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## ORIGINAL ARTICLE

# Elevated serum IgG4 levels in diagnosis and treatment response in patients with idiopathic retroperitoneal fibrosis

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**Abstract** Idiopathic retroperitoneal fibrosis (iRPF) may be a manifestation of IgG4-related disease. Measuring serum IgG4 (sIgG4) may be of value in monitoring iRPF, but this has scarcely been evaluated. It is unknown if tamoxifen (TMX) affects sIgG4 levels. We performed a prospective inception cohort study of 59 patients with untreated (re)active iRPF stratified by elevated (>1.4 g/L) or normal sIgG4 level. Changes in sIgG4 levels following TMX initiation and, if treatment failed, during subsequent corticosteroid (CS) treatment were analyzed. The median sIgG4 level was 1.1 g/L (interquartile range (IQR) 0.4–2.2); 24 patients (40%) had elevated sIgG4 level. Patients with elevated sIgG4 tended to present with higher ESR (46 vs. 34 mm/h; $P = 0.08$) and more frequent locoregional lymphadenopathy adjacent to the mass (41.7 vs. 20.0%; $P = 0.08$). sIgG4 also correlated with ESR ($\rho = 0.26$; $P = 0.05$) and serum creatinine (SC) ($\rho = 0.26$; $P = 0.04$). Following TMX initiation, sIgG4 level decreased, particularly when achieving treatment success ($P < 0.01$). Odds ratio for TMX treatment success in patients with elevated sIgG4 level was 0.77 (95% CI 0.53–1.14; $P = 0.19$). After adjusting for age, sex, and SC, the odds ratio was 0.78 (95% CI 0.51–1.18; $P = 0.24$). ROC curve analyses of sIgG4 on a continuous scale and treatment success showed an AUC of 0.62. Treatment success and concurrent sIgG4 decrease ($P < 0.01$) were achieved in 78% of patients who converted to CS therapy. Patients with elevated sIgG4 level may be more inflammatory than patients with normal sIgG4 level, but this needs further study. TMX affects sIgG4 levels, but to a lesser extent than CSs. sIgG4 cannot be used as an outcome prediction tool, irrespective of which cutoff value was chosen.

**Keywords** Corticosteroids · IgG4 · Retroperitoneal fibrosis · Tamoxifen

## Introduction

Idiopathic retroperitoneal fibrosis (iRPF) is a rare disorder characterized by chronic non-specific inflammation of the retroperitoneum, which leads to fibrosis. If unrecognized or left untreated, the fibrotic mass may entrap and obstruct retroperitoneal structures [1–4].

Hypotheses about the etiology of iRPF remain diverse. It may be caused by a local disease process based on an inflammatory reaction to substances of atherosclerotic plaques [5–7] or be a manifestation of a systemic disease [8, 9]. In both hypotheses, auto-immune mechanisms are likely to be important in inducing the chronic inflammatory reaction [1, 2, 4, 6, 7].

In a subset of patients, iRPF may be a manifestation of IgG4-related disease (IgG4-RD) [10–17]. IgG4-RD is an increasingly recognized systemic disease entity affecting a large group of different organs and tissues, notably the pancreas, salivary glands, and lung. The pathogenesis of IgG4-RD is

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unclear, but the involvement of an auto-immune process or an underlying allergic reaction is often assumed [18, 19]. Elevated serum IgG4 (sIgG4) levels are suggestive for IgG4-RD [15].

Histopathological features characteristic for IgG4-RD have been shown to be present in retroperitoneal fibrotic lesions [14, 16, 20]. However, routine biopsy is not performed in suspected retroperitoneal fibrosis (RPF) as this diagnosis can usually be made with near certainty with combined clinical and radiological findings [1–4].

Patients with IgG4-RD often have elevated sIgG4 levels, which tend to be higher when multiple organs are involved [15, 20, 21]. There is no consensus as to whether sIgG4 concentration is a reliable indicator for disease activity in IgG4-RD [15, 20], but treatment with corticosteroids (CSs) often results in a decrease of serum levels [22]. As elevated sIgG4 levels are also described in iRPF [16], it would be of interest of these specific cases of RPF which differ from those cases without elevated sIgG4 levels, particularly because biopsy material is typically not available.

Therefore, we assessed whether iRPF patients with normal range sIgG4 levels differ in presentation from iRPF patients with elevated sIgG4 levels. We also assessed whether tamoxifen (TMX), a safe and commonly used alternative for CSs [23], affects sIgG4 levels, which has not yet been investigated, and similarly for CS treatment in those who failed TMX treatment.

