Beyond diagnosis: exploring the significance of IgG4+ plasma cell count through immunostaining in IgG4-related disease

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ABSTRACT

Objective. *To evaluate whether the grade of IgG4+ plasma cell infiltration in biopsies is associated with clinical or serologic outcomes in IgG4-RD.*

Methods. *We included 57 patients with biopsy proven IgG4-RD according to the Comprehensive Diagnostic Criteria and/or the 2019 ACR/EULAR Classification Criteria. We collected histological, clinical (disease duration, phenotype, remission and relapses) and serological variables.*

Results. *29 (50.9%) patients were men, mean age 49.9 years, with a median disease duration of 22 months. The distribution among clinical phenotypes were 14% pancreato-hepato-biliary, 12.3% retroperitoneal/aortic, 29.8% head and neck-limited, 29.8% Mikulicz/systemic and 14% undefined. Thirty-nine patients had a proliferative and 18 a fibrotic phenotype. Most biopsies were from lacrimal gland, lymph node, pancreas, orbit, kidney, retroperitoneum and thyroid gland. Thirty-nine (68.4%) patients had <100 IgG4+ plasma cells/HPF and 18 (31.6%) ≥100 IgG4+ plasma cells/HPF. Patients with ≥100 IgG4+ plasma cells/HPF were more likely to belong to the pancreato-hepato-biliary and the proliferative phenotypes, had fewer relapses and a higher remission rate. On multivariate analysis, the OR for remission at last follow-up was 6.7, 95% CI 1.1–4.42, p=0.03. The log-rank test showed a difference in relapse-free survival between the two groups (HR 2.6, 95% CI 1.2–5.6, p=0.01). According to the ROC analysis, patients with more than 61 IgG4+ plasma cells were less likely to relapse.*

Conclusion. *A count of ≥100 IgG4+ plasma cells/HPF may identify patients with a proliferative phenotype, fewer relapses and a higher remission rate.*

Introduction

Immunoglobulin G4-related disease (IgG4-RD) is a fibroinflammatory condition that presents with single or multi-organ involvement, and which histopathological findings includes the presence of dense lymphoplasmacytic infiltrate, obliterative phlebitis, storiform fibrosis and marked IgG4+ plasma cell infiltration observed by immunostaining (1-3). Indeed, the current classification criteria assigned the maximum score for the histological item when an IgG4+/IgG+ ratio ≥71% and number of IgG4+ cells/HPF (high-power field) ≥51 are present (4). However, this histological cut-off has different sensitivities and specificities among the affected organs. In addition, the IgG4+ plasma cell number and IgG4+/IgG+ cell ratio may be affected according to the presence or not of fibrosis (5).

Up to date, IgG4+ plasma cell number has been used in clinical practice only as a diagnostic tool. Whether the number of IgG4+ plasma cells in tissue is associated with any clinical or serological feature, or with the presence of relapse and remission has not been established. Thus, our objective was to evaluate if the grade of IgG4+ plasma cell infiltration in biopsies from patients with IgG4-RD is associated with any clinical or serological characteristics, or clinical outcomes.

Methods

Patients

This was a cross-sectional study conducted in a tertiary referral centre (2009-2023). All the patients fulfilled the 2020 Comprehensive Diagnostic Criteria CDC and/or the 2019 American College of Rheumatology/European League Against Rheumatism Classification Criteria for IgG4-RD (AECC) (2, 4), and have an available biopsy in which immunostaining for IgG4 and IgG were performed.

We retrospectively collected demographics, organ involvement, length of follow-up, remission (IgG4-RD Responder Index of zero at last follow-up) and time to first relapse and number of relapses. Patients were classified according to four clinical phenotypes, namely pancreato-hepato-biliary, retroperitoneal/aortic, head and neck-limited, and Mikulicz/systemic phenotypes (4). A fifth phenotype termed "undefined" was created for patients who could not be fitted in none of the aforementioned phenotypes (6-7). Patients were also classified into proliferative and fibrotic phenotypes (8). At the time of biopsy be registered the number and type of involved organs, the IgG4-RD

Table I. Involved organ distribution and serological features.

