Insights from comparison of serum IgG4 between T2-eosinophilic asthma and eosinophilic granulomatosis with polyangiitis/idiopathic hypereosinophilic syndrome

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Abstract

Objective

Eosinophilic granulomatosis with polyangiitis (EGPA) and idiopathic hypereosinophilic syndrome (iHES) are systemic hypereosinophilic diseases largely overlapping. Type 2 (T2)-eosinophilic asthma is documented in 100% and 44-95% of EGPA and iHES patients, respectively, probably representing the beginning of the systemic eosinophilic disorders, as suggested by mepolizumab, effective in both asthma and EGPA/iHES. In this respect, there are no predictive biomarkers of progression from T2-eosinophilic asthma into EGPA/iHES. Immunoglobulins G type 4 (IgG4) take part in T2-eosinophilic inflammation, and elevated serum IgG4 have been previously documented in asthma, EGPA and HES. The objective of this study was to compare serum IgG4 between T2-eosinophilic asthma and EGPA/iHES in order to identify significant differences that could represent a reasonable background for prospective studies on IgG4 as potential predictive biomarker of progression from asthma to EGPA/iHES.

Methods

In this retrospective/cross-sectional case-control study, patients affected by T2-eosinophilic asthma or EGPA/iHES were consecutively enrolled. All patients underwent blood tests for serum IgG4. Asthmatics and EGPA/iHES patients were stratified upon serum IgG4 values (normal or elevated).

Results

62 patients were enrolled (27 asthmatics, 35 EGPA/iHES). The frequency of patients with elevated serum IgG4 was higher in the EGPA/iHES group than in the asthmatics (45.7% vs. 11.1%, p=0.003), as well as the mean serum IgG4 value (p=0.010).

Conclusion

Elevated serum IgG4 seemed to discriminate between T2-eosinophilic asthma "alone" and T2-eosinophilic asthma evolved into EGPA/iHES. This finding could represent a reasonable background for prospective long-term studies on asthmatic naive patients aimed at investigating IgG4 as a potential predictive biomarker of progression from asthma to EGPA/iHES.

Key words

asthma, eosinophilic granulomatosis with polyangiitis, hypereosinophilic syndrome, IgG4, vasculitis

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Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA) and idiopathic hypereosinophilic syndrome (iHES) are systemic diseases characterised by hypereosinophilia-related organ damage. These two conditions largely overlap, being defined by some authors as "two sides of the same coin" (1). According to the latest classifications, EGPA is actually considered an immune-mediated reactive HES (2-4). In the last years, Mepolizumab, an anti-interleukin-5 (IL-5) monoclonal antibody originally approved for severe type 2 (T2)-eosinophilic asthma, showed efficacy in iHES (5-8) and EGPA (9, 10). The efficacy of Mepolizumab in both T2-eosinophilic asthma and iHES/EGPA suggests a common IL-5-driven pathogenesis, raising the question whether IL-5 overexpression in iHES/EGPA may be the result of T2-eosinophilic inflammation evolving from asthma to a systemic disease. This hypothesis is based on a strong rationale for EGPA, typically developing in patients with T2-eosinophilic asthma, which is documented in about 100% of EGPA cases (11-13) and included in American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria (14, 15). On the other hand, the hypothesis of a "T2-driven" HES originating from asthma would be conceivable mostly in those patients affected by Mepolizumab-responsive iHES with documented asthma/pulmonary involvement, the percentage of which ranges from 44 to 95% in clinical trials (5-8); interestingly, Kuang *et al*. found that iHES patients with pulmonary involvement are significantly more prone to respond to Mepolizumab (7).

In this respect, there are no predictive biomarkers of progression from T2-eosinophilic asthma to EGPA/iHES. Anti-neutrophil cytoplasm antibodies (ANCA) are specific for EGPA, but they are detected only in 30–40% of patients (11-13). Moreover, positive ANCA are mainly associated to the vasculitic phase of EGPA (11-13), suggesting the development of an overt autoimmune pathogenic mechanism in addition to the T2-eosinophilic inflammation, constantly featuring in the first two phases, allergic/asthmatic and systemic eosinophilic, of EGPA (16-21).

Immunoglobulins G type 4 (IgG4) take part in the complex network of T2-eosinophilic inflammation, being produced by B cells in response to IL-4 and IL-13, as well as IgE (22). Elevated serum IgG4 have been previously documented in T2-eosinophilic asthma (23-25), HES (26, 27) and EGPA (28-31), seeming associated with severity/activity of such diseases (24-25, 29).

