IgG4-related disease in Italy: clinical features and outcomes of a large cohort of patients

C Campochiaro1,2a, GA Ramirez1,2a, EP Bozolo2, M Lanzillotta1, A Berti1,2, E Baldissera2, L Dagna1,2, L Praderio2, R Scotti2, M Tresoldi2, L Roveri3, A Mariani4, G Balzano5, R Castoldi5, C Doglioni5, MG Sabbadini1,2, E Della-Torre1,2

1Unit of Medicine and Clinical Immunology, 2Department of Neurology, 3Division of Gastroenterology and Gastrointestinal Endoscopy, 4Pancreas Unit, Department of Surgery, and 5Pathology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

Objectives: To describe the clinical features, treatment response, and follow-up of a large cohort of Italian patients with immunoglobulin (Ig)G4-related disease (IgG4-RD) referred to a single tertiary care centre.

Method: Clinical, laboratory, histological, and imaging features were retrospectively reviewed. IgG4-RD was classified as ‘definite’ or ‘possible’ according to international consensus guidelines and comprehensive diagnostic criteria for IgG4-RD. Disease activity was assessed by means of the IgG4-RD Responder Index (IgG4-RD RI).

Results: Forty-one patients (15 females, 26 males) were included in this study: 26 with ‘definite’ IgG4-RD and 15 with ‘possible’ IgG4-RD. The median age at diagnosis was 62 years. The median follow-up was 36 months (IQR 24–50). Ninety percent of patients (14/15) underwent surgery. The most frequently involved organs. Serum IgG4 levels were elevated in 68% of cases. Thirty-six patients were initially treated with glucocorticoids (GCs) to induce remission. IgG4-RD RI decreased from a median of 7.8 at baseline to 2.9 after 1 month of therapy. Relapse occurred in 19/41 patients (46%) and required additional immunosuppressive drugs to maintain long-term remission. Multiple flares occurred in a minority of patients. A single case of orbital pseudotumour did not respond to medical therapy and underwent surgical debulking.

Conclusions: IgG4-RD is an elusive inflammatory disease to be considered in the differential diagnosis of isolated or multiple tumefactive lesions. Long-term disease control can be achieved with corticosteroids and immunosuppressive drugs in the majority of cases.

Immunoglobulin (Ig)G4-related disease (IgG4-RD) is a rare systemic fibro-inflammatory condition characterized by expansile lesions with well-defined histological features. These features include a lymphoplasmocytic infiltrate rich in IgG4+ plasma cells, a dense storiform fibrosis, obliterative phlebitis, and a moderate tissue eosinophilia (1). Serum IgG4 elevation and a prompt response to glucocorticoids (GCs) are also observed in the majority of patients (1).

IgG4-RD was originally described in the pancreas as type 1 autoimmune pancreatitis (AIP) (1, 2), and subsequently reported in every organ system owing to the recognition of common histopathological features (3–13). Indeed, histopathological examination is the current gold standard for the diagnosis of IgG4-RD because clinical manifestations as well as serological findings are largely non-specific, and overlap with various inflammatory and neoplastic conditions (11–13). IgG4-RD swiftly responds to GCs, but disease-modifying anti-rheumatic drugs (DMARDs) are often required to maintain remission because relapse occurs in 30–50% of patients (14–18). Rapid and long-lasting clinical responses have also been obtained recently with the anti-CD20 monoclonal antibody rituximab (RTX) (19–21).

Few data exist on the global incidence of IgG4-RD because almost all epidemiological studies come from Japan (where the condition was first described) or the USA (1), and focus on AIP rather than on systemic manifestations (1). In Europe, lack of familiarity with IgG4-RD is probably the main reason for the underestimation of its prevalence and for the exiguity of population studies (22, 23). Similarly, apart from pancreatic involvement (24), IgG4-RD in Italy has been seldom described, with scant reports representing the only available source of clinical data (5–7, 10, 25–28).

In the present retrospective work we aimed to review the clinical, serological, and histological features of a large cohort of Italian patients affected by IgG4-RD with systemic manifestations referred to a single tertiary care centre.

*These authors contributed equally to this work.

Emanuel Della-Torre, Unit of Medicine and Clinical Immunology, IRCCS San Raffaele Scientific Institute, via Olgettina 60, 20132 Milan, Italy.
E-mail: dellatorre.emanuel@hsr.it

Accepted 25 May 2015
centre. Treatment outcomes and follow-up are also reported.