Meninges, n (%)	3 (5.3)
Hypophysis, n (%)	2 (3.5)
Lacrimal gland, n (%)	25 (43.9)
Orbit, n (%)	17 (29.9)
Parotid gland, n (%)	19 (33.3)
Submandibular gland, n (%)	26 (45.6)
Sublingual gland, n (%)	10 (17.5)
Paranasal sinus, n (%)	17 (29.9)
Thyroid, n (%)	3 (5.3)
Lymph nodes, n (%)	28 (49.1)
Mediastinum, n (%)	3 (5.3)
Pericardium, n (%)	3 (5.3)
Trachea, m (%)	1 (1.8)
Lung, n (%)	16 (28.1)
Pancreas, n (%)	17 (29.9)
Biliary tract, n (%)	9 (15.8)
Gallbladder, n (%)	1 (1.8)
Mesentery, n (%)	2 (3.5)
Aorta, n (%)	5 (8.8)
Retroperitoneum, (%)	7 (12.3)
Kidney, n (%)	18 (31.6)
Prostate, n (%)	5 (8.8)
High IgG, n (%)	25/53 (47.2)
High IgG1, n (%)	18/49 (36.8)
High IgG4, n (%)	32/56 (57.1)
Hypocomplementaemia, n (%)	14/49 (28.6)
Eosinophilia, n (%)	13 (22.8)

Responder Index (IgG4-RD RI) (9), eosinophil count, and IgG1, IgG4, C3 and C4 serum levels.

Immunostaining

Four pathologists with expertise in IgG4-RD reviewed the tissues. The following variables were collected by light microscopy: dense lymphoplasmacytic infiltrate, storiform fibrosis, and obliterative phlebitis. Direct immunostaining with primary antibodies against IgG (Dako: 1000) and IgG4 (Zymed 1:200) was assessed; IgG4+ plasma cells were counted in three x40 fields with the highest number of IgG4+ plasma cells and the average number of IgG4+ plasma cells within these fields was calculated (3). The same three fields were used for counting IgG+ plasma cells for the purpose of calculating the IgG4/IgG ratio.

Statistical analysis

We used descriptive statistics. Categorical variables were analysed with Chi square test or Fisher exact test as appropriate and numerical variables with Student's t-test or Mann-Whitney U-test. We used logistic regression analysis reporting OR and 95% CI, Kaplan-Meier survival curves and log-rank tests. Correlations among variables were evaluated using Spearman's test. We plotted receiver operating characteristics (ROC) curves to discern if IgG4+ plasma cell count was able to distinguish patients who relapsed. We chose the cut-off value with the highest Youden's J statistic. All analyses were performed using the SPSS 20.0 and GraphPad Prism 10.1.1. We obtained approval from our Institutional Review Board.