The objective of this study was to compare serum IgG4 between T2-eosinophilic asthma and EGPA/iHES, in order to identify significant differences that could represent a reasonable background for prospective studies on IgG4 as potential predictive biomarker of progression from T2-eosinophilic asthma to EGPA/iHES.

Materials and methods

In this retrospective/cross-sectional case-control study, patients affected by T2-eosinophilic asthma or EGPA/iHES were consecutively enrolled. All EGPA/iHES patients had T2-eosinophilic asthma in the context of their systemic disease. Diagnosis of T2-eosinophilic asthma was based on clinical symptoms, lung function tests, fractional exhaled nitric oxide (FeNO) and peripheral blood eosinophilia (PBE) (32). Diagnosis of iHES was based on PBE, evidence of multiorgan damage attributable to hypereosinophilia and exclusion of primary and secondary causes of hypereosinophilia (2-4). For EGPA, patients matching ACR/EULAR 2022 classification criteria (15) and/or MIRRA trial criteria (9) were included. The following baseline data were collected:

- *for all patients*: age; sex; asthma duration; presence of chronic rhinosinusitis (CRS) and nasal polpys (CRSwNP); atopy; history of previous allergen immunotherapy (AIT); history of NSAIDs-exacerbated respiratory disease (NERD); maximum levels of PBE, serum total IgE and fractional exhaled nitric oxide (FeNO) reported in patients' history before the study and/or before therapy with anti-T2 monoclonal antibodies (mAbs), if used; ongoing and previous therapy with oral

corticosteroids (OCS), anti-T2 mAbs and/or immunosuppressants (for any reason);

- *for EGPA/iHES patients*: disease duration; time between asthma onset and development of EGPA/iHES; type of organ involvement; ANCA status at EGPA diagnosis; results of organ biopsies, if performed (evidence of eosinophils and/or vasculitis); Five Factor Score (FFS) at EGPA diagnosis; Birmingham Vasculitis Activity Score (BVAS) at EGPA diagnosis and at the time of the study; disease status at the time of the study (activity or remission) and type of active clinical manifestations.

In 2023, serum IgG, IgA, IgM, IgE, IgG1, IgG2, IgG3, IgG4, ANCA and serum immunofixation were tested in all patients. ANCA were detected by chemilunescence immunoassay (CLIA), evaluating serum reactivity to myeloperoxidase (MPO, p-ANCA) and proteinase 3 (PR3, c-ANCA). Since these tests are routinely performed in clinical practice, no approval from right ethic board (REB) was needed for the study. Informed consent was obtained for using patients' data.

Primary analysis

Asthmatics and EGPA/iHES patients were stratified upon the results of the former blood tests, particularly serum IgG4 value (normal or elevated). Patients with normal *versus* elevated IgG4 were compared for baseline characteristics, as well as for the result of other blood tests.

Serum IgG4 cut-off

Elevated serum IgG4 were defined according to the reference value of local laboratory, *i.e.* >201 mg/dl.

Secondary analysis

A further comparison was made between EGPA and iHES patients.

Statistical methods

Descriptive statistics were reported as mean ± standard deviation or frequency (%), as appropriate. Comparisons between continuous and categorical variables were investigated with t-test and chi-squared test, respectively. Correlations were made using the Spearman's correlation test. STATA v. 18 (StataCorp-College Station, Texas, USA) was used for statistics, at a 0.05 statistical significance.

Table I. Baseline characteristics, asthmatics *vs*. EGPA/iHES patients.

