

# Elevated Immunoglobulin G4 Levels in Patients with Thyroid Eye Disease and Their Clinical Implications

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**PURPOSE.** The purpose of this study was to assess the relationship between thyroid eye disease (TED) in patients undergoing orbital decompression and immunoglobulin G4 (IgG4) levels.

**METHODS.** A prospective observational cohort study was conducted among 185 consecutive patients who were diagnosed with TED and underwent orbital decompression. Serum levels of IgG4 were measured, and immunohistochemical staining for IgG and IgG4 was performed in orbital adipose tissue. Data related to clinicopathologic features were analyzed.

**RESULTS.** Among the 185 enrolled patients with TED, 64 (34.6%) were IgG4-positive. The IgG4-positive patients were older, had higher clinical activity scores (CAS), and had worse best-corrected visual acuity (BCVA) than the IgG4-negative patients. Higher thyrotropin receptor antibody (TRAb) levels, histopathological IgG4 counts, IgG4/IgG ratios, and dense lymphocyte infiltration were more frequently observed in IgG4-positive than in IgG4-negative patients. Definitive and probable IgG4 subtypes were independently associated with the active stage in patients with TED.

**CONCLUSIONS.** Our data suggest that the IgG4 subtype in TED is common. IgG4-positive patients with TED may be older, have more severe disease, and have higher clinical activity scores. IgG4 may play an important role in the pathogenesis of TED.

**Keywords:** thyroid eye disease, immunoglobulin G4, clinicopathological features

Immunoglobulin G4-related disease (IgG4-RD) has recently been increasingly recognized as a multisystem fibro-inflammatory disorder characterized by typical immunoglobulin G4 (IgG4)-positive lymphoplasmacytic infiltration.<sup>1</sup> Ophthalmic involvement is common in IgG4-RD and has always been described as orbital inflammatory disease or a pseudotumor that can affect nearly every orbital structure.<sup>2</sup> Thyroid eye disease (TED) is one of the most common orbital inflammatory diseases. Studies of elevated serum IgG4 levels in the presence of TED compared with their absence in Graves' disease (GD) have suggested that IgG4 levels are associated with the development of TED in patients with GD.<sup>3,4</sup> Additionally, in an American study of a small group of patients with TED (24 cases), 20.8% of patients demonstrated serum IgG4 levels consistent with IgG4-RD without any additional systemic disease, and Irwin et al reported a patient with TED with significant IgG4 staining in the levator palpebrae superioris.<sup>5</sup> The above findings suggest that there may be a possible subtype of patients with TED with elevated pathological or serological IgG4 levels.<sup>6</sup> However, studies based on a large number of cases with IgG4 levels in the TED subgroup remain limited. Moreover, data regarding patients with TED with both pathological and serological IgG4 levels measured have not been previously reported.

To determine whether there is a subtype of patients with TED with elevated IgG4 levels and identify the clin-

icopathological features of this subtype, we prospectively and simultaneously assessed the levels of serum IgG4 and IgG4-positive plasma cells in orbital tissues obtained in 185 consecutive patients with TED and assess the possible association between IgG4 levels and TED.

## SUBJECTS AND METHODS

### Patients

This prospective observational cohort study was performed in consecutive patients with TED who underwent orbital decompression and were simultaneously assessed for blood and orbital adipose tissue between October 2017 and December 2018. The diagnosis of TED was based on their clinical, laboratory, and imaging findings.<sup>7</sup> The exclusion criteria of the study were as follows: (1) prior surgery on the operative eye, (2) steroid or immunosuppressive agent use within the last 6 months, (3) pregnancy or lactation, (4) active infection, (5) with any kind of immunodeficiency disorder, and (6) evidence of any unresolved medical problem that can affect IgG4 levels. Approval was obtained in accordance with the Declaration of Helsinki and the Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University. Written informed consent was obtained from each patient.

## Laboratory Evaluation

Serum levels of total triiodothyronine (TT3), total thyroxine (TT4), free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), thyrotropin receptor antibody (TRAb), and thyroid peroxidase antibody (TPOAb) were measured by electrochemiluminescent immunoassays (Roche, Rotkreuz, Switzerland). Antithyroglobulin antibody (TgAb) levels were measured with a chemiluminescent immunoassay (Architect i2000; Abbot Japan, Tokyo, Japan). Serum IgG4 and complement C3 and C4 concentrations were measured by turbidimetric assay (SRL, Tokyo, Japan).

