfide bonds between the heavy chains of the IgG4 molecule are unstable. In an IgG4 molecule without disulfide bonds between the heavy chains, dissociations of the noncovalent bonds permit the chains to separate*

and recombine randomly, such that asymmetric antibodies with two different antigen-combining sites are formed. The resulting bispecific (functionally monovalent) IgG4 molecules are unable to crosslink antigens, thereby losing the ability to form immune complexes.

***Figure 12. T cell and B cell crosstalk in IgG4-related disease**. Following germinal centre reactions, affinity- matured B cells leave the follicle and interact with IL-4- secreting BATF+ T follicular helper (TFH) cells. In conjunction with IL-10, IgM+ B cells class- switch to become IgG4+ memory B cells and plasmablasts. Short- lived IgG4+ and IgE+ plasmablasts secrete self-reactive IgG4 and IgE, respectively, circulate and compete for survival niches within the bone marrow as long- lived plasma cells (LLPCs). Both plasmablasts and LLPCs contribute to the marked increases in serum IgG4 and IgE that are often found in IgG4- RD. CD40L, CD40 ligand; ICOS, inducible T cell costimulator; ICOSL, ICOS ligand;PD1, programmed cell death protein 1; PDL1, PD1 ligand 1; RTX, rituximab; SLOs, secondary lymphoid organs; TCR, T cell receptor; TLOs, tertiary lymphoid organs. Adapted from Perugino C et al, Nat Rev Rheum,2020.*

***Figure 13. Active fibrosis**. Fibrogenesis starts with lymph proliferation and inflammation (active fibrosis) when resident fibroblasts or recruited fibrocytes are exposed to fibrotic cytokines. Fibroblasts differentiate into myofibroblasts and produce ECM. Profibrotic signals include TGFβ produced by CD4+ CTCs and macrophages, and PDGFB produced by plasmablasts or plasma cells. Macrophages efferocytose apoptotic bodies and interact with plasmablasts and CD4+ CTCs through the MerTK-ProS1 axis. Plasmablasts contribute to the formation of ECM and to its organization through the production of LOLXL2. Myofibroblasts in turn secrete IL-6, which supports B cell and germinal centre processes. (B) End-stage fibrosis. When the inflammatory triggers are cleared and ECM has largely replaced the lymphocytic infiltrate (end-stage fibrosis), scarring tissue remains with few immune cells (mainly scattered macrophages, memory B cells, and memory T cells) enmeshed in a dense collagenous matrix. (C, D) Waves of lymphocytic inflammation (active fibrosis; CD19+ B-cell infiltrate [arrows] localized in proximity of pancreatic ducts; C), preceding areas of dense fibrosis (end-stage fibrosis; sequential matched haematoxylin and eosin staining showing fibrotic areas [arrows] probably generated as a consequence of the periductal inflammatory infiltrate; D). CD4+ CTCs=CD4+ cytotoxic T cells. ECM=extracellular matrix. PDGFB=platelet-derived growth factor.*

***Figure 14. IgG1 and IgG4 bind autologous fibroblasts.** In the lower panel the saturation curve of the II antibody is reported. Abbreviations: ab: antibody; TCZ: tocilizumab; DAR: Daratumumab; Vol: Volociximab. The correlation coefficient is represented by ρ and reflects positive correlation.*

***Figure 15. Heterologous binding of IgG1 and IgG4 from healthy controls and IgG4-RD patients to pancreatic fibroblasts, compared to negative controls.** In the lower panel values from HC and IRD are grouped together and compared to negative controls, pooled together. Abbreviations: ab: antibody; HC: healthy controls; TCZ: tocilizumab; DAR: Daratumumab; Vol: Volociximab; IRD: IgG4-related disease. IgG1 are reported in boxes with uniform colors whereas IgG4 are reported in those with striped colors. * $p<0.05$; ** $p< 0.01$.*

***Figure 16. Flow cytometry analysis testing IgG1 (yellow) and IgG4 (striped, yellow) binding on pancreatic fibroblasts obtained from IRD-P.** Secondary antibody alone represents the negative control (grey curve), while volociximab (red) and anti-vimentin antibody (blue) represent positive controls. The black solid line represents the threshold of negative control.*

Figure 17. Changes in RNA concentration upon stimulation of pancreatic and orbital fibroblasts with IgG1 and IgG4 from healthy controls (HC), and IgG4-RD patients (IRD)

compared to not stimulated fibroblast (NS). *Abbreviation: ns: not significant. IgG1 are reported in boxes with uniform color whereas IgG4 are reported in those with striped colors. * p<0.05; **p< 0.01*

Figure 18. IgG1- and IgG4-induced fibroblasts' transcriptional changes*. Changes in the transcription of ACTA 2 (A), LAM (B), PDGFR (C), KI67 (D), and IL-6 are reported as ΔΔct. Control is represented by not stimulated fibroblasts at D4. In the left half of the figure are reported the changes in the transcription profile upon stimulation of IgG4-RD fibroblast with IgG1 and IgG4 from each healthy controls (HC) and IgG4-RD patients (IRD). In the right half of the figure, the stimuli from HC and IRD patients are grouped and compared to not stimulated (NS) fibroblast. IgG1 is reported in boxes with uniform color (yellow) whereas IgG4 are reported in those with striped colors. The analysis was performed on both orbital as well as pancreatic IgG4-RD fibroblasts. Given the comparable findings, graphs were merged together in this picture. * p<0.05. Abbreviations: ACTA 2: smooth muscle actin alpha 2. LAM: Laminin 1. PDGFR: Platelet-derived growth factor receptor.*

Figure 19. Elisa sandwich showing the concentration of collagen (A) and IL 6 (B) following the stimulation of pancreatic fibroblasts with IgG1 and IgG4 from healthy controls (HC) and from IgG4-RD patients (IRD). *In panel C a proliferation test is reported. The rate of proliferation is reported at D3 and D5 after stimulation. IgG1 are reported in boxes with uniform color whereas IgG4 are reported in those with striped colors.*

Figure 20. Immunoblot testing the link of IgG1 and IgG4 from IRD1 patient on lysate of pancreatic (P-FB) and orbital (O-FB) IgG4-RD fibroblast*. IgG1 binds a protein heavier than 62 kDA on pancreatic lysate. Volociximab (VOL) is used as a positive control. The two bands in the VOL column represent the heavy and light chain of antibodies.*

Figure 21. Immunoprecipitation of IgG1 and IgG4 and volociximab (Volo) on pancreatic fibroblasts.

Figure 22. Cell-based ELISA performed on not fixed (left) and fixed (right) fibroblasts following stimulation with IgG1 (yellow) and IgG4 (striped, yellow) from two IgG4-RD patients.

Figure 23. Potential pathogenetic model in light of our findings. *B and T cells cross-talk ultimately lead to IgG1 and IgG4 production by plasmablast. IgG1 and IgG4 bind resident fibroblast, increasing collagen production via PDGFR upregulation while IgG4 reduce cell proliferation via Ki-67 down regulation. Adapted from Lanzillotta M, et al. Emerging therapy options for IgG4-related disease, Expert Review of Clinical Immunology.*

Figure 24. Fibroblasts gating strategy. *Abbreviations: FSC: forward scatter; SSC: side scatter; L/D: live and dead. PDGFR: platelet-derived growth factor receptor, FAP: fibroblast activation protein.*

Introduction

1.1 Introduction

IgG4-Related Disease (IgG4-RD) is an increasingly recognized systemic fibroinflammatory disorder characterized by the presence of numerous IgG4-positive plasma cells in affected tissues and elevated IgG4 levels in patients' blood (Lanzillotta et al, 2020). The disease typically manifests with tumefactive lesions that lead to permanent organ injury and death if left untreated. Yet, disease response to corticosteroids is remarkable, although relapses often occur upon immunosuppressive treatment withdrawal(Della-Torre *et al*, 2015b).

Initially described in 2001 as "type I IgG4-related autoimmune pancreatitis" (AIP)(Hamano *et al*, 2001), IgG4-RD was soon identified as a systemic condition due to its distinctive histopathological features found across various organs(Kamisawa *et al*, 2003). Now, IgG4-RD has been reported in nearly every organ system, including the biliary tree, salivary glands, periorbital tissues, kidneys, lungs, lymph nodes, meninges, aorta, retroperitoneum, breast, prostate, thyroid, pericardium, and skin(Kamisawa *et al*, 2015). However, individual manifestations of the disease were identified as early as the 1800s. Since then, several previously distinct conditions—such as Mikulicz syndrome, Küttner tumor, type 1 autoimmune pancreatitis, and Ormond's disease—have been unified under this single diagnosis(Lanzillotta et al, 2020).

Due to its recent appraisal, IgG4-RD immunopathogenesis remains incompletely understood. The prevailing hypothesis is that an antigen-driven immune response leads to B cell activation and IgG4 production, alongside T lymphocyte-mediated secretion of pro-fibrotic cytokines(Lanzillotta *et al*, 2023b). The condition has garnered interest within immunology due to its responsiveness to treatment, particularly corticosteroids, which can reverse fibrotic processes. Unlike other fibrotic diseases, such as systemic sclerosis or idiopathic pulmonary fibrosis, IgG4-RD responds favorably to rituximab, an anti-CD20 monoclonal antibody, which induces rapid clinical improvement, indicating a significant role of B cells in the disease's pathogenesis(Lanzillotta *et al*, 2017b).

Advancing our understanding of the immune dysregulation in IgG4-RD may improve insights into the human immune system and offer therapeutic options for other fibrotic diseases

1.2 Epidemiology

The global incidence and prevalence of IgG4-RD remain significantly underestimated. Studies from Japan indicate that the incidence of autoimmune pancreatitis (AIP) rose from 0.8 to 3.1 cases per 100,000 people between 2007 and 2016, highlighting a rapid increase in IgG4-RD awareness within less than a decade(Nishimori *et al*, 2007; Masamune *et al*, 2020). However, AIP represents only one of the many organs that can be affected by this condition. Among 8000 IgG4-RD patients referred to Japanese hospitals in 2009, 5190 did not show pancreatic involvement, emphasizing the limited understanding of the disease's varied epidemiology. IgG4-RD typically affects middle-aged and older adults, with a male-to-female ratio ranging from 1.6:1 for head and neck manifestations to 4:1 for other organ involvements(Löhr *et al*, 2022). Moreover, IgG4-RD predominantly affects middle-aged to older males, although there are occasional reports in children(Lanzillotta et al, 2020).

Although no epidemiological data are currently available for Europe, it is estimated that AIP constitutes around 6% of all chronic pancreatitis cases, with a prevalence of 10–40 cases per 100,000 individuals. This suggests a prevalence of AIP of approximately 1–2 cases per 100,000 people, which is slightly lower than estimates from Japan(Löhr *et al*, 2022). A recent study by Wallace ZS et al. reported an incidence of IgG4-RD in the USA at 1.39 per 100,000 person-years and a prevalence of 5.3 per 100,000 individuals(Wallace *et al*, 2023).

1.3 Clinical presentation and phenotypes of IgG4-RD

Clinical presentation is usually indolent, with signs and symptoms becoming evident over months or even years. Notably, it does not commonly present with high fever or other signs of systemic inflammation, albeit elevation of ESR and CRP can be present in patients with retroperitoneum involvement(Lanzillotta et al, 2020; Zen et al, 2009). Approximately 30 to 40% of patients have a history of allergies, though symptoms resembling allergic reactions, such as asthma or chronic rhinitis, may also appear in individuals without a history of atopy (Sanders *et al*, 2020; Della Torre *et al*, 2014). IgG4-RD is marked by the presence of pseudotumor-like lesions that can affect one or multiple organs in a synchronous or metachronous fashion. The clinical symptoms are often non-

specific and heavily depend on the organs involved. The disease can be asymptomatic or present with symptoms due to *ab estrinseco* compression exerted by fibrotic masses on surrounding tissues.(Lanzillotta *et al*, 2024b)

It is crucial to recognize atypical forms of IgG4-RD that do not exhibit pseudotumor-like growths, such as tubulo-interstitial nephritis, glomerulonephritis, midline destructive lesions, interstitial lung disease, and pleural or pericardial effusions. Renal involvement, in particular, can manifest with proteinuria, hematuria, renal impairment, or hypocomplementemia. Rarely, life-threatening presentations such as ruptured inflammatory aneurysms, acute coronary syndrome, and acute neurological accidents have been documented (Della-Torre *et al*, 2021b).

1.3.1 IgG4-RD Phenotypes

The broad presentation of IgG4-RD contributes to its difficult diagnosis, which relies upon clinical, laboratory, radiological, and histopathological features. The tendency of IgG4-RD to affect certain organs has been recognized since the disease's early descriptions(Stone *et al*, 2012). However, consistent patterns in clinical manifestations were only thoroughly evaluated recently, when latent class analysis was applied to an international multicentric cohort of IgG4-RD patients that ultimately led to the development of the ACR/EULAR classification criteria. By examining organ involvement in nearly 800 patients, latent class analysis identified four distinct phenotypes of IgG4-RD, offering physicians clinical frameworks to improve disease recognition and potentially guiding treatment(Wallace *et al*, 2019). These phenotypes include pancreato-hepato-biliary disease (31%), retroperitoneal fibrosis with or without aortitis (24%), head and neck-limited disease (24%), and classic Mikulicz's syndrome with systemic involvement (22%). Patients grouped into each phenotype displayed unique clinical, epidemiological, and serological characteristics(Wallace *et al*, 2019; Lanzillotta *et al*, 2020b, 2020a). Indeed, patients with limited head and neck disease (group 3) were predominantly female and of Asian origin, unlike those in other categories (**Figure 1**). This group also tended to be younger and required histological evidence for a definitive diagnosis more frequently than the other groups. On the contrary, more frequent referrals to the emergency department due to symptoms related to IgG4-RD onset (e.g. jaundice) were noted in patients with pancreato-hepato-biliary phenotype (group 1). On the other hand, inflammatory markers (CRP, ESR) elevation was detected

specifically in group 2. Additionally, individuals with Mikulicz's syndrome and systemic involvement (group 4) exhibited the highest median serum IgG4 levels, likely reflecting the increased disease burden(Lanzillotta *et al*, 2020a). This classification will potentially help in the identification of significant biological variations between IgG4-RD phenotypes, in evaluating the efficacy of disease biomarkers within consistent patient cohorts and disease subtypes, and in developing tailored patient monitoring and treatment plans.

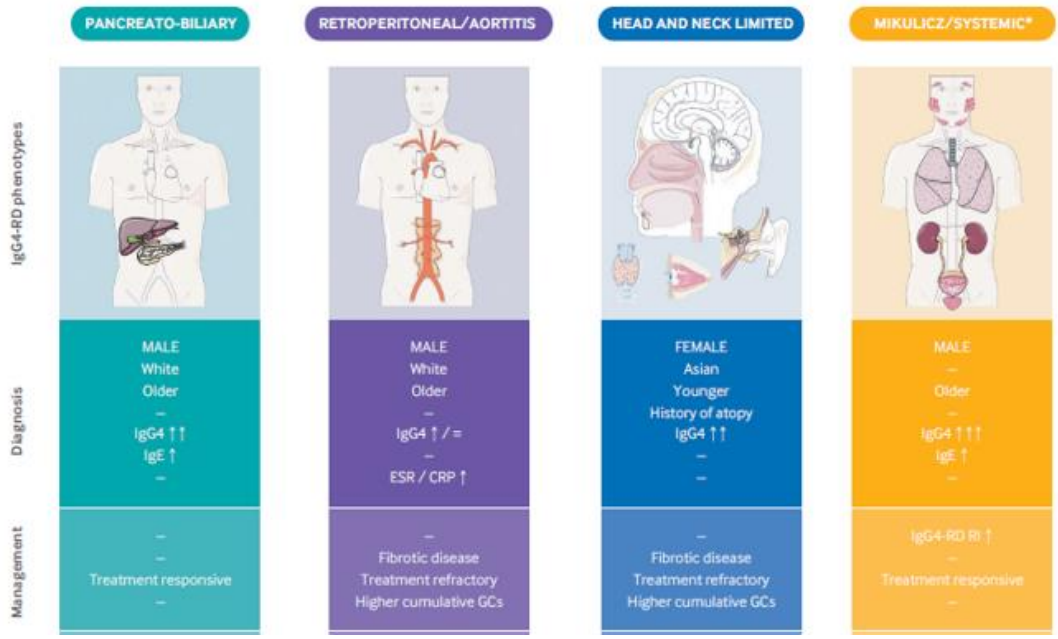
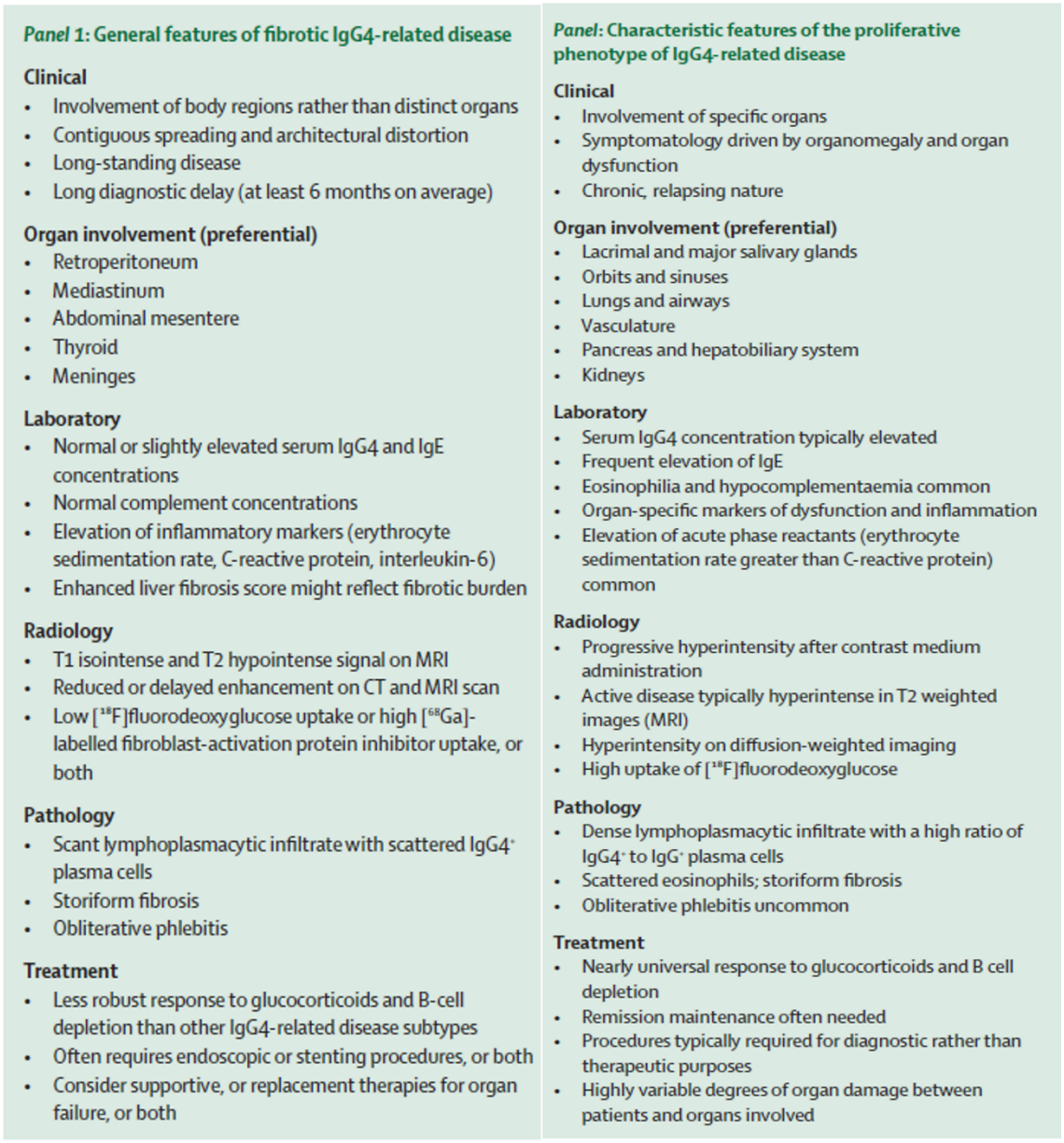


***Figure 1*. Differences and similarities among IgG4 related disease (IgG4-RD) phenotypes in clinical and serological features, outcomes, and therapeutic approaches**. *IgG4-RD has a strong male predominance. Compared with white patients, Asian patients are at higher risk of developing IgG4-RD in the head and neck region. Patients with head and neck involvement also seem to more often have atopic manifestations. Patients with Mikulicz's/ systemic disease have more organs involved, higher values of IgG4-RD responder index (RI), and serum IgE concentrations. Patients with retroperitoneal and head and neck involvement seem more prone to have a fibrotic outcome than those with other IgG4-RD phenotypes..*

1.3.2 Proliferative and Fibrotic Phenotype

Aside, another classification emerged very recently building upon the evidence that some clinical, pathological and laboratory features cluster together(Zhang & Stone, 2019). In addition, despite response to GCs represents a hallmark sign of IgG4-RD, certain manifestations of IgG4-related disease, which are marked by significant tissue fibrosis and minimal lymphocytic infiltration upon histological analysis, respond less effectively to glucocorticoid treatment compared to other disease types(Lanzillotta *et al*,

2024b). Such manifestations include retroperitoneal fibrosis, Riedel thyroiditis, and hypertrophic pachymeningitis among others. The reasons why various manifestations, that shall belong to a unique disease spectrum, have such different responses to glucocorticoids remain to be delineated. To interpret this dichotomy, a provisional distinction between proliferative and fibrotic phenotypes of IgG4- related disease has been proposed. In contrast to the proliferative phenotype, fibrotic IgG4-related disease preferentially affects extra-glandular tissues, such as anatomical regions (e.g., the retroperitoneum and mediastinum), as opposed to distinct organs, as the pancreas, kidneys, or extraocular muscles(Lanzillotta *et al*, 2024b; Katz *et al*, 2024). The fibrotic phenotype of IgG4-related disease also tends to involve single organs or single districts of the body (e.g., the retroperitoneum) rather than multiple organs simultaneously. Serological hallmarks of lymphoproliferation and plasma cell activation, such as a polyclonal hypergammaglobulinemia and elevated serum IgG4 level, are seldom observed in patients with disease features linked to the fibrotic subset. Similarly, the dense lymphoplasmacytic infiltrate observed during overt inflammation in affected organs is replaced by fibrotic features, such as predominant collagen deposition, scant numbers of immune cells, and fewer IgG4+ plasma cells compared with patients with proliferative disease. General features of the proliferative and fibrotic phenotypes of IgG4-related disease are summarized in **Figure 2**(Lanzillotta *et al*, 2024b; Katz *et al*, 2024)**.**



***Panel 1*: General features of fibrotic IgG4-related disease**

Clinical
- Involvement of body regions rather than distinct organs
- Contiguous spreading and architectural distortion
- Long-standing disease
- Long diagnostic delay (at least 6 months on average)

Organ involvement (preferential)
- Retroperitoneum
- Mediastinum
- Abdominal mesentere
- Thyroid
- Meninges

Laboratory
- Normal or slightly elevated serum IgG4 and IgE concentrations
- Normal complement concentrations
- Elevation of inflammatory markers (erythrocyte sedimentation rate, C-reactive protein, interleukin-6)
- Enhanced liver fibrosis score might reflect fibrotic burden

Radiology
- T1 isointense and T2 hypointense signal on MRI
- Reduced or delayed enhancement on CT and MRI scan
- Low [^{18}F]fluorodeoxyglucose uptake or high [^{68}Ga]-labelled fibroblast-activation protein inhibitor uptake, or both

Pathology
- Scant lymphoplasmacytic infiltrate with scattered IgG4$^+$ plasma cells
- Storiform fibrosis
- Obliterative phlebitis

Treatment
- Less robust response to glucocorticoids and B-cell depletion than other IgG4-related disease subtypes
- Often requires endoscopic or stenting procedures, or both
- Consider supportive, or replacement therapies for organ failure, or both

***Panel*: Characteristic features of the proliferative phenotype of IgG4-related disease**

Clinical
- Involvement of specific organs
- Symptomatology driven by organomegaly and organ dysfunction
- Chronic, relapsing nature

Organ involvement (preferential)
- Lacrimal and major salivary glands
- Orbits and sinuses
- Lungs and airways
- Vasculature
- Pancreas and hepatobiliary system
- Kidneys

Laboratory
- Serum IgG4 concentration typically elevated
- Frequent elevation of IgE
- Eosinophilia and hypocomplementaemia common
- Organ-specific markers of dysfunction and inflammation
- Elevation of acute phase reactants (erythrocyte sedimentation rate greater than C-reactive protein) common

Radiology
- Progressive hyperintensity after contrast medium administration
- Active disease typically hyperintense in T2 weighted images (MRI)
- Hyperintensity on diffusion-weighted imaging
- High uptake of [^{18}F]fluorodeoxyglucose

Pathology
- Dense lymphoplasmacytic infiltrate with a high ratio of IgG4$^+$ to IgG$^+$ plasma cells
- Scattered eosinophils; storiform fibrosis
- Obliterative phlebitis uncommon

Treatment
- Nearly universal response to glucocorticoids and B cell depletion
- Remission maintenance often needed
- Procedures typically required for diagnostic rather than therapeutic purposes
- Highly variable degrees of organ damage between patients and organs involved

***Figure 2**. Clinical, radiological, laboratory, and pathological features of fibrotic (left) and proliferative (right) phenotype of IgG4-RD.*

It is crucial to understand that both phenotypes are classified based on shared patterns of clinical, laboratory and pathological features. These phenotypes should be seen as two extremes of the classical IgG4-RD presentation; patients with the proliferative type often exhibit characteristics typical of the fibrotic type, and vice versa. Furthermore, individuals with the former may respond positively to immunosuppressive treatment (Lanzillotta *et al*, 2024b; Katz *et al*, 2024).

IgG4-related disease often affects the pancreas, biliary tree, orbits, lacrimal and salivary glands, nasal sinuses, lungs, large blood vessels, pancreas, the kidneys and the retroperitoneum. These features are typically caused by organ enlargement and dysfunction due to inflammatory infiltrate, while manifestation due to fibrosis are mainly related to contiguous organs compression(Katz *et al*, 2024; Peng *et al*, 2023).

1.3.3 IgG4-related Head and Neck disease

IgG4-RD typically displays symmetrical involvement of the lacrimal, parotid, and submandibular glands. Indeed, this represents one of the most common presentation of IgG4-RD, previously acknowledged as Mikulicz syndrome. Of note, as part of the IV phenotype, head and neck disease is frequently coupled with visceral organ involvement such as pancreas and bile duct. Thus, it is prudent to perform cross-sectional imaging in all patients, especially in the context of elevated serum IgG4 (Katz *et al*, 2024; Zhang & Stone, 2019). Notably, in stark contrast with Sjogren syndrome, sarcoidosis, and sialodochititis, sicca symptoms are usually absent. Yet, in the latter phases of the disease, fibrosis can replace glandular parenchyma, thus leading to reduced saliva production. (Peng *et al*, 2023). The importance of glandular involvement has been underlined both in the AIP diagnostic criteria as well as in the ACR/EULAR Classification criteria, where the presence of glandular disease represents a strong clue toward IgG4-RD diagnosis(Shimosegawa *et al*, 2011).

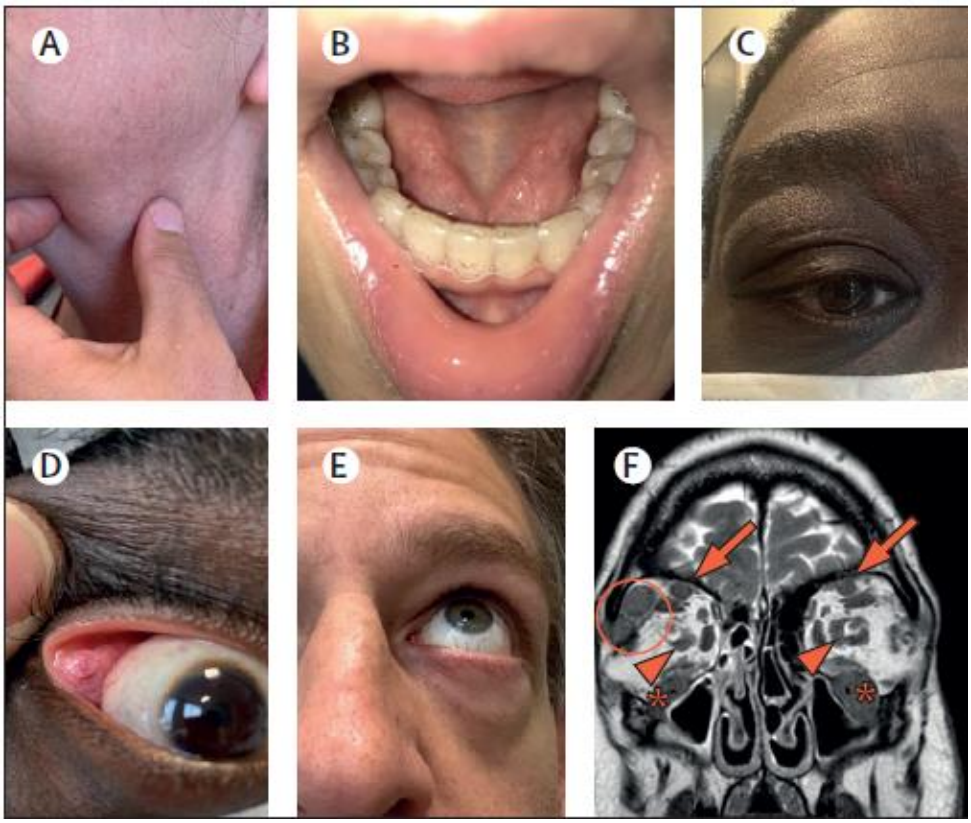


Figure 3. ***Head and Neck disease in IgG4-RD***. *A) Left submandibular gland involvement. (B) Bilateral swelling of sublingual glands. (C) Lacrimal gland involvement leading to orbital pseudotumor. (D) IgG4-related dacryoadenitis. (E) Left-sided infra-orbital swelling due to soft tissue mass infiltrating the pterygopalatine fossa. (F) Synchronous head and neck IgG4-RD involving lacrimal glands (red circle), frontal nerves (red arrows), infraorbital nerves (red asterisk), and optic nerve sheath (arrowhead). Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol.*

Unilateral or bilateral orbital inflammation characterize the third phenotype and is usually present in younger females, compared to other phenotypes. Symptoms include periorbital swelling, pain, proptosis, ultimately leading to impairment of eye movement, and vision loss if not treated promptly nerves(Katz *et al*, 2024; Della-Torre *et al*, 2014).