	Asthmatics	EGPA/iHES	Total	*p*-value
Number of patients (%)	27 (43.5%)	35 (56.5%)	62 (100.0%)	
Age, mean value (SD)	59.926 (17.663)	59.657 (11.358)	59.774 (14.314)	0.942
Sex				
Female (%)	16 (59.3%)	19 (54.3%)	35 (56.5%)	0.695
Male (%)	11 (40.7%)	16 (45.7%)	27 (43.5%)	
Extra-respiratory manifestations (%)	0 (0.0%)	34 (97.1%)	35 (56.5%)	**<0.001**
Asthma (%)	27 (100%)	35 (100%)		
Asthma duration (years), mean value (SD)	20.5 (14.5)	20.8 (14.6)	20.7 (14.4)	0.949
Chronic rhinosinusitis (%)	27 (100%)	35 (100%)		
Nasal polyposis (%)	19 (70.4%)	23 (65.7%)	42 (67.7%)	0.697
Atopy (%)	14 (51.9%)	18 (56.2%)	32 (54.2%)	0.735
Previous AIT (%)	0 (0.0%)	2 (11.1%)	2 (6.2%)	0.198
NERD (%)	4 (14.8%)	4 (11.4%)	8 (12.9%)	0.693
Maximum PBE (cells/mmc), mean value (SD)	1247.963 (558.869)	5447.059 (4,851.655)	3,588.443 (4,183.791)	**<0.001**
Maximum serum IgE (IU/ml), mean value (SD)	422.018 (379.246)	841.564 (897.517)	647.140 (729.721)	0.066
Maximum FeNO (ppb), mean value (SD)	62.769 (51.042)	67.870 (49.566)	65.368 (49.876)	0.714
Ongoing therapies				
OCS (%)	7 (25.9%)	14 (40.0%)	21 (33.9%)	0.246
OCS daily dose (prednisone-equivalent), mean value (SD)	4.529 (1.518)	4.374 (1.819)	4.426 (1.688)	0.849
Anti-T2 mAbs (%)	23 (85,2%)	22 (62,9%)	45 (72,6%)	0.051
Mepolizumab 100 mg/month (%)	5 (18.5%)	6 (17.1%)	11 (17.7%)	0.888
Mepolizumab 300 mg/month (%)	0 (0.0%)	4 (11.4%)	4 (6.5%)	0.069
Benralizumab 30 mg/2 months (%)	5 (18.5%)	6 (17.1%)	11 (17.7%)	0.888
Dupilumab 300 mg/2 weeks (%)	13 (48.1%)	6 (17.1%)	19 (30.6%)	**0.009**
Immunosuppressants (%)	1 (3.7%)	2 (5.7%)	3 (4.8%)	0.715
Previous therapies				
Corticosteroids (%)	27 (100%)	35 (100%)	62 (100.0%)	
Anti-T2 mAbs (%)	11 (40.7%)	14 (40.0%)	25 (40.3%)	0.952
Omalizumab (%)	3 (11.1%)	0 (0.0%)	3 (4.8%)	**0.043**
Mepolizumab 100 mg (%)	3 (11.1%)	4 (11.4%)	7 (11.3%)	0.969
Benralizumab (%)	3 (11.1%)	6 (17.1%)	9 (14.5%)	0.504
Dupilumab (%)	2 (7.4%)	4 (11.4%)	6 (9.7%)	0.595
Immunosuppressants (%)	1 (3.7%)	21 (60.0%)	22 (35.5%)	**<0.001**

AIT: allergen immunotherapy; ANCA: anti-neutrophil cytoplasmic autoantibodies; EGPA: eosinophilic granulomatosis with polyangiitis; FeNO: fractional exhaled nitric oxide; iHES: idiopathic hypereosinophilic syndrome; mAbs: monoclonal antibodies; NERD: non-steroidal anti-inflammatory drugs-exacerbated respiratory disease; OCS: oral corticosteroids; PBE: peripheral blood eosinophilia; ppb: parts per billion; T2: type 2.

Results

Baseline characteristics

62 patients (35 F, 27 M) were enrolled, of which 27 asthmatics and 35 affected by EGPA/iHES (18 EGPA, 17 iHES). The median age of the entire cohort was 59.774 years. Asthma and CRS were present in all patients, while extra-airway clinical manifestations occurred only in EGPA/iHES group ($p<0.001$). Median asthma duration was 20.7 years, with no significant differences between asthmatics and EGPA/iHES patients, while median EGPA/iHES duration was 10.1 years. Median latency between asthma onset and development of EGPA/iHES was 11.9 years. Maximum documented PBE was 1247.963 cells/mmc in asthmatics *versus* 5447.059 cells/mmc in EGPA/iHES patients ($p<0.001$), with significant higher values in EGPA than in iHES (6817.778 *vs*. 3905.000, $p=0.080$). Peripheral neuropathy was significantly more frequent in EGPA than in iHES ($p<0.001$), while purpura and renal involvement were observed exclusively in EGPA ($p<0.001$ and 0.039, respectively). Only EGPA patients showed evidence of vasculitis