## Histopathological Evaluation and Immunohistochemistry

After surgical resection of the orbital adipose tissue, formalin-fixed and paraffin-embedded sections were subjected to hematoxylin-eosin (HE) and immunohistochemical staining. All sections of surgical specimens were double-blind reviewed by two senior pathologists who evaluated the histopathological findings. The intensities of fibrosis and lymphocytic infiltration were semiquantified as 3+: severe, 2+: moderate, 1+: mild, or -: negative.

Immunohistochemical staining for IgG4 (mouse monoclonal antibody, MRQ-44; Gene Tech, Shanghai, China), IgG (rabbit monoclonal antibody; Gene Tech), CD20 (mouse monoclonal antibody; Gene Tech), and CD3 (mouse monoclonal antibody; Gene Tech) were performed. For each section, the mean numbers of IgG4-positive and IgG-positive cells were counted in three high-power fields (HPFs), the counts in the region with the highest density of immunostained cells were calculated, and the IgG4/IgG ratio was recorded. The area of each high-power region was approximately 0.24 mm<sup>2</sup> (Olympus BX50 microscope).

## Diagnostic Criteria for the IgG4-positive and IgG4-negative Groups

According to the diagnostic criteria for IgG4-related ophthalmic disease (ROD) defined in 2014,<sup>8</sup> patients with TED were categorized into the IgG4-positive group if they fulfilled either of the following criteria: (1) histopathological IgG4-positive indicating a histopathological examination showing an IgG4/IgG-positive cell ratio > 40% or an IgG4-positive plasma cell density  $\geq$  50/HPF, or (2) serum IgG4-positive indicating a blood test showing elevated serum IgG4 levels ( $\geq$  135 mg/dl). In particular, diagnosis was classified as “definitive” when (1) and (2) were satisfied; “probable” when only (1) was satisfied; and “possible” when only (2) was satisfied. Patients who did not meet any of these requirements were considered IgG4-negative TED (IgG4-negative group).

## Ophthalmological Evaluation

The severity of TED was assessed according to the European Group on Graves' Orbitopathy (EUGOGO) criteria, which was categorized as mild, moderate to severe, or sight threatening.<sup>9</sup> In addition, TED activity was assessed by the EUGOGO seven-item clinical activity score (CAS) criteria, in which CAS scores of 3 or higher are defined as active stage, whereas those with CAS scores below 3 are defined as stable stage.<sup>10</sup>

## Treatment

The enrolled patients underwent orbital decompression, which was conducted by the same experienced doctor, followed by intravenous glucocorticoid administration. Importantly, after surgery, patients with sight-threatening TED or active eye disease received methylprednisolone pulse therapy (MPT) at a dose of 1 g of methylprednisolone per day for three consecutive days, which has been proven effective for improving visual function and relieving clinical symptoms compared with MPT alone in a recent study,<sup>11</sup> whereas all other patients received methylprednisolone at a dose of 80 mg per day for three consecutive days. Prednisone was subsequently taken orally at a dose of 30 mg/day and gradually reduced to decrease the active immune attack stimulated by surgical stress and postoperative chemosis.<sup>12,13</sup> The clinical features of the patients, including best corrected visual acuity (BCVA) and CAS before surgery, were analyzed.

## Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). The means and standard deviations (SDs) of the quantitative variables were calculated. The paired *t*-test or analysis of variance (ANOVA) with Bonferroni's test for comparisons was used to analyze the quantitative variables when the assumptions of normality and homogeneity of variance were satisfied; otherwise, the Mann-Whitney *U*-test or Kruskal-Wallis test were used. Differences between rates or ratios were determined using the  $\chi^2$  test. Logistic regression analysis was used to estimate the influence of variables on CAS in patients with TED. Two-tailed Spearman's rank correlation analysis was used to evaluate the correlations between two variables. Any *P* value < 0.05 was considered to be statistically significant.

## RESULTS

Of the 185 total patients who agreed to participate in the study, 64 patients (34.6%) were included in the IgG4-positive group (histopathological or serum IgG4 positive), including 15 patients (8.1%), 27 patients (14.6%), and 22 patients (11.9%) who were allocated to the definitive (both histopathological and serum IgG4-positive), probable (only histopathological IgG4-positive), and possible (only serum IgG4-positive) groups, respectively, and 121 patients (65.4%) were included in the IgG4-negative group. The median age was 49.4 (range, 17.0–76.0) years. There were 100 male (54.1%) and 85 female patients (45.9%). All patients had normal TT3, TT4, FT3, and FT4 levels before surgery. In addition, IgG4-RD was not observed in other organs during the general examination.