## Patients and methods

### Patients

In this prospective inception cohort study, we included all untreated patients with first or recurrent presentation of active iRPF disease who were seen at our tertiary care referral center from September 2010 to February 2016. Patients without active disease or patients who already received medical treatment at the time of referral to our center were excluded from this study. After careful exclusion of malignancy, the diagnosis of iRPF was based on the typical clinical picture and the presence of characteristic computed tomographic (CT) findings. We included patients with peri-aneurysmal fibrosis because this condition is also considered idiopathic [2, 4]. In seven patients, the diagnosis was confirmed histologically. Unless suspected with systemic disease, patients received TMX 20 mg twice daily as primary treatment ($n = 52$, 88%). The prescribed duration of TMX treatment was 2 years. Seven (12%) patients were treated primarily with initial high-dose prednisone (PDN) (60 mg/day) because of suspected (associated) systemic disease (IgG4-RD, $n = 4$; vasculitis, $n = 1$; polymyalgia rheumatic, $n = 2$). In addition to specific baseline

examination, the prescribed follow-up included periodic clinical and laboratory examinations at 6 weeks and at 4 and 8 months of treatment, respectively, and repeated CT scanning at 4 and 8 months of treatment. If indicated, (emergency) renal drainage was also performed, either with placement of a percutaneous nephrostomy tube or a double-J splint, sometimes in sequence. Ureteral stents were changed every 4–6 months until definitive removal. $^{18}$F-fluorodeoxyglucose (FDG)-PET scanning was performed in selected cases only, since the recent study showed no additional value of FDG-PET scanning in the routine work-up of iRPF disease [24]. Other organ involvement was suspected on the patients' history and/or high sIgG4 levels and documented by additional imaging studies (i.e., thoracic CT and/or whole-body nuclear scanning). TMX treatment failures received initial high-dose PDN (60 mg/day), either alone or combined with mycophenolate mofetil (MMF, 750–1000 mg twice daily). The protocol was approved by the ethical committee of the Albert Schweitzer Hospital. Patients provided informed consent prior to the start of treatment. The study was in accordance with the principles of the Helsinki Declaration.

### Computed tomography

All CT scans were independently reviewed by one experienced radiologist who was unaware of the patient's status. If initial CT scan was performed at the referral center, imaging studies were retrieved. The following variables from these imaging studies were assessed: localization of the soft-tissue mass; presence of hydro-ureteronephrosis; presence of infrarenal aneurysmal aortic dilation, defined as an aortic diameter ≥30 mm; presence of locoregional lymphadenopathy adjacent to the retroperitoneal mass; and any other relevant intra-abdominal findings. The maximal thickness of the retroperitoneal mass was measured in three different view directions (i.e., anterior-posterior, left lateral, and transversal directions). For follow-up CT scans, measurements were made at the same levels and by using the same method used for the first CT scan. To further evaluate absolute and percentual changes of the mass within patients during follow-up, the largest value of the initial three different measurements of maximal thickness was compared to the thickness of the mass at each follow-up CT scan; for this comparison, we used the same measurement direction and the same level from which the largest value was obtained on the first CT scan.

### Pathologic examination

From seven study patients (16%), retroperitoneal tissue samples were available, of which the original hematoxylin and eosin slides were reviewed for the presence of the following specific histopathologic features: lymphoplasmacytic infiltrate, storiform fibrosis, obliterative phlebitis, tissue

eosinophilia (>5 hpf), neutrophilic infiltration (>10 hpf), and lymph follicles with a germinal center, granuloma, and multinucleated giant cells. We also examined the samples for the degree of inflammation defined as follows: 0, minimal inflammatory cell infiltration; 1+, focal accumulation of inflammatory cells; 2+, between 1+ and 3+; and 3+, diffuse inflammatory cell infiltration [16]. In addition, immunostaining for IgG4 and IgG was performed using a mouse monoclonal antibody against human IgG4 (Zymed Laboratory, Inc., San Francisco, CA) and a rabbit monoclonal antibody against IgG (Dako Cytomation, Glostrup, Denmark). The degree of IgG4$^+$ and IgG$^+$ plasma cells and the IgG4$^+$/IgG$^+$ plasma cell ratio were assessed in three non-overlapping hpfs with intense infiltration (10× eyepiece and 40× lens). We specifically assessed the presence of >30 IgG4$^+$ plasma cells/hpf and a IgG4$^+$/IgG$^+$ plasma cell ratio of >40% in tissue samples, as these are well-recognized features of RPF as part of IgG4-RD [25]. Immunohistochemical investigation was performed to assess the presence of estrogen and progesterone receptors (CONFIRM anti-ER [SP1] and anti-PR [1E2], Ventana Medical Systems, Tucson, USA).

## Measurements

Age, sex, clinical signs and symptoms, duration of symptoms, VAS scores for pain and discomfort (e.g., significant loss of subjective well-being), weight (kg), length (cm), body mass index (kg/m$^2$), weight loss (kg/months), specific laboratory parameters, CT scan findings, and if performed, nuclear scanning results were recorded as baseline. Laboratory parameters included the erythrocyte sedimentation rate (ESR, mm/h), C-reactive protein (CRP) level (mg/L), serum creatinine (SC) (μmol/L), and total sIgG and sIgG4 subclass level (g/L). sIgG4 subclass levels were measured by nephelometry (Siemens Healthcare Diagnostics, the Siemens BN™ II System), using validated reference ranges with 1.4 g/L being the upper limit of normal sIgG4 concentration [26]. Follow-up measurements included clinical improvement, changes in laboratory parameters, and follow-up CT scan findings. The primary endpoint was remission induced by TMX therapy. Remission was defined as significant clinical improvement within 6 weeks of treatment, documented stable or decreasing mass size on the prescribed follow-up CT scan at 4 months, and documented definitive decrease in mass size on the prescribed follow-up CT scan at 8 months. All three criteria had to be met. Because of unclear predictive value, results of follow-up acute-phase reactant (APR) levels were not part of the predefined criteria for remission [27]. Treatment outcome was analyzed in study patients receiving TMX monotherapy who had at least 8 months of follow-up. Secondary endpoints included changes in follow-up clinical, laboratory, and radiological variables. Patients who received primary treatment with initial high-dose PDN and who had sufficient follow-up

were included in the treatment outcome analysis of patients who received CS-based second-line treatment.