**Method**

**Patients**

The present study included 41 patients with IgG4-RD referred between November 2007 and March 2015 to our tertiary care centre. IgG4-RD was diagnosed according to the following comprehensive diagnostic criteria (29): (a) clinical/radiological examination showing characteristic diffuse/localized swelling or masses in single or multiple organs; (b) haematological examination showing elevated serum IgG4 concentrations (> 135 mg/dL); (c) histopathological examination showing (i) marked lymphocyte and plasmacyte infiltration, (ii) storiform fibrosis, and (iii) infiltration of IgG4+ plasma cells with a ratio of IgG4+/IgG+ plasma cells ≥ 40%, and a total of ≥ 10 IgG4+ plasma cells/high power field (HPF). Organ-specific histopathological criteria were adopted instead of criteria ‘c’ for those organs included in the ‘Consensus statement on the pathology of IgG4-RD’ (13). IgG4-RD was thus classified as ‘definite’ [in the presence of a highly suggestive histology (criteria ‘c’) with either typical clinical/radiological (criteria ‘a’) or serological (criteria ‘b’) features] or ‘possible’ (in the presence of criteria ‘a’ and ‘b’, when histological examination was not performed or inconclusive for IgG4-RD). Patients with pancreatic involvement who did not undergo pancreatic resection were diagnosed with definite IgG4-related AIP according to the international consensus diagnostic criteria (ICDC) for AIP (30). All participants had active disease as defined by the IgG4-RD Responder Index (IgG4-RD RI) (31). Patients were classified as either atopic or non-atopic according to the definitions of the European Academy of Allergy and Clinical Immunology (EAACI) (32). All patients provided written informed consent for the analyses and treatments described in the present study.

**Clinical assessment**

Disease activity was evaluated through the IgG4-RD RI, a validated instrument for monitoring clinical, laboratory, and radiological outcomes of IgG4-RD (30). An IgG4-RD RI score ≥ 3 was recently used to identify patients with active disease (33). Disease response was defined as an improvement (i.e. decline) in the IgG4-RD RI by ≥ 2 points over baseline (20). Complete response was defined as an IgG4-RD RI < 3. Partial response was defined as an IgG4-RD RI that remained ≥ 3. Patients who achieved an IgG4-RD RI < 3 and successfully completed a GC taper were considered in disease remission (i.e. the condition of inactive disease). Disease relapse was diagnosed if clinical symptoms recurred or imaging findings worsened after improvement with treatment.

Patients were evaluated 1 and 6 months after the institution of GC therapy, and then according to clinical priorities. In case of disease remission, patients were evaluated every 6 months.

**Haematological and serological assessments**

All patients underwent complete blood counts, liver and renal function assessment, and serological testing for C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and complement levels. Antinuclear antibodies (ANA), anti-Ro/SSA and La/SSB antibodies, antineutrophil cytoplasmic antibodies (ANCA), total IgE, IgG, IgG1, IgG2, and IgG3 were tested when necessary for the differential diagnosis. Total IgG concentrations were determined by nephelometry (BN II System, Siemens, Germany) and IgG1-4 subclasses by enzyme-linked immunosorbent assay (ELISA; Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturers’ instructions. Serial dilutions of the serum (1:2500 as a starting point) were used to avoid the prozone effect. All samples were drawn before the institution of specific immunosuppressive therapies.

**Imaging studies**

Imaging studies were performed according to the specific pattern of clinical organ involvement, and included a computed tomography (CT) scan, magnetic resonance imaging (MRI), and a fluorodeoxyglucose positron emission tomography/CT scan (FDG-PET/CT). Radiological studies were performed in all patients at baseline and repeated 1 and 6 months after initiation of GC therapy. Evaluations at earlier time points were performed if clinically appropriate. In case of disease remission, imaging studies were scheduled every year.

**Statistical analysis**

Statistical analysis was performed using SPSS version 21.0. Categorical variables were compared using Fisher’s exact test. Normal distribution of continuous variables was assessed with the Kolmogorov–Smirnov algorithm. Normally distributed variables were compared using the Student’s t-test. Non-normally distributed variables were compared using the Mann–Whitney U-test. Categorical and continuous variables were assessed for their relative contribution to disease relapse using Kaplan–Meier’s curves, adjusted for the follow-up time. Parametric correlations were calculated using Spearman’s correlation test. p < 0.05 was considered statistically significant. Values are presented as median and interquartile range (IQR), unless otherwise specified.
### Results

**Patients’ characteristics and organ involvement**

Forty-one patients with a median age of 62 (IQR 55–67) years at diagnosis were included in the study. The male to female ratio was 1.9:1 (26 males and 15 females). Twelve patients (30%) reported a history of atopy, characterized by urticaria, conjunctivitis, rhinitis, bronchial asthma, or gastrointestinal symptoms, together with positive skin prick tests or specific serum IgE for a given allergen. The median value of the IgG4-RD RI at baseline was 7.5 (IQR 6–9) (Table 1). Twenty-six patients were diagnosed with definite IgG4-RD and 15 with possible IgG4-RD. ‘Definite’ cases of IgG4-RD included 21 patients with histological findings ‘highly suggestive’ of IgG4-RD according to the ‘Consensus statement on the pathology of IgG4-RD’, and five patients with AIP diagnosed according to the ICDC for AIP (13, 30) (online Supplementary Table S1). ‘Possible’ cases of IgG4-RD were diagnosed by means of clinical, serological, and radiological ‘Comprehensive criteria’ because biopsies were not performed (six cases) or did not reach the ‘Consensus statement criteria’ (nine cases) (29) (online Supplementary Table S2). Epidemiological features including age, male to female ratio, history of atopy, IgG4-RD RI, and organ involvement at diagnosis did not differ significantly between ‘definite’ and ‘possible’ cases of IgG4-RD (p-value > 0.05) (Table 1).