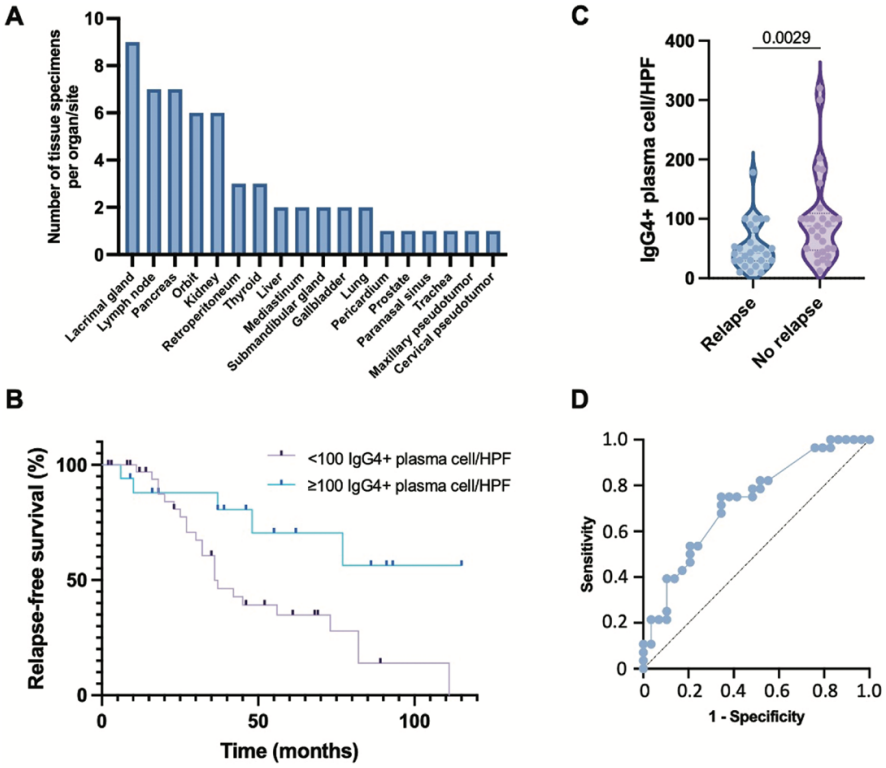

Fig. 1. A: Organs and sites of biopsies in 57 IgG4-related disease patients. **B**: The Kaplan-Meier survival analysis of relapse-free survival according to the IgG4+ plasma cell counts per high power field. **C**: The IgG4+ plasma cell counts in patients who relapsed vs. those that did not relapse. **D**: Receiver operating characteristic curve analysis to assess the predictive performance of the IgG4+ plasma cell counts for relapse.

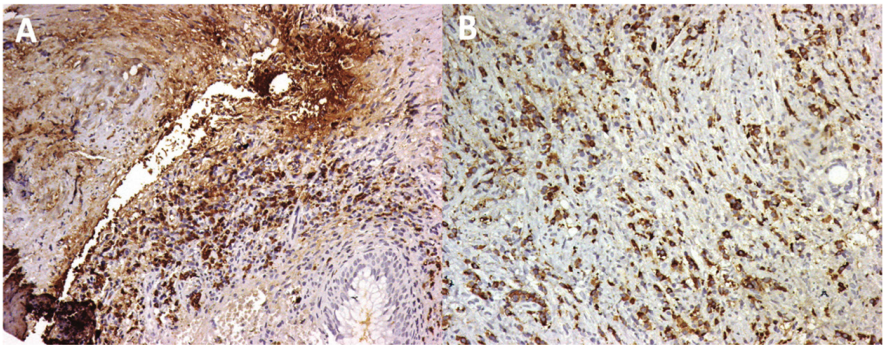

Fig. 2. A: Tracheal biopsy showing <100 IgG4+ plasma cell/HPF (40x). **B**: Orbital biopsy showing >100 IgG4+ plasma cell/HPF (40x).

Results

We included 57 patients with a mean of age 49.9±15.8 years, 29 (50.9%) were male, with a median disease duration of 22 (IQR 6-60) months. All the patients fulfilled the 2020 CDC whereas 39 (68.4%) fulfilled the AECC for IgG4-RD. Eight (14%) belonged to the pancreato-hepato-biliary, 7 (12.3%) to the retroperitoneal/aortic, 17 (29.8%) to the head and neck-limited, 17 (29.8%) to the Mikulicz/systemic and 8 (14%) to the undefined phenotypes.

Moreover, 39 (68.4%) were assigned to the proliferative and 18 (31.6%) to the fibrotic phenotypes. Table I depicts the organ distribution involvement of the patients.

We evaluated 57 tissue specimens, 41 corresponding to incisional biopsies and 16 to excisional biopsies or surgical specimens. Figure 1A shows the distribution of biopsies according to the organs and sites, being the most frequent the lacrimal gland, lymph node, and pancreas. All the biopsies had dense lymphoplasmacytic infiltrates, whereas fibrosis was found in 48 (84.2%), arranged in a storiform pattern only in 22 (38.6%), and obliterative phlebitis in 12 (21.1%).

The median IgG4+ plasma cells count was 50 (IQR 31–100) and the median IgG4+/IgG+ ratio was 67% (IQR 49–97). Thirty-nine (68.4%) patients had <100 IgG4+ plasma cells/HPF and 18 (31.6%) patients ≥100 IgG4+ plasma cells/HPF. Figure 2 shows a representative immunohistochemical staining of the groups.