## Baseline Characteristics of Patients With IgG4-positive and IgG4-negative TED

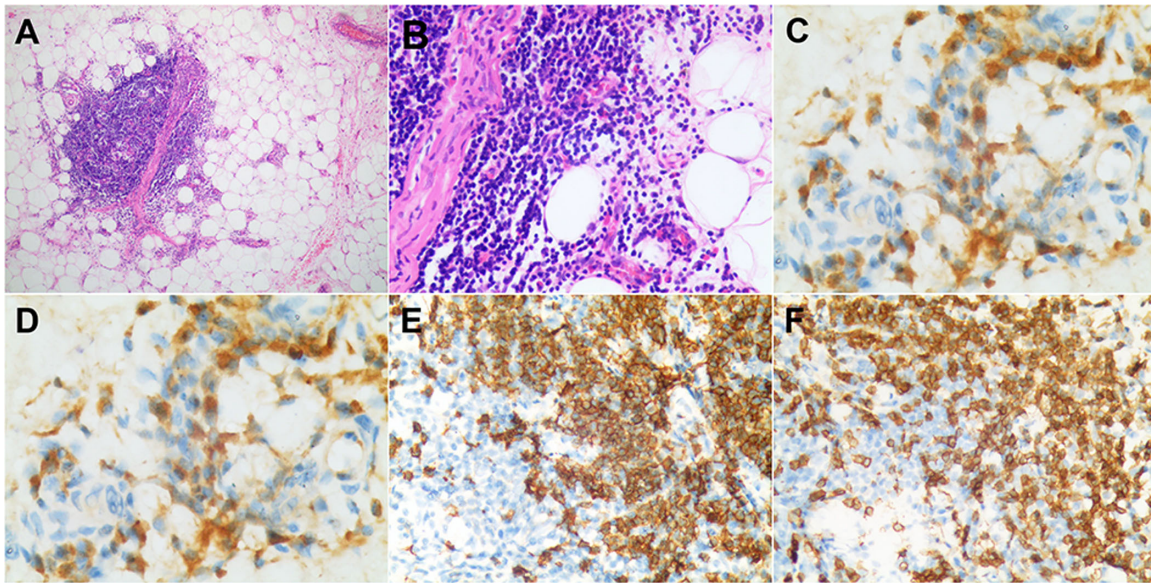
The baseline characteristics of the IgG4-positive and IgG4-negative patients with TED were compared and are summarized in Table 1. Although sex, laterality, and smoking history were not different among the four groups, patients were older in the IgG4-positive group than in the IgG4-negative group (*P* = 0.008). Specifically, patients were oldest in the possible IgG4-positive group (*P* = 0.021), and were also

TABLE 1. Baseline Characteristics of IgG4-Positive and IgG4-Negative Patients with TED