The majority of patients presented with single organ involvement (24 cases); 13 patients had IgG4-RD in two organs, and four patients in three organs. The pancreas was the most commonly affected organ (17 cases) in both ‘definite’ and ‘possible’ cases of IgG4-RD. Retroperitoneum and major salivary glands (submandibular or parotid) (eight cases each) were the second most common site of involvement in definite and possible cases of IgG4-RD, respectively. Less frequent disease localizations included lymph nodes (five cases); aorta and biliary tree (four cases each); pachymeninges and orbit (three cases each); and nasal sinuses, lacrimal glands, and pharynx (two cases each). The kidneys, nasal septum, lungs, gallbladder, and pleura were involved in one case each.

The clinical spectrum of IgG4-RD at presentation was broad, with the involvement of different anatomical sites leading to protean manifestations (Table 2). Patients with AIP were typically referred to the emergency department because of subacute onset of abdominal pain and/or jaundice. By contrast, the majority of subjects with extra-pancreatic manifestations were evaluated by different specialists before being referred to our outpatient clinic; on average, the delay between the onset of symptoms and IgG4-RD diagnosis was 11 (range 2–26) months. Differential diagnosis varied according to the specific clinical manifestation and included solid malignancies, lymphomas, histiocytosis, large-vessel vasculitis, ANCA-associated vasculitides, Sjögren’s syndrome, sarcoidosis, primary sclerosing cholangitis, and chronic infections, among others.

### Table 1. Differences in epidemiological features, serological characteristics, and outcomes between patients with ‘definite’ and ‘possible’ IgG4-RD.

| Parameter | Definite IgG4-RD (n = 26) | Possible IgG4-RD (n = 15) | p-value | IgG4-RD cohort (n = 41) |
|-----------|---------------------------|---------------------------|---------|------------------------|
| **Epidemiological features at baseline** | | | | |
| Age (years), median (IQR) | 64 (58–68) | 59 (53–63) | 0.33 | 62 (55–67) |
| Gender (male/female) | 16/10 | 10/5 | 1 | 26/15 |
| Atopy, n (%) | 8 (35) | 4 (27) | 1 | 12 (30) |
| Single/multi-organ involvement | 16/10 | 8/7 | 0.74 | 24/17 |
| **Organ involvement (n)** | | | | |
| Pancreas | 10 | 7 | 17 |
| Retroperitoneum | 6 | 2 | 8 |
| Aorta | 4 | – | 8 |
| Salivary glands | 2 | 6 | 8 |
| IgG4-RD RI, median (IQR) | 9 (6–9) | 9 (6–9) | 0.61 | 7.5 (6–9) |
| Starting dose of GCs (mg/day), median (IQR) | 37.5 (25–50) | 37.5 (25–50) | 0.8 | 37.5 (25–50) |
| **Serological features at baseline, median (IQR)** | | | | |
| Eosinophils (n/μL) (normal range ≤ 500/μL) | 325 (175–525) | 250 (200–452) | 0.69 | 300 (200–500) |
| CRP (mg/L) (normal range ≤ 5 mg/L) | 18 (2–36.8) | 5.6 (0–22.8) | 0.49 | 8 (0–27.5) |
| ESR (mm/h) (normal range ≤ 20 mm/h) | 44 (20–55) | 12 (8–23) | 0.06 | 21.5 (8–48.7) |
| IgG4 (mg/dL) (normal range 3–135 mg/dL) | 262 (121–577) | 300 (209–461) | 0.36 | 284 (132–545) |
| **Outcomes** | | | | |
| Complete/partial response to GCs | 18/8 | 7/8 | 0.19 | 25/16 |
| Remission/non-remission | 18/8 | 11/4 | 1 | 29/12 |
| Relapse/non-relapse | 14/12 | 5/10 | 0.33 | 19/22 |
| Follow-up (months), median (IQR) | 36 (26–51) | 30 (24–48) | 0.80 | 36 (24–51) |

IgG4-RD RI, IgG4-RD Responder Index; GC, glucocorticoid; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range.

Organ involvement refers to the three most frequently involved anatomical sites.
Peripheral blood eosinophilia (> 500 eosinophils/μL) was present in eight (22%) patients (median 840 eosinophils/μL; IQR 682–907), of whom four were atopic. Serum IgE concentrations were measured in 15 subjects, and were elevated in 10 (67%) (three atopic and seven non-atopic), with a median value of 277 IU/mL (IQR 245–579; normal < 100). CRP was increased in 19/33 patients (63%), with a median value of 23 mg/L (IQR 19.5–46.1; normal < 5). ESR was elevated in 17/32 patients (51%), with a median value of 50 mm/h (IQR 38–60; normal < 20). Serum protein electrophoresis showed polyclonal hypergammaglobulinaemia in 15/41 subjects (36%). The serum IgG4 level was increased in 30/41 patients (73%), with a median value of 402 mg/dL (IQR 261–629; normal 3–135). The other IgG subclasses were measured in 22/41 patients. In particular, IgG1 concentration was within the normal range in all subjects whereas the IgG2 level was increased in seven cases; the serum IgG3 level was elevated in a single case (data not shown). No significant correlation was found between serum IgG4 level and IgE concentration ($R^2 = 0.04$, $p = 0.54$), eosinophils ($R^2 = 0.03$, $p = 0.37$), or IgG4-RD RI ($R^2 = 0.06$, $p = 0.16$). The median serum IgG4 level was similar in patients with single (315 mg/dL; IQR 135–511) and multi-organ (284 mg/dL; IQR 135–315) IgG4-RD involvement ($p > 0.05$).