Patients with ≥100 IgG4+ plasma cells/HPF were more frequently from the pancreato-hepato-biliary and the proliferative phenotypes (Table II). Moreover, patients with ≥100 IgG4+ plasma cells/HPF were more likely to be in remission at last follow-up and less likely to relapse. We did not find any difference regarding age, sex, number of involved organs, IgG4-RD RI, serology, or treatment. At the multivariate analysis, the variable that remained associated with ≥100 IgG4+ plasma cells/HPF was remission at last follow-up (OR 6.7, 95% CI 1.11–40.42, p=0.03).

At a median follow-up after biopsy of 61 months (IQR 35-88), 28 (49.1%) patients relapsed (median number of relapses of 1 [range: 1–9]). Relapse occurred in 23 (59%) patients in the group with <100 IgG4+ plasma cells/HPF and in 5 (27.8%) patients in the ≥100 IgG4+ plasma cells/HPF group (p=0.02). The Kaplan-Meier survival analysis of relapse-free survival is shown in Figure 1B. The log-rank test showed a significant difference in the relapse-free survival between both groups (p=0.01) with a HR of 2.6 (95% CI 1.2–5.6).

The median IgG4+ plasma cell count in patients who did not relapse was 88 (IQR 50–100) whereas in those who relapsed was 42 (IQR 29–71) (p=0.002) (Fig. 1C). In the ROC analysis, the best cut-off value was 61 IgG4+ plasma cells/HPF (AUC 0.73, sensitivity 75%, specificity 65.5%, positive predictive value 67%, negative predictive value 68%, positive likelihood ratio 2.2, negative likelihood ratio 0.38, p=0.003), suggesting that patients with more than 61 IgG4+ plasma cells/HPF are less likely to relapse (Fig. 1D).

Finally, the IgG4+ plasma cell count did not correlate with the number of involved organs, the IgG4-RD RI, or the levels of IgG1, IgG4, C3 and C4 (data not shown).

Table II. Demographic, clinical, serological features and outcomes of IgG4-related disease patients according to the number of IgG4+ plasma cells/HPF.

Variable	<100 IgG4+ plasma cell/HPF (n=39)	≥100 IgG4+ plasma cell/HPF (n=18)	p-value
Male, n (%)	18 (46.2)	11 (61.1)	0.29
Age, mean ± SD	50.3 ± 14.2	49.2 ± 19.3	0.80
Pancreato-hepato-biliary, n (%)	3 (7.7)	5 (27.8)	0.04
Retroperitoenal/aortic, n (%)	6 (15.4)	1 (5.6)	0.41
Head and neck-limited, n (%)	14 (35.9)	3 (16.7)	0.14
Mikulics/systemic, n (%)	9 (23.1)	8 (44.4)	0.10
Undefined, n (%)	8 (20.5)	1 (5.6)	0.15
Proliferative, n (%)	23 (59)	16 (88.9)	0.02
Fibrotic, n (%)	16 (41)	2 (11.1)	0.02
Mono-organic (ever), n (%)	5 (12.8)	3 (16.7)	0.69
Two involved organs (ever), n (%)	3 (7.7)	4 (22.2)	0.12
≥3 involved organs (ever), n (%)	31 (79.5)	11 (61.1)	0.14
Number of involved organs (at biopsy), median (IQR)	5 (3.5-6)	5 (4-9)	0.85
IgG4-RD RI (at biopsy), median (IQR)	10 (6-14)	12 (6-20)	0.99
High IgG1, n (%)	12/33 (36.4)	6/16 (37.5)	0.93
High IgG4 (at biopsy), n (%)	19/33 (57.6)	8/13 (61.5)	0.80
Low C3, n (%)	7/24 (29.2)	3/9 (33.3)	0.81
Low C4, n (%)	7/26 (26.9)	4/9 (44.4)	0.32
Complete remission (ever), n (%)	24 (61.5)	15 (83.3)	0.10
Partial remission (ever), n (%)	14 (35.9)	2 (11.1)	0.05
Remission at last follow-up, n (%)	21 (53.8)	16 (88.9)	0.01
Relapse (ever), n (%)	23 (59)	5 (27.8)	0.02
Number of relapses, median (IQR)	1 (1-2.5)	1 (1-3)	0.90
Damage at last follow-up, n (%)	24 (61.5)	14 (77.8)	0.22
2019 AECC fulfillment, n (%)	28 (71.8)	11 (61.1)	0.42
Prednisone, n (%)	35 (89.7)	14 (77.8)	0.22
Immunosuppressors, n (%)	30 (76.9)	12 (66.7)	0.41
Azathioprine, n (%)	20 (51.3)	7 (38.9)	0.38
Mycophenolate mofetil, n (%)	4 (22.2)	12 (30.8)	0.50
Rituximab, n (%)	6 (15.4)	3 (16.7)	0.90
Methotrexate, n (%)	2 (5.1)	0	0.32
Leflunomide, n (%)	1 (2.6)	1 (5.6)	0.56
Cyclophosphamide, n (%)	1 (2.6)	0	0.49
Tacrolimus, n (%)	0	1 (5.6)	0.13
Tamoxifen, n (%)	1 (2.6)	0	0.49