on organ biopsies and ANCA-positivity (p<0.001), with 16/18 (88.9%) ANCA-positive patients at EGPA diagnosis (p-ANCA/anti-MPO). The median BVAS at EGPA diagnosis was 19.056; 5/18 (27.8%) patients had FFS >0. At the time of the study, 17/35 (48.6%) EGPA/iHES patients had active disease (9 EGPA, 8 iHES), with a median BVAS of 0.833 in EGPA patients. Disease activity was almost exclusively represented by persistent asthma and/or CRS. 21/62 patients (33.9%) were on OCS therapy (average daily prednisone-equivalent dose 4.4 mg), 45/62 patients (72.6%) received anti-T2 mAbs and 3/62 patients (4.8%) were on immunosuppressants, with no significant differences in ongoing treatment regimens between asthmatics and EGPA/iHES patients, except for Dupilumab, used more frequently in asthmatics (p=0.009). Regarding previous therapies, significant differences were observed for Omalizumab, used more frequently in asthmatics (p=0.043), and for immunosuppressants, used almost exclusively in EGPA/iHES patients (p<0.001) (Tables I-II).

Table II. Baseline characteristics, EGPA *vs*. iHES patients.

	EGPA	iHES	Total	*p*-value
Number of patients (%)	18 51.4%)	17 (48.6%)	35 (100.0%)	
Age, mean value (SD)	64.389 (7.785)	54.647 (12.565)	59.657 (11.358)	**0.009**
Sex				
Female (%)	10 (55.6%)	9 (52.9%)	19 (54.3%)	0.877
Male (%)	8 (44.4%)	8 (47.1%)	16 (45.7%)	
EGPA/iHES duration (years), mean value (SD)	8.7 (5.8)	11.0 (8.1)	10.1 (6.8)	0.406
Asthma (%)	18 (100.0%)	17 (100%)	35 (100.0%)	
Asthma duration (years), mean value (SD)	20.1 (16.3)	21.5 (13.0)	20.8 (14.6)	0.800
Latency from asthma to EGPA/iHES (years), mean value (SD)	11.6 (16.8)	11.5 (12.6)	11.9 (14.6)	0.995
Chronic rhinosinusitis (%)	18 (100.0%)	17 (100%)	35 (100.0%)	
Nasal polyposis (%)	14 (77.8%)	9 (52.9%)	23 (65.7%)	0.122
Atopy (%)	8 (53.3%)	10 (58.8%)	18 (56.2%)	0.755
Constitutional symptoms (%)	14 (77.8%)	5 (29.4%)	19 (54.3%)	0.004
Peripheral neuropathy (%)	12 (66.7%)	1 (5.9%)	13 (37.1%)	**<0.001**
Purpura (%)	9 (50.0%)	0 (0.0%)	9 (25.7%)	**<0.001**
Urticaria-angioedema (%)	4 (22.2%)	7 (41.2%)	11 (31.4%)	0.227
Myocarditis (%)	3 (16.7%)	5 (29.4%)	8 (22.9%)	0.369
Gastrointestinal involvement	(%) 1 (5.6%)	3 (17.6%)	4 (11.4%)	0.261
Renal involvement (%)	4 (22.2%)	0 (0.0%)	4 (11.4%)	**0.039**
Maximum PBE (cells/mmc), mean value (SD)	6817.778 (5,033.464)	3905.000 (4,275.495)	5,447.059 (4,851.655)	**0.080**
Maximum serum IgE (IU/ml), mean value (SD)	898.058 (884.002)	773.770 (956.612)	841.564 (897.517)	0.755
Maximum FeNO (ppb), mean value (SD)	67.771 (52.734)	67.977 (48.066)	67.870 (49.566)	0.992
ANCA+ (%)	16 (88.9%)	0 (0.0%)	16 (45.7%)	**<0.001**
Evidence of vasculitis on biopsy (%)	10 (90.9%)	0 (0.0%)	10 (55.6%)	**<0.001**
Evidence of eosinophils on biopsy (%)	9 (81.8%)	5 (71.4%)	14 (77.8%)	0.605
Active disease at the time of the study (%)	9 (50.0%)	8 (47.1%)	17 (48,6%)	0.862

ANCA: anti-neutrophil cytoplasmic autoantibodies; EGPA: eosinophilic granulomatosis with polyangiitis; FeNO: fractional exhaled nitric oxide; iHES: idiopathic hypereosinophilic syndrome; PBE: peripheral blood eosinophilia; ppb: parts per billion.

Primary analysis

The mean value of serum IgG4 in the entire cohort was 171.339 mg/dl, with a mean total IgG value of 984.902 mg/dl. HypoIgG was found in 7/62 (11.5%) patients, with a mean total IgG value of 577 mg/dl. Elevated IgG4 were found in 19/62 (30.6%) patients.