ANA were positive at low titre (1:80–1:320) in seven patients (two patients with salivary gland involvement, two with pancreatic and two with retroperitoneal involvement, and one patient with sclerosing cholangitis). When tested, ANCA, anti-Ro/SSA and anti-La/SSB were negative. C3 and C4 complement fractions were assessed in 14 patients and were slightly reduced in two (14%). Definite and possible cases of IgG4-RD did not differ significantly in the median values of eosinophils, CRP, ESR, and serum IgG4 levels ($p$ value > 0.05) (Table 1).

### Imaging

Apart from pulmonary, aortic, and nasal septum involvement, IgG4-RD presented with an abnormal swelling of the affected organ. Pancreatic involvement led to a ‘sausage-like’ appearance of the gland (Figure 1A). Pachymeningeal disease was confined to localized areas such as the meninges overlying the supratentorial hemispheres, skull base, or spinal cord, and appeared either as a linear dural thickening or as a bulging mass (Figure 1B). Salivary gland involvement caused a typical symmetrical enlargement of the parotids and/or submandibular glands (Figure 1C). Pharyngeal disease led to an irregular soft tissue thickening along the pharyngeal walls that extended into the tongue base in one case and towards the nasopharynx in another (Figure 1D). Lung involvement, instead, consisted of thickening of the bronchovascular bundles, bronchiectasia, and peripheral honeycombing (Figure 1E)
to thoracic or abdominal inflammatory aneurisms (Figure 1F), whereas nasal septum involvement was characterized by a septal perforation.

Imaging studies were performed according to the specific pattern of organ involvement. A CT scan was preferred in the case of pulmonary disease, retroperitoneal fibrosis, and potential bone involvement (e.g. pseudotumour orbitae and nasal septum perforation). MRI was used for a better anatomical evaluation of the optic chiasm, nerve roots, brainstem, and skull base in the case of meningeal and orbital disease. AIP was well documented by both CT and MRI, with the latter preferred in cases of concomitant cholangitis. An FDG-PET/CT scan was performed in 10 patients and represented a useful tool for assessing both disease activity in the case of multi-organ involvement and disease response to immunosuppressive therapies (Figures 2A–2C).

**Histological findings**

Histological analysis was performed in 30 patients. Five patients with AIP underwent only cytological examination through endoscopic ultrasonographic fine-needle aspiration as part of the diagnostic work-up for pancreatic malignancies. Biopsies were not performed in six cases because of safety concerns (such as in the case of meningeal, aortic, or salivary gland involvement) or because patients refused surgical procedures.

Lymphoplasmacytic infiltration was the most frequently reported histopathological finding. Extranodal lymphoid follicles were noted in five patients with IgG4-RD involvement of the gallbladder, retroperitoneum, salivary glands, aorta, and pancreas, respectively. A mild to moderate eosinophilic infiltrate was observed in all biopsies, while histiocytes, but not granulomas, were documented in two patients. Immunostaining for kappa and lambda chains was consistent with a polyclonal lymphocyte expansion.

Immunohistochemical studies of IgG4+ plasma cells were performed in 25 out of 30 patients. The mean value of the IgG4+/IgG+ plasma cell ratio was 0.4 (range 0.08–0.8). The lowest IgG4+/IgG+ plasma cell ratio was detected in a labial salivary gland from a patient with pancreatic, lacrimal gland, and lymph node involvement; the highest IgG4+/IgG+ plasma cell ratio was calculated in a patient with inflammatory aneurism of the ascending aorta (Figure 2D). The total number of IgG4+ plasma cells/HPF ranged from 10 to 100 (mean value 51/HPF). The lowest count was described in a labial
salivary gland from a patient with salivary gland, lacrimal gland, and nasal septum involvement; the highest count was found in a case of IgG4-related cholecystitis.

Labial salivary glands were biopsied in six subjects with systemic IgG4-RD involvement but characteristic pathological criteria were described in only two cases. Moderate to severe fibrosis was described in all tissue samples (Figure 2E). Obliterative phlebitis was not observed. Overall, histopathological analysis met the ‘Consensus statement on the pathology of IgG4-RD’ criteria in 21 cases (13).

Follow-up, therapeutic strategies, and outcomes

Thirty-six patients (87%) were treated with immunosuppressive therapies. Ten patients with AIP and obstructive jaundice also received biliary tract stenting. Five patients were not treated: four of them because the single organ affected by IgG4-RD (gallbladder in one case, pancreas in three cases) was removed surgically as part of the diagnostic work-up; in one of them (an asymptomatic case of inflammatory abdominal aneurism), watchful waiting was preferred. To date, none of these five untreated subjects has experienced disease relapse. Patients were monitored for a median follow-up period of 36 months (IQR 24–51). Follow-up did not differ between definite and possible cases of IgG4-RD (Table 1).