## Statistical analyses

Because of non-Gaussian distribution of most parameters, continuous variables were reported as median and 25th to 75th percentiles (interquartile range, IQR). Differences between continuous variables were analyzed by using the Mann-Whitney or Wilcoxon signed-rank sum tests, where appropriate. Categorical variables were expressed as proportions and compared with Fisher's exact test. Correlations were analyzed using Spearman's rank correlation coefficient. The association between TMX treatment success and elevated sIgG4 levels according to the commonly used threshold of 1.4 g/L was determined using univariate and multivariate binary logistic regressions. The independent variables in the multivariate regression model were chosen because of their clinical relevance and included age, sex, and pretreatment SC level. Variance inflation factors were calculated to assess the degree of multicollinearity among these independent variables in the multivariate logistic regression model. In addition, a receiver operating characteristic (ROC) curve was constructed to visualize the utility of sIgG4 levels in predicting treatment success. The baseline sIgG4 level was used on a continuous scale in the ROC curve to investigate the possibility of an optimum sIgG4 threshold to predict treatment success. All reported $P$ values are two-sided. A $P$ value of <0.05 was considered significant. All statistical analyses were performed with SPSS software (version 17.0; SPSS Inc., Chicago, IL).

## Results

### Baseline characteristics

Demographic and clinical characteristics of the 59 study patients according to the sIgG4 levels at presentation are depicted in Table 1. The median sIgG4 level was 1.1 g/L (IQR 0.4–2.2); 24 patients (40%) had elevated sIgG4 level. Male percentage was higher in patients with elevated sIgG4 levels compared to that in patients with normal range sIgG4 levels, albeit not statistically significant (Table 1). Overall, male patients tended to have higher sIgG4 levels compared to female patients (males, 1.13 g/L [IQR 0.6–2.3] vs. females, 0.69 g/L [IQR 0.4–0.6]; $P = 0.10$).

Groups with normal range or elevated baseline sIgG4 levels had a similar duration of symptoms and similar VAS scores for pain and discomfort. Median ESR level at presentation tended to be higher in patients with elevated sIgG4 levels compared to that in patients with normal range sIgG4 levels (Table 1). Baseline sIgG4 levels also correlated significantly with the ESR ($\rho = 0.26$; $P = 0.05$). In addition, baseline

**Table 1** Demographic, clinical, and radiological characteristics of study patients

| | All patients | Serum IgG4 level at presentation | | P value |
| | | Normal range | Elevated[a] | |
| --- | --- | --- | --- | --- |
| No. of patients, n | 59 | 35 | 24 | |
| First presentation/recurrence | 53/6 | 30/5 | 23/1 | 0.38 |
| Age, years | 61.0 (57.0–69.0) | 62.0 (54.5–69.0) | 61.0 (58.0–66.8) | 0.71 |
| Male gender, n (%) | 39 (66.1) | 21 (60.0) | 18 (75.0) | 0.27 |
| Duration of symptoms, months | 4.5 (2.0–11.0) | 4.0 (2.0–11.0) | 5.0 (3.0–10.0) | 0.58 |
| Visual analog scale score, mm | | | | |
| Pain | 70.0 (44.8–85.0) | 70.0 (43.0–80.0) | 70.0 (42.5–90.0) | 0.47 |
| Discomfort | 60.0 (39.5–77.5) | 55.0 (33.0–70.0) | 60.0 (50.0–85.0) | 0.40 |
| Weight loss, kg | 4.0 (0.0–7.0) | 4.0 (0.0–8.0) | 1.0 (0.0–7.0) | 0.84 |
| Acute-phase reactant levels | | | | |
| ESR, mm/h | 39.5 (20.8–62.5) | 34.5 (16.0–56.3) | 46.0 (32.3–64.0) | 0.08 |
| CRP, mg/L | 7.0 (5.0–23.5) | 6.0 (5.0–23.5) | 9.0 (5.0–26.0) | 0.44 |
| Immunoglobulin G, g/L | | | | |
| Total IgG | 14.5 (12.1–17.4) | 12.7 (10.1–14.5) | 17.5 (15.7–23.1) | 0.00 |
| IgG4 | 1.1 (0.4–2.2) | 0.6 (0.3–0.9) | 2.7 (1.7–6.6) | 0.00 |
| IgG4/IgG ratio | 0.08 (0.04–0.13) | 0.05 (0.02–0.07) | 0.15 (0.10–0.27) | 0.00 |
| Renal function | | | | |
| Creatinine, μmol/L | 92.0 (74.0–122) | 86.0 (73.0–114.0) | 99.5 (77.8–137.0) | 0.16 |
| Hydro-ureteronephrosis, n (%) | 31 (54.2) | 18 (51.4) | 14 (58.3) | 0.79 |
| D-J splint or PNS, n (%) | 16 (27.1) | 9 (25.7) | 7 (29.1) | 0.77 |
| Abdominal CT findings | | | | |
| Thickness RPF mass, mm | 29.5 (20.3–48.0) | 32.0 (17.0–49.5) | 29.0 (22.0–45.0) | 0.73 |
| Abdominal infrarenal aortic diameter, mm | 22.0 (19.3–29.8) | 24.5 (18.0–33.8) | 21.5 (20.0–24.5) | 0.19 |
| Atypical retroperitoneal localization, n (%) | 11 (18.6) | 6 (17.1) | 5 (20.8) | 0.74 |
| Locoregional lymphadenopathy, n (%) | 17 (28.8) | 7 (20.0) | 10 (41.7) | 0.08 |
| Extra-retroperitoneal localization, n (%) | 10 (16.9) | 4 (11.4) | 6 (25.0) | 0.28 |
| Success tamoxifen therapy[b], n (%) | 27/47 (57.4) | 19/31 (61.3) | 8/16 (50.0) | 0.54 |