Chronic rhinosinusitis and midline destructive lesions are common features of head and neck disease. Notably, it is difficult to distinguish IgG4-RD rhinosinusitis with idiopathic allergic polyposis and usually reflect an atopic background. These phenotypes are usually couple with peripheral eosinophilia, elevated serum IgE, and even IgG4-rich plasma cell infiltrates, and concomitant asthma(Lanzillotta et al, 2017; Della-Torre et al, 2014).

IgG4-related hypertrophic pachymeningitis is one of the most dreadful complications of IgG4-related disease. Symptoms can range from headache to visual disturbance or seizures due to local compression of adjacent nerves and anatomical structures. Spinal involvement is relatively uncommon and can present with neck stiffness, back discomfort, and neurological dysfunctions caused by spinal cord compression(AbdelRazek & Stone, 2017; Hwang *et al*, 2016). Due to the high clinical overlap between IgG4-related hypertrophic pachymeningitis and other causes of hypertrophic pachymeningitis, the diagnosis requires a multimodal approach, possibly including the results of cerebrospinal fluid tests(Della-Torre *et al*, 2018b).

Notably, many conditions can mimic head and neck disease, such as sarcoidosis, lymphoma, Castleman Disease, eosinophilic granulomatosis with polyangiitis (EGPA), granulomatosis with polyangiitis (GPA),, extraintestinal manifestations of inflammatory bowel disease, and Rosai-Dorfman. Therefore, a comprehensive multimodal evaluation is mandatory to rule out these conditions.

1.3.4 IgG4-related lung disease

IgG4-related lung disease can have several presentations. Imaging often reveals bronchial wall thickening, which is commonly linked to asthma symptoms like shortness of breath, wheezing, or coughing. Pulmonary involvement may include peri-bronchovascular thickening, interlobular septal enlargement, lung nodules, or interstitial lung disease(Zen *et al*, 2009a). IgG4-related pulmonary masses can be large, display [^{18}F]fluorodeoxyglucose uptake on PET scans, and possess concerning radiological traits such as irregular edges, spiculation, or calcification, leading them to be frequently misdiagnosed as cancer (Katz *et al*, 2024; Nikolic *et al*, 2020). In these patients, interstitial lung disease typically appears as ground-glass or consolidative opacities, mimicking an organizing pneumonia pattern, although rare instances of more fibrotic interstitial lung disease forms can occur. In one third of the patients, lung disease is coupled with pleural involvement, that often manifests as asymptomatic pleural thickening or exudative pleural effusions, presenting clinically with exertional dyspnea. Additionally, pleural involvement in IgG4-related disease may be associated with pericardial thickening or effusion(Dragos *et al*, 2024; Corcoran *et al*, 2017).

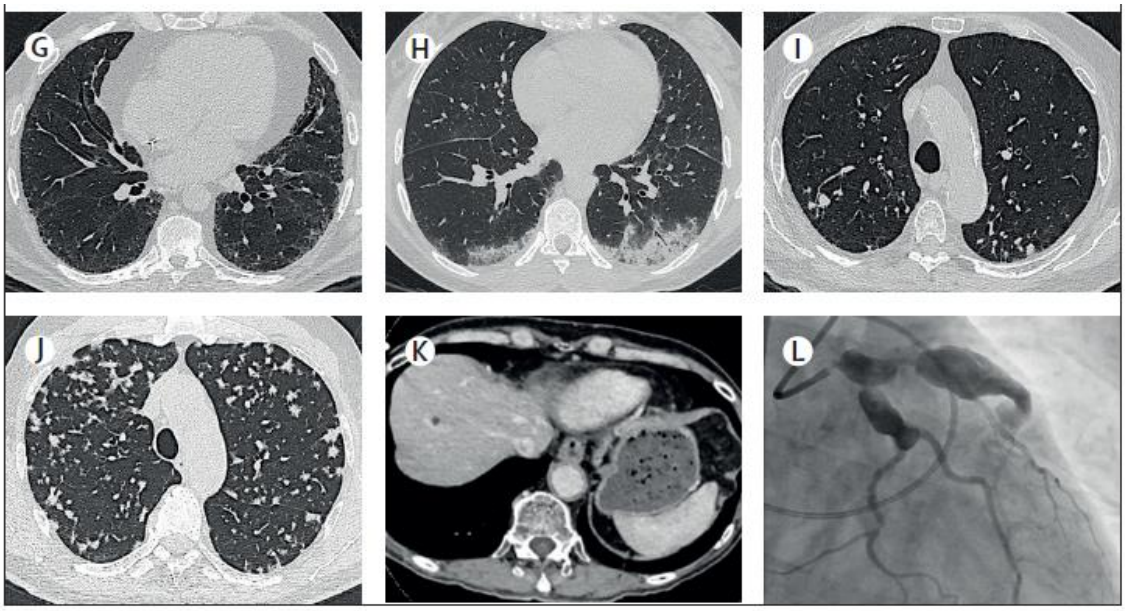


Figure 4. ***Lung involvement in IgG4-RD***. *(G) Bronchovascular subtype, with thickening of interlobular septa mainly in peripheral zones; (H) IgG4 related ground-glass opacity; and (I,J) a solid nodular subtype. (K) circumferential aortic wall thickening (aortitis). (L) Coronary aneurysms due to IgG4-related coronary arteritis. Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol*

1.3.5 IgG4-related Pancreato-biliary disease

Autoimmune pancreatitis (AIP) represents an inflammatory disease of the pancreas that can lead to chronic pancreatitis and that is often coupled with bile duct involvement (IgG4-related cholangitis). To date, two types of AIP have been identified: type 1 and type 2 AIP. The former directly stems from the seminal findings from Japanese investigators (Kamisawa *et al*, 2003; Hamano *et al*, 2001) who unveiled the link between serum IgG4 level and the so called 'lymphoplasmacytic pancreatitis' (Sarles et al, 1961). Indeed, type 1 AIP is now considered the most frequent manifestation of IgG4-related disease, a multi-organ fibro-inflammatory disorder characterized by dense lymphoplasmacytic infiltrate and storiform fibrosis in the affected organs that typically involves elderly males(Zamboni *et al*, 2004; Okazaki *et al*, 2017). On the contrary, type 2 AIP emerged as a distinct entity in the early 2000s after acknowledging a different pathological scenario featured by neutrophilic infiltration within the lumen and epithelium of the interlobular ducts a finding designated as 'granulocyte epithelial lesion' (GEL)(de Pretis *et al*, 2024; Kamisawa *et al*, 2011). In contrast to type 1 AIP, type 2 AIP is limited to the pancreas and involves younger patients with no sex predominance(Hart *et al*, 2013; Löhr *et al*, 2021). AIP involving the pancreatic head or the entire gland, for

instance, may lead to obstructive jaundice and abdominal pain whereas involvement of the uncinate process may be incidentally discovered. Additional manifestations may include signs and symptoms of endocrine and exocrine pancreatic insufficiency, such as steatorrhea, postprandial bloating, increased stool frequency, and weight loss(Lanzillotta et al, 2020; Overbeek et al, 2024).

Up to 50% of patients with autoimmune pancreatitis have concurrent biliary involvement in the form of IgG4-related cholangitis, and the frequency of biliary involvement in autoimmune pancreatitis varies widely across reports(Culver *et al*, 2019).

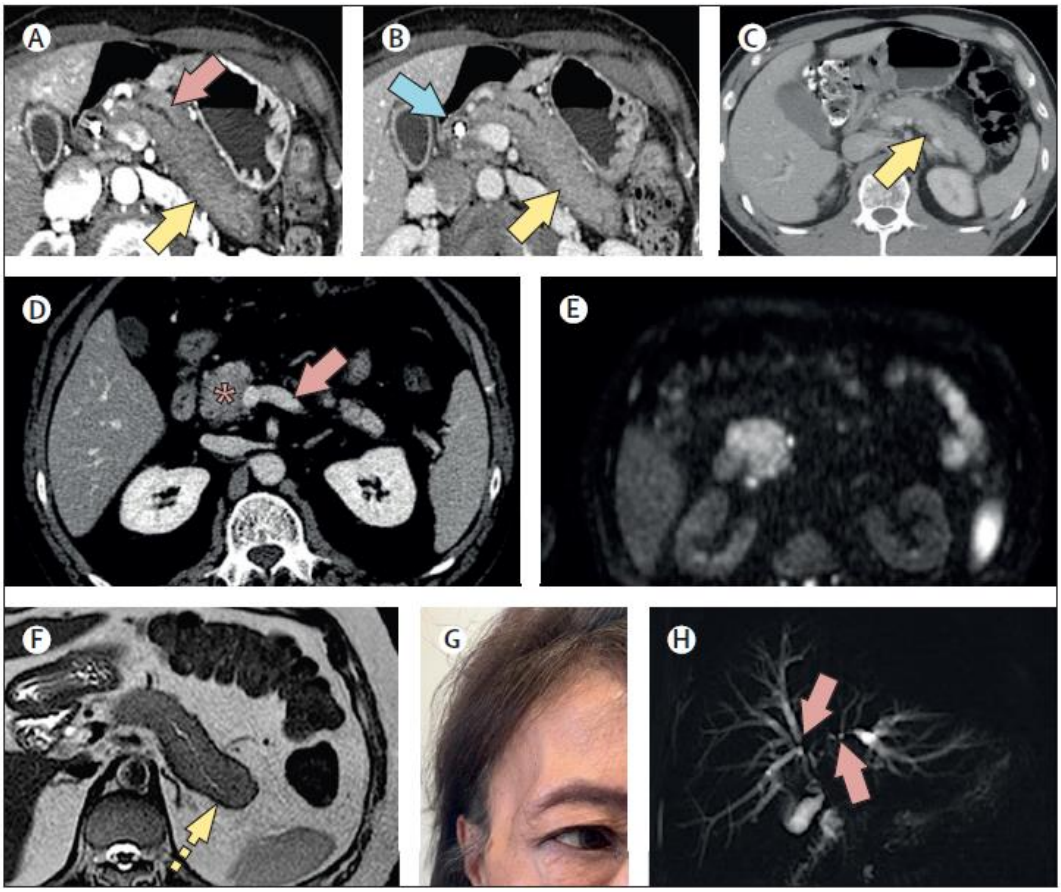


Figure 5 Pancreato-biliary manifestations of IgG4-related disease. *(A–C) Diffuse pancreatic swelling during (A) late arterial phase, (B) portal venous phase, and (C) late phase of contrast administration. Hyperattenuation of pancreatic parenchyma with hypoattenuating pancreatic rim (halo sign) indicated by the yellow arrow (A–C). The red arrow (A) indicates a dilated pancreatic duct, the blue arrow (B) indicates concentric wall thickening of the common bile duct with a plastic stent within the duct lumen. (D) Pancreatic mass (asterisk) and adjacent atrophy (red arrow) due to autoimmune pancreatitis. Iso-hyperintensity of the pancreatic mass during the portal venous phase on CT scan (D) and low apparent diffusion coefficient (E) help to differentiate mass-forming autoimmune pancreatitis from pancreatic adenocarcinoma. (F) Capsule-like rim (halo sign) is typically hypointense in T2 weighted images (dashed yellow arrow). (G) Temporal wasting associated with PEI. (H) Intrahepatic biliary stenosis (red arrows) connected by*

segments of less stenotic or normal caliber biliary ducts (skip lesions). Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol

1.3.6 IgG4-related Kidney disease

Renal involvement is frequently seen in patients with IgG4-RD, being represented by tubulo-interstitial nephritis and, to a lesser extent, by glomerulonephritis, both often asymptomatic(Vujasinovic *et al*, 2019; Kawano *et al*, 2011). IgG4-related tubulointerstitial nephritis may display normal or slightly reduced renal function alongside minor proteinuria. Since this proteinuria is not glomerular, it typically lacks albumin and is better identified using the urine protein–creatinine ratio rather than standard urinalysis(Katz *et al*, 2024). Roughly half of those with IgG4-related kidney disease also show C3 or C4 hypocomplementaemia, or both. Imaging such as cross-sectional CT or MRI can reveal IgG4-related tubulointerstitial nephritis as hypoenhancing, round or wedge-shaped lesions at the kidney's periphery or as diffuse enlargement of the kidney(Vujasinovic *et al*, 2019). Because IgG4-related kidney disease often lacks symptoms, patients may exhibit kidney damage by diagnosis, potentially progressing to chronic or end-stage kidney disease. Key risk factors for renal damage include older age, elevated peak serum creatinine, and higher serum IgG4 levels. Prompt treatment can enhance renal function and prevent chronic kidney disease(Alexander *et al*, 2013).

Membranous glomerulonephritis occurs in less than 10% of patients with IgG4-related tubulointerstitial nephritis. Notable proteinuria should raise suspicions of membranous glomerulonephritis. Unlike idiopathic membranous glomerulonephritis, the IgG4-related form typically lacks anti-phospholipase A2 receptor antibodies(Buglioni *et al*, 2024; Alexander *et al*, 2013).

Beyond tubulointerstitial nephritis and membranous glomerulonephritis, IgG4-related disease can present varied radiological patterns in the renal or genitourinary systems. Renal parenchymal masses can mimic cancer, needing biopsy for clear differentiation. Masses at the ureteropelvic junction or inflammation of the ureter may also occur, at times resulting in hydronephrosis. Prostate involvement can present as lower urinary tract symptoms and focal radiological changes, both generally improving with treatment(Liu *et al*, 2020b; Huggett *et al*, 2014).

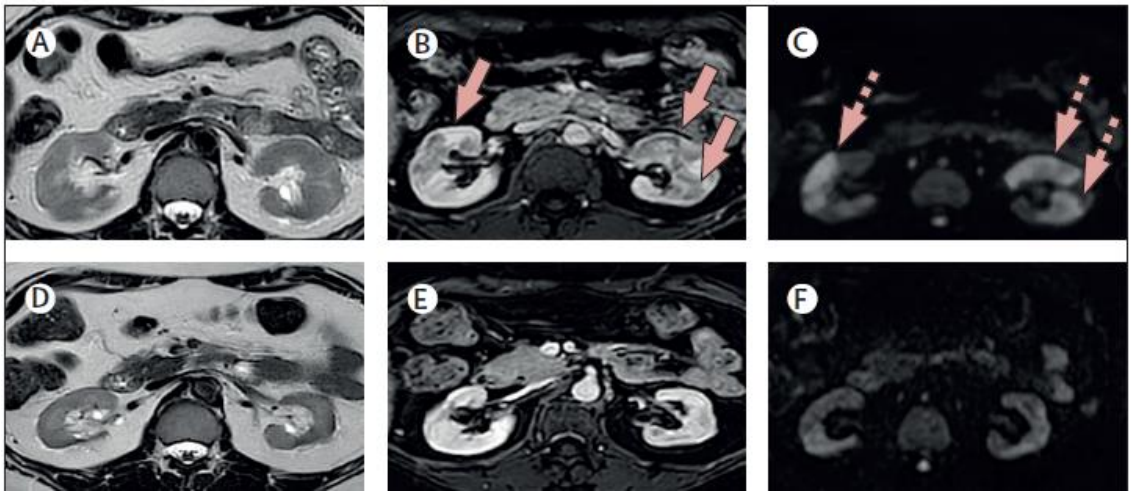


Figure 6. Renal involvement in IgG4-RD*. (A) Diffuse bilateral kidney swelling. (B,C) Multiple, hyperintense parenchymal lesions (red arrows in B) and substantial reduction of apparent diffusion coefficient value in diffusion weighted image (dashed arrows in C). (D–F) Full disappearance of all lesions and normalization of renal size after 1 month of glucocorticoid treatment. Images courtesy of dr Katz and adapted from Katz et al. Proliferative manifestation of IgG4-RD. Lancet Rheumatol.*

1.3.7 IgG4-related Retroperitoneum involvement and aortitis

IgG4-related retroperitoneal fibrosis is characterized by extensive fibroinflammatory tissue that tends to encase the abdominal aorta and is estimated to account for nearly 40–50% of all retroperitoneal fibrosis(Vaglio *et al*, 2006; Fenaroli *et al*, 2021). IgG4-related retroperitoneal fibrosis can involve contiguous vascular, ureteral, and lymphatic structures, leading to complications due to *ab extrinseco* compression of inferior vena cava or hydronephrosis through ureteral entrapment. Patients with IgG4-related retroperitoneal fibrosis tend to have fewer organs or body areas affected by the disease compared to other disease phenotypes(Lanzillotta *et al*, 2024b). Nevertheless, the occasional co-occurrence of disease in another IgG4- related disease organ, such as the submandibular gland, kidney, orbits, or lung, strongly supports the inclusion of retroperitoneal fibrosis and other primarily fibrotic presentations of the disease under the same IgG4-related disease diagnosis. The clinical presentation of IgG4- related retroperitoneal fibrosis includes variable manifestations that appear gradually over time, such as lower back or flank pain, leg oedema, oligoanuria, and weight loss(Vaglio *et al*, 2006; Fenaroli *et al*, 2021; Zen *et al*, 2009b). Furthermore, inflammatory markers are elevated in two-thirds of the patients, while serum IgG4 concentration is normal 40–50% of patients. For these reasons, IgG4-retroperitoneal fibrosis is rarely identified at disease

onset, showing longer diagnostic delay compared with other disease phenotypes(Kasashima *et al*, 2018).

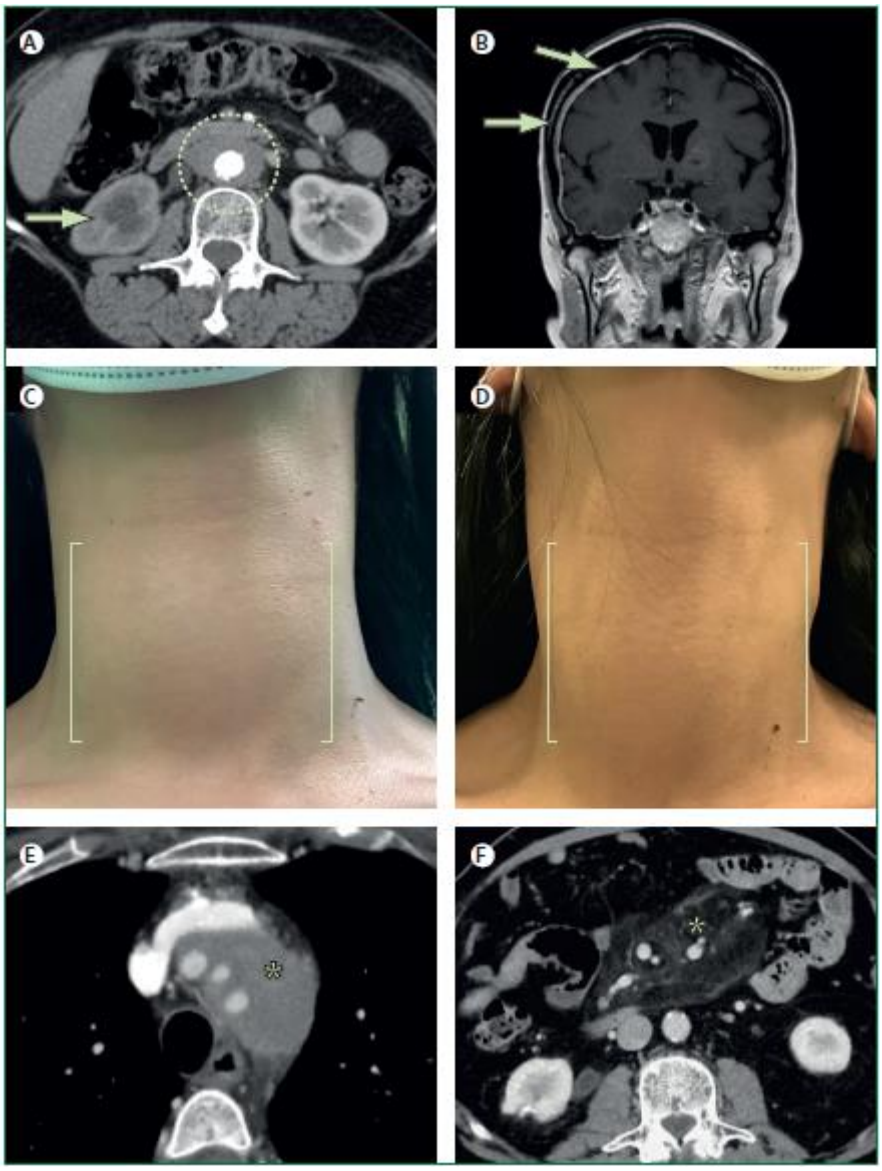


Figure 7. ***Fibrotic lesions in IgG4-RD***. *Retroperitoneal fibrosis: soft tissue circumferential to the abdominal aorta (dashed circle) and the right ureter causing right kidney hydronephrosis and functional exclusion (arrow). Right kidney does not accumulate or excrete iodinated contrast media on CT scan. (B) Hypertrophic pachymeningitis: thickening of the dura mater (arrowheads) showing uptake of the contrast media on MRI. (C, D) IgG4-related thyroid disease in a patient before and after treatment. Before treatment, the thyroid gland has a hard, woody feel (brackets; C). The patient was treated with rituximab with only minor dimensional improvement after 4 months (brackets; D). (E) Fibrosing mediastinitis: soft tissue encasing branches of the thoracic aorta on CT scan (asterisk). (F) Sclerosing mesenteritis: hypodense thickening of the mesentery (asterisk).*

1.3.8 IgG4-related sclerosing mesenteritis

Sclerosing mesenteritis affects the bowel mesentery, and can lead to a broad range of symptoms, with a variable response to immunosuppressive treatments(Danford *et al*, 2019; Akram *et al*, 2007). It has been linked to multiple factors, including prior surgeries, infections, cancer, and autoimmune disorders. IgG4-related disease likely contributes to about 30% of cases, as indicated by significant fibrosis found in biopsy samples. In most instances, sclerosing mesenteritis is associated with normal serum IgG4 levels and seldom involves multiple organs, often causing delays in diagnosis. The most common symptom upon presentation is abdominal pain, followed by bloating, abdominal distension, diarrhea, and weight loss(Akram *et al*, 2007; Danford *et al*, 2019; Lanzillotta *et al*, 2024b). If left untreated, the condition can lead to long-term issues such as bowel obstruction, chylous ascites, and chronic mesenteric ischemia**.**

1.4 Diagnosis

Definite diagnosis of IgG4-RD necessitates a thorough clinical-pathological correlation, as clinical evaluations, lab tests, and imaging alone are often inadequate to differentiate it from disease mimickers, such as neoplastic, inflammatory, and infectious diseases(Lanzillotta *et al*, 2020b). In IgG4-RD patients serological results tend to be non-specific. The ESR might show a moderate increase, while CRP levels generally remain normal, except in certain conditions like retroperitoneal and aortic involvement, where they may slightly rise(Lanzillotta *et al*, 2020a). A significant increase in acute phase reactants should prompt suspicion of other diseases closely resembling IgG4-RD, including ANCA-associated vasculitis and multicentric Castleman disease(Batani *et al*, 2023a). Yet, patients with very high IgG4 level might show increased ESR due to increase blood viscosity. About 30% of patients experience peripheral blood eosinophilia and elevated serum IgE levels. Some individuals may exhibit low-titer antinuclear antibodies or test positive for rheumatoid factor, or both. Serum IgG4 levels are elevated in 55-97% of cases, particularly among Asian individuals, and mirror the disease burden in terms of organ involvement and disease activity(Aalberse *et al*, 2009; Rispens *et al*, 2011).

Although serum IgG4 elevation can serve as a helpful initial screening tool, its diagnostic value is limited due to low specificity as it may occur in a wide range of neoplastic, infectious, and autoimmune diseases. Additionally, measuring serum IgG4 is prone to analytical errors. Most global laboratories measure IgG4 levels through

turbidimetry or nephelometry, but the former can yield falsely normal values due to antigen excess, also known as the "prozone phenomenon"(Culver *et al*, 2017). Besides IgG4, other IgG subclasses such as IgG1, IgG2, and IgG3 are frequently elevated, although typically to a lesser extent than IgG4. Notably, IgG1 elevation may contribute to complement consumption in about 25% of patients with active IgG4-RD. In such cases, a baseline urinalysis is advisable, as reduced serum C3 and C4 levels might suggest subclinical or overt kidney involvement(Yue *et al*, 2022). Autoantibodies specific to other diseases, such as ANCA, SSA/Ro, SSB/La, double-stranded DNA, RNP, and Sm, are usually absent in IgG4-RD, steering diagnosis towards other autoimmune disorders(Lanzillotta *et al*, 2020b). Radiological findings are generally non-specific in most affected organs, with IgG4-related AIP as an exception. This condition typically presents on CT and MRI as a diffusely enlarged, "sausage-shaped" pancreas surrounded by a halo of swollen tissue (rim sign). PET scan emerged as a powerful tool for IgG4-RD diagnosis both for staging and identifying areas that can be more easily sampled(Hart *et al*, 2015; Gallo *et al*, 2024; Chari *et al*, 2006).

Given the limitations of serological and radiological features, histological examination is crucial for a definitive diagnosis and should be performed whenever feasible(Deshpande *et al*, 2012). Obtaining high-quality biopsy samples can be challenging, especially in the case of retroperitoneum and aortitis phenotype, which might necessitate invasive procedures. Five pathological hallmarks of IgG4-RD were identified: lymphoplasmacytic infiltrate, a tissue fibrosis with “storiform” pattern, obliterative phlebitis, a number of infiltrating IgG4 positive plasma cells per high power field that varies according to the affected organ, and an IgG4 positive to IgG positive plasma cell ratio exceeding 40% on immunohistochemistry staining(Zen & Deshpande, 2019; Deshpande *et al*, 2012). Notably, the first three histopathological features are critical for diagnosis, whereas immunohistochemistry is secondary in importance because mimicker neoplastic and inflammatory disorders can also show increased tissue IgG4 counts. Neutrophils, leukocytoclastic vasculitis, granulomas, and tissue necrosis do not belong to the spectrum of pathological features of IgG4-RD and, even if accompanied by a prominent IgG4 positive plasma cell infiltrate, should prompt exclusion of other inflammatory conditions such as ANCA associated vasculitis and sarcoidosis(Wallace *et al*, 2020b, 2020a).

Overall, IgG4-RD diagnosis is thus achieved upon the presence of suggestive clinical, laboratory, radiological, and pathological findings. The comprehensive diagnostic criteria were proposed in 2011 by a consensus of Japanese experts to classify IgG4-RD as “definitive,” “probable,” or “possible” depending on a combination of clinical, serological, and pathological features. In particular, a “possible” diagnosis of IgG4-RD is formulated in the absence of pathological confirmation, which is instead mandatory to fulfill a definitive diagnosis(Umehara *et al*, 2017, 2012).

1.4.1 ACR/EULAR classification criteria

The classification criteria for IgG4-RD were released in 2019 by experts from the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) specifically for classification purposes. These criteria were formulated based on consensus exercises and validated using over 1,000 cases of IgG4-RD and around 800 cases involving similar conditions. Experts employed multi-criterion decision analysis to pinpoint, score, and test criteria with optimal specificity. This led to the development of a three-step classification procedure composed by a main entry criterion, exclusion criteria, and a group of weighted inclusion criteria(Wallace *et al*, 2020b, 2020a).

Following this classification process, at least one of 11 possible organs typically targeted by IgG4-RD must be involved in order to proceed through the algorithm (entry criteria). The exclusion criteria are then applied; if any is fulfilled, the patient is ruled out for IgG4-RD classification. Subsequently, inclusion criteria based on clinical, serological, radiological, and pathological findings are weighed and summed. A patient is classified in case of a combined score of 20 or higher. It is important to highlight that a patient can still be diagnosed with IgG4-RD even in the presence of exclusion criteria or if the 20-points threshold is not achieved. This classification algorithm demonstrates remarkable accuracy in differentiating IgG4-RD from other conditions it might mimic—such as malignancies, granulomatous diseases, and vasculitides—with a specificity of 97.8% and a sensitivity of 82.0%(Wallace *et al*, 2020b; Lanzillotta *et al*, 2020b).

Notably, features that weighed heavily in IgG4-RD classification within the inclusion criteria included involvement of multiple sets of salivary and lacrimal glands (14 points), thoracic paravertebral band-like soft tissue (10 points), diffuse pancreatic

enlargement with capsule-like rim and biliary tree involvement (19 points), low-density areas in bilateral renal cortex (10 points), and soft tissue around the infra-renal aorta (8 points), thus reflecting the high positive predictive value of this findings in the proper context. A recent retrospective analysis found that 39 out of 40 patients (98%) who met the comprehensive diagnostic criteria for definite IgG4-RD were also classified using the classification criteria, highlighting the promising utility of this new algorithm. Consequently, it is likely that the 2019 ACR/EULAR classification criteria will be widely adopted globally, not only for classifying IgG4-RD patients but also as a useful framework for guiding diagnosis. However, these criteria are not designed for diagnosing but rather for identifying uniform patient groups for clinical trials, research, and observational studies(Lanzillotta *et al*, 2020b; Wallace *et al*, 2020b).

1.4.2 Imaging features of IgG4-RD

Differentiating the fibrotic and inflammatory aspects of IgG4-related disease through imaging is complex due to their coexistence(Katz *et al*, 2024; Lanzillotta *et al*, 2024b). Indeed, they likely represent a continuum where both features can be appreciated in the same organ or in different organs until end-stage fibrosis occurs, where extracellular matrix (ECM) entirely replaces the cellular infiltrate. Notably, while ultrasound cannot effectively distinguish between fibrosis and inflammation since both result in hypoechoic signals, MRI and CT can effectively guide the diagnosis. Indeed, MRI and CT offer valuable insights for distinguishing fibrotic and inflammatory features via the combination of specific sequences and contrast enhancement. In MRI, fibrosis produces T1 isointense and T2 hypointense signals compared to muscle, while inflammation presents with T2 hyperintensity(Lanzillotta *et al*, 2024b; Löhr *et al*, 2021). Additionally, fibrotic tissues show no restriction on diffusion-weighted images due to decreased cellularity. On MRI and CT, dynamic contrast infusion can highlight reduced or delayed enhancement in relatively avascular fibrotic tissue, whereas inflammation shows early and rapid post-contrast enhancement. A heavily fibrotic phenotype might be indicated by T2-weighted sequences showing bile duct strictures and wall thickening in IgG4-related sclerosing cholangitis, compared to the liver parenchyma's low signal intensity on MR cholangiopancreatography(Luo *et al*, 2019; Berti *et al*, 2017).