- Asthmatics vs. EGPA/iHES patients

The frequency of patients with elevated IgG4 was significantly higher in EGPA/iHES group than in asthmatics (16/35, 45.7% *vs*. 3/27, 11.1%, p=0.003) (Table III). Moreover, the mean IgG4 value was higher in EGPA/iHES than in asthma alone (224.057 *vs*. 103 mg/dl, p=0.010). No significant differences were observed for IgG, IgA, IgM, IgE, IgG1-2-3 values or frequency of monoclonal gammopathy and hypoIgG. At the time of IgG4 sampling, ANCA positivity was found only in EGPA patients (p 0.024, 6 patients).

- Patients with normal vs. elevated IgG4

In the group of patients with elevated IgG4, those affected by EGPA/iHES were significantly more represented (84.2 *vs*. 44.2%, p=0.003), especially EGPA patients (p=0.034) with positive ANCA at diagnosis (p=0.010) (Tables IV-V). Maximum documented PBE was significantly higher in patients with elevated IgG4 (5357.789 *vs*. 2788.024 cells/mmc, p=0.025). Peripheral neuropathy and renal involvement were significantly more frequent in patients with elevated IgG4 (p=0.041 and 0.047, respectively), as well as ongoing therapy with OCS at the time of the study (p=0.038). No significant differences were observed for other ongoing or previous therapies. The mean IgG4 value was 393.211 mg/dl in patients with elevated IgG4, while patients with normal IgG4 had a mean IgG4 value of 73.302 mg/dl (p<0.001). The mean total IgG value was significantly higher in patients with elevated IgG4 (1159.444 *vs*. 911.837 mg/dl, p<0.001), as well as mean values of IgG1 and IgG2 (p=0.013 and 0.002, respectively). ANCA-positivity was found exclusively in patients with elevated IgG4 (p<0.001). HypoIgG was found only in patients with normal IgG4, but without statistical significance (p=0.069).

Secondary analysis

Comparing EGPA with iHES patients the results of blood tests (Table VI) did not show significant differences except for ANCA positivity, found exclusively in EGPA patients (p=0.009). Regarding disease activity, a significant difference was observed only between active *versus* non-active EGPA patients, with the former showing a higher mean value

Table III. Results of study blood tests, asthmatics *vs*. EGPA/iHES patients.

	Asthmatics	EGPA/iHES	Total	*p*-value
Number of patients (%)	27 (43.5%)	35 (56.5%)	62 (100.0%)	
Patients with elevated IgG4 (%)	3 (11.1%)	16 (45.7%)	19 (30.6%)	**0.003**
IgG4 (mg/dl), mean value (SD)	103.000 (95.365)	224.057 (219.071)	171.339 (185.170)	**0.010**
IgG (mg/dl), mean value (SD)	931.000 (231.400)	1,027.706 (302.419)	984.902 (275.409)	0.175
Patients with hypoIgG (%)	4 (14.8%)	3 (8.8%)	7 (11.5%)	0.466
IgA (mg/dl), mean value (SD)	210.630 (99.288)	182.912 (85.074)	195.180 (91.898)	0.245
IgM (mg/dl), mean value (SD)	96.259 (53.195)	126.147 (121.494)	112.918 (97.819)	0.239
IgE (IU/ml), mean value (SD)	209.911 (302.042)	162.791 (165.930)	183.648 (235.016)	0.441
IgG1 (mg/dl), mean value (SD)	555.259 (156.318)	597.697 (152.681)	578.600 (154.483)	0.294
IgG2 (mg/dl), mean value (SD)	286.852 (107.936)	338.171 (138.635)	315.823 (127.814)	0.118
IgG3 (mg/dl), mean value (SD)	26.370 (14.036)	30.914 (16.123)	28.935 (15.298)	0.249
Monoclonal gammopathy (%)	2 (7.4%)	9 (25.7%)	11 (17.7%)	0.061
ANCA+ (%)	0 (0%)	6 (17.1%)	6 (9.7%)	**0.024**

ANCA: anti-neutrophil cytoplasmic autoantibodies; EGPA: eosinophilic granulomatosis with polyangiitis; iHES: idiopathic hypereosinophilic syndrome.

Table IV. Baseline characteristics, patients with normal *vs*. patients with elevated IgG4.