The values are median and interquartile range (25th to 75th percentiles) or counts and percentage, where appropriate

[a] Defined as serum IgG4 level >1.4 g/L

[b] Treatment success was analyzed in study patients with at least 8 months of follow-up

sIgG4 levels correlated significantly with the SC level ($\rho = 0.26$; $P = 0.04$). CT scanning in patients with elevated sIgG4 levels more often showed locoregional lymphadenopathy adjacent to the mass compared to that in patients with normal range sIgG4 levels, albeit of borderline significance (Table 1). CT-documented retroperitoneal mass thickness did not differ between groups (Table 1) nor did sIgG4 levels correlate with the CT-documented mass thickness ($\rho = -0.12$, $P = 0.93$).

FDG-PET scanning was performed in 10 patients (16.9%). All scans showed pathologic uptake at the level of the CT-documented retroperitoneal mass of which three patients had normal range serum IgG4 levels and seven patients had elevated sIgG4 levels. Six patients who underwent FDG-PET scanning had extra-retroperitoneal pathologic FDG uptake, including the lungs, oropharyngeal area, soft tissues, pancreas,

and biliary system, of which two (33.3%) patients had normal range sIgG4 levels and four (66.6%) had elevated sIgG4 levels ($P = 0.80$).

**Follow-up**

Two of 59 patients were lost to follow-up after 6 weeks and 4 months of TMX therapy, respectively; three TMX-treated patients had insufficient follow-up time to analyze treatment success or failure. Of 47 patients with sufficient follow-up who were receiving TMX as primary treatment, 20 patients (42.6%) had TMX treatment failure. The median overall follow-up was 24 months (IQR 12–24). The percentage of patients who had treatment failure did not differ significantly between groups with normal range or elevated sIgG4 levels at presentation (Table 1). However, patients who had TMX

treatment failure tended to have higher sIgG4 levels and had significantly higher ESR and CRP levels (both $P < 0.05$) at baseline compared to patients who had treatment success (Table 2). Eighty percent of patients with treatment failure were males. This differed from the percentage of males in patients who had TMX treatment success (51.9%) ($P = 0.06$). The odds ratio for treatment success in male patients was 0.27 (95% CI 0.07–1.02; $P = 0.05$). The dose of TMX per kilogram body weight did not differ between patients with treatment success or treatment failure (0.59 mg/kg [IQR 0.45–0.58] vs. 0.46 mg/kg [IQR 0.43–0.55]; $P = 0.29$). The odds ratio for TMX treatment success in patients with elevated sIgG4 level was 0.77 (95% CI 0.53–1.14; $P = 0.19$). After adjusting for age, sex, and SC (Table 3), the odds ratio was 0.78 (95% CI 0.51–1.18; $P = 0.24$). All variation inflation factors were less than 1.5; hence, no multicollinearity existed between variables used in the multivariate logistic regression. ROC curve analyses of sIgG4 level and treatment outcome showed an AUC of 0.62 (Fig. 1).

Following TMX initiation, total IgG and IgG4 subclass levels decreased significantly, which persisted during follow-up in patients who had treatment success, but not in patients who had treatment failure (Table 2). There was also a significant decrease in ESR and CRP levels following the initiation of TMX in both groups (Table 2). In both the success and the failure groups, there were no significant correlations between the absolute or percentual decreases of sIgG4 levels and the absolute or percentual decreases in the ESR (data not shown).

Eighteen patients switched to second-line treatment (prednisone, $n = 15$; prednisone/MMF, $n = 3$). The median time to

treatment switch was 7.0 months (IQR 4.0–9.0). One patient had failure of TMX treatment but chose not to start second-line treatment. At the time of treatment switch in patients who had TMX treatment failure, the median sIgG4 level did not differ from the sIgG4 level at presentation ($P = 0.86$). Four of the seven patients who received primary treatment with prednisone had sufficient follow-up and were included in the outcome analysis of immunosuppressive treatment. Following the initiation of second-line treatment, there was a notable and significant decrease in total sIgG and sIgG4 levels (Table 4). Fourteen of 18 patients (77.8%) had treatment success with second-line treatment. Two patients had insufficient follow-up time to conclude success or failure of second-line treatment. We found no significant correlation between sIgG4 levels and CT-documented mass thickness at subsequent follow-up moments after switch to second-line therapy, nor did we find a correlation between absolute and percentual decreases in sIgG4 levels and absolute and percentual decreases of the mass thickness (data not shown). Two (3.4%) patients died during follow-up: one from septicemia and one from hemorrhagic shock.