The initial therapeutic approach in all treated patients was based on GCs. Oral prednisone (PDN) was started at a daily dose of 0.5–0.6 mg/kg in patients with single organ involvement, and 1 mg/kg in patients with multi-organ involvement (median 37.5 mg; IQR 25–50). Two patients with biliary tree and pachymeningeal involvement, respectively, received 1 g of methylprednisolone intravenously for three consecutive days before shifting to oral PDN. GCs were gradually tapered over a period of 6 months. After the first month of therapy, disease response was obtained in all 36 patients with a median IgG4-RD RI that decreased to 2.9 (IQR 1–4) from a baseline of 7.5. In particular, 20 patients (55%) achieved complete response (CR) and 16 patients (45%) achieved partial response (PR). Epidemiological and serological features at the time of diagnosis did not differ between complete and partial responders, except for a higher incidence of orbital involvement in the latter (three cases vs. none) (Table 3). The starting dose of GCs as well as the rate of CR and PR did not differ between definite and possible cases of IgG4-RD (Table 1). Seventeen subjects (12 who achieved CR and five who achieved PR) were able to complete GC taper without experiencing further flares.

Disease relapse occurred in 19/41 (46%) patients (14 cases of definite IgG4-RD and five cases of possible IgG4-RD) at a median time of 8 months (IQR 6–13.5) after the institution of GC therapy. Nine patients relapsed

Table 3. Differences in epidemiological features, serological characteristics, and outcomes between patients with IgG4-RD achieving ‘complete’ and ‘partial’ response after corticosteroid therapy.

| Parameter                                      | Complete response (n = 25) | Partial response (n = 16) | p-value |
|------------------------------------------------|---------------------------|---------------------------|---------|
| Epidemiological features at baseline           |                           |                           |         |
| Age (years), median (IQR)                      | 61 (56–66)                | 63 (55–68)                | 0.92    |
| Gender (male/female)                           | 15/10                     | 11/5                      | 0.74    |
| Atopy, n (%)                                   | 8 (32%)                   | 4 (25%)                   | 0.73    |
| Single/multi-organ involvement                | 15/10                     | 9/7                       | 1       |
| Organ involvement (n)                          |                           |                           |         |
| Pancreas                                       | 9                         | 8                         |         |
| Retroperitoneum                                | 6                         | –                         |         |
| Salivary glands                                | 5                         | 3                         |         |
| Orbital pseudotumour                           | –                         | 3                         |         |
| Definite/possible IgG4-RD                      | 18/7                      | 8/8                       | 0.19    |
| IgG4-RD RI, median (IQR)                       | 6 (6–9)                   | 9 (6–9)                   | 0.31    |
| Starting dose of GCs (mg/day), median (IQR)    | 31.25 (25–50)             | 38.75 (37.5–50)           | 0.18    |
| Serological features at baseline, median (IQR) |                           |                           |         |
| Eosinophils (n/μL) (normal range ≤ 500/μL)     | 215 (200–500)             | 400 (300–500)             | 0.81    |
| CRP (mg/L) (normal range ≤ 5 mg/L)             | 14 (0–29.7)               | 7.3 (0–22.5)              | 1       |
| ESR (mm/h) (normal range ≤ 20 mm/h)            | 20 (7–44)                 | 20 (16–50)                | 0.24    |
| IgG4 (mg/dL) (normal range 3–135 mg/dL)        | 300 (137–534)             | 260 (129–610)             | 0.83    |
| Outcomes                                       |                           |                           |         |
| Remission/non-remission                        | 21/4                      | 8/8                       | 0.03    |
| Relapse/non-relapse                            | 8/17                      | 11/5                      | 0.54    |
| Follow-up (months), median (IQR)*              | 36 (24–51)                | 30 (25–61)                | 0.72    |

IgG4-RD RI, IgG4-RD Responder Index; GC, glucocorticoid; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range.

Organ involvement refers to the three most frequently involved anatomical sites.

Significant P-values are shown in bold.
during GC taper and 10 after GC withdrawal. Twelve/19 (63%) patients relapsed within 1 year after GC introduction, 5/19 (26%) patients within 2 years, and 2/19 (11%) patients within 3 years. Relapsing patients had significantly higher levels of ESR at the time of diagnosis compared to non-relapsing patients (p < 0.01) (Table 4). A significantly higher incidence of flares was found in patients who did not achieve disease remission (i.e., a condition of inactive disease after GC withdrawal) (p < 0.01) (Figure 3B); none of the other epidemiological and serological variables shown in Table 4 increased the risk of disease relapse (p > 0.05) (data not shown). When calculated on the entire cohort of 41 patients and adjusted for the length of the follow-up, IgG4-RD relapse occurred on average 48 months [Confidence Interval (CI) 36–61] after GC introduction (Figure 3A).