Observing adjacent structures can further aid in differentiating between inflammation and fibrosis. Masses that displace or constrict anatomical structures suggest

inflammation, whereas those causing architectural distortion and tissue retraction indicate fibrosis. Indeed, the medial deviation and dilation of ureters seen in retroperitoneal fibrosis is an example of fibrosis-driven structure alterations(Fenaroli *et al*, 2021; Maritati *et al*, 2019). Fibrosis is suspected when abnormalities persist on follow-up imaging despite immunosuppressive treatment. For example, a 25-50% reduction in periaortic soft tissue after glucocorticoids or rituximab in retroperitoneal fibrosis suggests a residual fibrotic component unlikely to respond further to current therapies(Lanzillotta *et al*, 2024b). Similarly, recurring or persistent strictures after treatment point to a more fibrotic nature in IgG4-related sclerosing cholangitis. Notably, alternative diagnosis, such as cancers, should be sought if lesions enlarge or remain stable after treatment.

Non-invasive molecular techniques, like [^{18}F]FDG PET, might provide additional insights into the fibrotic and inflammatory components of IgG4-related disease(Berti *et al*, 2017). Generally, [^{18}F]FDG uptake indicates increased glucose metabolism during active inflammation and resolves with treatment response. However, glucose metabolism is absent in ECM components of IgG4-related disease and significantly reduced in both resting and activated fibroblasts, complicating the assessment of tissue fibrosis dynamics. Recent research explored the use of 68Ga-prolyl endopeptidase FAP inhibitor (FAPI), a novel tracer specific for prolyl endopeptidase FAP known as fibroblast activation protein-α, widely used in oncology for studying sarcomas and tumor stroma(Kessler *et al*, 2022; Mori *et al*, 2023; Luo *et al*, 2019). FAP expression by activated fibroblasts in IgG4-related disease tissues was found, with 68Ga-FAPI positivity linked to prominently fibrotic lesions with no [^{18}F]FDG uptake. Conversely, [^{18}F]FDG-positive lesions exhibited dense lymphoplasmacytic infiltration of IgG4+ plasma cells, corroborated by clinical responses to immunosuppressive treatment being less pronounced in fibrotic (68Ga-FAPI positive and [^{18}F]FDG negative) lesions compared to inflammatory ([^{18}F]FDG positive) ones(Schmidkonz *et al*, 2020).

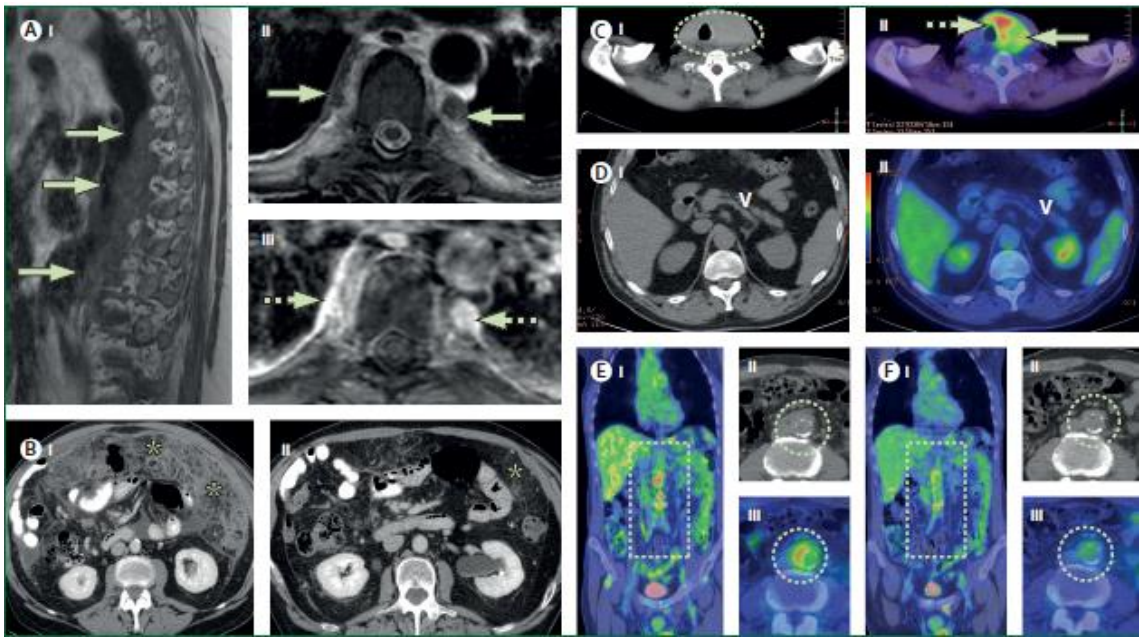


Figure 8. ***Radiological findings of fibrotic IgG4-related disease.*** *(A) Paravertebral soft tissue showing T1-weighted isointense (i; arrows) and T2-weighted low-signal intensity (ii; arrows) suggesting fibrosis. Gadolinium enhancement (iii; dashed arrows) suggesting a state of active rather than end-stage fibrosis. (Bi) CT scan showing mesenteric nodularity (asterisks). (Bii) Residual fibrosis remains (asterisk) after glucocorticoid therapy. (C) Riedel's thyroiditis (i; dashed circle) showing patchy [18F]-FDG accumulation with areas of intense uptake (ii; dashed arrow, active inflammation) and areas of physiological uptake (arrow, unaffected thyroid tissue or areas of end-stage fibrosis). (Di, ii) Atrophic pancreas after glucocorticoid therapy showing absent [18F]-FDG uptake reflecting fibrotic evolution (arrowhead). (Ei–iii, Fi–iii) [18F]-FDG avid soft tissue surrounding the infrarenal aorta (dashed frame). (Ei–iii) Axial and coronal CT scan shows extensive soft tissue anterolateral to the aorta (dashed circle and frame). (Fi–iii) Follow-up CT after glucocorticoid therapy shows minimal [18F]-FDG-PET avidity (dashed frame) and residual retroperitoneal fibrosis (dashed circle and frame). [18F]-FDG=[18F] fluorodeoxyglucose.*

These preliminary findings underscore a pathogenic transition from inflammation to advanced fibrosis, suggesting that non-invasive functional monitoring of IgG4-related disease progression from a proliferative to a fibrotic phenotype may become feasible through a combination of 68Ga-FAPI and [^{18}F]FDG-PET and CT findings. A positive FAPI signal, without [^{18}F]FDG-PET uptake, might indicate ongoing fibrotic activity in IgG4-related disease lesions and justify using anti-fibrotic instead of anti-inflammatory treatments. Further validation of this approach for IgG4-related disease and other fibrotic conditions is necessary(Schmidkonz *et al*, 2020; Lanzillotta *et al*, 2020b, 2024b).

In summary, imaging techniques remain the most effective strategy for assessing tissue fibrosis in IgG4-related disease lesions. Given the chronic course of the disease and the necessity for long-term follow-up, we prefer widely available imaging methods

without ionizing radiation, such as ultrasound and MRI. The optimal timing for repeat imaging is yet to be defined. Typically, if a 6-month glucocorticoid regimen is administered, we conduct a baseline contrast-enhanced MRI, then repeat it at 2 and 6 months to evaluate therapy response, followed by MRI every 6 months for 2 years to monitor disease activity. Ultrasound helps confirm IgG4-related disease relapses promptly and guides additional imaging assessments.

1.4.3 Histopathology

Fibrotic IgG4-related disease, developing de novo or secondary to chronic proliferative IgG4-related disease, is marked by extensive fibrosis and minor scattered lymphoplasmacytic infiltrates compared to proliferative phenotype. Notably, key histological characteristics of IgG4-related disease, like storiform fibrosis and obliterative phlebitis, change distinctly as the disease progresses from a proliferative to a fibrotic state(Lanzillotta *et al*, 2024b; Zen & Nakanuma, 2010; Zhang *et al*, 2020; Deshpande *et al*, 2012).

In contrast to proliferative IgG4-related disease, the inflammatory infiltrate in fibrotic IgG4-related disease primarily consists of a limited number (<10/HPF) of mature lymphocytes and plasma cells. The formation of lymphoid follicles is less frequent in fibrotic IgG4-related disease compared to its proliferative counterpart, and the IgG4+ plasma cells tend to be sparse, with occasional clusters having more than 10 cells per high-power field and an IgG4 to IgG+ cell ratio exceeding 40%(Deshpande *et al*, 2012; Lanzillotta *et al*, 2020b, 2024b).

Fibrosis is consistently present, often with a storiform pattern. Unlike the non-specific fibrosis seen in other conditions, the collagen fibers in IgG4-related disease exhibit a distinct irregular whorled arrangement with significant amounts of type IV collagen. In the context of proliferative IgG4-related disease, storiform fibrosis is associated with numerous CD168+ M2-type macrophages coupled with sparse fibroblasts and myofibroblasts(Rovati *et al*, 2021; Chinju *et al*, 2022). Conversely, in fibrotic IgG4-related disease, storiform fibrosis appears as a process rich in fibroblasts and myofibroblasts with a reduced macrophages infiltrate.

Vascular channels in IgG4-related disease may become partially or entirely obstructed by the sclerosing inflammatory process, leading to obliterative phlebitis. While vascular outlines can be observed in proliferative IgG4-related disease, in its fibrotic form, veins become completely encased in connective tissue, necessitating elastin stains for vascular identification(Kamisawa *et al*, 2015). The ongoing presence of obliterated veins in post-treatment cases of fibrotic IgG4-related disease indicates that obliterative phlebitis is an irreversible histological feature. Therefore, fibrotic IgG4-related disease can be seen as a severe and persistent variant of IgG4-related disease, requiring thorough histological evaluation for accurate recognition, as the distinctive microscopic features of proliferative IgG4-related disease become less clear over time(Della-Torre *et al*, 2015b).

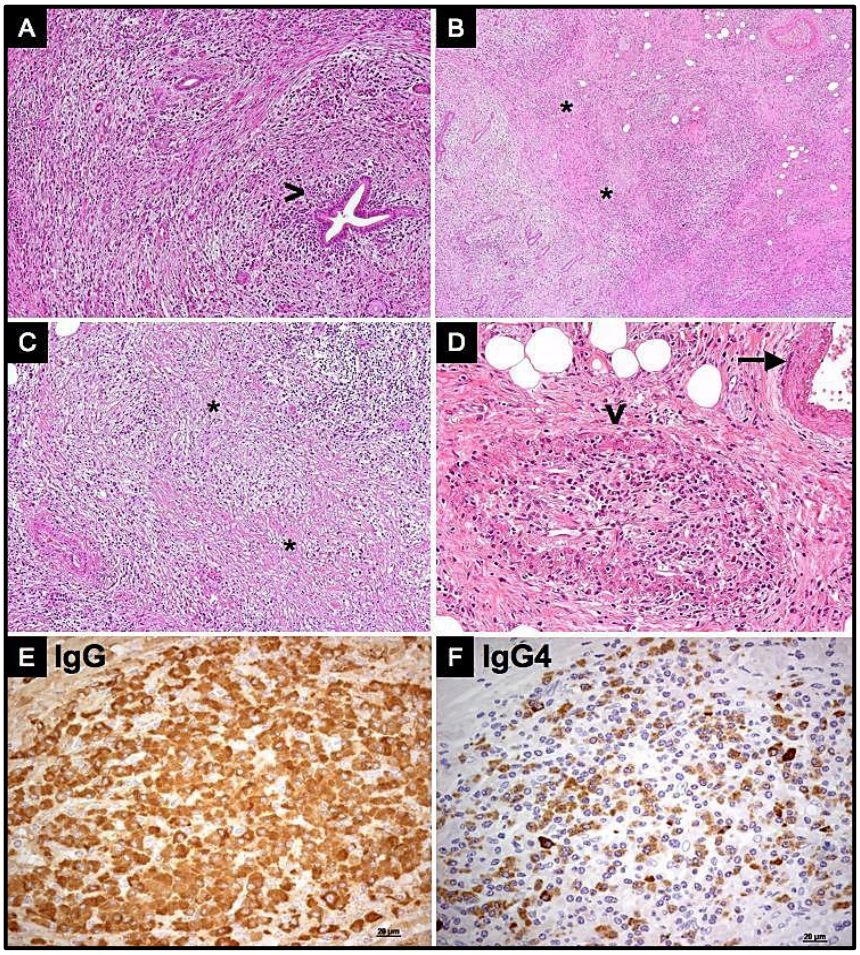


***Figure 9**. Pathological features of IgG4-RD. A. Pancreatic ducts are not affected by the fibroinflammatory infiltrate in IgG4-related autoimmune pancreatitis (arrowhead). B-C. Areas of storiform fibrosis in IgG4-related autoimmune pancreatitis (asterisks). D. Obliterative phlebitis: an obliterated vein surrounded by an inflammatory nodule (arrowhead), next to an intact artery (arrow). E-F. Immunohistochemistry for IgG (E) and IgG4 (F) on sequential sections shows an IgG4/IgG ratio > 40%.*

1.4.4 Novel biomarkers for IgG4-RD diagnosis

Understanding the immunological changes associated with IgG4-RD has paved the way for proposing several new serological and cellular biomarkers. These biomarkers are currently undergoing validation in prospective multicenter studies. For example, higher ratios of serum IgG4 to total IgG (over 10%) or to IgG1 (over 24%) have been found to enhance diagnostic specificity, even when IgG4 levels are only slightly elevated(Yue *et al*, 2022; Lin *et al*, 2017; Dugic *et al*, 2022; Iaccarino *et al*, 2022).

Quantitative PCR assessing the IgG4 to IgG RNA ratio in peripheral blood has effectively differentiated IgG4-related cholangitis from malignant and inflammatory conditions of the hepatobiliary system, with a sensitivity of 94% and a specificity of 99%(Iaccarino *et al*, 2022). Techniques such as multicolor flow cytometry, next-generation sequencing, and gene expression analysis have helped identify specific B and T cell subpopulations that are expanded in both the blood and affected tissues of IgG4-RD patients(Lanzillotta *et al*, 2020b). Notably, oligoclonally expanded hypermutated CD19lowCD20negCD27+CD38hi plasmablasts are elevated in individuals with elevated or normal serum IgG4, providing better diagnostic sensitivity and specificity for IgG4-RD compared to serum IgG4 alone(Lin *et al*, 2017; Wallace *et al*, 2015; Mattoo *et al*, 2014b; Lanzillotta *et al*, 2017b). Next-generation sequencing of circulating plasmablasts revealed that in patients with IgG4-related cholangitis, class-switched IgG4 positive clones are preferentially expanded compared to other biliary tract disorders, making it a promising diagnostic marker for pancreato-biliary IgG4-R(Löhr *et al*, 2022)D.

Lastly, while several autoantigens in IgG4-RD, such as prohibitin, annexin A11, laminin 511, and galectin-3, have been identified, the autoantibodies against these proteins are infrequently found in patient serum and can also appear in some healthy individuals and similar conditions, thereby offering limited specificity and sensitivity for diagnosis(Perugino & Stone, 2020; Liu *et al*, 2020a). Yet, it has been noted that the presence of multiple auto antigens correlated with a higher disease activity and a more aggressive disease(Liu *et al*, 2020a).

1.6 Treatment of IgG4-RD

A consensus statement on the treatment of IgG4-RD was issued 2015, outlining seven overarching principles for managing and treating the disease. However, the

evidence level (ranging from 2b/B-5) and recommendation strength (ranging from B-D) were generally low (Khosroshahi *et al*, 2015). There was a strong consensus among experts on the necessity of a thorough clinical-pathological evaluation for diagnosing IgG4-RD and the use of glucocorticoids as the primary treatment (with agreement levels between 94-96%). In contrast, opinions varied regarding the use of DMARDs for initiating and sustaining IgG4-RD remission (with agreement levels between 46-81%), likely due to differing practices in various countries(Khosroshahi *et al*, 2015; Lanzillotta *et al*, 2020b).

The consensus recommends IgG4-RD treatment for all symptomatic patients and for asymptomatic patients with (I) persistent pancreatic focal mass at imaging, (III) liver test abnormalities in case of concomitant IgG4-related cholangitis, or (III) subclinical situations that could lead to irreversible organ failure (34,46). It is still debated whether prompt treatment alters the progression toward chronic pancreatitis (Khosroshahi *et al*, 2015; Okazaki *et al*, 2017).

Glucocorticoids (GCs) are considered the first-line therapy for inducing remission due to their high remission rates, reported to range from 95% to 100% in various cohorts (95%-100% according to the described cohorts) (Okazaki *et al*, 2017; Hart *et al*, 2013; López-Serrano *et al*, 2016; Overbeek *et al*, 2024). The recommended starting dose of prednisone is typically between 0.6 and 0.8 mg/kg for 2–4 weeks (Okazaki *et al*, 2017; Hart *et al*, 2017, 2015). If a response to treatment is confirmed within this period, the dose is gradually tapered by 5 mg every two weeks and discontinued within 3–6 months(Hart *et al*, 2013; Okazaki *et al*, 2017). However, several studies, including a randomized trial, have emphasized the importance of maintenance therapy to prevent relapses of type 1 autoimmune pancreatitis (AIP), with relapse rates reaching as high as 70% (Della-Torre et al, 2015; Campochiaro et al, 2020; Majumder et al, 2018; Masamune et al, 2017). Maintenance therapy is particularly important for patients at higher risk of relapse, such as those with a history of relapses, involvement of the proximal biliary tree, multi-organ disease, or significantly elevated baseline IgG4 levels(Yue *et al*, 2022; Tacelli *et al*, 2019). Culver et al. demonstrated that an IgG4 serum level of ≥2.8 g/L at diagnosis is associated with an increased risk of relapse, with higher levels correlating with greater risk(Culver *et al*, 2017). Additionally, Lee et al. found that the involvement of the proximal and distal biliary tree in IgG4-related disease is associated with distinct long-

term outcomes, with proximal involvement significantly increasing the risk of relapse (Lee *et al*, 2018). Moreover, patients for whom relapse could lead to organ failure benefit from extended treatment duration.

A pan-European retrospective study involving 735 patients with type 1 IgG4-related autoimmune pancreatitis (AIP) found no significant difference in remission rates between higher doses (≥0.4 mg/kg/day) and lower doses (<0.4 mg/kg/day) of corticosteroids for induction therapy (Lanzillotta *et al*, 2020c; Overbeek *et al*, 2024). Similarly, maintaining the initial dose of GCs for more than two weeks did not prove more effective than shorter courses (<2 weeks) in achieving remission. Furthermore, remission induction was not influenced by the duration of steroid tapering or the total cumulative steroid dose. These findings align with earlier research by Buijs et al., which showed comparable response rates in patients treated with low-dose glucocorticoids (<20 mg/day) and high-dose regimens (>40 mg/day) (Buijs *et al*, 2014). Collectively, these data underscore the high sensitivity of IgG4-RD to GCs and suggest that aggressive corticosteroid regimens offer no additional benefit over lower doses while increasing the risk of steroid-related toxicity(Lanzillotta *et al*, 2022).

To reduce the risk of relapse, various strategies have been implemented, including low-dose GCs, immunosuppressive agents, and rituximab (Yunyun *et al*, 2017, 2019; Lanzillotta *et al*, 2023b, 2023a, 2024c). In a randomized controlled trial (RCT), Masamune et al. demonstrated the superiority of long-term (3 years) low-dose GCs over induction therapy alone(Masamune *et al*, 2017). However, extended GC therapy may exacerbate metabolic complications, particularly in an already vulnerable patient population(Omar *et al*, 2020; Lanzillotta *et al*, 2022). Immunosuppressive agents are typically used alongside GCs and include azathioprine, mycophenolate mofetil, methotrexate, leflunomide, tacrolimus, ciclosporin A, iguratimod, and cyclophosphamide(Wang *et al*, 2018; Yunyun *et al*, 2017, 2019; Lanzillotta *et al*, 2020b). The rationale for employing disease-modifying antirheumatic drugs is to reduce the cumulative GC dosage, enhance remission rates, and lower relapse rates compared to GCs alone. This approach has been supported by findings from two prospective trials and a meta-analysis(Omar *et al*, 2020; Yunyun *et al*, 2017, 2019). A detailed overview of the different maintenance treatment strategies is provided in **Table 1**.

Drug	***Initial Dose***	***Tapering***	***Maintenance***	***Study design***
Glucocorticoids	*Po PDN 0.6 mg/kg/day* *Po PDN 30-60 mg/day* *Iv MPDN 250-500 (3-5days) →switch po* *Po PDN 0.5 v 1 mg/kg day (4 weeks)* *Po PDN 0.6 mg/kg/day*	*5 mg/1-2 weeks (2-6 months)* *5mg/1-2 weeks (2-6 months)* *5-10% every 2 weeks* *12 weeks*	*2.5-10 mg/day (6-26 months)* *2.5-10 mg/day (6-26 months)* *7.5-10 mg/day (24 weeks)* *5-7.5 mg/day v0 (36 month)*	*Cohort studies* *RCT* *RCT*
DMARDs *-Azathioprine* *- Methotrexate* *-Mycophenolate mofetil* *- CYC* *- Cyclosporin A* *- Tacrolimus* *- 6 Mercapto-p* *-Iguratimod*	*-0.5-2.5 mg/kg/day* *-po/sc15-20 mg/week* *-1-1.5 g/day (6 months)* *- po 50-100 mg/day* *- po 100 mg/day* *- po 1-2.6 mg/day* *- po 1-2.6 mg/kg/day* *- po 50 mg/day*	*-0.5-1g /day*	*-0.5-2.5 mg/kg/day* *-po/sc15-20 mg/week* *-1-1.5 g/day (6 months)* *- po 50 mg/day* *- po 100 mg/day* *- po 1-2.6 mg/day* *- po 1-2.6 mg/kg/day* *- po 50 mg/day*	*Case series* *Case series* *RCT* *Cohort studies* *Case series* *Case series*
Rituximab	*Iv 1 gr (2 infusion 15 days apart* *Iv 375 mg/mq (4 weekly infusions)*		*Iv 1 gr (2 infusion 15 days apart or single infusion every 6 months)*	*Retrospecti ve cohort studies*

Table 1*. Available therapeutic strategies for inducing and maintaining remission of IgG4 related disease (IgG4-RD). Adapted from Lanzillotta et al, 2020.*

Given the central role of B cells in IgG4-related disease (IgG4-RD), B-cell depletion therapy using rituximab (administered either as 375 mg/m² weekly for four weeks or as two 1000 mg infusions 15 days apart) has been shown to achieve high remission rates, even in the absence of concomitant glucocorticoids (GCs), across multiple international cohorts(Khosroshahi *et al*, 2012; Carruthers *et al*, 2015; Hart *et al*; Campochiaro *et al*, 2020). This approach is particularly suitable for patients with GC intolerance, GC resistance, or recurrent relapses. Notably, several retrospective studies and a recently published meta-analysis revealed that patients maintained on periodic

rituximab infusions experienced lower relapse rates. Additionally, monitoring total CD19+ cells and memory B cells in peripheral blood has been shown to aid in predicting autoimmune pancreatitis (AIP) recurrence and optimizing the interval for rituximab infusions(Majumder *et al*, 2018; Lanzillotta *et al*, 2021). Building on the demonstrated efficacy of B-cell depletion therapy in IgG4-RD, two randomized controlled trials are currently underway to evaluate the potential of other B-cell-targeting agents, such as inebilizumab and obexelimab, in the treatment of type 1 AIP (Stone *et al*, 2024; Perugino *et al*, 2023; Do *et al*, 2023). A summary of biologic therapies potentially effective in IgG4-RD is provided in **Table 2**, while **Figure 10** outlines a treatment algorithm reflecting current management strategies for IgG4-RD.

***Table 2**. Established, emerging, and novel potential biological therapies for IgG4 related disease. Adapted from Lanzillotta et al, 2020.*

Target	***Mechanism of action***	***Agent***	***Development stage***
B cells	*CD20 targeting* *CD19 targeting* *CD38 targeting* *Proteasome degradation* *B cells inhibition*	*Rituximab, obinutuzumab* *Inebilizumab* *Daratumumab* *Bortezomib* *Obexelimab*	*Open label trial* *RCT* - *Case report* *RCT*
T cells	*Prevention of CD 28 mediated T cell activation by targeting CD80*	*Abatacept*	*Open label trial*
B and T cells	*SLAMF7 targeting*	*Elotuzumab*	-
Complement	*Inhibtion of complement pathway*	*Eculizumab, avacopan*	-
Cytokines	*IL-1 blockade* *IL-6 blockade* *IL 4/13 blockade*	*Anakinra* *Tocilizumab* *Dupilumba*	*Case reports*
Fibrosis	*LOXL2 targeting*	*Simtuzumab*	

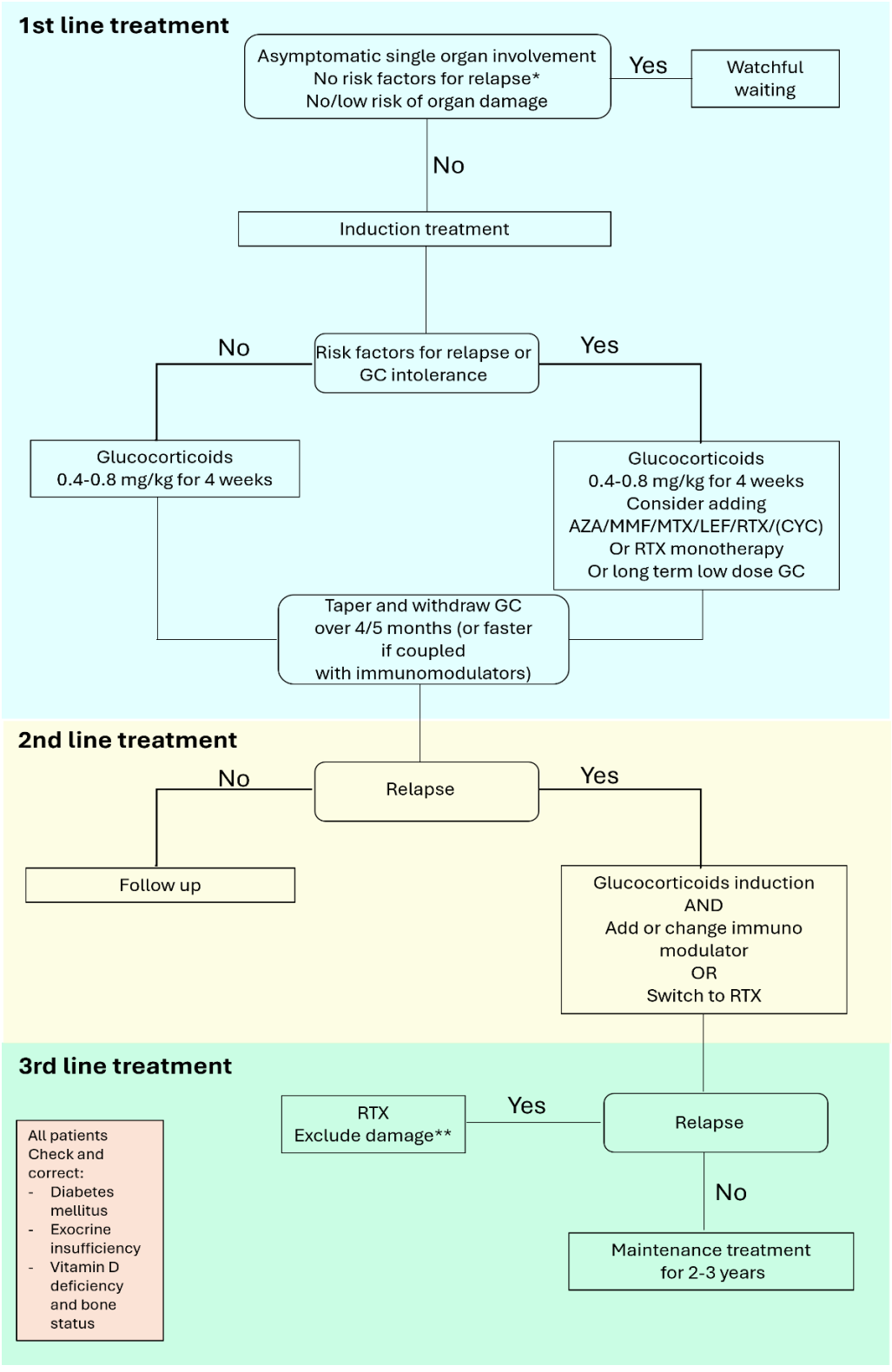


Figure 10. IgG4-RD treatment algorithm. *Abbreviations. AZA: azathioprine; MMF: mycophenolate mofetil; MTX: methotrexate; LEF: leflunomide; RTX: rituximab; CYC: cyclophosphamide. Risk factors : *Previous relapses; very high serum IgG4 at baseline; very high IgE at baseline; multiorgan involvement; proximal biliary tree involvement **Damage is not reverted by immunosuppressive treatment.*

1.7 Novel disease activity biomarkers

Typically, no single disease biomarker can precisely reflect disease activity, as they may appear normal at the onset of the disease, making them less effective in tracking treatment responses or predicting relapses(Lanzillotta *et al*, 2020b). However, as familiarity with managing IgG4-RD patients increases, we identify specific situations where these biomarkers prove beneficial. For instance, monitoring IgG4 levels is helpful in assessing disease activity, especially in patients with initially high serum IgG4. Serum IgG4 often decreases with clinical improvement and rises again if the disease relapses(Iaccarino *et al*, 2022). Nonetheless, relying solely on serum IgG4 levels for treatment decisions is not advisable, as they do not normalize in 63% of cases after glucocorticoid treatment and fail to rise during disease flares in about 10% of cases. Similarly, elevated serum IgE and eosinophil levels are found in 30% to 40% of cases, regardless of atopic background, and often show a stronger correlation with disease activity than IgG4. In some patients with asthma, increased serum IgE or eosinophils may signal worsening respiratory symptoms and a potential IgG4-RD flare(Della Torre *et al*, 2014; Culver *et al*, 2017). High levels of serum IgG4, IgE, and eosinophilia at disease onset are independent risk factors for relapses, with hazard ratios of 6.2, 8.2, and 7.9, respectively(Culver *et al*, 2017). Low to normal inflammatory markers are common in IgG4-RD, making ESR and CRP unreliable for follow-up, though they remain important for monitoring conditions like retroperitoneal fibrosis and large vessel involvement due to their association with elevated ESR and CRP levels(Peng *et al*, 2020).