## Pathology

The main results of histopathologic examination are depicted in Table 5. All tissue samples exhibited a dense lymphoplasmacytic infiltrate with fibrosis, in three cases accompanied by tissue eosinophilia. A storiform-type pattern of fibrosis was observed in one case only; none of the tissue samples exhibited obliterative phlebitis. In patients who exhibited >50

**Table 2** Changes in acute-phase reactant, serum IgG and IgG4 subclass levels, and IgG4/IgG ratio during tamoxifen therapy

|  | Presentation | Follow-up | | |
|---|---|---|---|---|
|  |  | 6 weeks | 4 months | 8 months |
| Success tamoxifen, $n$ | 27 | 27 | 26 | 23 |
|    ESR, mm/h | 31.0 (15.0–50.0) | 13.0 (6.0–26.0)** | 6.5 (5.0–15.0)** | 5.0 (5.0–15.5)** |
|    CRP, mg/L | 5.0 (5.0–23.5) | 5.0 (5.0–10.0)** | 5.0 (5.0–5.0)* | 5.0 (5.0–5.0)** |
|    Total IgG, g/L | 12.9 (10.2–17.1) | 13.9 (11.1–16.6) | 12.1 (10.0–14.3)** | 11.7 (9.2–14.1)** |
|    IgG4, g/L | 0.7 (0.3–1.5) | 0.8 (0.3–1.7) | 0.7 (0.1–1.3)* | 0.6 (0.2–1.1)** |
|    IgG4/IgG ratio | 0.05 (0.02–0.09) | 0.06 (0.03–0.10) | 0.06 (0.02–0.09) | 0.05 (0.02–0.08)* |
| Failure tamoxifen, $n$ | 20 | 18 | 18 | 17 |
|    ESR, mm/h | 64.0 (22.0–85.0) | 31.0 (21.3–49.0)** | 27.0 (12.0–37.0)** | 26.5 (7.3–37.3)** |
|    CRP, mm/h | 9.5 (5.0–50.8) | 5.0 (5.0–17.0)** | 5.0 (5.012.0)** | 5.0 (5.0–13.8)** |
|    Total IgG, g/L | 14.9 (12.3–18.3) | 13.8 (11.1–18.5) | 12.5 (10.6–16.3) | 11.4 (8.0–13.3)** |
|    IgG4, g/L | 0.9 (0.5–2.8) | 0.9 (0.5–2.0) | 0.8 (0.3–2.3)* | 0.6 (0.3–1.2) |
|    IgG4/IgG ratio | 0.06 (0.04–0.18) | 0.06 (0.04–0.13) | 0.06 (0.02–0.15) | 0.05 (0.02–0.11) |

The depicted values are levels of APR, IgG, and IgG4 subclass levels while patients were on active tamoxifen treatment. The values are median and interquartile range (25th to 75th percentiles)

*APR* acute-phase reactant, *n* number of study patients

*$P < 0.05$, vs. similar variable at presentation; **$P < 0.01$, vs. similar variable at presentation

**Table 3** Association of elevated sIgG4 level at presentation and tamoxifen treatment success

| Variable | Unadjusted | | | Adjusted | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *P* value | OR | 95% CI | *P* value |
| Age | 0.95 | 0.90–1.02 | 0.14 | 0.98 | 0.91–1.05 | 0.55 |
| Male sex | 0.27 | 0.07–1.02 | 0.05 | 0.40 | 0.10–1.63 | 0.20 |
| Serum creatinine | 1.00 | 0.98–1.01 | 0.46 | 1.00 | 0.99–1.01 | 0.52 |
| Elevated sIgG4[a] | 0.77 | 0.53–1.14 | 0.19 | 0.78 | 0.51–1.18 | 0.24 |

*OR* odds ratio, *CI* confidence interval

[a] Defined as sIgG4 >1.4 g/L

plasma cells/hpf in tissue samples, median sIgG4 levels were higher compared to those in patients with ≤50 plasma cells/hpf in tissue samples (3.1 vs. 0.39 g/L; $P = 0.05$) (Table 4). In none of the tissue samples, neutrophilic infiltration, granulomas, or multinucleated giant cells were observed. In tissue samples of one patient, lymph follicles with a germinal center were observed. Estrogen and progesterone receptors were not detected immunohistochemically in tissue samples.