| Variable   | IgG4-positive TED (n = 64) |                   |                   | IgG4-negative (n = 121) |               |              |              |              |                |                |
|--|----------------------------|-------------------|-------------------|-------------------------|---------------|--------------|--------------|--------------|----------------|----------------|
|  | Definitive (n = 15)        | Probable (n = 27) | Possible (n = 22) | All (n = 64)            | P1            | P2           | P3           | P4           | P5             |                |
| Sex, Male:Female                                 | 10:5                       | 14:13             | 13:9              | 37:27                   | 63:58         | 0.285        | 0.984        | 0.544        | 0.456          | 0.698          |
| Age at diagnosis, y                              | 53.40 ± 12.11              | 50.44 ± 9.29      | 55.91 ± 10.03     | 53.02 ± 10.37           | 47.45 ± 13.46 | 1.000        | 1.000        | <b>0.021</b> | <b>0.008</b>   | <b>0.023</b>   |
| Laterality, bilateral:unilateral                 | 14:1                       | 26:1              | 22:0              | 62:2                    | 109:12        | 1.000        | 0.512        | 0.260        | 0.171          | 0.341          |
| Smoking history, n (%)                           | 8 (53.3)                   | 10 (37.0)         | 9 (40.9)          | 27 (42.2)               | 35 (28.9)     | 0.105        | 0.407        | 0.263        | 0.069          | 0.214          |
| Severity (moderate-to-severe: sight-threatening) | 9:6                        | 10:17             | 5:17              | 24:40                   | 70:51         | 0.847        | 0.050        | <b>0.002</b> | <b>0.008</b>   | <b>0.008</b>   |
| Pre-operative CAS                                | 2.53 ± 1.73                | 2.04 ± 1.53       | 1.68 ± 1.49       | 2.03 ± 1.57             | 1.05 ± 1.16   | <b>0.003</b> | <b>0.007</b> | 0.318        | < <b>0.001</b> | < <b>0.001</b> |
| Pre-operative BCVA logMAR                        | 0.56 ± 0.78                | 0.59 ± 0.73       | 0.88 ± 0.76       | 0.68 ± 0.75             | 0.43 ± 0.60   | 1.000        | 1.000        | <b>0.023</b> | <b>0.026</b>   | <b>0.023</b>   |
| Duration of TED, mo                              | 42.33 ± 64.11              | 22.37 ± 23.67     | 19.23 ± 23.91     | 25.97 ± 37.68           | 31.18 ± 31.84 | 0.987        | 0.499        | 0.259        | 0.322          | 0.087          |
| History of steroid use, n (%)                    | 12 (80.0)                  | 19 (70.4)         | 18 (81.8)         | 49 (76.6)               | 88 (72.7)     | 0.770        | 0.805        | 0.370        | 0.571          | 0.737          |
| Initial thyroid function                         |                            |                   |                   |                         |               |              |              |              |                |                |
| Hypothyroidism, n (%)                            | 12 (80.0)                  | 26 (96.3)         | 18 (81.8)         | 56 (87.5)               | 103 (85.1)    | 0.889        | 0.211        | 0.941        | 0.658          | 0.360          |
| Hypothyroidism, n (%)                            | 0 (0.0)                    | 1 (3.7)           | 2 (9.1)           | 3 (4.7)                 | 5 (4.1)       | 1.000        | 1.000        | 0.649        | 1.000          | 0.589          |
| Euthyroidism, n (%)                              | 3 (20.0)                   | 0 (0.0)           | 2 (9.1)           | 5 (7.8)                 | 13 (10.7)     | 0.532        | 0.159        | 1.000        | 0.522          | 0.183          |
| Hashimoto's thyroiditis, n (%)                   | 1 (6.7)                    | 0 (0.0)           | 0 (0.0)           | 1 (1.6)                 | 0 (0.0)       | 0.110        | NA           | NA           | 0.346          | 0.081          |
| Duration of thyroid disease, mo                  | 63.67 ± 66.62              | 34.11 ± 28.47     | 40.05 ± 59.96     | 42.14 ± 50.10           | 53.81 ± 60.38 | 1.000        | 0.657        | 1.000        | 0.207          | 0.278          |
| I-131 therapy history, n (%)                     | 2 (13.3)                   | 4 (14.8)          | 4 (18.2)          | 10 (15.6)               | 29 (24.0)     | 0.549        | 0.302        | 0.554        | 0.186          | 0.618          |
| Hypothyroidism after I-131 therapy, n (%)        | 1 (50.0)                   | 3 (75.0)          | 3 (75.0)          | 7 (70.0)                | 14 (48.3)     | 1.000        | 0.639        | 0.639        | 0.412          | 0.678          |

P1, definitive IgG4-positive versus IgG4-negative; P2, probable IgG4-positive versus IgG4-negative; P3, possible IgG4-positive versus IgG4-negative; P4, all IgG4-positive versus IgG4-negative; P5, definitive IgG4-positive versus probable IgG4-positive versus possible IgG4-positive versus IgG4-negative.

Data are shown as the means plus/minus SDs. IgG4, immunoglobulin G4; TED, thyroid eye disease; CAS, clinical activity score; BCVA, best corrected visual acuity; NA, not applicable. Significant P values are shown with bold characters.



**FIGURE.** Histopathology images of IgG4-positive patients with TED. (A) HE staining ( $\times 40$  magnification), (B) HE staining ( $\times 200$  magnification), (C) immunohistochemical staining for IgG4 ( $\times 400$  magnification), (D) immunohistochemical staining for IgG ( $\times 400$  magnification), (E) immunohistochemical staining for CD20 ( $\times 200$  magnification), and (F) immunohistochemical staining for CD3 ( $\times 200$  magnification). HE, hematoxylin and eosin.

older in the definitive and probable IgG4-positive groups, although these differences were not significant.