	Normal IgG4	Elevated IgG4	Total	*p*-value
Number of patients (%)	43 (69.4%)	19 (30.6%)	62 (100.0%)	
Age, mean value (SD)	58.581 (15.494)	62.474 (11.097)	59.774 (14.314)	0.328
Sex				
Female (%)	23 (53.5%)	12 (63.2%)	35 (56.5%)	0.479
Male (%)	20 (46.5%)	7 (36.8%)	27 (43.5%)	
Asthmatics (%)	24 (55.8%)	3 (15.8%)	27 (43.5%)	**0.003**
EGPA/iHES (%)	19 (44.2%)	16 (84.2%)	35 (56.5%)	**0.003**
EGPA (%)	9 (20.9%)	9 (47.4%)	18 (29.0%)	**0.034**
iHES (%)	10 (23.3%)	7 (36.8%)	17 (27.4%)	0.269
Asthma (%)	43 (100%)	19 (100%)	62 (100.0%)	
Chronic rhinosinusitis (%)	43 (100%)	19 (100%)	62 (100.0%)	
Nasal polyposis (%)	28 (65.1%)	14 (73.7%)	42 (67.7%)	0.506
Atopy (%)	25 (58.1%)	7 (43.8%)	32 (54.2%)	0.324
Previous AIT (%)	2 (8.0%)	0 (0.0%)	2 (6.2%)	0.440
NERD (%)	5 (11.6%)	3 (15.8%)	8 (12.9%)	0.652
Constitutional symptoms (%)	11 (25.6%)	8 (42.1%)	19 (30.6%)	0.193
Peripheral neuropathy (%)	6 (14.0%)	7 (36.8%)	13 (21.0%)	**0.041**
Purpura (%)	5 (11.6%)	4 (21.1%)	9 (14.5%)	0.331
Urticaria-angioedema (%)	6 (14.0%)	5 (26.3%)	11 (17.7%)	0.240
Myocarditis (%)	6 (14.0%)	2 (10.5%)	8 (12.9%)	0.711
Gastrointestinal involvement (%)	4 (9.3%)	0 (0.0%)	4 (6.5%)	0.169
Renal involvement (%)	1 (2.3%)	3 (15.8%)	4 (6.5%)	**0.047**
Maximum PBE (cells/mmc), mean value (SD)	2,788.024 (2,946.114)	5,357.789 (5,811.836)	3,588.443 (4,183.791)	**0.025**
Maximum serum IgE (IU/ml), mean value (SD)	546.426 (674.632)	864.062 (822.370)	647.140 (729.721)	0.198
Maximum FeNO (ppb), mean value (SD)	58.142 (47.743)	80.671 (52.269)	65.368 (49.876)	0.126
ANCA+ (%)	7 (16.3%)	9 (47.4%)	16 (25.8%)	**0.010**
Ongoing therapies				
OCS (%)	11 (25.6%)	10 (52.6%)	21 (33.9%)	**0.038**
OCS daily dose (prednisone-equivalent), mean value (SD)	4.814 (1.287)	3.999 (2.026)	4.426 (1.688)	0.280
Anti-T2 mAbs (%)	32 (74.4%)	13 (68.4%)	45 (72.6%)	0.625
Immunosuppressants (%)	2 (4.7%)	1 (5.3%)	3 (4.8%)	0.918
Previous therapies				
Corticosteroids (%)	43 (100%)	19 (100%)	62 (100.0%)	
Anti-T2 mAbs (%)	14 (32.6%)	11 (57.9%)	25 (40.3)	0.060
Immunosuppressants (%)	13 (30.2%)	9 (47.4%)	22 (35.5%)	0.194

AIT: allergen immunotherapy; ANCA: anti-neutrophil cytoplasmic autoantibodies; EGPA: eosinophilic granulomatosis with polyangiitis; FeNO: fractional exhaled nitric oxide; iHES: idiopathic hypereosinophilic syndrome; mAbs: monoclonal antibodies; NERD: non-steroidal anti-inflammatory drugs-exacerbated respiratory disease; OCS: oral corticosteroids; PBE: peripheral blood eosinophilia; ppb: parts per billion; T2: type 2.

of serum IgG4 (304.2 *vs*. 134.4 mg/dl, *p*=0.044).

Discussion

In this study, we found that the frequency of patients with elevated serum IgG4, as well as the mean IgG4 value, were significantly higher in EGPA/iHES patients than in asthmatics.