New potential biomarkers for disease activity are emerging as our understanding of IgG4-RD develops, although none has been examined in prospective multicentric studies. Patients with antibodies against multiple autoantigens exhibit increased IgG subclasses, complement consumption, and visceral organ involvement, linking IgG4-RD severity to a broader autoimmunity burden. Elevated soluble interleukin 2 receptor levels, indicative of T cell activation, correlate with IgG4-RD activity and predict glucocorticoid response(Dugic *et al*, 2022).

Circulating plasmablasts labelled as CD19+CD20-CD27+CD38+ increase during active IgG4-RD and relapses and decrease after effective treatment, thus providing a more reliable disease activity indicator than serum IgG4 levels(Lin *et al*, 2017; Wallace *et al*, 2015; Lanzillotta *et al*, 2017b). Recently, memory B cells (CD19+CD20+CD27+CD38-)

appeared as a predictor of disease relapse. Indeed, raising memory B cells after treatment indicated patients at higher risk of relapse within two years(Lanzillotta *et al*, 2018).

We recently demonstrated in a population of relapsing IgG4-RD patients that total Tfh cells, Tfh1, Tfh2, Tfh17, follicular regulatory (Tfr), and peripheral helper (Tph) were not affected by RTX treatment despite the achievement of clinical remission (Mancuso *et al*, 2021). These findings suggest that the persistence of Tfh after RTX might sustain disease relapse by recruiting naïve B cells in affected organs, thereby orchestrating the re-expansion of pathogenic plasmablast clones.

The [^{18}F]FDG uptake on PET scans correlates with plasmablast expansion in blood and likely with inflammation at disease sites, making it valuable for assessing disease activity, as confirmed by several studies. Collaboration between clinicians and nuclear medicine specialists is crucial to accurately evaluate IgG4-RD activity, particularly in lymph node cases, since [^{18}F]FDG-PET uptake does not differentiate between reactive and affected nodes without clinical context(Berti *et al*, 2017).

Finally, various serum markers show promise as tissue fibrosis biomarkers, though they remain in early research stages. These include the ELF score, consisting of a signature composed by hyaluronic acid, procollagen-III N-terminal propeptide, and tissue inhibitor of matrix metalloproteinase-1, as well as mL-chemokine ligand 18 (CCL-18), RANKL, BAFF, and APRIL. Notably, the ELF score and CCL-18 are significantly higher in IgG4-RD patients compared to healthy individuals, correlating with the number of affected organs, likely indicating systemic collagen deposition and disease activity(Iaccarino *et al*, 2022; Lanzillotta *et al*, 2020b). Experience with these new biomarkers is limited to individual centers, necessitating larger longitudinal studies to establish their role in monitoring IgG4-RD activity and quantifying the burden of IgG4-RD fibrosis.

1.8 Pathogenesis

Multiple immune-mediated mechanisms contribute to the fibroinflammatory process of IgG4-RD.

1.8.1 Genetic Risk Factors

Given the recent appraisal of IgG4-RD only few genetic studies are available. Thanks to population studies mainly conducted in Japan in AIP framework, few susceptibility loci has been highlighted, such as HLA serotypes DRB1*0405 and DQB1*0401 and DQβ1-57 in Korean populations (Kawa *et al*, 2002). Non-HLA genes have been involved too, including CTLA4, TNF, and Fc receptor–like 3 (Della-Torre *et al*, 2015b). Recently, a Genome Wide Association Study conducted in Japan from Terao et al, confirmed HLA-DRB1 and unveiled FCGR2B regions as susceptibility loci for IgG4-RD. The single nucleotide variant in *FCGR2B* (rs1340976) was coupled with higher expression of *FCGR2B*, as well as with specific clinical features of IgG4-related disease(Terao *et al*, 2019).

1.8.2 Bacterial Infection and Molecular Mimicry

Molecular mimicry following bacterial infections has been postulated to be part of IgG4-RD pathogenesis, especially in AIP. Indeed, substantial homology exists between human carbonic anhydrase II expressed in pancreatic tissues and the ubiquitin-protein ligase E3 with the α-carbonic anhydrase of Helicobacter pylori (Frulloni *et al*, 2009). As such, antibodies targeting these bacterial components might act as autoantibodies via molecular mimicry in genetically predisposed patients, thus targeting self-structures and leading to autoimmunity.

1.8.3 Environmental risk factors

In parallel, several environmental risk factors emerged in the pathogenesis of IgG4-RD. In a bicentric cohort study, Huberts et al reported a three-fold risk increase of developing IgG4-RD pancreato-biliary disease in patients exposed to industrial compound, such as biological dust, mineral dust, gas fumes, VDGF (vapors, dusts, gases and fumes), all pesticides, herbicides, insecticides, fungicides, aromatic solvents, chlorinated solvents, other solvents, and metals. The delay between occupational exposure and the development of disease/ symptoms in IgG4-RD suggests that effects outlast exposure(Hubers *et al*, 2021). The authors speculate that pollutants may cause direct damage to tissues, resulting in release of autoantigens that can induce B cell responses. The injured tissues may further be targeted by auto-reactive B and T cells upon alterations of the structure of self-proteins or by a process of molecular mimicry. Ultimately, a chronic inflammatory would lead to immune cells accrual and lymphoid

organ follicles formation. In this context it is likely IgG4 antibodies are formed by highly expanded and affinity-matured B cell clones to dampen an uncontrolled auto-inflammatory response.

In addition, in a study conducted by Wallace ZS et al, an association between smoking habit and retroperitoneal fibrosis due to igG4-RD was observed(Wallwork *et al*, 2021).

1.8.4 B cells

The role of B cells in the pathogenesis of IgG4-RD has been widely investigated since its appraisal. In particular, the characteristic elevation of the serum IgG4 level in most patients and the observation of IgG4+plasma cells in virtually every affected organ quickly brought attention to the role of humoral immunity in IgG4-RD pathophysiology (Lanzillotta *et al*, 2017b). In addition, the exceptional response to B cell depleting regimens highlighted the key role of this player in IgG4-RD pathogenesis. Multiple studies have confirmed the oligo-clonal expansion of somatically hypermutated IgG4+ B cells in IgG4-RD, consistent with the concept that this condition is an antigen-driven, immune-mediated process (Lin *et al*, 2017; Wallace *et al*, 2015; Mattoo *et al*, 2014b; Lanzillotta *et al*, 2019). This theory is all supported by the demonstration of several autoantibodies targeting a plethora of autoantigens, including carbonic anhydrase, plasminogen binding protein, lactoferrin, pancreatic secretory trypsin inhibitor, amylase alpha-2A, trypsinogen, annexin A11, laminin 511, and galectin 3(Perugino & Stone, 2020).

Yet, the pathogenic role of B cells in IgG4-RD is far from being limited to the sole production of autoantibodies. First, B cells can act as antigen-presenting cells, activating cognate T lymphocytes in the context of the appropriate cytokine milieu [1(Perugino & Stone, 2020)]. In this view, antigen-primed tissue-infiltrating B cells present the (auto)antigen to naïve T cells, promoting their expansion and differentiation into CD4+ or CD8+ cytotoxic T lymphocytes (CTLs). CTLs are a recently identified cluster of T cells that bear a cytotoxic signature due to the presence of IL-1, perforin and granzyme B(Mattoo *et al*, 2016; Perugino *et al*, 2021; Della-Torre *et al*, 2018a). CTLs migrate then to affected sites and possibly lead to apoptosis and fibrosis via interleukin (IL)-1 and transforming growth factor (TGF)-b production (Perugino *et al*, 2021). Second,

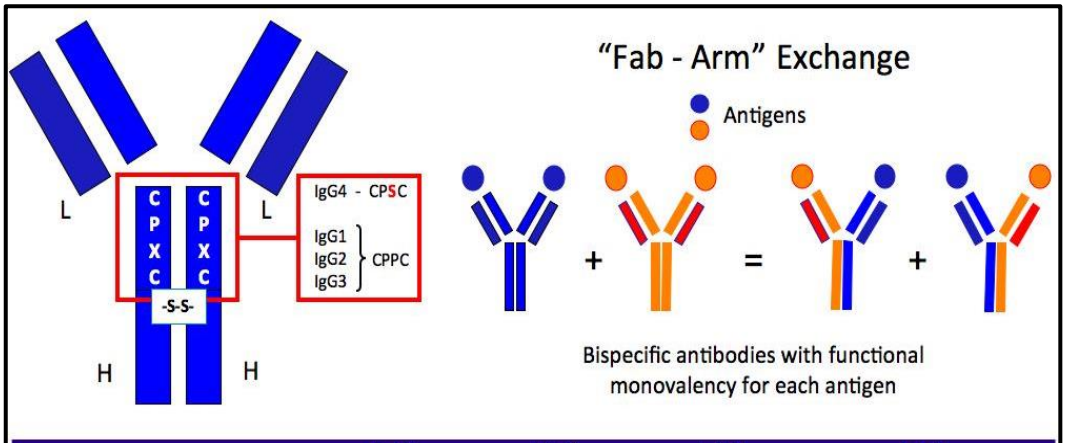
oligoclonally expanded plasmablasts and plasma cells can secrete IgG4 antibodies with unexpected pathologic properties. The IgG4 subclass of human immunoglobulins has, in fact, been considered an anti-inflammatory molecule due to a reduced capacity to fix complement proteins and to bind activating Fc receptors on innate immune cells (Aalberse *et al*, 2009). Yet, as outlined below in details some evidence for a direct contribution of IgG4 to tissue damage exist. Finally, recent studies performed by our group demonstrated a direct pro-fibrotic role of B cells in IgG4-RD. Once co-cultured with patients' derived fibroblasts, B cells were shown to promote collagen secretion via platelet derived growth factor (PDGF)-β signaling (Della-Torre *et al*, 2020, 2015a). Notably, this PDGF-β mediated pro-fibrotic role has been demonstrated even in the context of other fibrotic diseases, such as pancreatic cancer(Minici *et al*, 2020; Öhlund *et al*, 2017). Moreover, it has been shown that B cell depletion with RTX results in amelioration of organ fibrosis and normalization of serum fibrosis biomarkers in these patients(Della-Torre *et al*, 2015a). In conclusion, B cells might exert multiple tasks in the context of IgG4-RD pathogenesis, from antigen presentation through tissue fibrosis.

1.8.5 The role of IgG4 antibodies

IgG4 antibodies represent the least abundant IgG subclass in healthy individuals (1 to 4 % of total IgG), and their role in IgG4-RD is still controversial. IgG4 antibodies are known to be produced after long-term antigen exposure in response to IL-4 and IL-10 (Aalberse *et al*, 2009). but the molecular mechanisms that drive the IgG4 class switch are not fully understood. Indeed, part of these mechanisms (e.g. Th2 cytokines) might also increase serum IgE levels, as exhibited by many IgG4-RD patients. Indeed, high IgG4 levels are linked to various autoimmune, malignant, infectious, and allergic conditions(Della-Torre *et al*, 2015b). While any persistent inflammatory disease can trigger a B-cell response that switches to the IgG4 class, the presence of IgG4-producing plasma cells in the affected areas together with the above-mentioned histopathological features, is a crucial indicator of IgG4-related disease. In the context of allergic disease, IgG4 is considered a neutralizing antibody, diminishing allergens and inhibiting an IgE-driven immune reaction, thereby preventing IgE-mediated anaphylaxis(Aalberse *et al*, 2009; Rispens *et al*, 2011; van der Neut Kolfschoten *et al*, 2007). In addition, IgG4 antibodies also have limited ability to form immune responses because of their low

affinity C1 complement molecule. Furthermore, the IgG4 molecule contains an immune inhibitory Fc portion that poorly engages activating Fc receptors(Aalberse *et al*, 2009).

Another striking feature of IgG4 antibodies take advantage from its peculiar structure. In contrast to other IgG subclasses, heavy chains in each IgG4 molecule have non-covalent associations and inefficient disulfide bridges due to a single–amino acid difference in the hinge region (a serine in lieu of a proline) (Rispens *et al*, 2011). As a result, hemi-IgG4 molecules have the propensity to dissociate from each other and re-associate randomly with distinct hemi-IgG4 molecules that have different antigen-binding specificities. This phenomenon is known as "Fab - Arm exchange" (**Figure 11**). This half-antibody exchange, unique to the IgG4 isoclass, generates functionally "bi-specific" antibodies that are capable of binding two different antigens but rarely associate with each other to form large immune complexes (Aalberse *et al*, 2009). For these reasons IgG4 has been traditionally viewed as a "non-inflammatory" molecule, the primary function of which is to dampen rather than to incite or accelerate chronic immune activation. Thus, the increase in serum IgG4 in IgG4- RD might represent a failing counter- regulatory response in which serum IgG4 concentrations rise higher and higher in an attempt to (unsuccessfully) dampen the primary immune response(Aalberse *et al*, 2009; van der Neut Kolfschoten *et al*, 2007).



	IgG1	IgG2	IgG3	IgG4
Biological target	Protein	Carbohydrate	Protein	Protein
Functional form	Monomeric, bivalent	Dimeric, bivalent or tetravalent	Monomeric, bivalent	Bispecific, monovalent for each antigen
Serum levels (mg/dl)	5 - 11	1.5 - 6	0.2 - 1	0.08 - 1.4
Proportion of total IgG (%)	43 - 75	16 - 48	1.7 - 7	0.8 - 11.7
Complement fixation	+++	+	+++	-
Binding to FC gamma receptors				
FC gamma RI	++	+	+++	+
FC gamma RII	++	+	+++	-
FC gamma RIII	++	+	++	-

Figure 10 *IgG4 Fab – Arm exchange. IgG4 forms disulfide bonds within the heavy chains in its hinge region because, in contrast to the other IgG subclasses, the disulfide bonds between the heavy chains of the IgG4 molecule are unstable. In an IgG4 molecule without disulfide bonds between the heavy chains, dissociations of the noncovalent bonds permit the chains to separate and recombine randomly, such that asymmetric antibodies with two different antigen-combining sites are formed. The resulting bispecific (functionally monovalent) IgG4 molecules are unable to crosslink antigens, thereby losing the ability to form immune complexes.*

Nevertheless, some evidence suggests some potential for tissue injury induced by IgG4. IgG4 immune complexes have been identified in IgG4-related tubulointerstitial nephritis likely entailing the complement activation via the lectin pathway. Interestingly, IgG4 antibodies directed against desmoglein 1 and the metalloproteinase ADAMTS13 have been also implicated in the pathogenesis of pemphigus vulgaris and thrombotic thrombocytopenic purpura, respectively(Motta & Culver, 2024; Aalberse *et al*, 2009). Furthermore, IgG4 as well as IgE might theoretically bind to Fc receptors on macrophages and eosinophils, foster internalization of extracellular antigens, and facilitate their presentation to CD4 + T lymphocytes.

In this view, the potential role of IgG4 in IgG4-RD pathogenesis cannot be excluded a priori. Indeed, at least two mechanistic studies explored the pathogenicity of

IgG4 in IgG4-RD context. In a mouse model of IgG4-RD, Shiokawa et al employed IgG1 and IgG4 adoptive transfer and observed that in both cases the pancreatic tissues of the recipient mice showed changes characteristic of human AIP, in lesser extent with IgG1 compared to IgG4. Of note, when co administered, IgG4 dampened the damage induced by IgG1(Shiokawa *et al*, 2016). Similarly, Huber et al observed that IgG4 impaired the binding of IgG1 antibodies to Annexin A11, suggesting that IgG1-mediated pro-inflammatory autoreactivity against annexin A11 in patients with IgG4-RD may be attenuated by formation of annexin A11-specific IgG4 antibodies supporting an anti-inflammatory role of IgG4 in IgG4-RD(Hubers *et al*, 2018). Yet, findings from animal studies cannot be readily translated into human pathology as IgG4 are not secreted in mice and because mice IgG1, the functional equivalent of human IgG4, have a markedly higher affinity for the inhibitory receptor FcγRIIB compared to human ones(Perugino & Stone, 2020).

1.8.6 Autoantibodies response in IgG4-RD

A number of autoantigens have been tied to IgG4-RD, mainly in the pancreato-biliary phenotypes, including carbonic anhydrase, plasminogen binding protein, lactoferrin, pancreatic secretory trypsin inhibitor, amylase alpha-2A, trypsinogen and annexin A11. Yet, these marked exhibited a very low positive predictive value for diagnosing IgG4-RD when evaluated in other cohorts, thus limiting their usefulness in clinical scenarios(Perugino & Stone, 2020). Indeed, only three auto antibodies have been consistently observed among different cohorts and in different organs involvement, namely anti Annexin A11, anti-Galectin 3, and anti-Laminin 511-E8.

Anti-Annexin A11 antibodies were identified in 20% of patients from a cohort of IgG4-RD patients with pancreas and bile duct involvement by means of immunoprecipitation followed by mass spectrometry analysis. Notably, antibodies titers decreased following successful treatment. Moreover, IgG4 inhibited IgG1 binding to AnnexinA11 from cholangiocytes. In vivo, these findings translated into an impaired function of AnnexinA11, leading to impaired cholangiocytes function and increased apoptosis due to bile acid toxicity.

Anti-galectin 3 antibodies were identified via single-cell immunoglobulin sequencing(Perugino *et al*, 2019). These antibodies were mainly of class IgG4 and IgE

tightly reflecting serological features of IgG4-RD patients. Notably, the presence of anti-galectin-3 antibodies reflected the level of galectin-3, likely due to an alteration of peripheral tolerance due to the increase of this antigen in IgG4- RD(Perugino & Stone, 2020).

Anti Laminin 511-E8 antibodies were identified building upon the study from Shiokawa et al outlined above. Indeed, after highlighting IgG1 and IgG4 mediated damage, the ligand was represented by laminin-511- E8. As confirmation, the transfer of laminin-511- E8 protein led to a B cell antibody mediated reaction entailing the pancreas and the salivary gland, thus supporting the role of auto antigens in IgG4-RD pathogenesis (Shiokawa *et al*, 2018).

Last, patients with IgG4-RD and autoantibody responses against Annexin A11, Galectin 3 and Laminin 511 E8 showed a higher disease burden compared to patients who have little evidence of reactivity against these same antigens(Liu *et al*, 2020a). This evidence shed further light on the key role of autoantibodies in IgG4-RD pathogenesis(Perugino & Stone, 2020).

1.8.7 T cells

Mechanistic studies entailing T cells were performed shortly after those focused on b cells, leading to the appraisal of different T cell subsets in the context of IgG4-RD pathogenesis. Yet, these two populations are obviously tightly linked as witnessed by the co presence in the affected tissue. PD1+ Follicular helper T cells (Tfh) are pivotal in B cells development within the germinal centres, leading to B cells expansion and plasmablast differentiation(Akiyama *et al*, 2016; Lu *et al*, 2022). Notably, the number of circulating PD1+ Tfh cells correlates with the number of organs involved and serim IgG4 and IL4 concentrations. Indeed, IL -4 is a key cytokine in IgG4-RD pathogenesis, driving B cells class switching toward IgG4 and IgE production and is mainly produced by TFH. Moreover, IL-4 producing TFH cells are characterized by BATF transcription factor expression and often infiltrate IgG4-RD lesions, being the most prominent TFH population in this setting. Their presence correlates with the number of infiltrating B cells and with serum IgG4 concentration(Cargill *et al*, 2019; Akiyama *et al*, 2015).

IL 10 represents another pivotal cytokine in igG4-RD pathogenesis being mostly secreted by T follicular regulatory T cells (TFR). As such, IL 10 is increased in patients'

serum and in the affected tissues and has been tied to germinal centre formation, IgG4 class switching in combination with IL4. The role of Tfh in the pathogenesis of IgG44-RD is summarized in **Figure 12**.

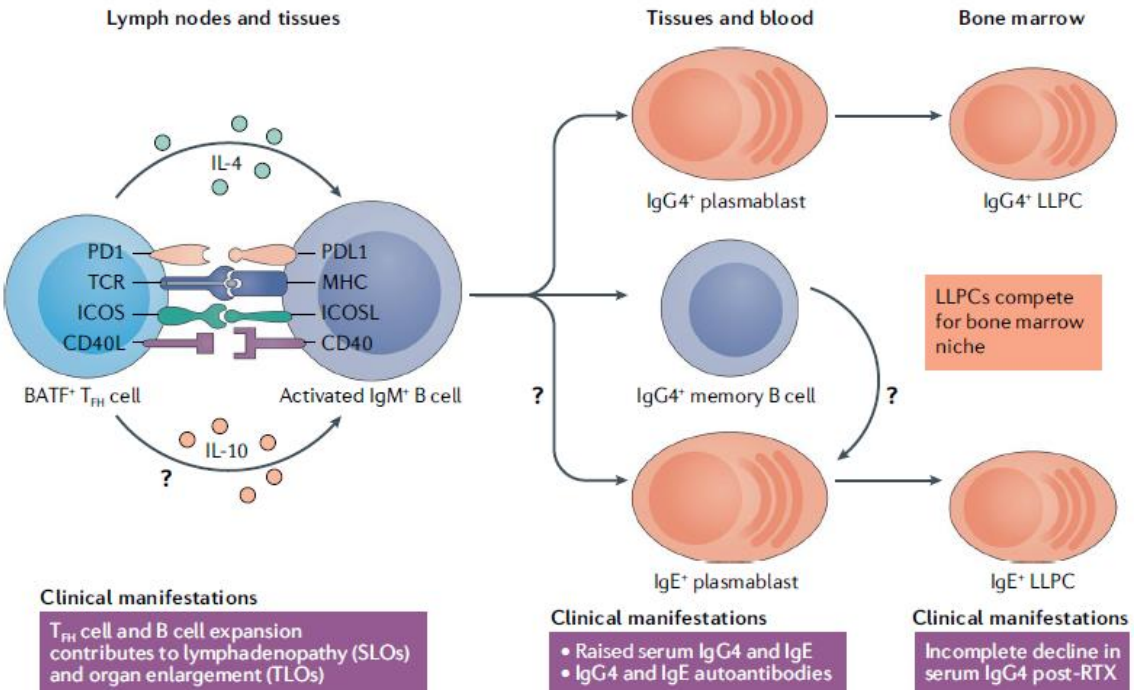


Figure 12. T cell and B cell crosstalk*. cell–B and immunoglobulin class switching in IgG4-related disease. Following germinal centre reactions, affinity- matured B cells leave the follicle and interact with IL-4- secreting BATF+ T follicular helper (TFH) cells. In conjunction with IL-10, IgM+ B cells class- switch to become IgG4+ memory B cells and plasmablasts. Short- lived IgG4+ and IgE+ plasmablasts secrete self- reactive IgG4 and IgE, respectively, circulate and compete for survival niches within the bone marrow as long- lived plasma cells (LLPCs). Both plasmablasts and LLPCs contribute to the marked increases in serum IgG4 and IgE that are often found in IgG4- RD. CD40L, CD40 ligand; ICOS, inducible T cell costimulator; ICOSL, ICOS ligand;PD1, programmed cell death protein 1; PDL1, PD1 ligand 1; RTX, rituximab; SLOs, secondary lymphoid organs; TCR, T cell receptor; TLOs, tertiary lymphoid organs. Adapted from Perugino C et al, Nat Rev Rheum,2020.*

Another key player in the pathogenesis of IgG4-RD is represented by CD4 T cells. Early studies focused on TH2 cells as crucial in the disease pathogenesis due to the abundance of tH2 associated cytokines, such as IL-4, IL-5, and IL-13. Yet, subsequent studies highlighted Tfh2 as the most likely source of these mediators. In parallel, in population level gene expression analysis a population of CD4+ cytotoxic T cells emerged with a specific signature(Mattoo *et al*, 2014a, 2016). These cells are markedly clonally expanded in patients' sera, and are characterized by upregulation of genes associated with

cytotoxicity, including granzymes, perforin and granulysin, while lacking any features of TH2 cells. Moreover, the latter exhibited a widely diverse TCR repertoire, likely deriving from antigen exposure and being expanded only in patients with concurrent atopic disease. Notably, CD4+ CTLs represent the most dominant T cells subset in the affected tissues and their number correlated with the disease burden(Della-Torre *et al*, 2018a). Moreover, the same group identified a similar population of CD8+ CTL that might exert a synergistic effect, being activated by presentation of self- peptides on MHC class I molecules that would normally have been sequestered but had not been because of the cytotoxic effect of CD4+ CTLs(Perugino *et al*, 2021).

1.8.8 Possible pathogenetic model for fibrosis in IgG4-RD

Our understanding of the pathophysiology of IgG4-RD suggests that it progresses through phases of persistent proliferative inflammation and recurring tissue damage, eventually leading to tissue fibrosis(Lanzillotta *et al*, 2024b). Generally, fibrogenesis acts as a primary mechanism for resolving tissue injury, beginning and continuing during inflammation. Upon exposure to fibrogenic cytokines, such as transforming growth factors (TGFs), fibroblast growth factors (FGFs), and platelet-derived growth factors (PDGFs), fibroblasts develop into myofibroblasts and produce extracellular matrix (ECM). Once inflammation subsides, myofibroblasts undergo apoptosis, with scavenger cells eliminating excess ECM components. B cells play a key role in orchestrating fibrogenesis. Depleting B cells from affected tissues has shown potential in reversing myofibroblast activation and reducing the overall ECM content. SLAMF7+ cytotoxic T cells are also thought to be involved in the fibrotic process due to their abundance in affected lesions(Della-Torre *et al*, 2018a). Although these cells can produce TGF-β in situ, no direct evidence currently links SLAMF7+ cytotoxic T cells with stromal cells in terms of profibrotic interactions. Yet, CD4+ CTL mediated cell death has been suggested as a mechanism leading to tissue remodeling and fibrosis, in which T cell mediated killing is followed by fibroblasts activation to fill the space left by the cytotoxic process.

Plasmablasts and IgD and CD27 double-negative type 3 B cells have demonstrated a profibrotic signature in vitro. Indeed, these include (I) releasing pro fibrotic cytokines, (II) inducing an epithelial-to-mesenchymal transition signature in cocultured fibroblasts, (III) secreting collagenous proteins, and enzymes involved in ECM organization, and (IV) aiding in recruiting and activating other inflammatory cells,

such as SLAMF7+ cytotoxic T cells, which could further maintain fibroblast activation or cause fibrosis following resident cells apoptosis(Della-Torre *et al*, 2020; Lanzillotta *et al*, 2024b).

Notably, the pathways that lead to inflammation resolution likely contribute to the development of fibrosis too. The overproduction of IgG4, recruitment of scavenger macrophages, and hyperactivation of fibroblasts are all contributors to fibrosis in IgG4-RD(Rovati *et al*, 2021). Indeed, given the anti-inflammatory properties of IgG4 antibodies, their secretion mitigates chronic inflammation. MerTK+ macrophages, abundant in IgG4-related disease lesions, phagocytose apoptotic bodies, interact with B and T cells, and produce fibrotic cytokines like interleukin (IL)-10 and TGFs.

Preliminary evidence suggests that fibroblasts not only respond to profibrotic signals by secreting ECM but also transform into myofibroblasts and release IL-6, further intensifying the inflammatory cascade. IL-6 may sustain B-cell activation and play a role in germinal center formation. These findings primarily come from patients with aortic and retroperitoneal involvement, which are linked with atypically high levels of inflammatory markers, possibly indicating additional mechanisms of tissue fibrosis specific to this disease phenotype(Batani *et al*, 2023b).

Notably, TGF-β represent the master regulator in orbital fibrogenesis too, switching fibroblasts to myofibroblast phenotype and enhancing extracellular matrix deposition via upregulation of αSMA, collagen, and fibronectin, ultimately leading to fibrotic lesions and organ fibrosis. TGF-β is secreted by infiltrating cytotoxic T cells, resident macrophages and even by fibroblasts themselves, in an autocrine loop, coupled with inflammatory mediators, such as IL-1 and IL-6 (Chang *et al*, 2024).

A potential distinction between active and terminal-stage fibrosis has been proposed, carrying implications for treatment. Active fibrosis may begin early in the disease's proliferative phase, characterized by ongoing interactions between lymphocytes and myofibroblasts that result in collagen secretion. Active fibrosis could then progress into a scarring, terminal stage, where lymphocytic infiltrates are gradually replaced by inert ECM, with few immune cells and quiescent fibroblasts. Immunohistochemical studies of pancreatic involvement in IgG4-related disease revealed that waves of lymphocytic inflammation are progressively superseded by dense collagen deposition

areas, reinforcing the idea of a spatiotemporal shift from proliferative to end-stage fibrotic IgG4-related disease. Whether these concepts apply universally across all potentially affected organs or if they accelerate in specific locations, such as the retroperitoneum, thyroid, and meninges, remains to be clarified. Understanding the fibrogenesis mechanisms in IgG4-related disease has clinical significance, as exploiting the therapeutic window offered by active fibrosis appears to be the most logical strategy to halt rampant collagen deposition and fibroblast activation before they become entrenched and challenging to reverse, thereby preserving organ function(Lanzillotta *et al*, 2024b).