Pre-operative CAS tended to be elevated as a function of advancement in patients with IgG4-positive TED (definitive IgG4-positive versus probable IgG4-positive versus possible IgG4-positive versus IgG4-negative,  $2.53 \pm 1.73$  vs.  $2.04 \pm 1.53$  vs.  $1.68 \pm 1.49$  vs.  $1.05 \pm 1.16$ ;  $P < 0.001$ ). When patients with TED were classified as having inactive ( $n = 146$ ; 78.9%) or active ( $n = 39$ ; 21.1%) ophthalmopathy status, the proportion of active patients was higher in the IgG4-positive group than in the IgG4-negative group (39.1% vs. 11.6%;  $P < 0.001$ ).

In addition, IgG4-positive patients with TED had worse BCVA than IgG4-negative patients with TED ( $P = 0.026$ ). Specifically, possible IgG4-positive patients with TED had worst BCVA ( $P = 0.023$ ), and BCVA was also worse in the definitive and probable IgG4-positive groups than in the IgG4-negative group, although these differences were not significant. Moreover, disease was most severe in the possible IgG4-positive group ( $P = 0.002$ ). The four groups did not differ regarding steroid history, initial thyroid function, I-131 therapy history, hypothyroidism after I-131 therapy, and duration of TED or thyroid disease.

### Laboratory and Pathological Characteristics of Patients With IgG4-positive and IgG4-negative TED

Table 2 shows a comparison of the laboratory and pathological findings obtained in patients with IgG4-positive and IgG4-negative TED. Histopathological IgG4 levels tended to be elevated as a function of advancement in patients with IgG4-positive TED (definitive IgG4-positive versus probable IgG4-positive versus possible IgG4-positive versus IgG4-negative,  $10.47 \pm 14.94$  vs.  $3.33 \pm 4.84$  vs.  $1.18 \pm 1.82$  vs.  $0.66 \pm 0.94$ ;  $P < 0.001$ ). In addition, IgG4/IgG ratios

were higher in the IgG4-positive groups than in the IgG4-negative group, with both  $P < 0.001$  in definitive and probable IgG4-positive subgroups, and was also higher in the possible IgG4-positive group, although this difference was not significant. Furthermore, definitive and possible IgG4-positive patients with TED had higher serum IgG4 levels ( $P < 0.001$  and  $< 0.001$ , respectively) than IgG4-negative patients with TED. In addition, serum IgG4 levels were positively correlated with histopathological IgG4 and the IgG4/IgG ratio in all 185 patients (Pearson  $r = 0.186$ ,  $P = 0.011$ ;  $r = 0.262$ ,  $P < 0.001$ ).

TRAb levels, lymphocytic infiltrates, and CD20+ lymphocyte counts were higher IgG4-positive patients with TED than in the IgG4-negative patients with TED, especially in the definitive IgG4-positive group ( $P = 0.039$ , 0.001, and 0.003, respectively). Moreover, TgAb and TPOAb levels were also higher in the IgG4-positive than in the IgG4-negative group, but this difference was not significant. However, other factors, including serum C3 and C4 levels, thyroid hormone levels, pathological IgG counts, the presence of fibrosis, and CD3+ lymphocytic infiltrate, were similar between the two groups. The Figure displays the representative pathologic findings of the IgG4-negative patients with TED in the present study.

### IgG4 Subtype is an Independent Factor Associated with Clinical Activity in Patients With TED

To identify whether IgG4 is an independent factor associated with higher clinical activity in patients with TED, we performed logistic regression analysis, including factors potentially related factors with clinical activity (Table 3). In the univariate analysis, age at diagnosis ( $P = 0.024$ ) and IgG4 subtype (definitive IgG4-positive,  $P < 0.001$ ; probable IgG4-positive,  $P < 0.001$ ) were significantly associated with active stage (CAS  $\geq 3$ ) in patients with TED. In the