Focusing on pathophysiology, a possible explanation for a stronger IgG4 immune response in EGPA/iHES may lie in the anti-inflammatory role of IgG4. IgG4 take part in the complex network of T2 inflammation, being produced by B cells in response to IL-4 and IL-13, as well as IgE. However, IgG4 arise as consequence of the so called "modified" or "biased" T2 response, characterised by the expression of IL-10 and other cytokines (e.g IL-21) produced by Th2 and Tfh2 cells and driving class switching to IgG4, suppressing IgE production (22, 33). This kind of T2 response occurs in case of repeated or prolonged exposure to antigens and leads to immune tolerance (22). Therefore, in the context of T2 inflammation, IgG4 may carry a protective role, in line with their unique antinflammatory properties, such as inability of forming immune complexes, lack of complement binding activity and high affinity for the inhibitory IgG receptor FcγRIIb (22). In this perspective, a stronger IgG4 immune response in EGPA/iHES may arise from a counterbalance of the immune system against an excessive T2-eosinophilic inflammation, evolving from asthma to a systemic disease and characterised by higher eosinophilia, documented in this study in EGPA/iHES patients and in asthmatic patients with elevated IgG4. These findings are in accordance with Flament *et al*. who observed that asthmatic patients with elevated serum IgG4 had significantly higher eosinophilia (24), and with Gutierrez *et al*. who documented that T2-eosinophilic asthma was significantly more severe in patients with elevated serum IgG4 (25). Moreover, we found that IgG4 seemed to discriminate active from inactive EGPA, as already suggested by Vaglio *et al*. (29). On the other hand, IgG4 have been demonstrated to exert a pro-inflammatory role.

Table V. Results of study blood tests, patients with normal *vs*. patients with elevated IgG4.

	Normal IgG4	Elevated IgG4	Total	*p*-value
Number of patients (%)	43 (69.4%)	19 (30.6%)	62 (100.0%)	
IgG4 (mg/dl), mean value (SD)	73.302 (51.269)	393.211 (187.464)	171.339 (185.170)	**<0.001**
IgG (mg/dl), mean value (SD)	911.837 (228.972)	1,159.444 (304.003)	984.902 (275.409)	**<0.001**
Patients with hypoIgG (%)	7 (16.3%)	0 (0.0%)	7 (11.5%)	0.069
IgA (mg/dl), mean value (SD)	187.419 (96.771)	213.722 (78.448)	195.180 (91.898)	0.312
IgM (mg/dl), mean value (SD)	114.140 (108.736)	110.000 (67.439)	112.918 (97.819)	0.882
IgE (IU/ml), mean value (SD)	169.293 (249.795)	217.939 (197.519)	183.648 (235.016)	0.466
IgG1 (mg/dl), mean value (SD)	545.195 (148.670)	650.684 (145.190)	578.600 (154.483)	**0.013**
IgG2 (mg/dl), mean value (SD)	283.070 (104.085)	389.947 (147.381)	315.823 (127.814)	**0.002**
IgG3 (mg/dl), mean value (SD)	28.163 (13.615)	30.684 (18.865)	28.935 (15.298)	0.554
Monoclonal gammopathy (%)	7 (16.3%)	4 (21.1%)	11 (17.7%)	0.650
ANCA+	0 (0%)	6 (31.6%)	6 (9.7%)	**<0.001**

ANCA: anti-neutrophil cytoplasmic autoantibodies.

Table VI. Results of study blood tests, EGPA *vs*. iHES patients.

	EGPA	iHES	Total	*p*-value
Number of patients (%)	18 (51.4%)	17 (48.6%)	35 (100.0%)	
Patients with elevated IgG4 (%)	9 (50.0%)	7 (41.2%)	16 (45.7%)	0.600
IgG4 (mg/dl), mean value (SD)	219.333 (182.139)	229.059 (258.232)	224.057 (219.071)	0.898
IgG (mg/dl), mean value (SD)	956.722 (259.968)	1,107.562 (334.310)	1,027.706 (302.419)	0.149
Patients with hypoIgG (%)	2 (11.1%)	1 (6.2%)	3 (8.8%)	0.618
IgA (mg/dl), mean value (SD)	177.222 (82.150)	189.312 (90.508)	182.912 (85.074)	0.686
IgM (mg/dl), mean value (SD)	143.944 (155.427)	106.125 (65.477)	126.147 (121.494)	0.373
IgE (IU/ml), mean value (SD)	156.559 (189.021)	169.024 (144.827)	162.791 (165.930)	0.830
IgG1 (mg/dl), mean value (SD)	555.706 (137.392)	642.312 (159.608)	597.697 (152.681)	0.104
IgG2 (mg/dl), mean value (SD)	330.889 (125.814)	345.882 (154.599)	338.171 (138.635)	0.754
IgG3 (mg/dl), mean value (SD)	30.611 (16.950)	31.235 (15.714)	30.914 (16.123)	0.911
Monoclonal gammopathy (%)	5 (27.8%)	4 (23.5%)	9 (25.7%)	0.774
ANCA+ (%)	6 (33.3%)	0 (0.0%)	6 (17.1%)	**0.009**

ANCA: anti-neutrophil cytoplasmic autoantibodies; EGPA: eosinophilic granulomatosis with polyangiitis; iHES: idiopathic hypereosinophilic syndrome.