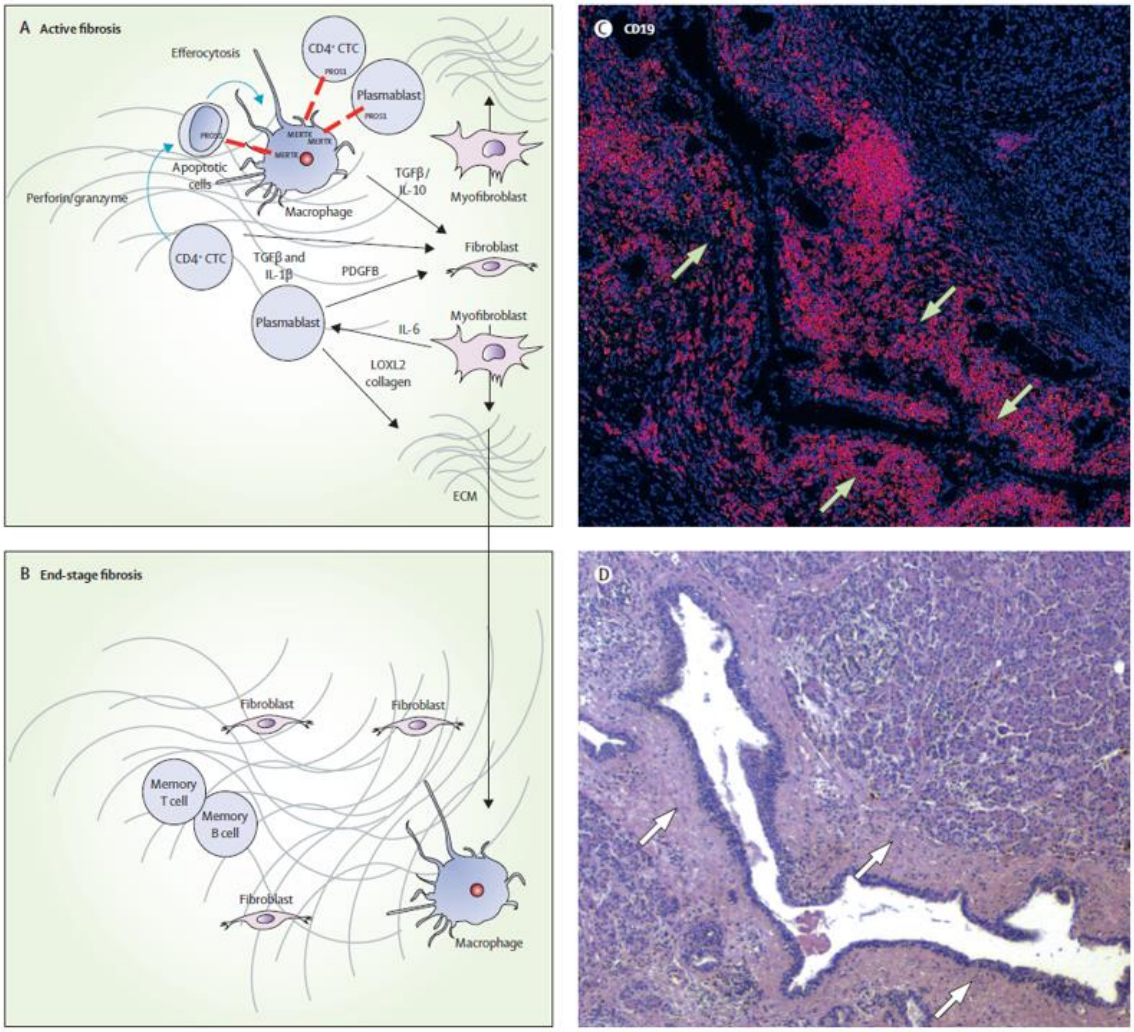


***Figure** 13 . **Active fibrosis**. Fibrogenesis starts with lymph proliferation and inflammation (active fibrosis) when resident fibroblasts or recruited fibrocytes are exposed to fibrotic cytokines. Fibroblasts differentiate into myofibroblasts and produce ECM. Profibrotic signals include TGFβ produced by CD4+ CTCs and macrophages, and PDGFB produced by plasmablasts or plasma cells. Macrophages efferocytose apoptotic bodies and interact with plasmablasts and CD4+ CTCs through the MerTK-ProS1 axis. Plasmablasts contribute to the formation of ECM and to its organization through the production of LOLXL2. Myofibroblasts in turn secrete IL-6, which supports B cell and germinal centre processes. (B) End-stage fibrosis. When the inflammatory*

triggers are cleared and ECM has largely replaced the lymphocytic infiltrate (end-stage fibrosis), scarring tissue remains with few immune cells (mainly scattered macrophages, memory B cells, and memory T cells) enmeshed in a dense collagenous matrix. (C, D) Waves of lymphocytic inflammation (active fibrosis; CD19+ B-cell infiltrate [arrows] localized in proximity of pancreatic ducts; C), preceding areas of dense fibrosis (end-stage fibrosis; sequential matched haematoxylin and eosin staining showing fibrotic areas [arrows] probably generated as a consequence of the periductal inflammatory infiltrate; D). CD4+ CTCs=CD4+ cytotoxic T cells. ECM=extracellular matrix. PDGFB=platelet-derived growth factor B.

Aim

Fibrosis represents a hallmark of IgG4-RD leading to end organ damage if left untreated. Indeed, one of the most relevant features of IgG4-RD consists in the dynamic status of fibrosis. Indeed, fibrosis resolves following prompt treatment, an unprecedented finding in autoimmunity whereby fibrosis had been regarded as an unmodifiable end-stage status. This finding underscores the close relationship between B cells, T cells, as well as resident fibroblast. Indeed, our group previously shown that B cells secrete pro-fibrotic biomarkers and B cell depletion ameliorates tissue fibrosis(Della-Torre *et al*, 2020). On the contrary, later phases of IgG4-RD are characterized by burnt-out fibrosis, characterized by a scant lymphoplasmacytic infiltrate and a dense collagen stroma with a poor response to immunosuppression. End-stage fibrosis represents an unmet need in IgG4-RD as well as in other rheumatic diseases, such as systemic sclerosis and pulmonary fibrosis, and in cancers.

Building upon accumulating evidence already published in literature, we hypothesize that IgG1 and IgG4 antibodies from IgG4-RD patients bear overlooked fibrotic properties and directly contribute to tissue fibrosis. Indeed, IgG4 antibodies display established anti-inflammatory activity, participate to the resolution of inflammation in both autoimmune diseases and cancers, and can mitigate IgG1 mediated damage, but their direct involvement in stromal cell activation and fibrogenesis has never been evaluated before (Aalberse *et al*, 2009).

With the present research project, we aim to (I) demonstrate a direct pro-fibrotic effect of IgG1 and /or IgG4 antibodies from IgG4-RD patients to (II) gauge a IgG1/IgG4-mediated drift in fibroblast phenotype, and to (II) identify fibrotic molecular properties of IgG1 and /or IgG4 antibodies from IgG4-RD patients.

Results

3.1 Clinical characteristic of the patients' cohort

Ten consecutive patients were included in this study. None of the patients received any immunosuppressive treatment before enrolment in the study. The male to female ratio was 1.5. The median age at diagnosis was 67 (IQR 55-77). The most common organs involved were the pancreas, the orbit, and the salivary glands (40%), followed by bile duct and kidney (30%), retroperitoneum and nasal sinuses (20%), lymph nodes and meninges (10%). Serum IgG4 was elevated in most patients (90%). Multiorgan disease was present in 9 patients. Patients belonged to pancreato-hepato-biliary disease, retroperitoneal fibrosis and/or aortitis, head and neck-limited disease, and classic Mikulicz's syndrome with systemic involvement phenotypes in 2,2,3,3 cases, respectively. The median IgG4-responder Index at diagnosis was 8 (range 2-12), witnessing active disease in all patients. Clinical and laboratory characteristics of enrolled patients are reported in **Table 3.** There was no difference in age and sex of healthy controls compared to IgG4-RD patients. All but one patient (IRD6) reported alcohol consumption less than 1 unit per week. Median diagnostic delay was 2 months (IQR 1-4.5 months).

Table 3. *Clinical and laboratory characteristics of enrolled patients. Abbreviations: F: female; M: male; IRD: IgG4-related disease; IgG4RD-RI: IgG4-RD Responder Index. Yrs: years; FBs: fibroblasts; I: immortalized; D: discarded*

Patient	Sex	Age (yrs)	Organ Involved	Diagnostic Delay (months)	IgG4-RD RI	Serum IgG4 (<864mg/l)	FBs
IRD-P	F	55	Pancreas – biliary tree - salivary gland	2	12	2103	I
IRD-O	M	58	Orbit-nose-pancreas-salivary gland	3	12	4570	I
IRD1	F	70	Orbit- Kidney – lymph nodes	1	8	1830	D
IRD2	M	69	Pancreas – biliary tree	1	6	2650	D
IRD3	M	81	Pancreas - biliary tree - salivary glands	2	10	1820	D
IRD4	F	25	Retroperitoneum - pancreas	3	8	1230	D
IRD5	M	53	Meninges - nasal sinused	8	6	1990	D

IRD6	M	80	Retroperitoneum-aorta-kidney	8	8	850	D
IRD7	F	65	Orbit -lung-salivary glands	1	6	5670	D
IRD8	M	76	Orbit	2	2	1785	D

All pancreatic samples for IgG4-RD patients were obtained via endoscopic ultrasound biopsy, while all orbital biopsies were retrieved during surgical procedures. Four pancreatic biopsies were performed. In three cases the samples were not suitable for further fibroblast isolation due to inadequate material. In one case, fibroblasts were successfully isolated, cultured, and immortalized as detailed in the protocol section. One sample was retrieved from CT-guided kidney and meningeal biopsies. Yet, fibroblasts failed to grow after isolation in both cases. Four samples from orbital biopsies were obtained. In all cases fibroblast were isolated but in three cases cell growth stopped after 2 passaged and samples were discarded. Fibroblasts obtained from the remaining patient were successfully isolated and immortalized, as detailed in the Method section.

Serum IgG1 and IgG4 were successfully isolated in nine out of ten IgG4-RD patients and in five healthy controls.

3.2 IgG1 and IgG4 from IgG4-RD patients bind autologous fibroblasts

In order to investigate the binding of serum IgG1 and IgG4 from IgG4-RD patients to autologous fibroblasts we performed cell-based ELISA. Specifically, IgG1 and IgG4 isolated from IRD-P and IRD-O patients were added to cultured pancreatic and orbital fibroblasts. Notably, pancreatic and orbital fibroblasts were obtained from IRD-P and IRD-O patients, respectively. Binding of IgG1 and IgG4 obtained from IgG4-RD patients was compared to negative controls, represented by daratumumab and tocilizumab, whose target (CD38 and IL6, respectively) are absent on fibroblasts' surface.

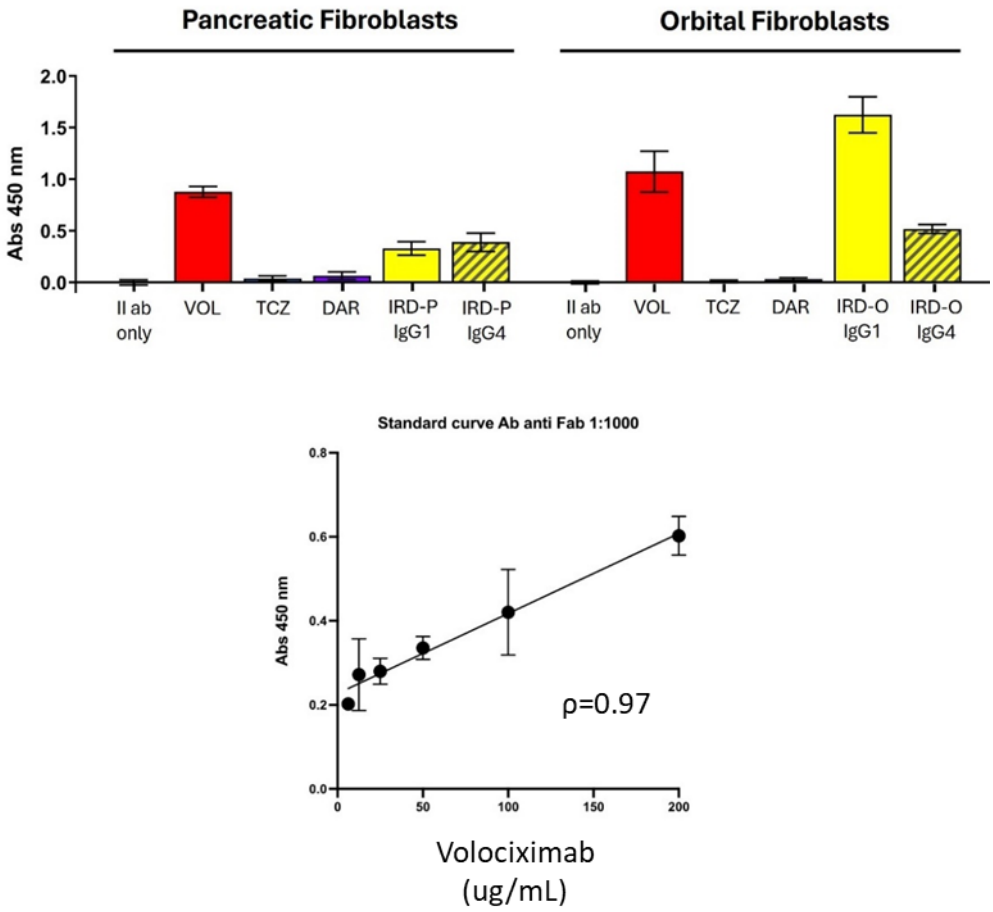


Figure 114. ***IgG1 and IgG4 bind autologous fibroblasts***. *In the lower panel the saturation curve of the II antibody is reported. Abbreviations: ab: antibody; TCZ: tocilizumab; DAR: Daratumumab; Vol: Volociximab; IRD-P: Patient from whom pancreatic fibroblast were immortalized; IRD-O: Patient from whom orbital fibroblast were immortalized. The correlation coefficient is represented by ρ (Speraman's coefficient) and reflects positive correlation*.

As shown in **Figure 14**, both IgG1 and IgG4 exhibited a stronger signal compared to negative controls in pancreatic as well as in orbital fibroblasts.

3.3 IgG1 and IgG4 from healthy controls and IgG4-RD patients bind heterologous fibroblasts

After observing an autologous binding between serum IgG1 and IgG4 and orbital or pancreatic fibroblast, we then explored the capacity of serum IgG1 and IgG4 to bind heterologous fibroblasts. Thus, by means of cell-based ELISA we tested the binding of IgG1 and IgG4 from healthy controls and IgG4-RD patients on pancreatic fibroblasts obtained from IRD-P patient. The concentration of IgG1 and IgG4 employed was the same for IgG4-RD patients and healthy controls (50 uL/well).

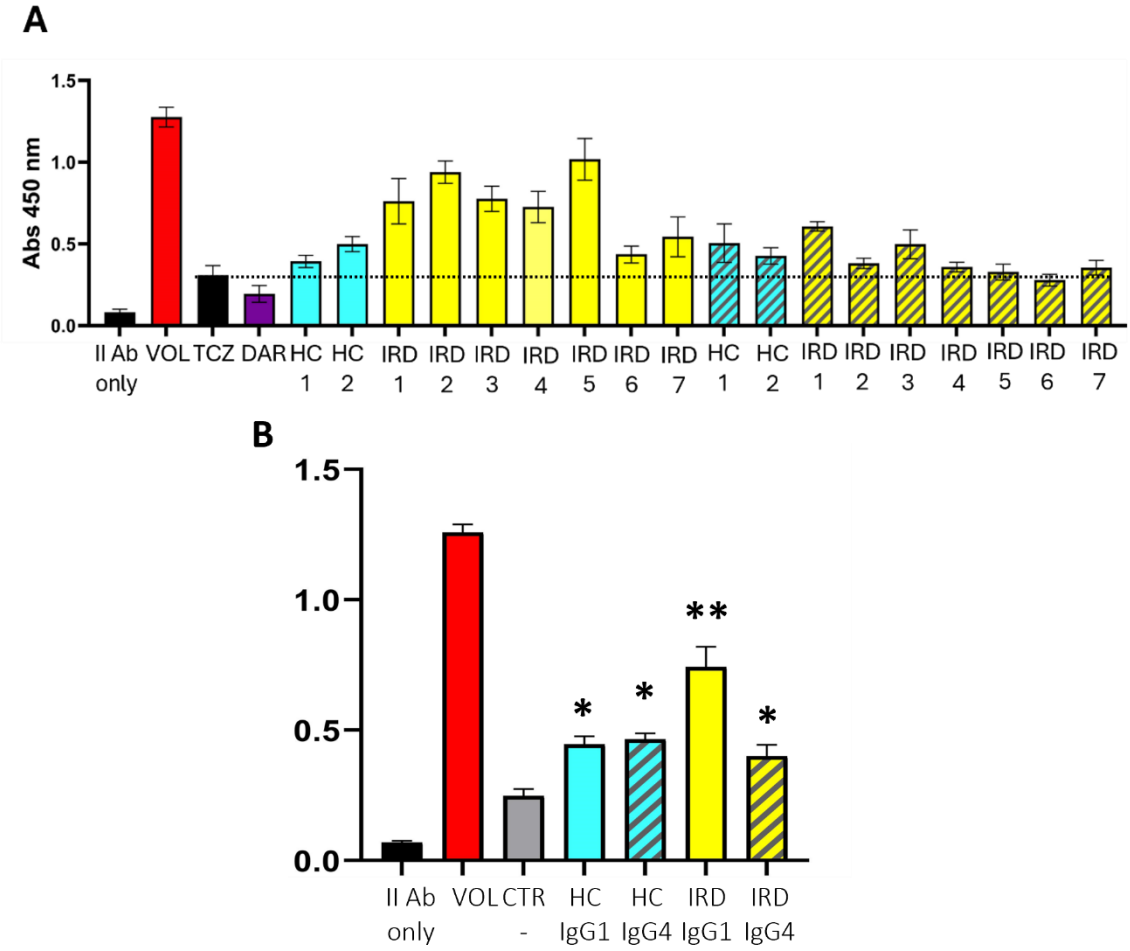


Figure 15. *Heterologous binding of IgG1 and IgG4 from healthy controls and from IgG4-RD patients to pancreatic fibroblasts, compared to negative controls. In the lower panel values from HC and IRD are grouped together and compared to negative control, pooled together. Abbreviations: ab: antibody; HC: healthy controls; TCZ: tocilizumab; DAR: Daratumumab; Vol: Volociximab; IRD: IgG4-related disease. IgG1 are reported in boxes with uniform colors whereas IgG4 are reported in those with striped colors. * p<0.05; **p< 0.01.*

Notably, we observed a higher absorbance from IgG1 and IgG4 derived from patients and healthy controls compared to negative controls. In panel B negative controls were pooled together to perform statistical analysis.

In order to further confirm the binding of IgG1 and IgG4 to fibroblast we performed flow cytometry analysis on one example patient (IRD1) comparing the signal with secondary antibody alone (negative control) and with volociximab and vimentin (positive controls). **Figure 16** shows that both IgG1 and IgG4 from IRD1 patient displayed a stronger signal compared to negative control. It is important to note that the

magnitude of signal amplification is reduced in flow cytometry compared to ELISA methods as only two antibodies are employed instead of three.

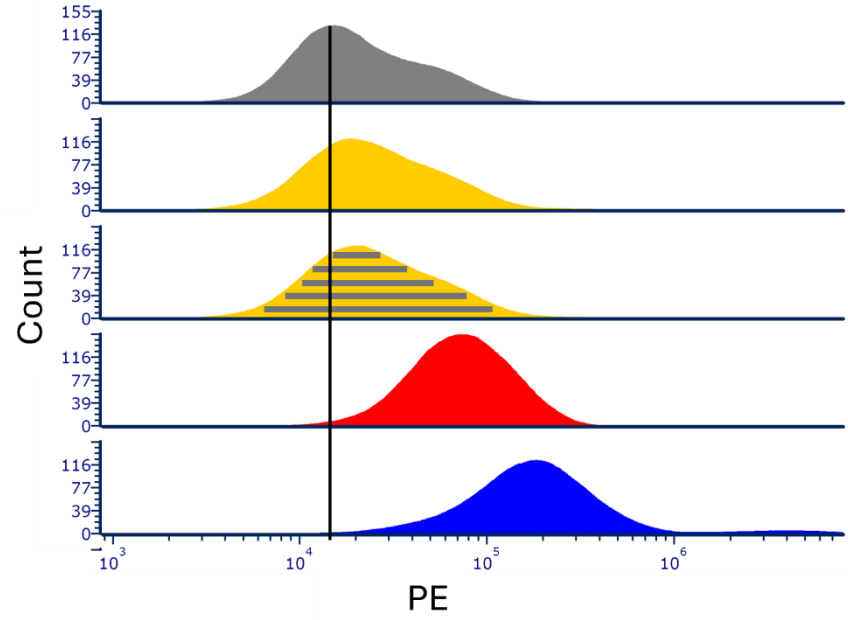


***Figure 16**. Flow cytometry analysis testing IgG1 (yellow) and IgG4 (striped, yellow) binding on pancreatic fibroblasts obtained from IRD-P. Secondary antibody alone represents the negative control (grey curve), while volociximab (red) and anti-vimentin antibody (blue) represent positive controls. The black solid line represents the threshold of negative control.*

3.4 Gene expression modification after IgG1 and IgG4 stimulation

After having observed an autologous and heterologous binding of serum IgG1 and IgG4 to IgG4-RD fibroblast, we assessed whether this bound determined a change in fibroblasts transcriptional profile.

In order to gauge the effect from IgG stimulation on fibroblasts we first performed total RNA quantification. Total RNA from pancreatic- and orbit-derived fibroblasts was measured 4 days (D) after stimulation with IgG1 and IgG4 obtained from IgG4-RD patients and healthy controls and compared to not stimulated fibroblasts. We did not observe any differences after 24 hours. On D4 we observed a trend toward increased RNA production in fibroblast stimulated with IgG1 and IgG4 from healthy controls and IgG4-RD patients compared to not stimulated fibroblasts. Indeed, significantly higher RNA concentration in pancreatic fibroblasts were observed upon stimulation with IgG4 from

healthy controls and IgG1 from IgG-RD patients. A higher RNA concentration in orbital fibroblasts was observed in all conditions except with IgG1 from IgG4-RD patients. We did not observe a significant difference in RNA production between (I) IgG4-RD patients and HC and (II) between IgG1 and IgG4 (**Figure 17**).

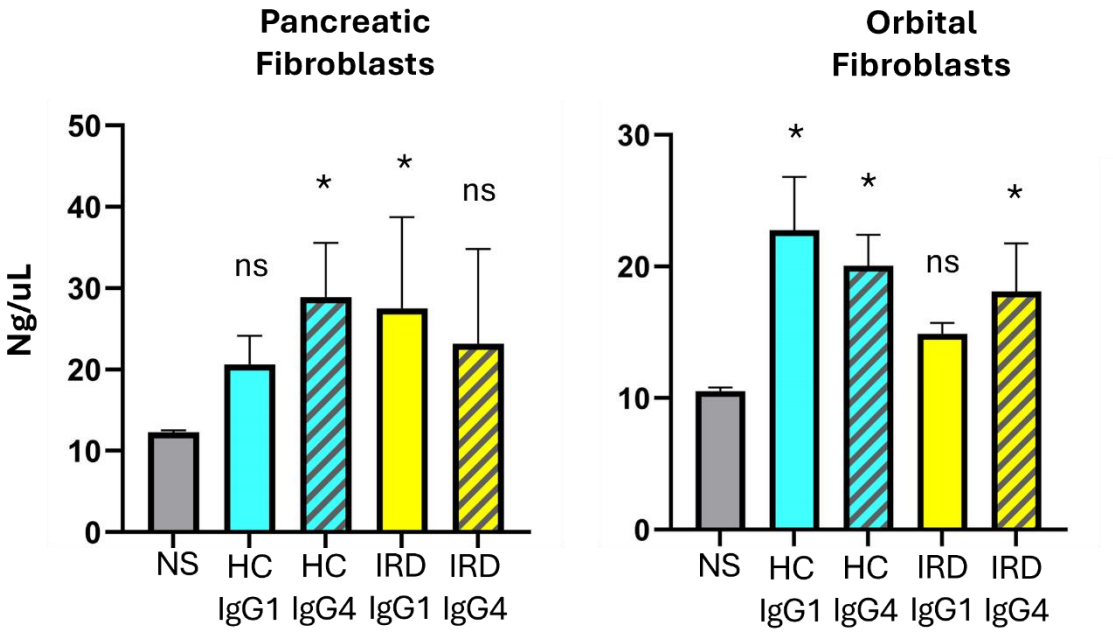


Figure 127*. Changes in RNA concentration upon stimulation of pancreatic and orbital fibroblasts with IgG1 and IgG4 from healthy controls (HC), and IgG4-RD patients (IRD) compared to not stimulated fibroblast (NS). Abbreviation: ns: not significant. IgG1 are reported in boxes with uniform colors whereas IgG4 are reported in those with striped colors. * p<0.05; **p< 0.01.*

To further explore the impact of IgG stimulation on fibroblasts we performed PCR analysis focusing on ACTA 2, Laminin-1, PDGFRα, Interleukin (IL)-6, and Ki-67 genes. These genes were selected as a proxy for key processes in IgG4-RD, such as fibrosis (ACTA 2, Laminin-1, PDGFRα), inflammation (IL-6), and proliferation (Ki67), respectively. This analysis was performed on both pancreatic and orbital fibroblasts. As results were comparable, we merged them together for graphical purposes.

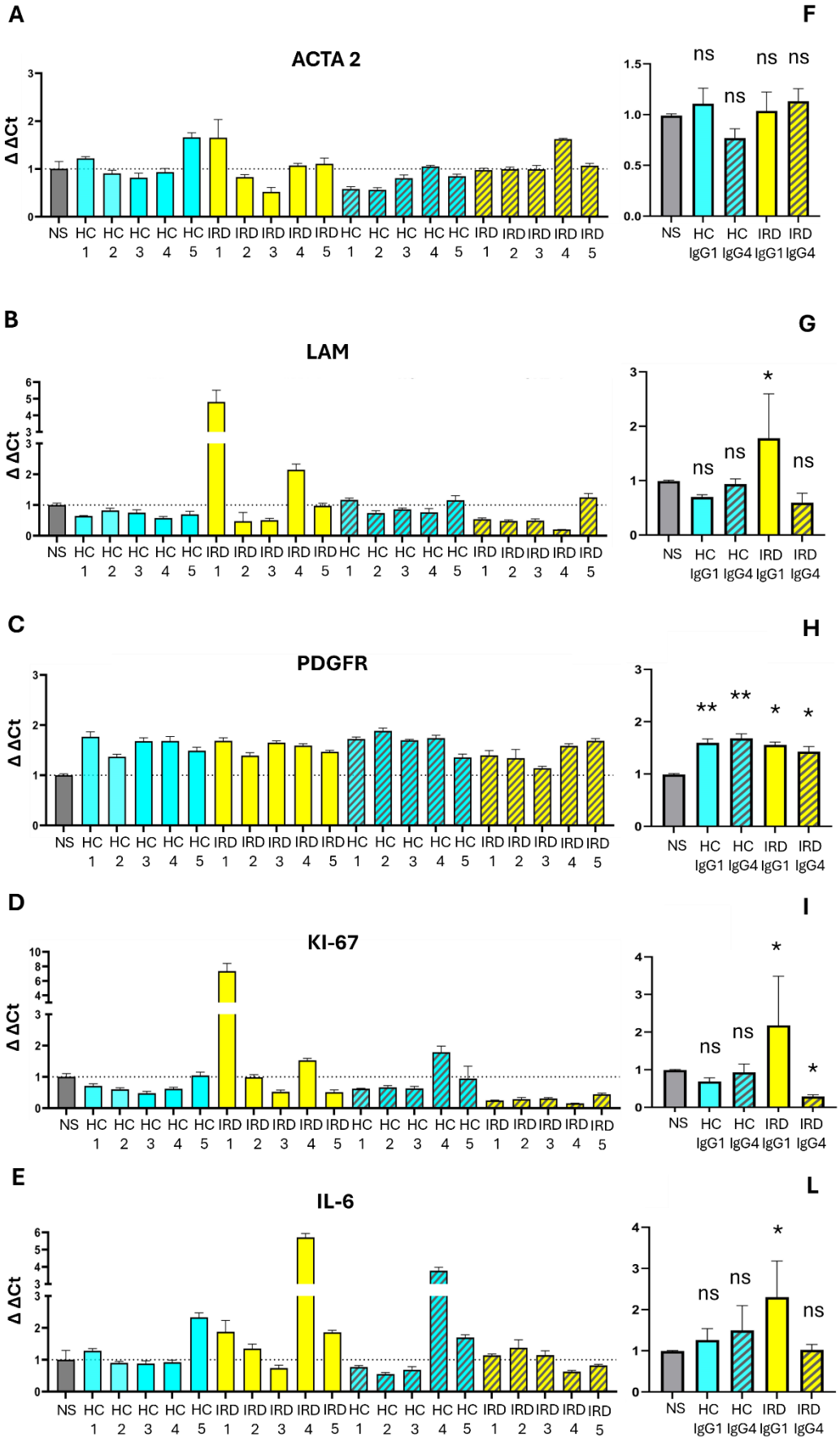

A
ACTA 2
Δ ΔCt
NS HC 1 HC 2 HC 3 HC 4 HC 5 IRD 1 IRD 2 IRD 3 IRD 4 IRD 5 HC 1 HC 2 HC 3 HC 4 HC 5 IRD 1 IRD 2 IRD 3 IRD 4 IRD 5
F
ns ns ns ns
NS HC IgG1 HC IgG4 IRD IgG1 IRD IgG4
B
LAM
G
* ns ns ns
C
PDGFR
H
** ** * *
D
KI-67
I
* ns ns *
E
IL-6
L
* ns ns ns

***Figure 18**. **IgG1- and IgG4-induced fibroblasts' transcriptional changes**. Changes in the transcription of ACTA 2 (A), LAM (B), PDGFR (C), KI67 (D), and IL-6 are reported as ΔΔct. Control is represented by not stimulated fibroblasts at D4. In the left half of the figure are reported the changes in the transcription profile upon stimulation of IgG4-RD fibroblast with IgG1 and IgG4 from each healthy controls (HC) and IgG4-RD patients (IRD). In the right half of the figure, the stimuli from HC and IRD patients are grouped and compared to not stimulated (NS) fibroblast. IgG1 is reported in boxes with uniform color (yellow) whereas IgG4 are reported in those with striped colors. The analysis was performed on both orbital as well as pancreatic IgG4-RD fibroblasts. Given the comparable findings, graphs were merged together in this picture. * p<0.05. Abbreviations: ACTA 2: smooth muscle actin. LAM: Laminin 1. PDGFR: Platelet-derived growth factor receptor.*

Figure 18 reports the fold increase in ACTA 2, LAM, PDGFRα, IL 6, and Ki67 transcripts following stimulation with IgG1 and IgG4 from healthy controls IgG4-RD patients compared to not stimulated fibroblasts. Notably, at D4 we unveiled a significantly higher level of PDGFRα upon stimulation with IgG1 and IgG4 from healthy controls IgG4-RD patients, and an increased transcription of laminin 1 following stimulation with IgG1 from IgG4-RD patients. Moreover, at D4 we observed a significant decrease of Ki67 transcript upon stimulation with IgG4 from IgG4-RD patients, while stimulation with IgG1 led to an opposite result. Of note, the increase in Ki67 and laminin 1 transcription were predominantly driven by IRD1 patient.

Overall, we (I) unveiled a trend toward a pro-fibrotic PDGFRα-based signature of fibroblasts upon IgG stimulation. In parallel, a down regulation of proliferation (II) was observed upon stimulation of IgG4 but not IgG1 from IgG4-RD patients. Yet, stark differences emerged with the different sources of IgG1 and IgG4, reflecting patient's level heterogeneity (III).

3.5 IgG1 and IgG4 increase collagen production from IgG4-RD pancreatic fibroblast

After unveiling IgG-mediated changes in fibroblasts' transcription profile, we explored the impact of stimulating IgG1 and IgG4 on fibroblasts' fibro-inflammatory signature at the protein level. Thus, we performed ELISA sandwich assessing the concentration of collagen I and IL-6 in the supernatant of cultured IgG4-RD pancreatic fibroblast following stimulation with IgG1 and IgG4 from healthy controls and IgG4-RD patients, compared to not stimulated fibroblast. Indeed, collagen secretion from fibroblasts is a key process in the formation of the extracellular matrix.