TABLE 2. Laboratory and Pathological Characteristics of IgG4-Positive and IgG4-Negative Patients with TED

| Variable                                 | IgG4-positive TED (n = 64) |                   |                   |                 | IgG4-negative TED (n = 121) | P1           | P2     | P3     | P4           | P5           |
|--|----------------------------|-------------------|-------------------|-----------------|-----------------------------|--------------|--------|--------|--------------|--------------|
|  | Definitive (n = 15)        | Probable (n = 27) | Possible (n = 22) | All (n = 64)    |                             |              |        |        |              |              |
| Serum IgG4, g/l                          | 2.44 ± 1.20                | 0.67 ± 0.32       | 3.16 ± 3.90       | 1.94 ± 2.59     | 0.58 ± 0.33                 | <0.001       | 1.000  | <0.001 | <0.001       | <0.001       |
| Serum C3, g/l                            | 1.15 ± 0.18                | 1.20 ± 1.54       | 1.23 ± 0.17       | 1.20 ± 0.16     | 1.24 ± 0.22                 | 0.804        | 1.000  | 1.000  | 0.215        | 0.449        |
| Serum C4, g/l                            | 0.36 ± 0.25                | 0.33 ± 0.12       | 0.27 ± 0.78       | 0.32 ± 0.15     | 0.30 ± 0.13                 | 0.869        | 0.277  | 0.412  | 0.587        | 0.670        |
| TT3, nmol/l                              | 1.63 ± 0.38                | 1.80 ± 0.78       | 1.71 ± 0.35       | 1.73 ± 0.57     | 1.75 ± 0.47                 | 1.000        | 1.000  | 1.000  | 0.798        | 0.762        |
| TT4, nmol/l                              | 80.79 ± 35.95              | 94.78 ± 27.85     | 99.18 ± 30.43     | 93.15 ± 31.06   | 97.63 ± 27.94               | 0.245        | 1.000  | 1.000  | 0.330        | 0.211        |
| FT3, pmol/l                              | 4.37 ± 1.12                | 5.11 ± 1.84       | 5.01 ± 0.82       | 4.90 ± 1.41     | 5.09 ± 1.27                 | 0.287        | 1.000  | 1.000  | 0.349        | 0.256        |
| FT4, pmol/l                              | 14.24 ± 6.43               | 15.53 ± 5.05      | 16.03 ± 4.58      | 15.40 ± 5.21    | 16.40 ± 4.89                | 0.709        | 1.000  | 1.000  | 0.198        | 0.421        |
| TSH, $\mu$ U/ml                          | 3.55 ± 4.89                | 3.67 ± 6.79       | 2.51 ± 3.79       | 3.25 ± 5.43     | 4.41 ± 8.29                 | 1.000        | 1.000  | 1.000  | 0.312        | 0.718        |
| TRAb, IU/l                               | 17.75 ± 21.41              | 17.66 ± 31.52     | 21.02 ± 44.55     | 18.93 ± 34.66   | 8.97 ± 13.97                | <b>0.039</b> | 0.063  | 0.550  | <b>0.031</b> | 0.103        |
| TgAb, IU/ml                              | 338.30 ± 838.15            | 145.10 ± 367.94   | 236.70 ± 645.32   | 225.10 ± 604.38 | 163.41 ± 463.91             | 1.000        | 1.000  | 1.000  | 0.452        | 0.614        |
| TPOAb, IU/ml                             | 140.74 ± 209.66            | 28.78 ± 27.48     | 166.45 ± 218.84   | 108.03 ± 178.75 | 69.23 ± 124.96              | 0.631        | 1.000  | 0.092  | 0.065        | <b>0.039</b> |
| No. of IgG4 (+) cells/HPF                | 10.47 ± 14.94              | 3.33 ± 4.84       | 1.18 ± 1.82       | 4.27 ± 8.56     | 0.66 ± 0.94                 | <0.001       | <0.001 | 1.000  | <0.001       | <0.001       |
| No. of IgG (+) cells/HPF                 | 15.87 ± 21.80              | 4.70 ± 8.88       | 3.91 ± 3.24       | 7.05 ± 12.88    | 4.33 ± 3.08                 | 0.053        | 0.085  | 0.559  | 0.548        | 0.054        |
| IgG4 (+)/IgG (+) cells (%)               | 81.11 ± 22.91              | 88.27 ± 23.49     | 25.00 ± 37.80     | 64.85 ± 40.90   | 14.04 ± 29.75               | <0.001       | <0.001 | 0.666  | <0.001       | <0.001       |
| Lymphocytic infiltrate, -/1+/2+/3+       | 0/12/3/0                   | 0/26/1/0          | 1/21/0/0          | 1/59/4/0        | 0/121/0/0                   | <b>0.001</b> | 0.182  | 0.154  | <b>0.008</b> | <0.001       |
| Degree of fibrosis, -/1+/2+/3+           | 2/13/0/0                   | 0/27/0/0          | 2/20/0/0          | 4/60/0/0        | 4/117/0/0                   | 0.131        | 1.000  | 0.231  | 0.578        | 0.129        |
| CD20+ lymphocytic infiltrate, -/1+/2+/3+ | 0/11/3/1                   | 0/26/1/0          | 1/20/1/0          | 1/57/5/1        | 0/116/3/2                   | <b>0.003</b> | 0.752  | 0.102  | <b>0.183</b> | <b>0.013</b> |
| CD3+ lymphocytic infiltrate, -/1+/2+/3+  | 0/13/2/0                   | 0/26/1/0          | 1/20/1/0          | 1/59/4/0        | 0/118/3/0                   | 0.094        | 0.557  | 0.053  | 0.166        | 0.066        |