Discussion

Tissue infiltration by IgG4 plasma cells is a distinct feature of IgG4-RD. However, the interpretation of IgG4+ cells count is complex as infiltration can also be observed in other inflammatory conditions, and because the count might vary across organs (3). For instance, the rate of fulfillment of the International Consensus Statement cut-off value is higher in ophthalmic, pancreatic, and renal lesions, and lower in submandibular and skin lesions (5).

Herein, we tested the hypothesis that the histologic number of IgG4+ plasma cells can be associated with clinical and serological outcomes. First, we observed that patients with ≥100 IgG4+ plasma cell/HPF corresponded to a proliferative rather than a fibrotic phenotype. The proliferative subtype involves lymph nodes, lacrimal glands, major salivary glands, pancreas, bile ducts, kidneys, lungs, pituitary, and paranasal sinuses, while the fibrotic subtype

involves the retroperitoneum, aorta and periaortic tissue, mesentery, mediastinum, pachymeninges, and thyroid (8). Interestingly, it has been described that tissues containing both fibrotic and non-fibrotic areas contain fewer IgG4+ cells in the fibrotic areas (10).

On the other hand, in a previous study that included patients with IgG4-related ophthalmic disease, the number of IgG4+ plasmacytes correlated positively with serum IgG4+ concentration, and the AUC for identifying extra-ophthalmic involvement was 0.75. In our study, we did not find a correlation with either IgG4, IgG1 or complement serum levels. However, the ≥100 IgG4+ plasma cell/HPF group had more pancreato-hepato-biliary involvement.

In addition, in the same study on IgG4-related ophthalmic disease, a pathological IgG4+plasmacyte count of >150 HPF was a risk factor for relapse (11). In contrast, in our cohort, now including different disease phenotypes, we observed that patients in the ≥100 IgG4+ plasma cell/HPF group had less relapses. This finding may also contradict a recent study that described a higher relapse rate in proliferative *versus* fibrotic patients and mixed patients (24%, 4.3% and 25.9%, respectively) (12). However, in that study, the histologic number of IgG4+ plasma cells was not evaluated in the proliferative and fibrotic phenotypes, and did not consider lymph nodes as proliferative organs. Finally, we also found a higher rate of remission in the group with ≥100 IgG4+ plasma cell/HPF regardless of treatment and number of affected organs.

Regarding the association between other immunohistochemical features and disease activity, only a pilot study reported that endothelial staining intensity of a Dna J homologue subfamily B member 9 (a co-chaperon protein that protects against cell death) negatively correlated with serum IgG4 concentrations and the number of treatments required to achieve remission. The authors claimed that this protein might be implicated int the pathogenesis of the fibrotic features of the disease (13).

Our study has some limitations. First, our sample size hampered the analysis of the results by site of biopsy or by each clinical phenotype. However, we were able to identify differences between the proliferative and fibrotic phenotypes. Second, we included some incisional biopsies that might have underestimated the IgG4+plasma cells. Third, the counting of IgG4+ plasma cell hotspots was performed manually. Lastly, the quantification of IgG4+ plasma cells relied on immunohistochemistry technique introducing the possibility of background staining that may overestimate the total cell count.

Summing up, we observed that a count of ≥100 IgG4+ plasma cell/HPF identified patients with a proliferative phenotype, fewer relapses and a higher rate of remission. However, further studies with larger samples are needed to replicate these results.