Relapsing patients increased or resumed corticosteroid therapy. One patient with retroperitoneal fibrosis and one patient with AIP were retreated with a longer course of GCs (a total of 12 months). The former achieved disease remission, the latter achieved CR and is still on low-dose PDN. The remaining 17 patients received additional treatments with DMARDs [e.g. azathioprine (AZA), methotrexate (MTX) mycophenolate mofetil (MMF), or cyclophosphamide (CTX)] or RTX, as specified in Table 5. Azathioprine was preferred in the case of AIP whereas MTX was frequently used for extra-pancreatic and multi-organ IgG4-RD involvement. Intravenous CTX was chosen in three cases of pachy meningeval involvement reported elsewhere (5, 7). RTX induced CR in a case of glomerulonephritis that was lost to follow-up. Disease remission was achieved in four patients who were able to complete a GC taper without further relapses on DMARDs; one of them switched from AZA to MTX because of liver toxicity. Three patients maintained CR and three maintained PR on DMARDs and low-dose PDN. Six patients experienced a third relapse (three on DMARDs alone and three on DMARDs plus low-dose PDN) and were treated with a new course of GCs together with a third-line therapeutic regimen. One of them failed RTX and ultimately underwent surgical excision of his orbital pseudotumour (Table 5).

Overall, disease remission was obtained in 29/41 (70%) patients, 21/25 (84%) with initial CR to GCs, and 8/16 (50%) with initial PR to GCs. Twelve patients are still on GC therapy (six on CR and six on PR). The median follow-up time of patients who did and who did not achieve remission was 33 months (IQR 15–50) and 40 months (IQR 29–57), respectively. At the longest available follow-up, serum IgG4 concentration decreased in all patients and normalized in 10. No major adverse events due to GCs were observed. GC-induced diabetes mellitus was reported in 10 patients. Nausea and abdominal discomfort were reported in five patients treated with oral MTX and in three patients treated with AZA. These patients were switched to subcutaneous injections of MTX and tolerated that route of administration better. Irreversible organ damage was reported in seven patients, and was related either to IgG4-RD per se (two cases of pachymeningitis with cranial nerve

| Parameter | Relapsing patients (n = 19) | Non-relapsing patients (n = 22) | p-value |
|-----------|-----------------------------|-----------------------------|---------|
| Age (years), median (IQR) | 63 (55–66) | 61 (57–69) | 0.78 |
| Gender (male/female) | 11/8 | 15/7 | 0.53 |
| Atopy, n (%) | 5 (26%) | 7 (32%) | 1 |
| Single/multi-organ involvement | 8/11 | 10/12 | 1 |
| Pancreas | 5 | 12 |
| Retroperitoneum | 5 | 3 |
| Salivary glands | 4 | 4 |
| Definite/possible IgG4-RD | 14/5 | 12/10 | 0.33 |
| IgG4-RD RI, median (IQR) | 9 (6–9) | 9 (6–9) | 0.69 |
| Starting dose of GCs (mg/day), median (IQR) | 37.5 (25–50) | 37.5 (25–50) | 0.89 |
| Complete/partial response to GCs | 8/11 | 12/10 | 0.54 |
| Remission/non-remission | 7/12 | 22/0 | < 0.01 |
| Eosinophils (n/μL) (normal range ≤ 500/μL) | 400 (200–600) | 230 (100–470) | 0.27 |
| CRP (mg/L) (normal range ≤ 5 mg/L) | 7.8 (6–34.3) | 14 (0–23) | 0.93 |
| ESR (mm/h) (normal range ≤ 20 mm/h) | 40 (20–55) | 11 (7–39) | < 0.01 |
| IgG4 (mg/dL) (normal range 3–135 mg/dL) | 360 (123–625) | 274 (160–423) | 0.97 |
| Follow-up (months), median (IQR) | 37 (26–51) | 33 (15–50) | 0.57 |

IgG4-RD RI, IgG4-RD Responder Index; GC, glucocorticoid; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range.

Organ involvement refers to the three most frequently involved anatomical sites. Significant P-values are shown in bold.
population (34, 35); of note, elevation of eosinophil count and serum IgE level was independent of the atopic background. Single-organ involvement was the most common presentation of IgG4-RD in our cohort. The pancreas, retroperitoneum, and major salivary glands were the most frequently affected anatomical sites. Clinical manifestations were protean and varied according to the specific pattern of organ involvement (Table 1). At the time of diagnosis, inflammatory markers were, in general, normal or only mildly elevated whereas serum IgG4 levels were increased in almost 70% of patients.