P1, definitive IgG4-positive versus IgG4-negative; P2, probable IgG4-positive versus IgG4-negative; P3, possible IgG4-positive versus IgG4-negative; P4, all IgG4-positive versus IgG4-negative; P5, definitive IgG4-positive versus probable IgG4-positive versus possible IgG4-positive versus IgG4-negative.

Data are shown as the means plus/minus SDs. IgG4, immunoglobulin G4; TED, thyroid eye disease; HPF, high-power field.

Significant P values are shown with bold characters.

TABLE 3. Univariate and Multivariate Analyses for Clinical Activity in Patients With TED

| Variable               | Univariate Regression Model |           |                  | Multivariate Regression Model |           |                  |
|------------------------|-----------------------------|-----------|------------------|-------------------------------|-----------|------------------|
|                        | OR                          | 95% CI    | P                | OR                            | 95% CI    | P                |
| IgG4 subtype           |                             |           |                  |                               |           |                  |
| Definite               | 8.73                        | 2.74-27.8 | <b>&lt;0.001</b> | 6.83                          | 2.07-22.6 | <b>&lt;0.001</b> |
| Probable               | 6.11                        | 2.38-15.7 | <b>&lt;0.001</b> | 5.83                          | 2.19-15.5 | <b>&lt;0.001</b> |
| Possible               | 2.25                        | 0.72-7.04 | 0.165            | 1.60                          | 0.48-5.30 | 0.445            |
| Female sex             | 0.59                        | 0.28-1.22 | 0.159            |                               |           |                  |
| Age at diagnosis, y    | 1.04                        | 1.00-1.07 | <b>0.024</b>     | 1.03                          | 0.99-1.07 | 0.086            |
| History of steroid use | 1.79                        | 0.73-4.36 | 0.204            | 1.80                          | 0.69-4.72 | 0.233            |
| Smoking history        | 1.98                        | 0.97-4.09 | 0.062            | 1.39                          | 0.49-3.93 | 0.538            |
| I-131 therapy history  | 0.48                        | 0.18-1.34 | 0.161            | 0.63                          | 0.21-1.90 | 0.414            |

TED, thyroid eye disease; IgG4, immunoglobulin G4; BCVA, best corrected visual acuity; OR, odds ratio; 95%CI, 95% confidence interval. Significant *P* values are shown with bold characters.

multivariate analysis, after adjusting for age, sex, smoking status, history of I-131 therapy, and steroid therapy in the multivariate analysis, the definite and probable IgG4 subtypes were independently associated with the active stage in patients with TED (both *P* < 0.001, odds ratio [OR] = 6.83; 95% confidence interval [CI] = 2.07–22.6 in the definitive IgG4-positive group; OR = 5.83; 95% CI = 2.19–15.5 in the probable IgG4-positive group).

## DISCUSSION

The purpose of this study was to demonstrate that there is a subset of patients with TED with elevated IgG4 levels that meets the IgG4-ROD criteria but shows no findings indicating systemic IgG4-RD. TED and IgG4-RD are both autoimmune fibrous inflammatory diseases in which Th2 lymphocytes play a dominant role in their development.<sup>14,15</sup> Therefore, some scholars have recently suggested that there are certain correlations between TED and IgG4-ROD, including evaluated serum or histopathological IgG4 levels.<sup>3,5,16,17</sup> However, the data from a large number of patients with TED with both pathological and serological IgG4 levels remain limited. Our aim was to prospectively gather valid data from patients with TED undergoing orbital decompression to determine whether there is a subpopulation of patients with TED with elevated IgG4 levels and to identify the clinicopathological features of this subtype.