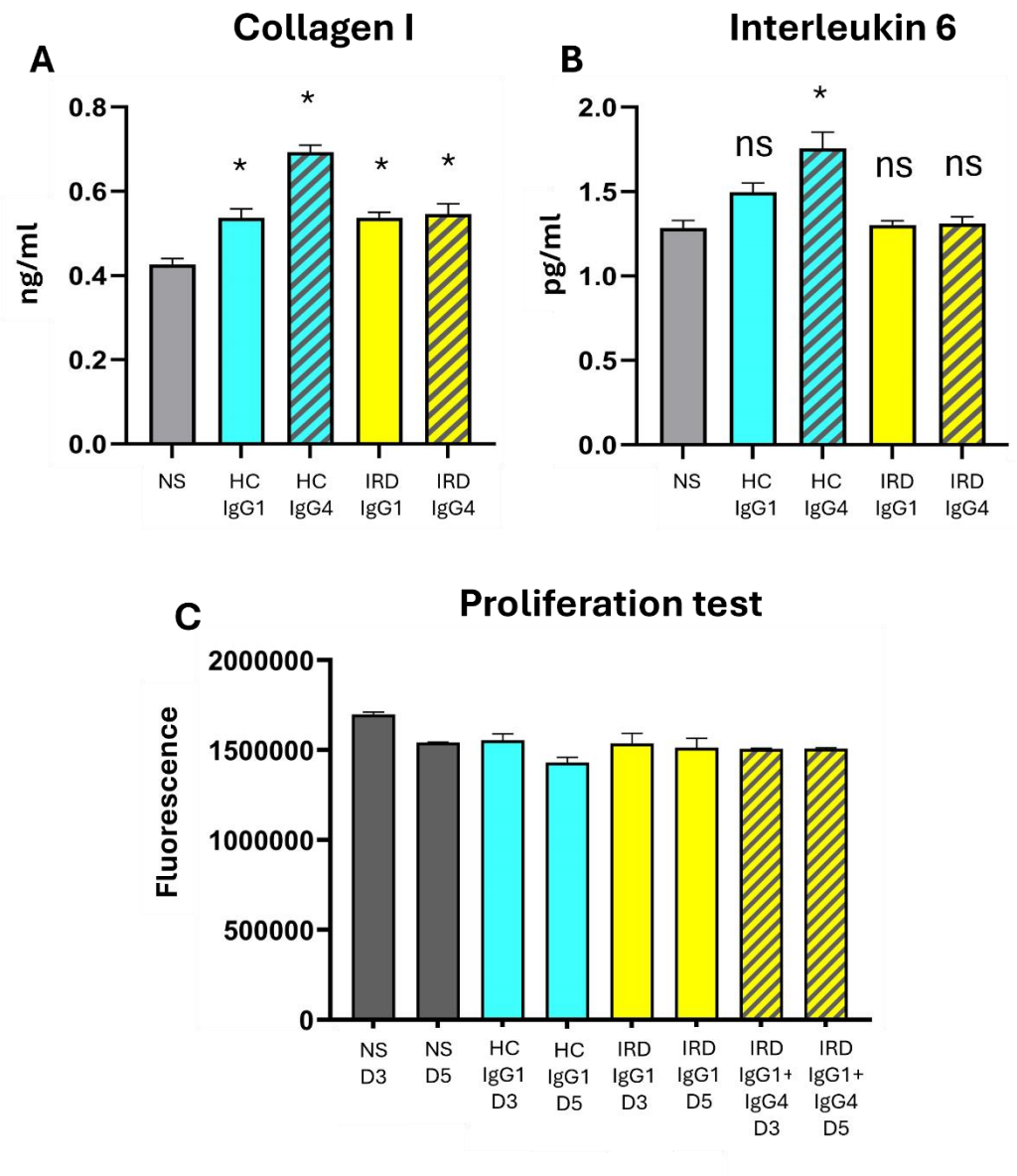


Figure 19. *Elisa sandwich showing the concentration of collagen (A) and IL 6 (B) following the stimulation of pancreatic fibroblast with IgG1 and IgG4 from healthy controls (HC) and from IgG4-RD patients (IRD). In panel C a proliferation test is reported. The rate of proliferation is reported at D3 and D5 after stimulation. IgG1 are reported in boxes with uniform colors whereas IgG4 are reported in those with striped colors.*

As evident in the above Figure (Panel A), we unveiled a significantly increased collagen production from IgG4-RD fibroblast once stimulated with either IgG1 or IgG4, both from patients and healthy controls. In parallel, we explored the effects of IgG stimulation on fibroblast inflammatory phenotype. On the contrary, we did not observe any differences in IL-6 concentrations following stimulation with IgG1 and IgG4 from

IgG4-RD patients, while a higher concentration was found after stimulation with IgG4 from healthy controls (Panel B).

Last, we performed a proliferation test (panel C), assessing the number of cultured fibroblasts 3- and 5-days following stimulation with IgG1 from healthy controls and IgG4-RD patients, compared to not stimulated fibroblasts. At D5 fibroblasts growth was reduced in not stimulated fibroblast, compared to those stimulated with IgG1 from IgG4-RD patient. Notably, the proliferation rate did not decrease adding IgG4 to IgG1.

In conclusion, we observed a higher collagen production following fibroblasts' stimulation with IgG1 and IgG4. In addition, a trend toward increased proliferation was observed in fibroblasts stimulated with IgG1 from IgG4-RD patients.

3.6 IgG1 from IgG4-RD patient bind an auto antigen on pancreatic lysate

In order to identify a potential auto antigen targeted by IgG1 and IgG4 antibodies, we performed an immunoblot testing the link between IgG1 and IgG4 from IRD1 patient on a lysate of IgG4-RD pancreatic and orbital fibroblast. Volociximab was used as a positive control and tested on pancreatic fibroblast. IRD1 patient was chosen given the strongest signals observed in cell-based ELISA and in the PCR analysis.

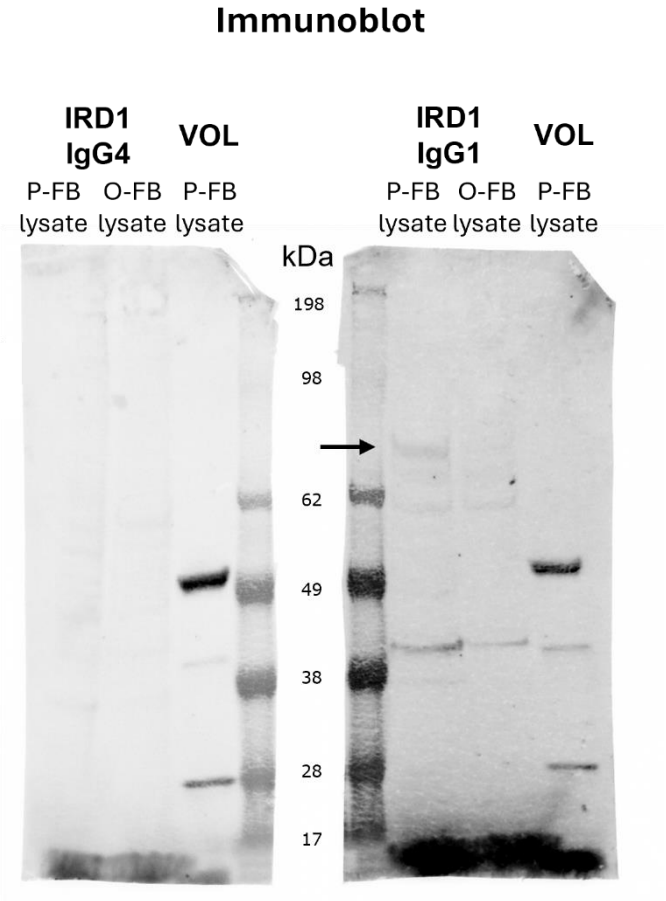


***Figure 130**. Immunoblot testing the link of IgG1 and IgG4 from IRD1 patient on lysate of pancreatic (P-FB) and orbital (O-FB) IgG4-RD fibroblast. IgG1 binds a protein heavier than 62 kDA on pancreatic lysate. Volociximab (VOL) is used as a positive control. The two bands in the VOL column represent the heavy and light chain of antibodies.*

As shown in Figure 20, we observed that IgG1 from patient IRD1 bound a protein contained in the pancreatic lysate heavier than 62 KDa that was not present in the Volociximab column. On the contrary, the signal observed around 38 kDa was deemed as contaminant, as present in all three conditions. The two bands in the volociximab column represent the heavy and light chain of the antibody that had separated following gel electrophoresis.

3.7 Immunoprecipitation confirms Immunoblot analysis

In order to determine the epitope bound by IgG1 antibodies from IRD 1 patient, we performed Immune-precipitation assay. Specifically, via immunoprecipitation we linked IgG1 from IRD 1 patient to magnetic beads and used to isolate antigen-containing/epitope-containing proteins lysate of pancreatic IgG4-RD fibroblast.

Immunoprecipitated proteins were plotted on to SDS-PAGE and probed using serum of the same patient and an HRP-labelled antibody against IgG1 for detection. As shown in **Figure 21**, the binding of IgG1 on pancreatic fibroblasts was confirmed.

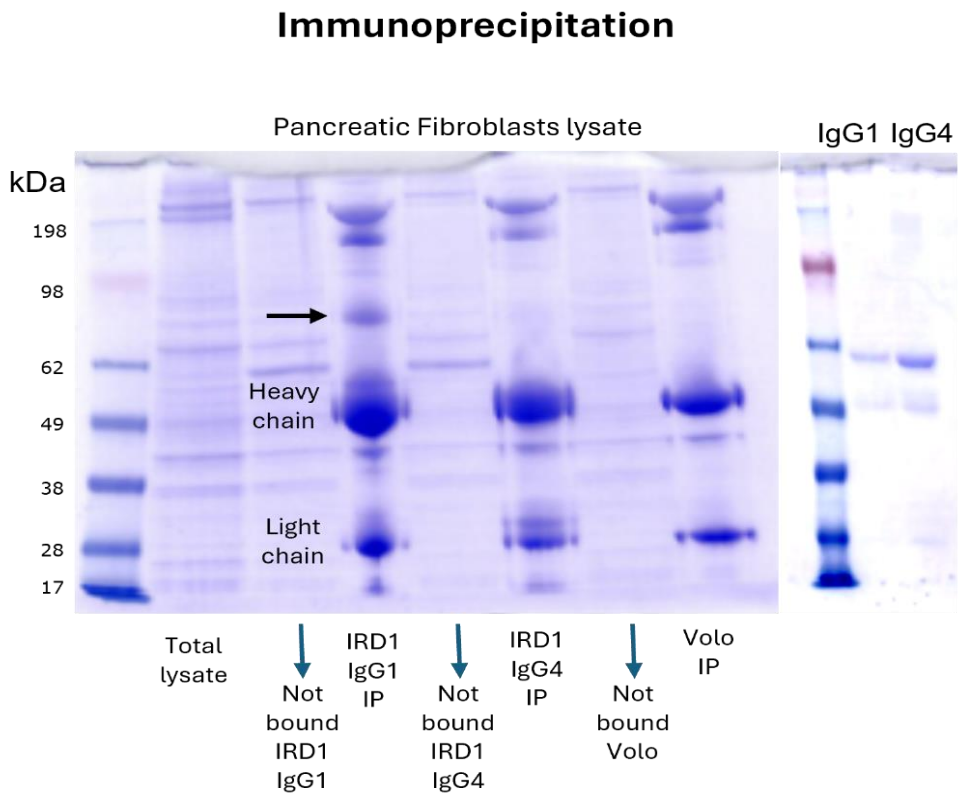


***Figure 141**. Immunoprecipitation of IgG1 and IgG4 and volociximab (Volo) on pancreatic fibroblasts. The last two columns represent the migration of IgG1 and IgG4 on gel electrophoresis and act as comparator in order to show the molecular weights of the heavy and light chains of both antibodies.*

A subsequent mass-spectrometry analysis is ongoing to determine the antigen bound.

3.8 IgG1 from patient IRD1 bind a surface antigen

After having observed a link between IgG1 from IRD1 patient and pancreatic fibroblasts lysate, we checked whether the antigen could be exposed on membrane or intracellular. Therefore, we performed a cell-based ELISA with 'fix and perm' technique leading to permeabilization of fibroblasts membrane. Thus, intracellular antigens can be actively bound by stimulating IgG as well as those presented on cells membrane. On the contrary, intracellular antigens are not available in not-fixed fibroblasts, whereas extracellular ones can be bound both on fixed and not fixed fibroblasts. Specifically, we

compared signals obtained following stimulation of fixed and not fixed fibroblast with IgG1 and IgG4 from an autologous (IRD-P) and a heterologous patient (IRD1).

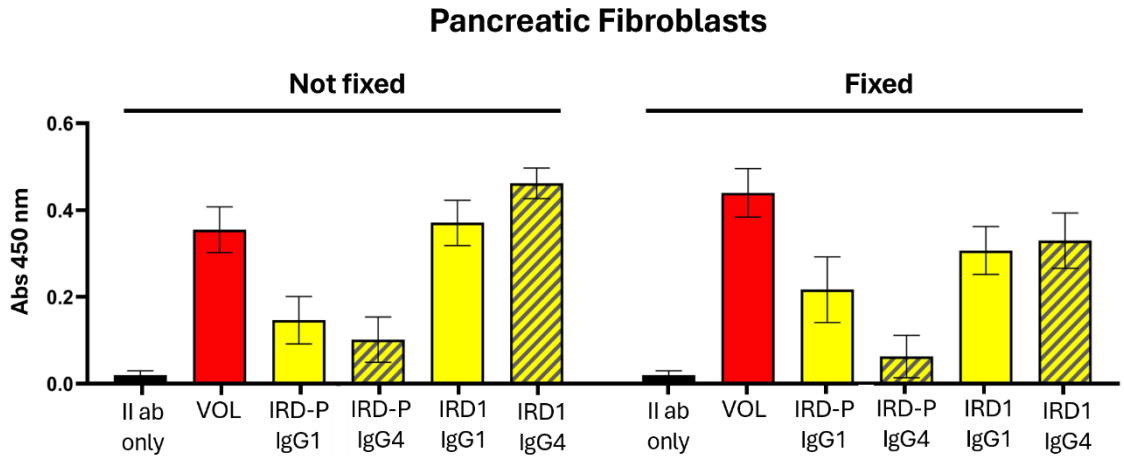


***Figure 22**. Cell-based ELISA performed on not fixed (left) and fixed (right) fibroblasts following stimulation with IgG1 (yellow) and IgG4 (striped, yellow) from two IgG4-RD patients.*

As reported in **Figure 22** we did not observe a difference between fixed and not fixed fibroblasts in terms of signal intensity following stimulation with IgG1 and IgG4 from patient IRD1, suggesting that the antigen has been bound in both conditions. This finding suggests that IgG1 and IgG4 from IRD1 patient bind a surface antigen.

Discussion

IgG4-related disease is an increasingly recognized disorder that is characterized by elevated serum IgG4 level and fibrotic lesions that can cause end organ damage if left untreated. However, the disease shows a remarkable response to immunosuppressive treatments which lead to shrinkage of the fibrotic lesions, an unprecedented finding in rheumatic diseases(Lanzillotta *et al*, 2020b). IgG4-RD pathogenesis is far from being solved but builds upon a complex crosstalk between B cells, T cells, and tissue resident fibroblasts. Indeed, the former (I) act as antigen presenting cells priming cognate T cells, (II) mirror disease activity, (III) decrease following effective treatment, and (IV) produce pro-fibrotic stimuli, shaping the fibrogenic milieu(Della-Torre *et al*, 2020). Indeed, B cells depletion has been shown to dramatically reduce the fibrotic burden in the targeted organ, a finding mirrored by the reduction of plasma cells infiltrate. T cells have a broad range of functions, ranging from B cells nurturing (Tfh cells) leading to affinity maturation and somatic hypermutation to effector killing (CD4 and CD8 cytotoxic T cells), ultimately leading to organ fibrosis(Perugino & Stone, 2020). Last, resident fibroblasts produce extracellular matrix components upon stimulation with pro fibrotic signals(Rockey *et al*, 2015).

In this view, IgG4 acted mainly as bystanders. Indeed, IgG4 were deemed as part of a counterregulatory response due to Fab-arm exchange, poor complement fixation and reduced ability to bind activating Fc receptor on effector cells. Yet, in a mouse model recapitulating autoimmune pancreatitis, Shiokawa et al observed a potential IgG4 driven damage on pancreatic tissues, yet to a lesser extent than compared to IgG1. Notably, co administration of IgG4 with IgG1 to Balb/c mice led to a mitigated damage, further supporting a protective role of IgG4 in dampening inflammation(Shiokawa *et al*, 2016). Similarly, Hubers et al unveiled that IgG4 from a subset of IgG4-RD patients impaired the binding of IgG1 to Annexin A11, likely impairing IgG1 ability to cause local damage(Hubers *et al*, 2018).

With our work we aimed to investigate a direct role of IgG from IgG4-RD patients in regulating fibrogenesis via modulation of fibroblasts' phenotype and to explore the mechanism underlying these alterations, ultimately unveiled a potential therapeutic target. Our work highlighted that (I) serum IgG1 and IgG4 isolated from IgG4-RD

patients can bind autologous and heterologous IgG4-RD fibroblasts; that (II) fibroblasts stimulation with IgG1 and IgG4 leads to a higher collagen I production and that stimulation via IgG1 increase fibroblast proliferation, while IgG4 stimulation negatively impact proliferation; that (III) these changes are mediated via upregulation of pro fibrotic genes such as PDGFRα and laminin and modulation of Ki67, and that (IV) tangible heterogeneity exists in terms of IgG1 and IgG4 mediated effect between different IgG4-RD patients. In addition, we unveiled that (V) even IgG1 and IgG4 from healthy controls can bind fibroblast, altering their phenotype in a pro-fibrotic fashion. Last, we tested the IgG1 and IgG4 of one patient for auto antigen recognition and we observed the binding of IgG1 to a lysate of IgG4-RD pancreatic fibroblast, suggesting auto-reactivity (VI). Given the similar results observed between permeabilized and not fixed fibroblasts, we speculate that this binding occurs on surface antigen (VII).

Altogether, these data offer new insights in the pathogenesis of IgG4-RD. Indeed, we observed that IgG1 and IgG4 tuned fibroblasts toward a more fibrotic phenotype, leading to enhanced collagen production via laminin and PDGFRα upregulation. This finding has multiple implications. Indeed, we broaden the role of B cells in IgG4-RD that can thus contribute to fibrosis directly via soluble mediators (PDGF), as previously shown by our group, and via IgG1 and IgG4 production, ultimately leading to increased collagen production. Moreover, these results underscore the importance of employing effective treatments that actively reduce serum IgG4 level, thus removing a risk factor for organ fibrosis, which can ultimately damage targeted organs. Last, it unveils a potential new target in IgG4-RD. As such, several drugs targeting PDGF/PDGFR axis are available, mainly in the oncological scenario(Ai *et al*, 2024). Nintedanib is a PDGFRα inhibitor that is now approved for idiopathic pulmonary fibrosis and for progressive lung fibrosis, regardless of the aetiology(Wollin *et al*, 2015). We thus speculate that nintedanib could be employed in IgG-RD, ameliorating tissue fibrosis via PDGFRα blockage.

Furthermore, we observed an opposite effect of IgG1 and IgG4 in terms of fibroblast proliferation. Indeed, while the former led to an increased proliferation compared to not stimulated fibroblasts, especially in patient IRD1, IgG4 stimulation negatively impacted fibroblasts growth, likely via Ki-67 downregulation. This novel finding well aligns with the proposed anti-inflammatory role of IgG4, whereby IgG4 dampens a chronic immune response due to prolonged antigenic trigger. In addition, this

evidence can be readily translated into other diseases where IgG4 infiltrate occurs, such as in pancreatic cancer(Minici *et al*, 2020). This disease is characterized by a dense peritumoral, IgG4 rich, stromal reaction surrounding the adenocarcinoma. In this view, the IgG4 response to cancer might represent an anti-inflammatory and anti-proliferative reaction.

We have previously shown that B cells can activate fibroblast via PDGFβ, can organize extracellular matrix via enzyme production, and that can induce the production of chemokines by resident fibroblast. Recently, Kitaoka et al. observed that MUM1+ plasma cells were in close contact with resident fibroblasts in IgG4-related sialadenitis, leading to fibroblasts activation via the PDGFβ production in a paracrine fashion(Kitaoka *et al*, 2024). Our work adds up to these findings, suggesting an adjunctive overlooked mechanism that takes part in fibroblasts activation in IgG4-RD.

Surprisingly, we observed a similar effect in collagen production from IgG obtained by healthy controls compared to IgG from IgG4-RD patients. Notably, Metcalfe et al observed a similar rate of glycosaminoglycan production after stimulation of dermal fibroblast with IgG from healthy controls and from patients with Graves' disease, that was higher compared to not stimulated fibroblasts(Metcalfe *et al*, 1993). This evidence would suggest a novel role of circulating IgG even in healthy controls that requires further investigation. Yet, under physiological conditions circulating IgGs do not colocalize with tissue fibroblasts, thus limiting this effect.

Last, we checked whether IgG induced pro-fibrotic properties might depend on auto antigen reactivity. Therefore, we performed an immunoblot adding IgG1 and IgG4 obtained from IRD1 patient to orbital and pancreatic lysate. IRD1 patient was chosen because his IgG1 and IgG4 showed the strongest signal in cell-based ELISA (**Figure 15**), PDGFR/Ki67 regulation (**Figure 18**), and collagen production (not shown). Notably, IgG1 bound a protein located between 62 and 98 kDa on pancreatic lysate that was absent in the volociximab column, thus suggesting auto reactivity. Thus, we performed immunoprecipitation analysis that confirmed this finding. Curiously, all the main four antigens so far identified in IgG4-RD, namely galectin 3, Annexin A11, Laminin 511 E8, prohibit have molecular weights that do not fit the range of our immunoprecipitation analysis, thus suggesting a potential new one.

Unfortunately, at the time of writing, Mass Spectrometry analysis to identify the targeted antigen is ongoing as well as immunoblot on the other patients included in the present study. Yet, to understand the location of the putative auto-antigen we performed cell-based ELISA with IgG1 from IRD1 patient on permeabilized pancreatic fibroblasts compared to not fixed ones. As shown in **Figure 22**, the signal intensity was similar in both conditions, suggesting that IgG1 binds a surface antigen. Indeed, an intracellular one would not be available in non-fixed fibroblasts. Yet, it would be intriguing to speculate that binding can occur both on cell surface or intracellularly depending on which portion of IgG (FC or Fab) mediates the binding and on antigen availability. As such, surface fibroblast antigens could be bound by FC segment of IgG1, while intracellular antigens can be targeted by the variable portion (Fab), when exposed (e.g. tissue necrosis). In this view, the FC binding epitope on fibroblast might be shared by IgG1 and IgG4 from patients and healthy controls, thus explained some the increased collagen production observed in the healthy control group. In addition, FC-mediated binding might represent a feature shared by the IgG1 and IgG4 from different patients, while Fab-mediated binding would suggest a patient-specificity given the high variability of the Fab region. In this view, by providing clues to the epitope bound, mass spectrometry will further inform us on the location of IgG1 and IgG4 binding.

Notably, we observed a discrete heterogeneity among IgG4-RD patients in terms of cell-based ELISA and PCR analysis. Several explanations can be proposed. First, IgG1 or IgG4 might be auto reactive only in a subset of patients. Indeed, Hubers et al observed Annexin A11 binding IgG1 only in 20% of the patients(Hubers *et al*, 2018). Moreover, it would be intriguing to speculate that IgG1 and IgG4 from patients with different disease phenotypes might exert different effects or bind different autoantigens. Last, such differences might be due to heterogeneity in post translation modifications on IgG1 and/or IgG4. Indeed, sialylation, fucosylation, and sumoylation are key to IgG functions and can modify their structure. Recently, Culver et al observed that patients with AIP had significantly higher sialylation ratios of IgG4 and increased fucosylation of IgG1 compared with pancreatic cancer patients and controls(Culver *et al*, 2019). Of note, sialylation has been proposed to have the greatest impact on the Fc domain of IgG, closing the binding site for FcγRs and opening a cryptic binding site for dendritic cell-specific

intercellular adhesion molecule-3-grabbing nonintegrin leading to anti-inflammatory activity.

Our study has some strengths. Indeed, it comes from a tertiary care center providing uniformity in patients selection and diagnosis. In addition, we employed IgG4-RD patient-derived fibroblast rather than other fibroblasts' sources, testing both autologous and heterologous IgG binding, thus investigating potential IgG4-RD patients' specific antigens. Moreover, we confirm the binding observed in cell-based ELISA with a second technique, thus adding robustness to our findings.

On the other hand, several weaknesses are present. First and foremost, the limited number of patients included. Indeed, we managed to immortalize fibroblast in only two patients starting from a cohort of 10 and isolated IgG1 and IgG4 from 9 patients. Although it will be important to confirm and extend these findings in larger studies, our statistical analyses suggest robust differences across many of the comparisons made. Moreover, we have not yet performed blocking experiments and ELISA sandwich to confirm the relevance of PDGFR axis in collagen production. Furthermore, our findings are based *on vitro* analysis, which might not reflect the complexity of ex vivo model. In this view, the procedure employed to immortalize fibroblast might add uncertainty to our results, as this does not represent a physiological condition. In addition, we had not considered the role of post translation modification of IgG, such as sialylation and fucosylation that can impact on their functions. Besides, we have not tested the hypothesis that IgG- induced changes to fibroblasts phenotype might be mediated by other cells type (e.g. macrophages) in vivo. In addition, we performed our experiments only on fibroblasts affected by IgG4-RD. As such a negative control represented by fibroblast from healthy controls or from unaffected organ of IgG4-RD thesis is missing. This element would have helped in distinguishing the pathogenic role and properties of fibroblast themselves. Last, we have not yet performed immunoprecipitation analysis on the whole cohort due to consumables and time constraints.

In conclusion, our work provides new evidence on the role of IgG1 and IgG4 in the context of IgG4-RD. Indeed, both induce a pro-fibrotic phenotype on stimulated fibroblasts due to laminin and PDGFRα upregulation, ultimately leading to increased collagen I secretion. Moreover, IgG4 but not IgG1 reduces fibroblasts proliferation. Last,

an extra cellular antigen between 62 and 98 kDa might represent an auto antigen targeted by IgG1 in some patients. In **Figure 23** a schematic cartoon of our findings is reported.

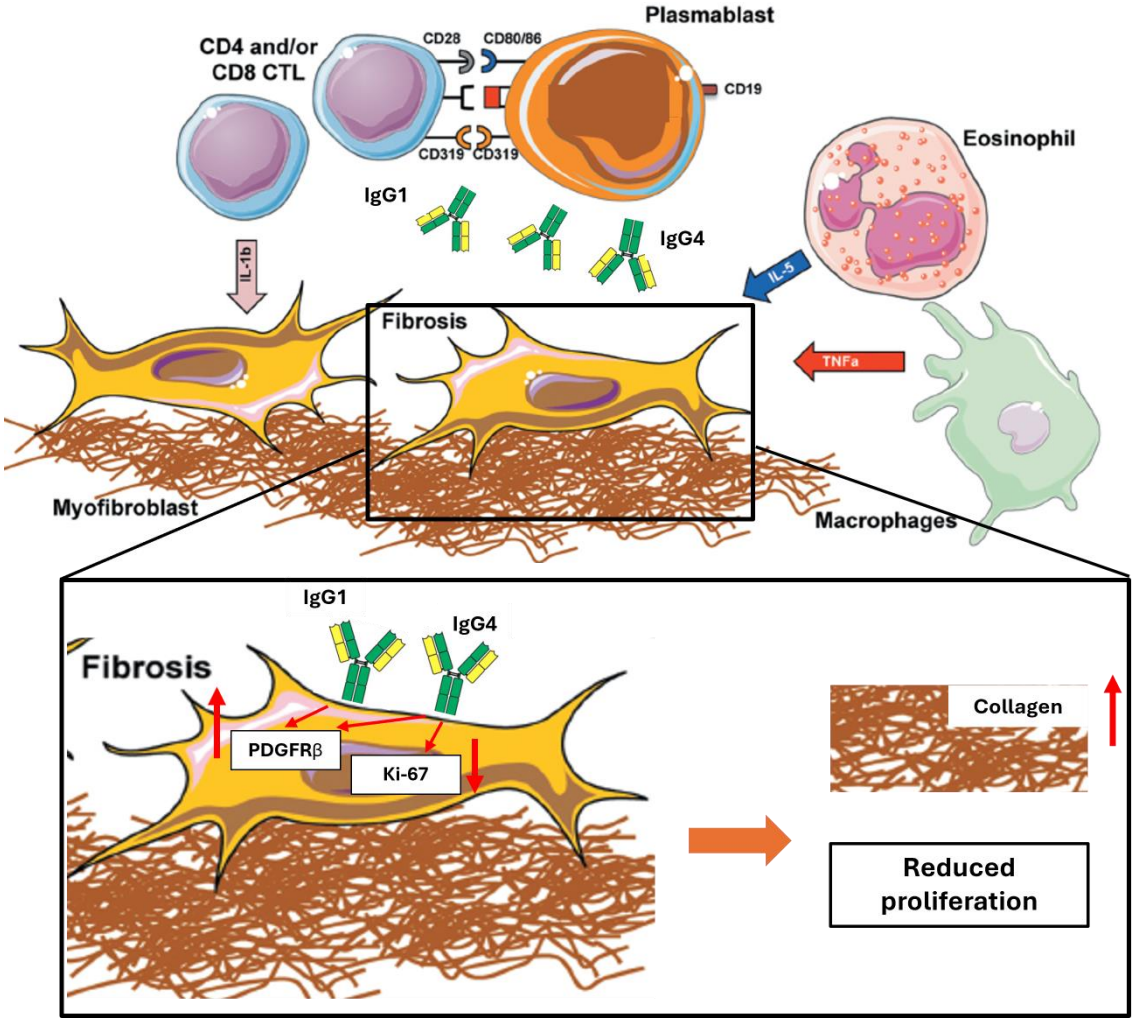


Figure 23. Potential pathogenetic model considering our findings. *B and T cells cross-talk ultimately lead to IgG1 and IgG4 production by plasmablast. IgG1 and IgG4 bind resident fibroblast, increasing collagen production via PDGFR upregulation while IgG4 reduce cell proliferation via Ki-67 down regulation. Adapted from Lanzillotta M, et al. Emerging therapy options for IgG4-related disease, Expert Review of Clinical Immunology.*

Building upon our findings, in the next months we will try to answer some of the questions raised by our experiments. First, we will increase the sample size of our cohort and will try to immortalize other fibroblasts, ideally from other IgG4-RD targeted organs, such as salivary glands or retroperitoneum. Then, we will perform mechanistic studies inhibiting IgG1 and IgG4 mediated signaling to confirm our findings in terms of collagen production and cells proliferation. Thirdly, we will perform immunoprecipitation on other fibroblast lysates such as IgG4-related orbital fibroblasts and fibroblasts from healthy

donors. In case of consistency in the immunoprecipitation analysis, we will screen our cohort for the presence of this auto antibodies in patients' sera and perform association analysis with this feature and patients' outcomes. Last, in case of further funding we will perform unbiased single cell RNA sequencing to further confirm our investigations and to unveil potential new pro-fibrotic pathways involved.