A ‘definite’ diagnosis of IgG4-RD required the fulfillment of the histological criteria reported in the ‘Consensus statement on the pathology of IgG4-RD’ (13), together with either typical clinical or serological features. In our cohort, however, adequate tissue samples for histological analysis were not obtained from all patients. In fact, parotid and submandibular glands were not routinely biopsied because the surgical procedure was considered at risk of causing permanent cranial nerve damage. Similarly, pancreatic tissue was available in a minority of AIP cases because cytological examination through fine-needle aspiration adequately ruled out pancreatic adenocarcinoma. In some other cases, patients refused surgical procedures because of concerns about the invasiveness of the surgery. Moreover, a thorough histological evaluation that focused on both the number of IgG4+ plasma cells/HPF and the IgG4+/IgG+ plasma cell ratio was not performed in all patients; for instance, in five cases, biopsies were performed in other hospitals, and there were no more slides to stain for IgG4. Finally, failure to observe diagnostic numbers of IgG4+ plasma cells in the tissue, despite the presence of other suggestive histological features, was not uncommon, probably because of the small sample size or the biopsy having been performed during a later stage of disease, where fibrosis predominates over the IgG4+ lymphoplasmocytic infiltration. Thus, in the absence of an informative histological examination, we took advantage of the ‘Comprehensive diagnostic criteria for IgG4-RD’, and considered a diagnosis of ‘possible’ IgG4-RD based on characteristic clinicoradiological findings and elevated serum IgG4 levels (> 135 mg/dL) (29). Similarly, the ICDC for AIP were adopted for the diagnosis of IgG4-related AIP when pancreatic resection was not performed (30). Of note, ‘definite’ and ‘possible’ cases of IgG4-RD did not differ with respect to epidemiological features, serological findings, and response to immunosuppressive therapy, suggesting that these two groups did indeed represent a unique pathological condition (Table 1).

These general and practical considerations raise important diagnostic implications: (i) pathology reports are still heterogeneous in the description of typical IgG4-RD histological features. Therefore, an increased awareness of the consensus guidelines is required among pathologists to enable immunohistochemical studies of IgG4+ plasma cells to be readily performed and to generate standardized reports. (ii) The ‘Consensus statement
on the pathology of IgG4-RD does not cover the full spectrum of IgG4-related disorders because other organs potentially affected by IgG4-RD have not been included. For instance, gallbladder, nasal sinuses, pharynx, nasal septum, and orbital pseudotumour might represent unique sites of IgG4-RD involvement but cannot yet be classified according to international guidelines. This was precisely our experience with several cases of atypical IgG4-RD manifestations. (iii) Finally, diagnostic biomarkers are urgently needed to overcome the limit of uninformative histopathological analyses as well as the risk of invasive surgical procedures. In this sense, labial salivary gland biopsies were proposed as a safe alternative diagnostic approach in patients with systemic IgG4-RD involvement, but their sensitivity and specificity were estimated to be no higher than 70% (36). Indeed, our experience confirmed the shortcomings of this procedure. Similarly, an increase in serum IgG4 concentration above 135 mg/dL has been considered as a potential disease biomarker for IgG4-RD, but larger population studies are required to validate this test (33).

Second-line treatment with DMARDs was introduced at disease relapse to reduce the cumulative steroid dose and to maintain remission. In the absence of standardized guidelines, the choice of a specific DMARD was largely empirical and guided by the pattern of organ involvement. For instance, AZA was preferred in cases of AIP, as suggested by the international consensus guidelines (14–16); MTX was frequently used for extra-pancreatic and multi-organ IgG4-RD involvement, according to our positive experience with this drug, described elsewhere (18); CTX measurement of circulating plasmablasts, the precursors of mature plasma cells (37), was proposed as a potential biomarker for IgG4-RD, but larger population studies are required to validate this test (33).

PDN, Prednisone; MTX, methotrexate (15–20 mg/week); AZA, azathioprine (2–2.5 mg/kg/day); CTX, cyclophosphamide [intravenous (iv) 1000 mg/m2/month; per os (po) 100 mg/day]; MMF, mycophenolate mofetil (2 g/day); RTX, rituximab (1000 mg in two intravenous doses 15 days apart); CR, complete response; PR, partial response.

*Not effective. †Drug-related toxicity.
administration was derived from the results obtained by the French Vasculitis Study Group on meningeal manifestations of systemic vasculitis (38).

Our experience with DMARDs in IgG4-RD is controversial (Table 5). In effect, although six patients were able to withdraw GCs and to maintain disease remission on DMARDs, six patients flared on DMARDs as well. Moreover, the relative contribution of DMARDs and low-dose PDN in maintaining CR or PR was difficult to assess when these two therapies were combined. Thus, while it is clear that IgG4-RD responds favourably to high-dose GCs, demonstration of the real effectiveness of DMARDs in this specific setting remains to be fully clarified. This would require case–control studies whereby DMARDs were administered alone as induction of remission therapies, similarly to experiments conducted recently by Carruthers et al with RTX (20). Indeed, RTX was used with opposite outcomes in our cohort: a case of renal involvement achieved CR promptly, but a case of orbital pseudotumour did not respond and underwent surgical debulking of the fibrotic mass.