## Discussion

As biopsy of the retroperitoneal lesion is not routinely performed in diagnosing RPF, we aimed to assess the usefulness of sIgG4 levels in differentiating possible IgG4-related RPF from iRPF in terms of clinical and radiological presentations.
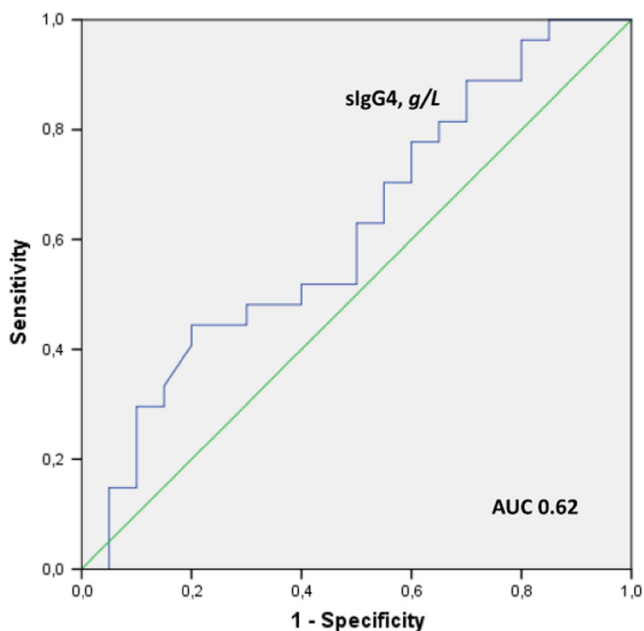


**Fig. 1** ROC curve analyses of sIgG4 levels on a continuous scale and tamoxifen treatment success. The area under the ROC curve is 0.62. The ROC curve shows no cutoff value of sIgG4 level with satisfying sensitivity and/or specificity

In addition, we evaluated if sIgG4 levels were affected during treatment with TMX and, if this treatment failed, subsequent second-line treatment.

Although patients with normal range or elevated sIgG4 baseline levels had comparable duration and intensity of symptoms, patients with elevated sIgG4 levels appear to present with a higher ESR and with more frequent locoregional lymphadenopathy adjacent to the mass. The baseline sIgG4 level also correlated significantly with the ESR and SC level. The higher ESR may, in part, be explained by the hemorheologic influence of plasma globulins on the ESR [28], but taken together, this may also mean that patients with elevated sIgG4 levels are more inflammatory. Baseline sIgG4 levels tended to be higher in men compared to women, which were also found by others [10, 16]. In addition, others found a male predominance in histopathologically confirmed IgG4-related RPF [10, 16, 29–32].

The fibrotic mass in iRPF is typically localized around the abdominal aorta and/or iliacal arteries [1, 3–5]. We found no difference in atypical localization of the retroperitoneal mass between patients with normal range and patients with elevated sIgG4 levels, which was also found by others [12, 13, 16, 32, 33]. As noted, patients with elevated sIgG4 levels more often had locoregional lymphadenopathy adjacent to the RPF mass compared to patients with normal range sIgG4 levels. This phenomenon has been noted at other affected organs in IgG4-RD, notably auto-immune pancreatitis [15, 34]. One patient had involvement of axillary, mediastinal, hilar, para-aortic, and infrarenal nodes and also had a typical peri-aortic and peri-iliacal lesion with an additional sIgG4 level of 7.40 g/L. Generalized lymphadenopathy can be the sole component of the clinical presentation in IgG4-RD [15].

Like others [13, 32], we found no correlation between sIgG4 levels and CT-documented RPF mass thickness. Hence, it appears that extensiveness of the retroperitoneal lesion cannot be determined on the basis of sIgG4 levels, but this may only be true for IgG4-related RPF. Others found that patients with active RPF disease more often have normal sIgG4 levels compared to other organ manifestations of IgG4-RD [29]. In auto-immune pancreatitis, Matsubayashi

**Table 4** Changes in acute-phase reactant, serum IgG and IgG4 subclass levels, and IgG4/IgG ratio after switch to immunosuppressive treatment

| | At the start of immunosuppressive therapy[a] | Follow-up[a] | |
| --- | --- | --- | --- |
| | | First follow-up visit | End of follow-up |
| No. of patients[b], n | 22 | 21 | 19 |
| ESR, mm/h | 39.00 (23.25–60.50) | 14.00 (8.00–28.50)** | 16.00 (8.00–27.00)** |
| CRP, mg/L | 5.00 (5.00–15.50) | 5.00 (5.00–5.00)* | 5.00 (5.00–5.00) |
| Total IgG, g/L | 51.31 (11.95–21.24) | 11.03 (7.79–12.51)** | 9.07 (6.89–11.53)** |
| IgG4, g/L | 1.00 (0.37–3.09) | 0.70 (0.30–1.61)** | 0.42 (0.20–1.04)** |
| IgG4/IgG ratio | 0.07 (0.04–0.15) | 0.06 (0.04–0.15)** | 0.05 (0.03–0.11)** |

The values are in median and interquartile range (25th to 75th percentiles)

*n* number of patients

[a] The median time from presentation to the start of second-line treatment was 4.0 months (IQR 1.0–7.0), and the time interval from switch of therapy to the first follow-up visit amounted to 3.0 months (IQR 1.0–4.0) and that to the last follow-up visit amounted to 7.0 months (IQR 4.0–12.0)

[b] Four patients who received prednisone as primary treatment were included in this analysis

*$P < 0.05$, vs. similar variable at the time of start of immunosuppressive treatment; **$P < 0.01$, vs. similar variable at time of start of immunosuppressive treatment

et al. found significantly larger pancreatic lesions in patients with high sIgG4 levels [35]. Other authors suggested that a reduction of the organs or tissues involved in IgG4-RD may contribute to a decrease of sIgG4 levels [36]. In the present study, the percentage of patients with extra-retroperitoneal lesions did not differ between patients with elevated or normal range sIgG4 levels. However, others found a positive correlation between the number of affected organs in histopathologically confirmed IgG4-RD with systemic disease and sIgG4 levels, notably in auto-immune pancreatitis [21, 29–31, 34, 37].