References

1. KAMISAWA T, ZEN Y, PILLAI S, STONE JH: IgG4-related disease. *Lancet* 2015; 385(9976):1460-71. https://doi.org/10.1016/S0140-6736(14)60720-0
2. UMEHARA H, OKAZIKI K, KAWA S *et al*.: The 2020 revised comprehensive diagnostic (RCD) criteria for IgG4-RD. *Mod Rheumatol* 2021; 31(3):529-533. https://doi.org/10.1080/14397595.2020.1859710
3. DESHPANDE V, ZEN Y, CHAN JK *et al*.: Consensus statement on the pathology of IgG4-related disease. *Mod Pathol* 2012; 25(9): 1181-92. https://doi.org/10.1038/modpathol.2012.72
4. WALLACE ZS, ZHANG Y, PERUGINO CA, NADEN R, CHOI HK, STONE JH; ACR/EULAR IgG4-RD Classification Criteria Committee: Clinical phenotypes of IgG4-related disease: an analysis of two international cross-sectional cohorts. *Ann Rheum Dis* 2019; 78(3): 406-12. https://doi.org/10.1136/annrheumdis-2018-214603
5. MIZUSHIMA I, YAMADA K, HARADA K *et al*.: Diagnostic sensitivity of cutoff values of IgG4-poistive plasma cell number and IgG4-positive/CD138-positive cell ratio in typica multiple lesions of patients with IgG4-related disease. *Mod Rheumatol* 2018; 28(2): 293-99. https://doi.org/10.1080/14397595.2017.1332540
6. FERNÁNDEZ-CODINA A, PINILLA B, PINAL-FERNÁNDEZ I *et al*.: Performance of the 2019 ACR/EULAR classification criteria for IgG4-related disease and clinical phenotypes in a Spanish multicentre registry (REERIGG4). *Rheumatology* (Oxford) 2021; 60(1): 217-23. https://doi.org/10.1093/rheumatology/keaa247
7. MARTÍN-NARES E, BAENAS DF, CUELLAR GUTIÉRREZ MC *et al*.: Clinical and serological features in Latin American IgG4-related disease patients differ according to sex, ethnicity, and clinical phenotype. *J Clin Rheumatol* 2022; 28(6): 285-92. https://doi.org/10.1097/rhu.0000000000001858
8. ZHANG W, STONE JH: Management of IgG4-related disease. *Lancet Rheumatol* 2019; 1(1): e55-e65. https://doi.org/10.1016/S2665-9913(19)30017-7
9. WALLACE ZS, KHOSROSHAHI A, CARRUTHERS MD *et al*.: An international multispecialty validation study of the IgG4-Related Disease Responder Index. *Arthritis Care Res* (Hoboken) 2018; 70(11): 1671-78. https://doi.org/10.1002/acr.23543
10. MASAKI Y, KUROSE N, YAMAMOTO M *et al*.: Cutoff values of serum IgG4 and histopathological IgG4+ plasma cells for diagnosis of patients with IgG4-related disease. *Int J Rheumatol* 2012: 2012: 580814. https://doi.org/10.1155/2012/580814
11. YUAN Y, MENG F, REN H, YUE H, XUE K, ZHANG R: Pathological count of IgG4-positive plasmacytes suggests extraophtlamic involvement and relapse in patients with IgG4-related ophthalmic disease: a retrospective study. *Arthritis Res Ther* 2022; 24(1): 80. https://doi.org/10.1186/s13075-022-02757-2
12. PENG L, ZHANG X, ZHOU J *et al*.: Comparison of clinical features and outcomes of proliferative, fibrotic, and mixed subtypes of IgG4-rewlated disease: a retrospective cohort study. *Chin Med J* (Engl) 2024; 137(3): 303-11. https://doi.org/10.1097/cm9.0000000000002755
13. STUCHFIELD-DENBY E, MATTUTZU V, GROBOS V, ANDRÉ M, PEREIRA B, RUIVARD M: Immunohistochemical expression of DnaJ homolog subfamily B member 9 in immunoglobulin G4-related disease: a pilot study. *Clin Exp Rheumatol* 2024; 42 (3): 718-25.