Overall, we currently lack the ability to predict disease response to treatments or to disease relapse. On one hand, our experience suggests that, once disease response is achieved with high-dose GCs, relapse might occur after a few months or even after years on DMARDs or off any specific treatment. In this sense, the role of DMARDs in maintaining disease remission remains controversial, and relapse-free survival might simply reflect the natural history of the disease. On the other hand, researchers have hypothesized that mass-forming IgG4-RD lesions are more likely to shrink in the presence of a prominent lymphoplasmocytic infiltrate (‘active fibroinflammation’), rather than in the presence of tightly organized collagen bundles in which both inflammatory cells and myofibroblasts are rare (‘acellular end-stage fibrosis’ or ‘fibrotic scar’) (21). This is precisely the case of our experience with organs prone to the development of extensive fibrosis (e.g. the retroperitoneum and retro-orbital tissue), where treatment response is known to be less predictable (17). Finally, our study has demonstrated that serum IgG4 level does not correlate with disease activity, and thus it cannot be used as a reliable marker to predict either disease response or disease relapse to treatment (1). In effect, more than 30% of patients with both single and multiple organ involvement had normal serum IgG4 levels at diagnosis. In addition, GCs were effective in the vast majority of patients regardless of the initial serum IgG4 concentration, and long-term remission was also obtained in patients with increased IgG4 levels at baseline.

In conclusion, we describe here the largest Italian cohort of patients with systemic manifestations of IgG4-RD. Our work provides additional insights into the clinical characteristics, natural history, and response to treatment of IgG4-RD in an understudied European population, fostering our knowledge about the epidemiology of this emerging and overlooked entity. Indeed, the absence of correlation between serum IgG4 concentration and disease activity (evaluated by means of the IgG4-RD RI) represents a novel observation in this scenario. Our study also has some limitations, given its retrospective nature, the inclusion of ‘possible’ cases of IgG4-RD, and the lack of standardized treatment in all patients. Further studies on this rare fibro-inflammatory condition are needed to dissect the driving pathogenic mechanisms and to identify optimal therapeutic strategies. To do that, awareness of IgG4-RD is advisable among clinicians and pathologists when facing the differential diagnosis of localized and systemic inflammatory disorders.

References

1. Stone JH, Zen Y, Deshpande V IgG4-related disease. N Engl J Med 2012; 366:539–51.
2. Hamano H, Kawa S, Horuchi A, Unno H, Furuya N, Akamatsu T, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 2001; 344:732–8.
3. Khosroshahi A, Carruthers MN, Stone JH, Shinagare S, Sainani N, Hasserjian RP, et al. Rethinking Ormond’s disease: ‘idiopathic’ retroperitoneal fibrosis in the era of IgG4-related disease. Medicine (Baltimore) 2013; 92:82–91.
4. Lu LX, Della-Torre E, Stone JH, Clark SW IgG4-related hypertrophic pachymeningitis: clinical features, diagnostic criteria, and treatment. JAMA Neurol 2014; 71:785–93.
5. Della-Torre E, Passerini G, Furlan R, Roveri L, Chieffo R, Anzalone N, et al. Cerebrospinal fluid analysis in immunoglobulin G4-related hypertrophic pachymeningitis. J Rheumatol 2013; 40:1927–9.
6. Della-Torre E, Galli L, Franciotta D, Bozzolo EP, Biani C, Furlan R, et al. Diagnostic value of IgG4 indices in IgG4-related hypertrophic pachymeningitis. J Neuroimmunol 2014; 266:82–6.
7. Della Torre E, Bozzolo EP, Passerini G, Doglioni C, Sabbadini MG. IgG4-related pachymeningitis: evidence of intrathecal IgG4 on cerebrospinal fluid analysis. Ann Intern Med 2012; 156:401–3.
8. Hamano H, Umemura T, Uehara T, Kawa S, Kiyosawa K IgG4-related sclerosing cholangitis should be included as an exclusion criterion for the diagnosis of primary sclerosing cholangitis. Am J Gastroenterol 2007; 102:691–2.
9. Wallace ZS, Khosroshahi A, Jakobiec FA, Deshpande V, Hatton MP, Ritter J, et al. IgG4-related systemic disease as a cause of ‘idiopathic’ orbital inflammation, including orbital myositis, and trigeminal nerve involvement. Surv Ophthalmol 2012; 57:26–33.
10. Della-Torre E, Mattoo H, Mahajan VS, Deshpande V, Krause D, Song P, et al. IgG4-related midline destructive lesion. Ann Rheum Dis 2014; 73:1436–4.
11. Carruthers MN, Khosroshahi A, Augustin T, Deshpande V, Stone JH The diagnostic utility of serum IgG4 concentrations in IgG4-related disease. Ann Rheum Dis 2015; 74:14–18.
12. Ngwa TN, Law R, Murray D, Chari ST Serum immunoglobulin G4 level is a poor predictor of immunoglobulin G4-related disease. Pancreas 2014; 43:704–7.
13. Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, et al. Consensus statement on the pathology of IgG4-related disease. Mod Pathol 2012; 25:1181–92.
14. Kamisawa T, Okazaki K, Kawa S, Shimosegawa T, Tanaka M Japanese consensus guidelines for management of autoimmune pancreatitis. III. Treatment and prognosis of AIP. J Gastroenterol 2010; 45:471–7.
15. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. Gastroenterology 2008; 134:706–15.
Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary Table 1. Epidemiological, clinical, and serological features of patients diagnosed with “definite” IgG4-RD.

Supplementary Table 2. Epidemiological, clinical, and serological features of patients diagnosed with “possible” IgG4-RD.

Please note that the editors are not responsible for the content or functionality of any supplementary material supplied by the authors. Any queries should be directed to the corresponding author.