As opposed to CS treatment, which is the first treatment of choice in IgG4-RD including IgG4-related RPF [15, 16], no studies have been published concerning TMX treatment of suspected or confirmed IgG4-related iRPF. The success percentage of TMX treatment did not differ between patients with elevated and normal range baseline sIgG4 levels. However, patients with TMX treatment failure had higher APR levels and non-significantly higher baseline sIgG4 levels compared to those in patients with TMX success. Patients with lower sIgG4 levels may respond better to TMX because they have early and/or limited RPF disease or that it concerns burned-out

**Table 5** Serum IgG4 levels and major pathologic findings in retroperitoneal tissue samples of patients with idiopathic retroperitoneal fibrosis

| Age/sex | Serum IgG4, g/L | Storiform fibrosis | Obliterative phlebitis | Tissue eosinophilia[a] | Degree of inflammation[b] | Plasma cells >50/hpf | Proposed cutoff immunostaining[c] | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | | | | IgG4+ plasma cells >30/hpf | IgG4+/IgG+ plasma cell ratio >40% |
| 55/M | 0.73 | No | No | Yes | 3+ | No | No | N/A |
| 51/F | 0.39 | No | No | No | 2+ | No | No | N/A |
| 45/F | 3.14 | No | No | No | 3+ | Yes | No | No |
| 65/M | 4.09 | No | No | No | 2+ | Yes | No | No |
| 48/M | 1.13 | Yes | No | Yes | 3+ | Yes | No | No |
| 65/F | 0.04 | No | No | Yes | 3+ | No | No | N/A |
| 62/F | 3.06 | No | No | No | 3+ | Yes | No | No |

*hpf* high power field, *N/A* insufficient material available for assessment of IgG positivity

[a] Defined as >5 eosinophils/hpf

[b] Defined as follows: 0, minimal inflammatory cell infiltration; 1+, focal accumulation of inflammatory cells; 2+, between 1+ and 3+; and 3+, diffuse inflammatory cell infiltration [16]

[c] Proposed as the appropriate cutoff for the number of IgG4+ plasma cells and IgG4+/IgG+ plasma cell ratio in diagnosing RPF as part of IgG4-related disease [38]

IgG4-related lesions [16, 17, 36, 38]. Another theory may be that iRPF disease in patients with low sIgG4 levels might not be a manifestation of IgG4-related disease at all. Of note, however, our analyses also showed that sIgG4 levels per se cannot be used as a predictive tool, irrespective of which cutoff value was chosen.

The female percentage of patients that responded satisfactorily to TMX treatment amounted to 48%, compared to only 9% of the patients who had TMX treatment failure. Estrogen and progesterone receptors were not detected immunohistochemically in tissue samples in the present study, underlining the hormone-independent action of TMX on RPF disease [39]. Female patients tended to have lower sIgG4 levels than male patients in our study. These combined findings suggest that female patients may have had successful TMX treatment because they had less severe or less extended inflammation or that IgG4-related disease is more frequent in men compared to women [10, 16]. Of note, treatment success or failure could not be explained by a difference in TMX dose per kilogram body weight.

sIgG4 levels were significantly decreased at 8 months of follow-up in patients who responded well on TMX, although baseline levels were relatively low in this group. sIgG4 levels also decreased, albeit not significantly at 8 months of follow-up, in patients who had TMX treatment failure. Patients with partial treatment response may have accounted for the overall decrease in sIgG4 levels in the TMX failure group, i.e., patients who experienced amelioration of symptoms and discrete or moderate but ultimately insufficient radiological mass regression. Our data illustrate that the presumed anti-inflammatory/immunologic activity of TMX includes an effect on sIgG4 levels. In all patients in whom TMX was converted to CSs, there was a notable and significant decrease in sIgG4 levels, which has previously been described in other manifestations of IgG4-RD [22, 36, 37], but also in non-IgG4-RD disease conditions [30]. The majority of our patients treated with CSs subsequently had treatment success. Hence, CSs should probably be used primarily in patients with high initial sIgG4 levels.