Methods

Patients

Consecutive patients referred to the IgG4-RD Clinic of the San Raffaele Hospital from January 2022 and December 2023 were evaluated for the participation in this study. Inclusion criteria consisted in (I) fulfilling the Comprehensive diagnostic criteria for IgG4-RD and the ACR/EULAR Classification criteria(Della-Torre *et al*, 2021a; Wallace *et al*, 2020b); (II) requiring a biopsy as part of the baseline assessment; (III) signing of the informed consent (Protocol Fab-4).

Biopsy were performed in the culprit IgG4-RD lesions following imaging with either PET or MRI as clinically indicated. Four vials were collected from peripheral blood for further analysis including IgG1/IgG4 isolation, PBMC storage, and B cells isolation.

Pancreatic and orbital tissue acquisition

Pancreatic samples were obtained during EUS as part of the diagnostic work up. 22-gauge needles were employed. Orbital biopsies were obtained during surgical excision of inflamed periorbital tissue for diagnostic purposes. The samples were processed as outlined below within the same day.

Isolation and Culture of Fibroblasts from Biopsy Samples

Fresh pancreatic or orbital biopsy samples were collected in sterile tubes containing ice-cold phosphate-buffered saline (PBS) supplemented with 1% penicillin-streptomycin to avoid bacterial growth. Samples were transported to the lab on ice and processed immediately to maintain cell viability. The biopsy tissue was transferred to a gentleMACS C tube with 5 mL of enzyme mix (Miltenyi Tumor Dissociation Kit) and processed according to manufacturer's instructions. Briefly, the C tube was placed on the gentle MACS Dissociator and processed using the appropriate program ("multi_C" followed by "Multi_B" twice). The sample was incubated in the dissociator's heating block at 37°C for 30–60 minutes, with periodic agitation to promote effective enzymatic digestion. This step was repeated twice. After dissociation, the resulting cell suspension was filtered through a 70 µm cell strainer to remove any undigested fragments. The filtrate was centrifuged at 300 g for 5 minutes at room temperature to pellet the cells, and the supernatant was discarded. The cell pellet was washed twice by resuspending in PBS and centrifuging again at 300 g for 5 minutes. The cell pellet was resuspended in fibroblast

culture medium (e.g., DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% non-essential amino acids). Cells were seeded in a T25 and incubated at 37°C with 5% CO_2. After 24 hours, the medium was changed to remove non-adherent cells (e.g. lymphocytes) and any remaining tissue debris. Fresh fibroblast culture medium was added to support cell growth. Medium was subsequently changed every 2–3 days, or as needed, until fibroblast colonies became visible. Once the fibroblasts reached 70–80% confluence, they were passaged using 0.05% trypsin-EDTA. Cells were incubated with trypsin for 2–3 minutes at 37°C, then neutralized with culture medium. Fibroblasts were further expanded in culture and used in experiments at passages 3–6 to ensure consistency and maintain cellular characteristics.

We employed anti fibroblast beads to further isolate cultured fibroblasts from epithelial (EPCAM+) and endothelial cells (CD31+). Fibroblast identity was confirmed by means of flow cytometry in which cells were stained for fibroblast markers (e.g., vimentin, FAP). The thresholds for FAP, IL-6 and αSMA were determined via Fluorescence Minus One (FMO) technique in which the fluorochrome-conjugated antibody for the respective marker was omitted. Then, a positive gate was established above the background signal detected in the FMO control. In **Figure 24** is reported fibroblasts' gating strategy.

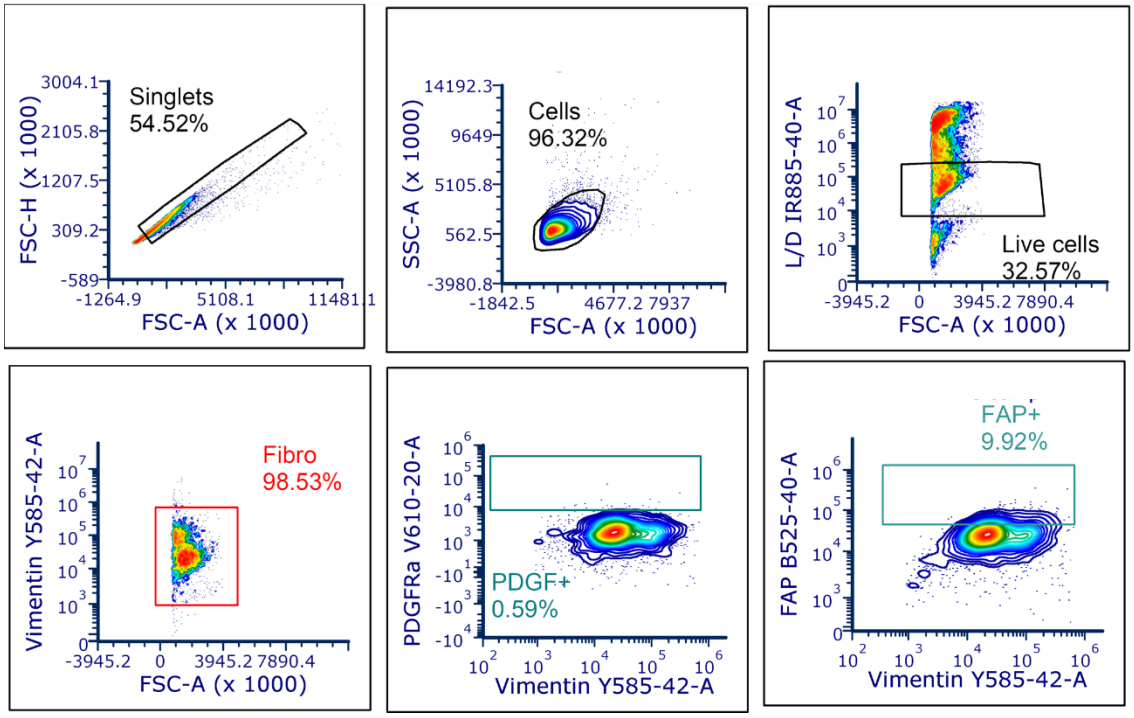


Figure 24. Fibroblasts gating strategy*. Abbreviations: FSC: forward scatter; SSC: side scatter; L/D: live and dead. PDGFR: platelet-derived growth factor receptor, FAP: fibroblast activation protein.*

Immortalization of Fibroblasts via hTERT Transduction

Cultured Fibroblasts were transduced with lentiviral particles encoding human telomerase reverse transcriptase (hTERT) under a constitutive promoter. Cells were incubated with the virus-containing medium for 24 hours before replacing it with fresh growth medium. After 48–72 hours, fibroblasts were subjected to selection with 2 ml/dish puromycin for 7–10 days to ensure stable integration of the hTERT construct. Non-transduced cells were eliminated during this period. Morphology and fibroblast markers (e.g., vimentin) were confirmed via flow cytometry as detailed above. Immortalized fibroblasts were cultured and passaged regularly and processed within 10–15 passages to avoid any clonal drift.

Flow Cytometry

Flow cytometry was employed to confirm IgG binding on IgG4-RD fibroblasts. Fibroblast obtained from pancreas samples (Patient IRD-P) were detached with TrypLE, counted and aliquoted in vials of 10^5 cells for each condition. Positive controls were represented by volociximab and anti-vimentin antibody, while negative controls consisted in unstained sample and II antibody alone (anti Human IgG-Novus). IgG1 and IgG4 from

IRD1 patient were tested (5 ug/50uL). Extracellular staining with I antibody was performed first. Samples were incubated at RT for 10 minutes. After 2 washes with PBS, II antibody PE-conjugated was added. Samples were incubated at RT 30 minutes. Then, cells were fixed and permeabilized (BD Perm/Wash) to allow intracellular staining. This passage is key as vimentin stains intracellularly. In addition, potential IgG antigens might reside intracellularly. Secondary antibody was added again following fix and per procedure. Samples were read with CytoFLEX LX and analyzed via FCS express software. Anti-vimentin antibody and volociximab were employed as positive controls.

Isolation of IgG1 and IgG4 from Blood Using Affinity Resin

Peripheral blood samples were collected from IgG4-RD patients and age and sex matched healthy controls and processed to obtain plasma. IgG and IgG4 were isolated from plasma using a protein-based affinity resin, following the manufacturer's instructions. Plasma was separated from whole blood by centrifugation at 1500 g for 10 minutes at 4°C and diluted 1:1 with binding buffer to reduce viscosity and optimize binding to the resin. The affinity resin specific for IgG and IgG4 was equilibrated with PBS. The resin was packed into chromatography columns and washed 3 times to ensure the removal of any storage preservatives and wet the columns. The diluted plasma was incubated with the resin at room temperature for 60 minutes with gentle rotation to facilitate binding. Following incubation, the resin was washed 5 times with binding buffer and pelleted by centrifugation at 500 g, and the supernatant was discarded. Bound IgG or IgG4 was eluted from the resin by adding elution buffer (0.1 M glycine-HCl, pH 2.7). Eluted IgG/IgG4 was immediately neutralized by adding 1/10th volume of neutralizing buffer (e.g., 1M Tris-HCl, pH 8.0) to prevent denaturation due to low pH. To concentrate the eluted IgG/IgG4 and exchange the buffer, eluates were applied to centrifugal filter units and centrifuged at 3000 g at 4°C until the desired volume was reached. The buffer was exchanged with PBS. Purified IgG and IgG4 were aliquoted and stored at -20°C or -80°C to avoid repeated freeze-thaw cycles. Quality and yield were assessed using SDS-PAGE to confirm the presence and integrity of the isolated antibodies. Standard curves were built to obtain the concentration of isolated IgG1 and IgG4.

RNA Isolation, Reverse Transcription (RT), and PCR for Gene Expression Analysis in Fibroblasts Stimulated with IgG

To evaluate the expression of genes related to cells proliferation (Ki-67), extracellular matrix production (PDGFRα, laminin, αSMA/ACTA 2), and inflammation (IL-6) in fibroblasts stimulated with IgG from patients and healthy controls, RNA was isolated from treated cells, reverse-transcribed, and analyzed by quantitative PCR. Fibroblasts were cultured in complete growth medium until they reached approximately 80% confluence. Cells were then stimulated with IgG1 and IgG4 isolated from IgG4-RD patients or healthy controls, added to the culture medium at a concentration of 5 ug/mL. After 24 hours and 96 hours, the medium was removed, and cells were washed twice with cold PBS to remove residual IgG. Total RNA was isolated from fibroblasts using a commercial RNA extraction kit (Qiagen RNeasy Kit) following the manufacturer's protocol. Cells were lysed directly in the culture dish with lysis buffer, and lysates were collected and processed according to the kit instructions. RNA concentration, quantity, and purity were assessed using a spectrophotometer (260/280 nm ratio, Nanodrop). RNA samples with an A260/280 ratio between 1.8 and 2.0 were considered pure and suitable for downstream analysis. 200 ng of total RNA from each sample was used as a template for cDNA synthesis. Reverse transcription was performed using a commercially available cDNA synthesis kit (e.g., Applied biosystem- Thermo Fischer) according to the manufacturer's instructions, with a reaction mixture containing RNA template, reverse transcriptase, buffer, and dNTPs. The reaction was incubated at 42°C for 30–60 minutes, followed by enzyme inactivation at 85°C for 5 minutes. Synthesized cDNA was stored at -20°C until PCR analysis. Gene expression levels of Ki-67, PDGFRα, laminin, ACTA 2, and IL-6 were quantified by qPCR using SYBR Green. Primers for each target gene and a GAPDH as housekeeping gene were employed. qPCR was performed in triplicate for each sample using a real-time PCR system, with the following cycling conditions: 95°C for 10 minutes (initial denaturation), followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The relative expression of each target gene was normalized to the housekeeping gene using the ΔΔCt method. Results were expressed as fold-change in gene expression in fibroblasts treated with patient IgG compared to those treated with IgG from healthy controls.

Cell-based ELISA for detecting IgG Antibody Binding on Fibroblasts

Isolated pancreatic fibroblasts were plated in a 24-wells flat-bottom cell culture plate and incubated at 37°C with 5% CO_2 overnight to ensure cell adherence. Cells were then blocked with 100 µL of 1% bovine serum albumin (BSA) in PBS for 30 minutes at 37°C to reduce nonspecific binding. After blocking, cells were incubated with primary IgG antibodies (IgG1 or IgG4 from patients, healthy controls, or positive and negative controls) at a concentration of 50 uL/well for each condition. The plate was incubated for 1 hour at 37°C. To fix and permeabilize cells 4% PAF was used, followed by permeabilization with 0.15% Triton X in PBS. Following primary antibody incubation, cells were washed three times with PBS. Next, cells were incubated with anti-goat II antibody 1:1000. The plate was incubated for 30 minutes at 37°C. After three washes a II antibody (chicken) anti-goat was added. The plate was incubated for 30 minutes at 37°C. Then, streptavidin 1:3000 was added 100 uL/well and incubated for 30 minutes at room temperature. Cells were washed again three times with PBS-T to remove any unbound secondary antibodies. For detection, 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added to each well, and color development was monitored for 5–10 minutes at room temperature in the dark. The reaction was stopped by adding 50 µL of 1M hydrochloric acid to each well, resulting in a color change from blue to yellow. Absorbance was measured at 450 nm using a microplate reader. Control wells, including those without primary antibody were included to account for background signal. Specifically, positive control was represented by volociximab, a monoclonal antibody binding alpha5 beta 1 integrin. Indeed, integrins are a key component of cell's membrane. Negative controls were represented by daratumumab and tocilizumab, two monoclonal antibodies that target CD38 receptor and IL-6 receptor, respectively. These receptor are absent on fibroblasts' membrane. Data were analyzed by comparing absorbance values across experimental and control groups.

Elisa sandwich for IL-6 and collagen I

Fibroblasts were seeded in a 96-well plate and incubated at 37°C in a humidified atmosphere with 5% CO_2. Cells were stimulated with IgG1 and IgG4 5 ug/mL and incubated for 4 days. Following the incubation period, cell culture supernatants were carefully collected and centrifuged at 300 g for 5 minutes to remove any cellular debris. The clear supernatants were stored at -80°C until analysis. The concentration of IL-6 and

collagen I in the supernatants was determined using a commercially available kit, following the manufacturer's instructions. Briefly, 100 µL of standards, controls, and samples were added to pre-coated wells in a 96-well ELISA plate and incubated at room temperature for 2 hours. Wells were then washed three times with wash buffer provided to remove unbound materials. Next, 100 µL of biotinylated anti-IL-6 antibody was added to each well and incubated for 1 hour at room temperature. Wells were washed again three times, followed by the addition of 100 µL of streptavidin-HRP conjugate and a 30-minute incubation at room temperature in the dark. After washing the wells thoroughly to remove unbound conjugate, 100 µL of TMB substrate solution was added to each well and incubated for 10–15 minutes at room temperature in the dark, allowing color development. The reaction was stopped by adding 50 µL of 1M sulfuric acid to each well, resulting in a color change from blue to yellow. Absorbance was measured at 450 nm using a microplate reader. IL-6 and collagen I concentrations in samples were quantified by interpolating from a standard curve generated from the known standards. Each sample was run in duplicate to ensure accuracy, and controls were included to validate assay performance.

Immunoblotting

Ice cold cell lysis buffer was added to cultured fibroblast. Lysates were collected and incubated on ice for 20 minutes and centrifuged. Lysates were resolved by SDS-PAGE on 10–12% gels and transferred onto nitrocellulose membranes using a wet transfer system at 300mA for 60 minutes. Membranes were blocked with 5% non-fat dry milk in TBST (TBS + 0.1% Tween-20) for 1 hour at room temperature. Membranes were incubated overnight at 4°C with primary antibodies (IgG1 and IgG4 from IRD1 patient and volociximab), 40mg/uL. After 3 washes, incubation with secondary antibody (mouse anti human IgG) diluted 1:20000 in TBST with 5% BSA or milk and incubated for 1 hour. After three washes incubation with tertiary antibody (goat anti mouse 1:1000) was performed. After three washes with TBST, membranes were incubated with HRP-conjugated secondary antibodies (1:5000) for 1 hour at room temperature. Following three washes with TBST, signals were developed using enhanced chemiluminescence (ECL) reagents and acquired at Typhoon. The anti-integrin monoclonal antibody volociximab was used as positive control as integrin are a fundamental protein of cells' membrane.

Immunoprecipitation

Cells were harvested and washed twice with ice-cold PBS, followed by lysis in ice-cold IP lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, protease, and phosphatase inhibitors) on ice for 20 minutes. Lysates were collected with scrapers and clarified by centrifugation at 5000 g for 15 minutes at 4°C, and the supernatant was collected and stored. 700uL of lysate were combined with 50ug IgG1 and IgG4 from IRD1 patient and volociximab. The reaction was incubated overnight at 4 °C on wheel. Protein A/G agarose beads were added and incubated at 4°C for 2 hours with gentle rotation to capture immune complexes. Beads were washed 3–5 times with ice-cold lysis buffer to remove unbound proteins. Immune complexes were eluted by boiling beads in SDS sample buffer (2× Laemmli buffer with β-mercaptoethanol) at 95°C for 5 minutes. The anti-integrin monoclonal antibody volociximab was used as positive control.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software 6.0. Normal distribution of continuous variables was assessed with the D'agostino & Pearson omnibus normality test. Normally distributed variables were compared using the Student's t-test or ANOVA test in case of analysis on more than two groups. Non-normally distributed variables were compared using the Mann-Whitney U-test or Kruskal-Wallis test in case of analysis on more than two groups. Tukey test was employed to correct for multiple comparisons. A p-value < 0.05 was considered statistically significant. Values are presented as mean and standard error of the mean (SEM), unless specified otherwise.

References

Aalberse RC, Stapel SO, Schuurman J & Rispens T (2009) Immunoglobulin G4: An odd antibody. *Clinical and Experimental Allergy* 39: 469–477

AbdelRazek M & Stone JH (2017) Neurologic Features of Immunoglobulin G4-Related Disease. *Rheum Dis Clin North Am* 43: 621–631

Ai JY, Liu CF, Zhang W & Rao GW (2024) Current status of drugs targeting PDGF/PDGFR. *Drug Discov Today* 29

Akiyama M, Suzuki K, Yamaoka K, Yasuoka H, Takeshita M, Kaneko Y, Kondo H, Kassai Y, Miyazaki T, Morita R, *et al* (2015) Number of circulating follicular helper 2 T cells correlates with IgG4 and interleukin-4 levels and plasmablast numbers in IgG4-related disease. *Arthritis and Rheumatology* 67: 2476–2481

Akiyama M, Yasuoka H, Yamaoka K, Suzuki K, Kaneko Y, Kondo H, Kassai Y, Koga K, Miyazaki T, Morita R, *et al* (2016) Enhanced IgG4 production by follicular helper 2 T cells and the involvement of follicular helper 1 T cells in the pathogenesis of IgG4-related disease. *Arthritis Res Ther* 18

Akram S, Pardi DS, Schaffner JA & Smyrk TC (2007) Sclerosing mesenteritis: clinical features, treatment, and outcome in ninety-two patients. *Clin Gastroenterol Hepatol* 5: 589–96; quiz 523–4

Alexander MP, Larsen CP, Gibson IW, Nasr SH, Sethi S, Fidler ME, Raissian Y, Takahashi N, Chari S, Smyrk TC, *et al* (2013) Membranous glomerulonephritis is a manifestation of IgG4-related disease. *Kidney Int* 83: 455–462

Batani V, Lanzillotta M, Mahajne J, Pedica F, Palumbo D, Venturini E, Mapelli P, Bossi P, Dagna L & Emanuel DT (2023a) Association of IgG4-related disease and systemic rheumatic disorders. *Eur J Intern Med* 111: 63–68

Batani V, Minici C, Sanvito F, Venturini E & Della-Torre E (2023b) Interleukin-6 inhibition for the treatment of IgG4 related vasculitis. *Eur J Intern Med* 118: 149–151

Berti A, Della-Torre E, Gallivanone F, Canevari C, Milani R, Lanzillotta M, Campochiaro C, Ramirez GA, Cassione EB, Bozzolo E, *et al* (2017) Quantitative measurement of 18F-FDG PET/CT uptake reflects the expansion of circulating plasmablasts in IgG4-related disease. *Rheumatology (United Kingdom)* 56: 2084–2092

Buglioni A, Jenkins SM, Nasr SH, Zhang P, Gibson IW, Alexander MP, Herrera Hernandez LP, Fidler ME, Takahashi N, Hogan MC, *et al* (2024) Clinicopathologic Features of IgG4-Related Kidney Disease. *Kidney Int Rep* 9: 2462–2473

Buijs J, Van Heerde MJ, Rauws EAJ, De Buy Wenniger LJM, Hansen BE, Biermann K, Verheij J, Vleggaar FP, Brink MA, Beuers UHW, *et al* (2014) Comparable efficacy

of low- versus high-dose induction corticosteroid treatment in autoimmune pancreatitis. *Pancreas* 43: 261–267

Campochiaro C, Della-Torre E, Lanzillotta M, Bozzolo E, Baldissera E, Milani R, Arcidiacono PG, Crippa S, Falconi M & Dagna L (2020) Long-term efficacy of maintenance therapy with Rituximab for IgG4-related disease. *Eur J Intern Med* 74: 92–98

Cargill T, Makuch M, Sadler R, Lighaam LC, Peters R, Van Ham M, Klenerman P, Bateman A, Rispens T, Barnes E, *et al* (2019) Activated t-follicular helper 2 cells are associated with disease activity in IGG4-related sclerosing cholangitis and pancreatitis. *Clin Transl Gastroenterol* 10

Carruthers MN, Topazian MD, Khosroshahi A, Witzig TE, Wallace ZS, Hart PA, Deshpande V, Smyrk TC, Chari S, Stone JH, *et al* (2015) Rituximab for IgG4-related disease: A prospective, open-label trial. *Ann Rheum Dis* 74: 1171–1177

Chang HH, Wu SB, Tsai CC (2024) A Review of Pathophysiology and Therapeutic Strategies Targeting TGF-β in Graves' Ophthalmopathy. *Cells* 5;13:1493

Chari ST, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, Zhang L, Clain JE, Pearson RK, Petersen BT, Vege SS, *et al* (2006) Diagnosis of Autoimmune Pancreatitis: The Mayo Clinic Experience. *Clinical Gastroenterology and Hepatology* 4: 1010–1016

Chinju A, Moriyama M, Kakizoe-Ishiguro N, Chen H, Miyahara Y, Haque ASMR, Furusho K, Sakamoto M, Kai K, Kibe K, *et al* (2022) CD163+ M2 Macrophages Promote Fibrosis in IgG4-Related Disease Via Toll-like Receptor 7/Interleukin-1 Receptor-Associated Kinase 4/NF-κB Signaling. *Arthritis Rheumatol* 74: 892–901

Corcoran JP, Culver EL, Anstey RM, Talwar A, Manganis CD, Cargill TN, Hallifax RJ, Psallidas I, Rahman NM & Barnes E (2017) Thoracic involvement in IgG4-related disease in a UK-based patient cohort. *Respir Med* 132: 117–121

Culver EL, van de Bovenkamp FS, Derksen NIL, Koers J, Cargill T, Barnes E, de Neef LA, Koeleman CAM, Aalberse RC, Wuhrer M, *et al* (2019) Unique patterns of glycosylation in immunoglobulin subclass G4-related disease and primary sclerosing cholangitis. *Journal of Gastroenterology and Hepatology (Australia)* 34: 1878–1886

Culver EL, Sadler R, Bateman AC, Makuch M, Cargill T, Ferry B, Aalberse R, Barnes E & Rispens T (2017) Increases in IgE, Eosinophils, and Mast Cells Can be Used in Diagnosis and to Predict Relapse of IgG4-Related Disease. *Clinical Gastroenterology and Hepatology* 15: 1444-1452.e6

Danford CJ, Lin SC & Wolf JL (2019) Sclerosing Mesenteritis. *Am J Gastroenterol* 114: 867–873

Della-Torre E, Bozzalla-Cassione E, Sciorati C, Ruggiero E, Lanzillotta M, Bonfiglio S, Mattoo H, Perugino CA, Bozzolo E, Rovati L, *et al* (2018a) A CD8α- Subset of

CD4+SLAMF7+ Cytotoxic T Cells Is Expanded in Patients With IgG4-Related Disease and Decreases Following Glucocorticoid Treatment. *Arthritis Rheumatol* 70: 1133–1143

Della-torre E, Campochiaro C, Bozzolo EP, Dagna L, Scotti R, Nicoletti R, Stone JH & Sabbadini MG (2015) Methotrexate for maintenance of remission in igg4-related disease. *Rheumatology (United Kingdom)* 54: 1934–1936

Della-Torre E, Campochiaro C, Cassione EB, Albano L, Gerevini S, Bianchi-Marzoli S, Bozzolo E, Passerini G, Lanzillotta M, Terreni M, *et al* (2018b) Intrathecal rituximab for IgG4-related hypertrophic pachymeningitis. *J Neurol Neurosurg Psychiatry* 89: 441–444

Della-Torre E, Feeney E, Deshpande V, Mattoo H, Mahajan V, Kulikova M, Wallace ZS, Carruthers M, Chung RT, Pillai S, *et al* (2015a) B-cell depletion attenuates serological biomarkers of fibrosis and myofibroblast activation in IgG4-related disease. *Ann Rheum Dis* 74: 2236–43

Della-Torre E, Lanzillotta M & Doglioni C (2015b) Immunology of IgG4-related disease. *Clin Exp Immunol* 181: 191–206

Della-Torre E, Lanzillotta M, Germanò T, Mancuso G, Ramirez GA, Capurso G, Falconi M & Dagna L (2021a) Utility of the '2019 ACR/EULAR classification criteria' for the management of patients with IgG4-related disease. *Semin Arthritis Rheum* 51: 761–765

Della-Torre E, Mancuso G, Lanzillotta M, Ramirez GA, Arcidiacono PG, Capurso G, Falconi M & Dagna L (2021b) Urgent manifestations of immunoglobulin G4-related disease. *Scand J Rheumatol* 50: 48–51

Della-Torre E, Mattoo H, Mahajan VS, Deshpande V, Krause D, Song P, Pillai S & Stone JH (2014) IgG4-related midline destructive lesion. *Ann Rheum Dis* 73: 1434–6

Della-Torre E, Rigamonti E, Perugino C, Baghai-Sain S, Sun N, Kaneko N, Maehara T, Rovati L, Ponzoni M, Milani R, *et al* (2020) B lymphocytes directly contribute to tissue fibrosis in patients with IgG4-related disease. *J Allergy Clin Immunol* 145: 968-981.e14

Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, Klöppel G, Heathcote JG, Khosroshahi A, Ferry JA, *et al* (2012) Consensus statement on the pathology of IgG4-related disease. *Mod Pathol* 25: 1181–92

Do P, Wallace ZS, Fernandes AD, Ragon) ;, Perugino A, Liu H, Phd K, Xing K, Xencor ;, Stone JH, *et al* (2023) Evaluation of the safety, efficacy, and mechanism of action of obexelimab for the treatment of patients with IgG4-related disease: an open-label, single-arm, single centre, phase 2 pilot trial

Dragos C, Joseph C, Elwell H, Dey M & Kouranloo K (2024) Pulmonary manifestations, treatments and outcomes of IgG4-related disease-a systematic literature review. *Rheumatol Int* 44: 1875–1886

Dugic A, Gil CV, Mellenthin C, Vujasinovic M, Löhr JM & Mühldorfer S (2022) The Clinical Utility of Soluble Serum Biomarkers in Autoimmune Pancreatitis: A Systematic Review. *Biomedicines* 10 doi:10.3390/biomedicines10071511 [PREPRINT]

Fenaroli P, Maritati F & Vaglio A (2021) Into Clinical Practice: Diagnosis and Therapy of Retroperitoneal Fibrosis. *Curr Rheumatol Rep* 23: 18

Frulloni L, Lunardi C, Simone R, Dolcino M, Scattolini C, Falconi M, Benini L, Vantini I, Corrocher R & Puccetti A (2009) Identification of a novel antibody associated with autoimmune pancreatitis. *N Engl J Med* 361: 2135–2142

Gallo C, Dispinzieri G, Zucchini N, Invernizzi P & Massironi S (2024) Autoimmune pancreatitis: Cornerstones and future perspectives. *World J Gastroenterol* 30: 817–832 doi:10.3748/wjg.v30.i8.817 [PREPRINT]

Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, Kamatsu AA, Ukushima AF, Ohzo K, Akayama N, *et al* (2001) HIGH SERUM IgG4 CONCENTRATIONS IN PATIENTS WITH SCLEROSING PANCREATITIS

Hart PA, Kamisawa T, Brugge WR, Chung JB, Culver EL, Czakó L, Frulloni L, Go VLW, Gress TM, Kim MH, *et al* (2013) Long-term outcomes of autoimmune pancreatitis: A multicentre, international analysis. *Gut* 62: 1771–1776

Hart PA, Krishna SG & Okazaki K (2017) Diagnosis and Management of Autoimmune Pancreatitis. *Curr Treat Options Gastroenterol* 15: 538–547

Hart PA, Topazian MD, Witzig TE, Clain JE, Gleeson FC, Klebig RR, Levy MJ, Pearson RK, Petersen BT, Smyrk TC, *et al* Treatment of relapsing autoimmune pancreatitis with immunomodulators and rituximab: the Mayo Clinic experience.