Namely, IgG4-related disease (IgG4-RD) is an immune-mediated condition characterised by fibroinflammatory masses in various organs, dense lymphoplasmacytic infiltration of IgG4-positive plasma cells, storiform fibrosis and elevated serum IgG4, with a suggested cut-off level of 135 mg/dl (34). Interestingly, in our study, patients with serum IgG4 >135 mg/dl were almost exclusively represented (19/24, 79.2%) by patients with elevated IgG4 according to the reference value of local laboratory (*i.e*. >201 mg/dl). Consistently with the T2 origin of IgG4, hallmarks of T2 inflammation such as blood/tissue eosinophilia and elevated serum IgE are frequently found in IgG4-RD, as well as co-occurrence of T2-allergic/eosinophilic diseases like CRS and asthma (35-40). Notably, ANCA have been demonstrated to be IgG4 autoantibodies (41, 42), and overlap between ANCA-associated vasculitis and IgG4-RD have been reported (43, 44). In this perspective, it might be interesting to speculate another hypothesis for the stronger IgG4 immune response in EGPA, with ANCA being IgG4 turned from anti-inflammatory to pro-inflammatory autoantibodies, maybe following changes in glycosylation pattern, as described in literature (36). Interestingly, in our study, EGPA patients with elevated IgG4 were all ANCA+ (9/9, 100%). In this sense, it might be interesting to speculate whether such patients could be better responder to rituximab, a very effective treatment in IgG4-RD (34) and used in our study in 8 patients (all ANCA+, 5/8 with elevated IgG4) with good results. Based on the above, in our study, elevated serum IgG4 seemed to discriminate between T2-eosinophilic asthma "alone" and T2-eosinophilic asthma evolved into EGPA/iHES. However, our study has several limitations that hinder prompt clinical implications, and it could only represent a reasonable background for prospective long-term studies on asthmatic naïve patients aimed to investigate IgG4 as potential predictive biomarker of progression from asthma to EGPA/iHES. Indeed, the first limitation is the retrospective nature of the study, *i.e*. the measurement of serum IgG4 after the diagnosis of asthma or EGPA/iHES. This retrospective correlation with clinical history may introduce bias and limit the ability to establish causality between elevated serum IgG4 values and progression from asthma to EGPA/iHES. In this sense, the finding of elevated serum IgG4 in asthmatic patients (11.1%) is of uncertain interpretation, since the future risk of developing EGPA/iHES cannot be assessed in absence of prospective data. However, asthmatic patients included in this study had long disease duration and follow-up (median asthma duration 20.7 years), so that asthmatic patients with normal IgG4 might be considered no longer at risk to develop EGPA/iHES. The second important limitation is that many patients were not treatment-naive at the time of IgG4 measurement, with potential negative influence on total IgG levels. HypoIgG was found in 11.5% of patients. However, the mean IgG value in patients with hypoIgG was >500 mg/dl and no significant difference was observed for hypoIgG frequency between asthmatic and EGPA/iHES patients, as well as between patients with normal and elevated IgG4. These findings could be explained by the low OCS average daily dose (<5 mg/day in both asthma and EGPA/iHES groups) and by the scarce use of immunosuppressants (used only in 3/62 patients). However, despite the low OCS average daily dose, 21/62 patients (33.9%) were on OCS therapy, and it is another important limitation. On the other hand, anti-T2 mAbs, despite widely used (85.2% and 62.9% of asthmatic and EGPA/iHES patients, respectively), seemed to not affect total IgG levels, maybe because of their action on selective targets (IL-5, IL-5R, IL4/IL-13Rα) probably unrelated

to IgG production by B cells. Finally, other limitations are represented by the single-centre nature of the study and by the small size of the cohort.

Conclusion

In our study, elevated serum IgG4 seemed to discriminate between T2-eosinophilic asthma "alone" and T2-eosinophilic asthma that evolved into EGPA/iHES. This finding could represent a reasonable background for prospective long-term studies on asthmatic naive patients aimed at investigating IgG4 as potential predictive biomarker of progression from T2-eosinophilic asthma to EGPA/iHES.

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