The value of sIgG4 levels for diagnosing IgG4-RD is still controversial. Previous studies have shown that up to 20–40% of patients with histopathologically confirmed IgG4-RD, notably auto-immune pancreatitis, have normal range sIgG4 levels, probably because of early and/or limited disease or because there is often a delay between the onset of inflammation and the performance of a biopsy [13, 27, 34, 40]. Also, in patients with biopsy-proven IgG4-related RPF, sIgG4 levels may be normal [29]. Conversely, elevated sIgG4 levels have been found in healthy individuals [15, 38, 40, 41]. Hence, elevated sIgG4 levels are suggestive but not sufficient for diagnosing IgG4-RD. However, reasonable sensitivity and specificity numbers have been demonstrated for the cutoff value of >1.4 g/L (67–97 or 73–100%) [22, 30, 38], but these cohorts were focussed on pancreatic lesions and represented hardly any IgG4-related RPF cases. There are no studies available on sensitivity and specificity of elevated sIgG4 levels in definite IgG4-related RPF. Such a study will be difficult to perform as routine biopsy material is not required in suspected RPF [3, 4, 16].

In our study cohort, biopsy was precluded for atypical cases, mainly to exclude malignancy [3, 4, 16], and patients with high suspicion of IgG4-related RPF were treated with CSs as primary therapy. As in IgG4-RD, CS therapy is also the most commonly used and effective therapy for iRPF [24]. Hence, histopathological confirmation of IgG4-related iRPF would not have therapeutic consequences. Consistent histopathological features of IgG4-RD include lymphoplasmacytic infiltrate, IgG4-positive plasma cells, storiform-type pattern of fibrosis, obliterative phlebitis, modest tissue eosinophilia, and the tendency to form tumefactive lesions, characteristic features that are also found in RPF lesions [15, 16]. Hypothetically, RPF lesions without IgG4-positive plasma cell infiltration may be long-standing, burned-out IgG4-related lesions [16, 17].

Although sometimes suggestive, in none of our available tissue samples, a histopathological diagnosis of definite IgG4-related RPF according to the recent consensus criteria could be made [38]. Two small comparative clinicopathological studies in IgG4-related RPF patients with respect to sIgG4 levels in 10 and 17 patients, respectively, revealed contradictory results [13, 16]. Khosroshahi et al. found no difference in sIgG4 levels between patients with histopathologically confirmed IgG4-related and non-IgG4-related RPF [13]. Conversely, Zen et al. found elevated sIgG4 levels in all patients with histopathologically confirmed IgG4-related RPF compared to none of the patients with non-IgG4-related RPF [16]. Differing findings may, in part, be explained by a different approach to accruing tissue samples, i.e., through percutaneous needle biopsy versus surgical biopsy, the first giving a higher chance of sampling error [16]. In the study of Khosroshahi et al. [13], absolute numbers of IgG4^{+} plasma cells were low in retroperitoneal biopsies (mean 13 IgG4^{+} plasma cells/HPF) compared to those at the other sites for IgG4-RD involvement, such as the lacrimal or submandibular glands. Indeed, RPF lesions are often highly fibrotic and therefore scarce in IgG4^{+} plasma cells [25], which might explain why none of our samples had >30 IgG4^{+} plasma cells per hpf despite the elevated sIgG4 level. We found that abundant plasma cell infiltration was associated with higher sIgG4 levels.

Given the abovementioned data and the typical absence of histological confirmation of presumed iRPF in clinical practice, unless part of suspected systemic disease, we argue that TMX may be used as primary treatment, particularly in cases of (relative) contraindications to long-

term CS use. Given our success rate with TMX, a substantial number of patients will not need prolonged CS treatment with its potentially hazardous complications. Whether patients with normal range sIgG4 levels are most responsive to TMX treatment needs further study. It may be that patients with higher sIgG4 levels are more inflammatory and more therapy-resistant, hence needing more intensive treatment [42, 43]. For this, we prefer to add MMF to CSs as accumulating data suggest this combination as efficacious and safe [44]. Although less data are available, adding methotrexate is also an option [45]. Mortality was low in this cohort of medically treated iRPF patients, which conforms other studies [46, 47].

The present clinical study has several limitations. Although this is the largest prospective study to date in investigating the role of sIgG4 levels in iRPF disease, the number of patients is still small. This can be explained by the low incidence of 1.3 per 100,000 inhabitants [4]. We did not routinely perform thoracic CT or whole-body nuclear scanning. Although medical history, physical examination, laboratory results, chest radiograph results, and results of abdominal CT scanning, including the lower pulmonary regions, were available in all cases, we may have missed possible other (i.e., extra-retroperitoneal) organ involvements in some patients. Another limitation is the absence of RPF tissue samples from all patients. As explained, however, it is commonly accepted that routine biopsy is not indicated in diagnosing suspected iRPF disease [2, 3]. Additionally, biopsy of IgG4-related RPF is often not conclusive because of the highly fibrotic character of these lesions [25].

In summary, 40% of patients with iRPF had elevated sIgG4 levels. Patients with elevated sIgG4 levels appear to present with higher ESR levels and more frequent locoregional lymphadenopathy adjacent to the mass, possibly indicating more intense inflammation. TMX affects sIgG4 levels as part of its presumed immunologic activity. Patients who had TMX treatment failure tended to have higher baseline sIgG4 levels, were more often male, and responded well to subsequent CS therapy with a concurrent notable decrease in sIgG4 levels, which suggest that CSs should be used primarily in these cases. Pretreatment sIgG4 levels cannot be used as an outcome prediction tool, irrespective of which cutoff value was chosen. Whether sIgG4 is able to sufficiently discriminate different clinical subsets of iRPF patients needs further study.

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