Hart PA, Zen Y & Chari ST (2015) Recent Advances in Autoimmune Pancreatitis. *Gastroenterology* 149: 39–51

Hubers LM, Schuurman AR, Buijs J, Mostafavi N, Bruno MJ, Vermeulen RCH, Huss A, van Buuren HR & Beuers U (2021) Blue-collar work is a risk factor for developing IgG4-related disease of the biliary tract and pancreas. *JHEP Rep* 3

Hubers LM, Vos H, Schuurman AR, Erken R, Oude Elferink RP, Burgering B, Van De Graaf SFJ & Beuers U (2018) Annexin A11 is targeted by IgG4 and IgG1 autoantibodies in IgG4-related disease. *Gut* 67: 728–735

Huggett MT, Culver EL, Kumar M, Hurst JM, Rodriguez-Justo M, Chapman MH, Johnson GJ, Pereira SP, Chapman RW, Webster GJM, *et al* (2014) Type 1 autoimmune pancreatitis and IgG4-related sclerosing cholangitis is associated with

extrapancreatic organ failure, malignancy, and mortality in a prospective UK cohort. *Am J Gastroenterol* 109: 1675–1683

Hwang G, Jin S-Y & Kim H-S (2016) IgG4-related disease presenting as hypertrophic pachymeningitis and compressive optic neuropathy. *Joint Bone Spine* 83: 601–2

Iaccarino L, Talarico R, Bozzalla-Cassione E, Burmester GR, Culver EL, Doria A, Ebbo M, van Hagen PM, Hachulla E, van Laar JAM, *et al* (2022) Blood biomarkers recommended for diagnosing and monitoring IgG4-related disease. Considerations from the ERN ReCONNET and collaborating partners. *Clin Exp Rheumatol*

Kamisawa T, Chari ST, Giday SA, Kim M-H, Bock Chung J, Lee KT, Werner J, Bergmann F, Lerch MM, Mayerle J, *et al* (2011) Clinical Profile of Autoimmune Pancreatitis and Its Histological Subtypes An International Multicenter Survey

Kamisawa T, Funata N, Hayashi Y, Eishi Y, Koike M, Tsuruta K, Okamoto A, Egawa N & Nakajima H (2003) A new clinicopathological entity of IgG4-related autoimmune disease. *J Gastroenterol* 38: 982–984

Kamisawa T, Zen Y, Pillai S & Stone JH (2015) IgG4-related disease. *Lancet* 385: 1460–71

Kasashima S, Kawashima A, Zen Y, Ozaki S, Kasashima F, Endo M, Matsumoto Y & Kawakami K (2018) Upregulated interleukins (IL-6, IL-10, and IL-13) in immunoglobulin G4-related aortic aneurysm patients. *J Vasc Surg* 67: 1248–1262

Katz G, Hernandez-Barco Y, Palumbo D, Guy T V, Dong L & Perugino CA (2024) Proliferative features of IgG4-related disease. *Lancet Rheumatol*

Kawa S, Ota M, Yoshizawa K, Horiuchi A, Hamano H, Ochi Y, Nakayama K, Tokutake Y, Katsuyama Y, Saito S, *et al* (2002) HLA DRB1*0405-dqb1*haplotype is associated with autoimmune pancreatitis in the Japanese population. *Gastroenterology* 122: 1264–1269

Kawano M, Saeki T, Nakashima H, Nishi S, Yamaguchi Y, Hisano S, Yamanaka N, Inoue D, Yamamoto M, Takahashi H, *et al* (2011) Proposal for diagnostic criteria for IgG4-related kidney disease. *Clin Exp Nephrol* 15: 615–626

Kessler L, Ferdinandus J, Hirmas N, Bauer S, Dirksen U, Zarrad F, Nader M, Chodyla M, Milosevic A, Umutlu L, *et al* (2022) 68Ga-FAPI as a Diagnostic Tool in Sarcoma: Data from the 68Ga-FAPI PET Prospective Observational Trial. *J Nucl Med* 63: 89–95

Khosroshahi A, Carruthers MN, Deshpande V, Unizony S, Bloch DB & Stone JH (2012) Rituximab for the treatment of IgG4-related disease: Lessons from 10 consecutive patients. *Medicine* 91: 57–66

Khosroshahi A, Wallace ZS, Crowe JL, Akamizu T, Azumi A, Carruthers MN, Chari ST, Della-Torre E, Frulloni L, Goto H, *et al* (2015) International Consensus Guidance

Statement on the Management and Treatment of IgG4-Related Disease. *Arthritis Rheumatol* 67: 1688–1699

Kitaoka T, Ohe R, Kabasawa T, Kaneko M, Sasahara N, Kono M, Suzuki K, Uchiyama N, Ogawa R & Futakuchi M (2024) Activation of fibroblasts by plasma cells via PDGF/PDGFR signaling in IgG4-related sialadenitis. *J Clin Exp Hematop* 64: 223–231

Lanzillotta M, Belli LI, Belfiori G, Palumbo D, Schiavo-Lena M, Capurso G, Arcidiacono PG, Dagna L, Falconi M, Crippa S, *et al* (2024a) Association of autoimmune pancreatitis and intraductal papillary mucinous neoplasm. A retrospective analysis from a tertiary care referral center. *Pancreatology* 24: 456–462

Lanzillotta M, Campochiaro C, Mancuso G, Ramirez GA, Capurso G, Falconi M, Dagna L & Della-Torre E (2020a) Clinical phenotypes of IgG4-related disease reflect different prognostic outcomes. *Rheumatology (Oxford)* 59: 2435–2442

Lanzillotta M, Campochiaro C, Trimarchi M, Arrigoni G, Gerevini S, Milani R, Bozzolo E, Biafora M, Venturini E, Cicalese MP, *et al* (2017a) Deconstructing IgG4-related disease involvement of midline structures: Comparison to common mimickers. *Mod Rheumatol* 27: 638–645

Lanzillotta M, Culver E, Sharma A, Zen Y, Zhang W, Stone JH & Della-Torre E (2024b) Fibrotic phenotype of IgG4-related disease. *Lancet Rheumatol* doi:10.1016/S2665-9913(23)00299-0 [PREPRINT]

Lanzillotta M, Della-Torre E, Milani R, Bozzolo E, Bozzalla-Cassione E, Rovati L, Arcidiacono PG, Partelli S, Falconi M, Ciceri F, *et al* (2018) Increase of circulating memory B cells after glucocorticoid-induced remission identifies patients at risk of IgG4-related disease relapse. *Arthritis Res Ther* 20

Lanzillotta M, Della-Torre E, Milani R, Bozzolo E, Bozzalla-Cassione E, Rovati L, Arcidiacono PG, Partelli S, Falconi M, Ciceri F, *et al* (2019) Effects of glucocorticoids on B-cell subpopulations in patients with IgG4-related disease. *Clin Exp Rheumatol* 37 Suppl 118: S159–S166

Lanzillotta M, Della-Torre E & Stone JH (2017b) Roles of plasmablasts and B cells in IgG4-related disease: Implications for therapy and early treatment outcomes. *Curr Top Microbiol Immunol* 401

Lanzillotta M, Della-Torre E, Wallace ZS, Stone JH, Karadag O, Fernández-Codina A, Arcidiacono PG, Falconi M, Dagna L & Capurso G (2021) Efficacy and safety of rituximab for IgG4-related pancreato-biliary disease: A systematic review and meta-analysis. *Pancreatology* 21: 1395–1401

Lanzillotta M, Mancuso G & Della-Torre E (2020b) Advances in the diagnosis and management of IgG4 related disease. *BMJ*: m1067

Lanzillotta M, Mapelli P, Dagna L & Della-Torre E (2023a) B-cell depletion with obinutuzumab for the treatment of IgG4-related disease. *Eur J Intern Med* 116: 155–156 doi:10.1016/j.ejim.2023.07.019 [PREPRINT]

Lanzillotta M, Ramirez GA, Milani R, Dagna L & Della-Torre E (2024c) B cell depletion after treatment with rituximab predicts relapse of IgG4-related disease. *Rheumatology*

Lanzillotta M, Stone JH & Della-Torre E (2023b) B-Cell depletion therapy in IgG4-related disease: State of the art and future perspectives. *Mod Rheumatol* 33: 258–265

Lanzillotta M, Tacelli M, Falconi M, Arcidiacono PG, Capurso G & Della-Torre E (2022) Incidence of endocrine and exocrine insufficiency in patients with autoimmune pancreatitis at diagnosis and after treatment: a systematic review and meta-analysis. *Eur J Intern Med* 100: 83–93

Lanzillotta M, Vinge-Holmquist O, Overbeek KA, Poulsen JL, Demirci AF, Macinga P, Löhr M & Rosendahl J (2020c) PrescrAIP: A Pan-European Study on Current Treatment Regimens of Auto-Immune Pancreatitis. *Front Med (Lausanne)* 7

Lee HW, Moon SH, Kim MH, Cho DH, Jun JH, Nam K, Song TJ, Park DH, Lee SS, Seo DW, *et al* (2018) Relapse rate and predictors of relapse in a large single center cohort of type 1 autoimmune pancreatitis: long-term follow-up results after steroid therapy with short-duration maintenance treatment. *J Gastroenterol* 53: 967–977

Lin W, Zhang P, Chen H, Chen Y, Yang H, Zheng W, Zhang X, Zhang F, Zhang W & Lipsky PE (2017) Circulating plasmablasts/plasma cells: a potential biomarker for IgG4-related disease. *Arthritis Res Ther* 19

Liu H, Perugino CA, Ghebremichael M, Wallace ZS, Montesi SB, Stone JH & Pillai S (2020a) Disease Severity Linked to Increase in Autoantibody Diversity in IgG4-Related Disease. *Arthritis and Rheumatology* 72: 687–693

Liu H, Wallace ZS, Harvey L, Crotty R, Wu CL, Deshpande V, O'Shea A, Harisinghani M, Ghebremichael M, Nehra A, *et al* (2020b) Prostate and pancreas involvement are linked in IgG4-related disease. *Semin Arthritis Rheum* 50: 1245–1251

Löhr JM, Beuers U, Vujasinovic M, Alvaro D, Frøkjær JB, Buttgereit F, Capurso G, Culver EL, de-Madaria E, Della-Torre E, *et al* (2021) European guideline on igg4-related digestive disease — ueg and sgf evidence-based recommendations. *Russian Journal of Gastroenterology, Hepatology, Coloproctology* 31: 80–114

Löhr JM, Vujasinovic M, Rosendahl J, Stone JH & Beuers U (2022) IgG4-related diseases of the digestive tract. *Nat Rev Gastroenterol Hepatol* 19: 185–197 doi:10.1038/s41575-021-00529-y [PREPRINT]

López-Serrano A, Crespo J, Pascual I, Salord S, Bolado F, Del-Pozo-García AJ, Ilzarbe L, De-Madaria E & Moreno-Osset E (2016) Diagnosis, treatment and long-term

outcomes of autoimmune pancreatitis in Spain based on the International Consensus Diagnostic Criteria: A multi-centre study. *Pancreatology* 16: 382–390

Lu H, Wu X, Peng Y, Sun R, Nie Y, Li J, Wang M, Luo Y, Peng L, Fei Y, *et al* (2022) TSLP promoting B cell proliferation and polarizing follicular helper T cell as a therapeutic target in IgG4-related disease. *J Transl Med* 20: 414

Luo Y, Pan Q & Zhang W (2019) IgG4-related disease revealed by 68Ga-FAPI and 18F-FDG PET/CT. *Eur J Nucl Med Mol Imaging* 46: 2625–2626

Majumder S, Mohapatra S, Lennon RJ, Piovezani Ramos G, Postier N, Gleeson FC, Levy MJ, Pearson RK, Petersen BT, Vege SS, *et al* (2018) Rituximab Maintenance Therapy Reduces Rate of Relapse of Pancreaticobiliary Immunoglobulin G4-related Disease. *Clin Gastroenterol Hepatol* 16: 1947–1953

Mancuso G, Jofra T, Lanzillotta M, Aiuti A, Cicalese MP, di Colo G, Dagna L, Fousteri G & Della-Torre E (2021) Persistence of circulating T-follicular helper cells after rituximab is associated with relapse of IgG4-related disease. *Rheumatology (Oxford)* 60: 3947–3949

Maritati F, Rocco R, Accorsi Buttini E, Marvisi C, Nicastro M, Urban ML, Fenaroli P, Peyronel F, Benigno GD, Palumbo AA, *et al* (2019) Clinical and Prognostic Significance of Serum IgG4 in Chronic Periaortitis. An Analysis of 113 Patients. *Front Immunol* 10: 693

Masamune A, Kikuta K, Hamada S, Tsuji I, Takeyama Y, Shimosegawa T, Okazaki K, Kanno A, Sano T, Uchida K, *et al* (2020) Nationwide epidemiological survey of autoimmune pancreatitis in Japan in 2016. *J Gastroenterol* 55: 462–470

Masamune A, Nishimori I, Kikuta K, Tsuji I, Mizuno N, Iiyama T, Kanno A, Tachibana Y, Ito T, Kamisawa T, *et al* (2017) Randomised controlled trial of long-term maintenance corticosteroid therapy in patients with autoimmune pancreatitis. *Gut* 66: 487–494

Mattoo H, Della-Torre E, Mahajan VS, Stone JH & Pillai S (2014a) Circulating Th2 memory cells in IgG4-related disease are restricted to a defined subset of subjects with atopy. *Allergy: European Journal of Allergy and Clinical Immunology* 69: 399–402

Mattoo H, Mahajan VS, Della-Torre E, Sekigami Y, Carruthers M, Wallace ZS, Deshpande V, Stone JH & Pillai S (2014b) De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. *Journal of Allergy and Clinical Immunology* 134: 679–687

Mattoo H, Mahajan VS, Maehara T, Deshpande V, Della-Torre E, Wallace ZS, Kulikova M, Drijvers JM, Daccache J, Carruthers MN, *et al* (2016) Clonal expansion of CD4+ cytotoxic T lymphocytes in patients with IgG4-related disease. *Journal of Allergy and Clinical Immunology* 138: 825–838

Metcalfe RA, Davies R & Weetman AP (1993) Analysis of fibroblast-stimulating activity in IgG from patients with Graves' dermopathy. *Thyroid* 3: 207–212

Minici C, Rigamonti E, Lanzillotta M, Monno A, Rovati L, Maehara T, Kaneko N, Deshpande V, Protti MP, De Monte L, *et al* (2020) B lymphocytes contribute to stromal reaction in pancreatic ductal adenocarcinoma. *Oncoimmunology* 9

Mori Y, Dendl K, Cardinale J, Kratochwil C, Giesel FL & Haberkorn U (2023) FAPI PET: Fibroblast Activation Protein Inhibitor Use in Oncologic and Nononcologic Disease. *Radiology* 306

Motta R V. & Culver EL (2024) IgG4 autoantibodies and autoantigens in the context of IgG4-autoimmune disease and IgG4-related disease. *Front Immunol* 15

van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martínez-Martínez P, Vermeulen E, den Bleker TH, Wiegman L, Vink T, Aarden LA, *et al* (2007) Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 317: 1554–7

Nikolic S, Brehmer K, Panic N, Valente R, Löhr JM & Vujasinovic M (2020) Cardiovascular and lung involvement in patients with autoimmune pancreatitis. *J Clin Med* 9

Nishimori I, Tamakoshi A, Otsuki M & Research Committee on Intractable Diseases of the Pancreas M of HL and W of J (2007) Prevalence of autoimmune pancreatitis in Japan from a nationwide survey in 2002. *J Gastroenterol* 42 Suppl 18: 6–8

Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, Corbo V, Oni TE, Hearn SA, Lee EJ, *et al* (2017) Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med* 214: 579–596

Okazaki K, Chari ST, Frulloni L, Lerch MM, Kamisawa T, Kawa S, Kim MH, Lévy P, Masamune A, Webster G, *et al* (2017) International consensus for the treatment of autoimmune pancreatitis. *Pancreatology* 17: 1–6

Omar D, Chen Y, Cong Y & Dong L (2020) Glucocorticoids and steroid sparing medications monotherapies or in combination for IgG4-RD: a systematic review and network meta-analysis. *Rheumatology (Oxford)* 59: 718–726

Overbeek KA, Poulsen JL, Lanzillotta M, Vinge-Holmquist O, Macinga P, Demirci AF, Sindhunata DP, Backhus J, Algül H, Buijs J, *et al* (2024) Type 1 Autoimmune Pancreatitis in Europe: Clinical Profile and Response to Treatment. *Clinical Gastroenterology and Hepatology* 22: 994-1004.e10

Peng L, Zhang P, Li J, Liu Z, Lu H, Zhu L, Wang X, Teng F, Li X, Guo H, *et al* (2020) IgG4-related aortitis/periaortitis and periarteritis: a distinct spectrum of IgG4-related disease. *Arthritis Res Ther* 22: 103

Peng L, Zhang X, Zhou J, Li J, Liu Z, Lu H, Peng Y, Fei Y, Zhao Y, Zeng X, *et al* (2023) Comparison of clinical features and outcomes of proliferative, fibrotic, and

mixed subtypes of IgG4-related disease: A retrospective cohort study. *Chin Med J (Engl)*

Perugino C, Culver EL, Khosroshahi A, Zhang W, Della-Torre E, Okazaki K, Tanaka Y, Löhr M, Schleinitz N, Falloon J, *et al* (2023) Efficacy and Safety of Inebilizumab in IgG4-Related Disease: Protocol for a Randomized Controlled Trial. *Rheumatol Ther* 10: 1795–1808

Perugino CA, AlSalem SB, Mattoo H, Della-Torre E, Mahajan V, Ganesh G, Allard-Chamard H, Wallace Z, Montesi SB, Kreuzer J, *et al* (2019) Identification of galectin-3 as an autoantigen in patients with IgG 4 -related disease. *Journal of Allergy and Clinical Immunology* 143: 736-745.e6

Perugino CA, Kaneko N, Maehara T, Mattoo H, Kers J, Allard-Chamard H, Mahajan VS, Liu H, Della-Torre E, Murphy SJH, *et al* (2021) CD4+ and CD8+ cytotoxic T lymphocytes may induce mesenchymal cell apoptosis in IgG4-related disease. *Journal of Allergy and Clinical Immunology* 147: 368–382

Perugino CA & Stone JH (2020) IgG4-related disease: an update on pathophysiology and implications for clinical care. *Nat Rev Rheumatol* 16: 702–714

de Pretis N, Carlin M, Calderini E, Caldart F, Conti Bellocchi MC, Amodio A, De Marchi G, Campagnola P, Crinò SF, Bernardoni L, *et al* (2024) Clinical features and long-term outcomes of patients with type 2 autoimmune pancreatitis. *United European Gastroenterol J* 12: 319–325

Rispens T, Ooijevaar-De Heer P, Bende O & Aalberse RC (2011) Mechanism of immunoglobulin G4 Fab-arm exchange. *J Am Chem Soc* 133: 10302–10311

Rockey DC, Bell PD & Hill JA (2015) Fibrosis--a common pathway to organ injury and failure. *N Engl J Med* 372: 1138–49

Rovati L, Kaneko N, Pedica F, Monno A, Maehara T, Perugino C, Lanzillotta M, Pecetta S, Stone JH, Doglioni C, *et al* (2021) Mer tyrosine kinase as a possible link between resolution of inflammation and tissue fibrosis in IgG4-related disease. *Rheumatology (United Kingdom)* 60: 4929–4941

Sanders S, Fu X, Zhang Y, Perugino CA, Wallwork R, Della-Torre E, Harvey L, Harkness T, Long A, Choi HK, *et al* (2020) Lifetime Allergy Symptoms in IgG4-Related Disease: A Case-Control Study. *Arthritis Care Res (Hoboken)*

Sarles H, Sarles J-C, ~iuratore R & Guien C Chronic Inflammatory Sclerosis of the Pancreas-An Autonomous Pancreatic Disease?

Schmidkonz C, Rauber S, Atzinger A, Agarwal R, Götz TI, Soare A, Cordes M, Prante O, Bergmann C, Kleyer A, *et al* (2020) Disentangling inflammatory from fibrotic disease activity by fibroblast activation protein imaging. *Ann Rheum Dis* 79: 1485–1491

Shimosegawa T, Chari ST, Frulloni L, Kamisawa T, Kawa S, Mino-Kenudson M, Kim M-H, Klöppel G, Lerch MM, Löhr M, *et al* (2011) International Consensus Diagnostic Criteria for Autoimmune Pancreatitis Guidelines of the International Association of Pancreatology

Shiokawa M, Kodama Y, Kuriyama K, Yoshimura K, Tomono T, Morita T, Kakiuchi N, Matsumori T, Mima A, Nishikawa Y, *et al* (2016) Pathogenicity of IgG in patients with IgG4-related disease. *Gut* 65: 1322–1332

Shiokawa M, Kodama Y, Sekiguchi K, Kuwada T, Tomono T, Kuriyama K, Yamazaki H, Morita T, Marui S, Sogabe Y, *et al* (2018) Laminin 511 is a target antigen in autoimmune pancreatitis. *Sci Transl Med* 10

Stone JH, Khosroshahi A, Zhang W, Della Torre E, Okazaki K, Tanaka Y, Löhr JM, Schleinitz N, Dong L, Umehara H, *et al* (2024) Inebilizumab for Treatment of IgG4-Related Disease. *N Engl J Med*

Stone JH, Zen Y & Deshpande V (2012) IgG4-related disease. *N Engl J Med* 366: 539–551

Tacelli M, Celsa C, Magro B, Barresi L, Guastella S, Capurso G, Frulloni L, Cabibbo G & Cammà C (2019) Risk Factors for Rate of Relapse and Effects of Steroid Maintenance Therapy in Patients With Autoimmune Pancreatitis: Systematic Review and Meta-analysis. *Clinical Gastroenterology and Hepatology* 17: 1061-1072.e8

Tacelli M, Zaccari P, Petrone MC, Della Torre E, Lanzillotta M, Falconi M, Doglioni C, Capurso G & Arcidiacono PG (2022) Differential EUS findings in focal type 1 autoimmune pancreatitis and pancreatic cancer: A proof-of-concept study. *Endosc Ultrasound* 0: 0

Terao C, Ota M, Iwasaki T, Shiokawa M, Kawaguchi S, Kuriyama K, Kawaguchi T, Kodama Y, Yamaguchi I, Uchida K, *et al* (2019) IgG4-related disease in the Japanese population: a genome-wide association study. *Lancet Rheumatol* 1: e14–e22

Della Torre E, Mattoo H, Mahajan VS, Carruthers M, Pillai S & Stone JH (2014) Prevalence of atopy, eosinophilia, and IgE elevation in IgG4-related disease. *Allergy: European Journal of Allergy and Clinical Immunology* 69: 269–272

Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, Matsui S, Yoshino T, Nakamura S, Kawa S, *et al* (2012) Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 22: 21–30

Umehara H, Okazaki K, Nakamura T, Satoh-Nakamura T, Nakajima A, Kawano M, Mimori T & Chiba T (2017) Current approach to the diagnosis of IgG4-related disease–Combination of comprehensive diagnostic and organ-specific criteria. *Mod Rheumatol* 27: 381–391

Vaglio A, Salvarani C & Buzio C (2006) Retroperitoneal fibrosis. *Lancet* 367: 241–51

Vujasinovic M, Mucelli RMP, Valente R, Verbeke CS, Haas SL & Löhr JM (2019) Kidney involvement in patients with type 1 autoimmune pancreatitis. *J Clin Med* 8

Vujasinovic M, Valente R, Maier P, von Beckerath V, Haas SL, Arnelo U, Del Chiaro M, Kartalis N, Pozzi-Mucelli RM, Fernandez-Moro C, *et al* (2018) Diagnosis, treatment and long-term outcome of autoimmune pancreatitis in Sweden. *Pancreatology* 18: 900–904

Wallace ZS, Mattoo H, Carruthers M, Mahajan VS, Della Torre E, Lee H, Kulikova M, Deshpande V, Pillai S & Stone JH (2015) Plasmablasts as a biomarker for IgG4-related disease, independent of serum IgG4 concentrations. *Ann Rheum Dis* 74: 190–195

Wallace ZS, Miles G, Smolkina E, Petruski-Ivleva N, Madziva D, Cook C, Fu X, Zhang Y, Stone JH & Choi HK (2023) Incidence, prevalence and mortality of IgG4-related disease in the USA: A claims-based analysis of commercially insured adults. *Ann Rheum Dis* 82: 957–962

Wallace ZS, Naden RP, Chari S, Choi H, Della-Torre E, Dicaire J-F, Hart PA, Inoue D, Kawano M, Khosroshahi A, *et al* (2020a) The 2019 American College of Rheumatology/European League Against Rheumatism Classification Criteria for IgG4-Related Disease. *Arthritis Rheumatol* 72: 7–19

Wallace ZS, Naden RP, Chari S, Choi HK, Della-Torre E, Dicaire J-F, Hart PA, Inoue D, Kawano M, Khosroshahi A, *et al* (2020b) The 2019 American College of Rheumatology/European League Against Rheumatism classification criteria for IgG4-related disease. *Ann Rheum Dis* 79: 77–87

Wallace ZS, Zhang Y, Perugino CA, Naden R, Choi HK, Stone JH & ACR/EULAR IgG4-RD Classification Criteria Committee (2019) Clinical phenotypes of IgG4-related disease: an analysis of two international cross-sectional cohorts. *Ann Rheum Dis* 78: 406–412

Wallwork R, Perugino CA, Fu X, Harkness T, Zhang Y, Choi HK, Stone JH & Wallace ZS (2021) The association of smoking with immunoglobulin G4-related disease: A case-control study. *Rheumatology (Bulgaria)* 60: 5310–5317

Wang L, Zhang P, Wang M, Feng R, Lai Y, Peng L, Fei Y, Zhang X, Zhao Y, Zeng X, *et al* (2018) Failure of remission induction by glucocorticoids alone or in combination with immunosuppressive agents in IgG4-related disease: A prospective study of 215 patients. *Arthritis Res Ther* 20

Wollin L, Wex E, Pautsch A, Schnapp G, Hostettler KE, Stowasser S & Kolb M (2015) Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. *Eur Respir J* 45: 1434–45

Yue Z, Tong F, Chen Y, Shen Y, Li J, Zeng C, Diao J, Chen X & Wei R (2022) Risk factors for IgG4-related disease relapse: A systematic review and meta-analysis. *Scand J Immunol* 96 doi:10.1111/sji.13200 [PREPRINT]

Yunyun F, Yu C, Panpan Z, Hua C, Di W, Lidan Z, Linyi P, Li W, Qingjun W, Xuan Z, *et al* (2017) Efficacy of Cyclophosphamide treatment for immunoglobulin G4-related disease with addition of glucocorticoids. *Sci Rep* 7

Yunyun F, Yu P, Panpan Z, Xia Z, Linyi P, Jiaxin Z, Li Z, Shangzhu Z, Jinjing L, Di W, *et al* (2019) Efficacy and safety of low dose Mycophenolate mofetil treatment for immunoglobulin G4-related disease: a randomized clinical trial. *Rheumatology (Oxford)* 58: 52–60

Zamboni G, Lüttges J, Capelli P, Frulloni L, Cavallini G, Pederzoli P, Leins A, Longnecker D & Klöppel G (2004) Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: A study on 53 resection specimens and 9 biopsy specimens. *Virchows Archiv* 445: 552–563

Zen Y & Deshpande V (2019) Tumefactive Inflammatory Diseases of the Pancreas. *Am J Pathol* 189: 82–93

Zen Y, Inoue D, Kitao A, Onodera M, Abo H, Miyayama S, Gabata T, Matsui O & Nakanuma Y (2009a) IgG4-related Lung and Pleural Disease: A Clinicopathologic Study of 21 Cases

Zen Y & Nakanuma Y (2010) IgG4-related disease: a cross-sectional study of 114 cases. *Am J Surg Pathol* 34: 1812–9

Zen Y, Onodera M, Inoue D, Kitao A, Matsui O, Nohara T, Namiki M, Kasashima S, Kawashima A, Matsumoto Y, *et al* (2009b) Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4. *Am J Surg Pathol* 33: 1833–9

Zhang P, Han X, Li J, Liu Z, Lu H, Luo X, Liu C, Peng L, Fei Y, Zeng X, *et al* (2020) IgG4-related fibrosing mediastinitis: clinical presentation, treatment efficacy and comparison with IgG4-RD without fibrosing mediastinitis. *Clin Exp Rheumatol* 38: 1206–1214

Zhang W & Stone JH (2019) Management of IgG4-related disease. *Lancet Rheumatol* 1: e55–e65

Yunyun F, Yu C, Panpan Z, Hua C, Di W, Lidan Z, Linyi P, Li W, Qingjun W, Xuan Z, *et al* (2017) Efficacy of Cyclophosphamide treatment for immunoglobulin G4-related disease with addition of glucocorticoids. *Sci Rep* 7

Yunyun F, Yu P, Panpan Z, Xia Z, Linyi P, Jiaxin Z, Li Z, Shangzhu Z, Jinjing L, Di W, *et al* (2019) Efficacy and safety of low dose Mycophenolate mofetil treatment for immunoglobulin G4-related disease: a randomized clinical trial. *Rheumatology (Oxford)* 58: 52–60

Zamboni G, Lüttges J, Capelli P, Frulloni L, Cavallini G, Pederzoli P, Leins A, Longnecker D & Klöppel G (2004) Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: A study on 53 resection specimens and 9 biopsy specimens. *Virchows Archiv* 445: 552–563

Zen Y & Deshpande V (2019) Tumefactive Inflammatory Diseases of the Pancreas. *Am J Pathol* 189: 82–93

Zen Y, Inoue D, Kitao A, Onodera M, Abo H, Miyayama S, Gabata T, Matsui O & Nakanuma Y (2009a) IgG4-related Lung and Pleural Disease: A Clinicopathologic Study of 21 Cases

Zen Y & Nakanuma Y (2010) IgG4-related disease: a cross-sectional study of 114 cases. *Am J Surg Pathol* 34: 1812–9

Zen Y, Onodera M, Inoue D, Kitao A, Matsui O, Nohara T, Namiki M, Kasashima S, Kawashima A, Matsumoto Y, *et al* (2009b) Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4. *Am J Surg Pathol* 33: 1833–9

Zhang P, Han X, Li J, Liu Z, Lu H, Luo X, Liu C, Peng L, Fei Y, Zeng X, *et al* (2020) IgG4-related fibrosing mediastinitis: clinical presentation, treatment efficacy and comparison with IgG4-RD without fibrosing mediastinitis. *Clin Exp Rheumatol* 38: 1206–1214

Zhang W & Stone JH (2019) Management of IgG4-related disease. *Lancet Rheumatol* 1: e55–e65