In the current study, a novel subgroup of patients with TED undergoing orbital decompression with elevated IgG4 levels was identified (34.6% of overall patients). Although only 8.1% of patients with TED were both histopathological and serum IgG4 positive, patients with either histopathological or serum IgG4 positivity still presented a series of unique clinicopathologic features compared with IgG4-negative patients with TED. They were older and had higher CAS, worse BCVA, a higher histopathological IgG4 count, a higher IgG4/IgG ratio, and more lymphocytic infiltrates than IgG4-negative patients.

The age of onset in patients with TED who were IgG4 positive was 53.02 years, which was similar to that in patients with IgG4-ROD (mean age 55.5 years)<sup>18,19</sup> and slightly younger than that in other patients with IgG4-RD (mean age 58 years).<sup>20</sup> Moreover, our study suggests that IgG4-positive patients with TED are older than IgG4-negative patients with TED (47.45 years), similar to what has been found in IgG4-positive patients with GD.<sup>21</sup> However, in a report from Korea, there was no difference in the age of onset of TED between the IgG4-negative and IgG4-positive subtypes when differ-

entiated by serology,<sup>3</sup> which may be related to the fact that all of their subjects were patients with GD and in a younger age group (37.20 years). In fact, IgG4 levels do not increase with age, suggesting that some unknown factor may be responsible for the abnormal expression of IgG4.<sup>22</sup>

We found that the IgG4-positive subtype was an independent factor associated with the active stage in patients with TED. There were more active patients and patients with a higher CAS in the IgG4-positive group than in the IgG4-negative group in our study, consistent with the conclusion presented in a previous Korean study.<sup>3</sup> In addition, our results show that IgG4-positive patients had more severe disease and worse BCVA than IgG4-negative patients. According to the natural course of TED, the early stage of TED is progressive and reflects the process of autoimmunity, with activity peaking at 13 to 24 months.<sup>23,24</sup> Then, the inflammatory response gradually subsides to the plateau stage, with increased intramuscular fatty degeneration and fibrosis.<sup>24,25</sup> This suggests that IgG4 may play an important role in the early onset of TED and that high IgG4 activity predicts the development of more severe muscle lesions and orbital fibrosis and even a worse visual prognosis. Moreover, in our study, the IgG4-positive group had higher levels of TRAb, TgAb, and TPOAb, and more orbital tissue lymphocytic infiltration than the IgG4-negative group, indicating that IgG4 is an asymmetric, bispecific antibody that is closely related to the activity of the disease from the perspective of autoimmune diseases caused by thyroid destruction.<sup>1,21</sup>

Similar to the research of a small group of patients with TED (24 cases) in America,<sup>6</sup> in our study of 185 Chinese patients, 37 patients (20.0%) with TED had elevated serum IgG4 levels. Furthermore, 42 patients (22.7%) with TED had an elevated histopathological IgG4/IgG ratio. In fact, similar to our study, other studies have found that the serum level of IgG4 is often but not always elevated in patients with IgG4-RD,<sup>26</sup> approximately only two-thirds of patients with IgG4-RD have elevated serum IgG4 levels,<sup>27</sup> and only 23% of pathologically identified patients with IgG4-positive IgG4-RD had elevated serum IgG4 levels.<sup>28</sup> The relative dys-synchrony of serum and pathologic IgG4 may provide two possible explanations for the overabundance of IgG4 antibodies: tissue-destructive immunoglobulins and the response to an unknown primary inflammatory stimulus.<sup>1</sup> However, we found that patients with either histopathological or serum IgG4 positivity still presented a series of unique clinicopathologic features compared with IgG4-negative patients with TED, indicating a more active and severe subtype.

Some limitations in this study need to be noted. We investigated only patients with TED undergoing orbital decompression, which involved a large number of patients with moderate-to-severe and even sight-threatening disease. As reported before,<sup>29</sup> the female-to-male ratio in these patients was significantly lower than that in Caucasian patients with TED and even overall patients with TED. Moreover, it is difficult to make comparison with other studies of patients with mild TED.

In conclusion, in the current study, we propose clinical, serological, and histopathological characteristics of the IgG4 subtype of patients with TED and confirm that elevated IgG4 levels are common in patients with TED and should be given consideration. IgG4-positive patients with TED are characterized by older age, higher activity, and more severe disease. IgG4-positive subtype was an independent factor associated with the active stage in patients with TED, indicating that IgG4 may also play an important role in the pathogenesis of TED.

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