**IMPORTANCE** Graves orbitopathy (GO) responds to immunosuppressive treatments when clinically active but poorly when inactive. In other autoimmune diseases, response has been ascribed to a reduction in lymphocytes infiltrating the target organ. It is not known whether active vs inactive GO differs in this regard, which would help in understanding the link between GO immunologic features and clinical behavior.

**OBJECTIVE** To investigate the association between orbital lymphocytic infiltrate and GO clinical features.

**DESIGN, SETTING, AND PARTICIPANTS** A cohort study aimed at assessing the extent and immunohistochemical phenotype of orbital lymphocytes and associating it with the ophthalmologic features of GO, especially its clinical activity score (CAS), was conducted at a tertiary referral center. Twenty consecutive patients with GO who underwent orbital decompression were included. The study was conducted from January 1 to May 31, 2017.

**EXPOSURES** Orbital tissue histology and immunohistochemistry testing as well as ophthalmologic evaluation.

**MAIN OUTCOMES AND MEASURES** Association between CAS and orbital lymphocytes, analyzed as total number of lymphocytes and main lymphoid subsets.

**RESULTS** The patient population included 8 men and 12 women, all of white race, with a mean (SD) age of 46 (13) years. With an established cutoff value of 300 lymphoid cells per tissue sample, lymphocytes above this value were found in orbital tissues of 9 of 20 patients (45%), often organized into distinct foci. The lymphocytes comprised a mixture of T (CD3-positive) and B (CD20-positive) cells, suggesting a mature, polyclonal autoimmune response. In a simple linear regression model, the total number of lymphocytes, as well as the number of CD3- and CD20-positive subsets, correlated with CAS (R = 0.63; 95% CI, 0.27-0.84; P = .003; R = 0.59; 95% CI, 0.20-0.82; P = .006; and R = 0.65; 95% CI, 0.30-0.85; P = .002, respectively). In a multiple linear regression model, lymphocytes maintained their effect on CAS when adjusted for 2 additional variables that were correlated with CAS—smoking and GO duration—highlighting even more the important role of orbital lymphocytes in affecting CAS (total number: R = 0.58; 95% CI, 0.18-0.82; P = .01; CD3-positive: R = 0.58; 95% CI, 0.17-0.82; P = .01; and CD20-positive: R = 0.59; 95% CI, 0.19-0.83; P = .01).

**CONCLUSIONS AND RELEVANCE** This study shows a correlation between T and B lymphocytes infiltrating orbital tissues and the activity of GO, possibly enhancing our understanding of the association between GO immunologic features and clinical expression.
Introduction

Graves orbitopathy (GO) is a chronic inflammatory disease associated with Graves hyperthyroidism in 95% of patients, with hypothyroid autoimmune thyroiditis in 3% to 4% and observed cases in 1% to 2% of patients who have thyroid autoimmunity but without overt thyroid dysfunction.1–3 Graves orbitopathy has an autoimmune pathogenesis, whereby the thyrotropin (TSH) receptor is considered the major autoantigen, thus establishing a direct link between the thyroid and orbital tissues.4 Unlike Graves hyperthyroidism, which is a classic example of humoral autoimmunity caused by TSH receptor–stimulating autoantibodies,5 GO is believed to reflect T-cell–mediated autoimmunity, although B-cell– and antibody-mediated autoimmunity, to some extent, may also participate in the pathogenic process.4,6,7 The role of B lymphocytes in the pathogenesis of GO is suggested by the finding that rituximab, a monoclonal antibody that destroys B cells by binding to CD20 on their surface, may ameliorate GO activity.8 The mechanism through which destruction of antibody-producing B cells ameliorates a T-cell–mediated disease is unknown but has been ascribed to the role that B cells have as professional antigen-presenting cells.

Rituximab has been used in other autoimmune diseases, such as type 1 diabetes,9 in which patients are known to be heterogeneous in terms of CD20-positive lymphocytes infiltrating the pancreatic islets.10 We hypothesized that something similar occurs in the orbits of patients with GO, a heterogeneity that may shed light on the link between GO immunologic features and clinical expression and possibly explain the variable response to immunosuppressive drugs. We thus designed this study to characterize in a relatively large cohort of patients the presence and composition of lymphocytes infiltrating orbital tissues and relate them to the clinical features of GO.

Methods

Patient Characteristics and Study Design

We arbitrarily chose a sample size of 20 consecutive patients with GO who underwent orbital decompressive surgery. In all patients, we recorded smoking habits; analyzed thyroid function through measurement of free thyroxine, free triiodothyronine (Vitros Immunodiagnosticis), and TSH (Immulite 2000; Siemens Healthcare); measured the presence of anti-TSH receptor antibodies (TRAbs) (Brahms); and performed a detailed ophthalmologic evaluation that included 6 factors: (1) exophthalmometry, (2) clinical activity score (CAS) (range, 0–7; GO active for CAS values ≥3),11 (3) diplopia according to the Gorman score (from absent to constant),11 (4) corneal status, (5) ocular fundus, and (6) visual acuity.

The study was conducted from January 1 to May 31, 2017. Written and signed informed consent was obtained from all patients. The study was approved by the local ethics committee (Comitato Etico Area Vasta Nord Ovest). The participants did not receive financial compensation.

Findings

In this cohort study conducted in 20 consecutive patients with Graves orbitopathy, an association between the clinical activity score and orbital lymphocytes was found, analyzed as total number and main lymphoid subsets, both in a simple and multiple regression model.

Meaning

These findings suggest a correlation between T and B lymphocytes infiltrating orbital tissue and Graves orbitopathy activity, possibly enhancing understanding of the association between Graves orbitopathy immunologic features and clinical expression.

Tissue Assays

Orbital tissues collected at surgery were fixed in formalin and embedded in paraffin for routine hematoxylin and eosin histology and immunohistochemistry (Ventana Benchmark system; Ventana Medical Systems) testing. Archival paraffin-embedded thyroid tissue samples from patients with Graves hyperthyroidism as well as archival orbital and abdominal fibroadipose tissue samples from patients without Graves hyperthyroidism and GO were used as controls. Sections 3- to 5-μm thick were deparaffinized in xylene, dehydrated, and processed using a diaminobenzidine detection system (Ventana), according to the manufacturer’s instructions. CD3, a pan T-lymphocyte marker, was detected using a rabbit monoclonal antibody (immunoglobulin [Ig]G) against the nonglycosylated ε chain of the CD3 molecule expressed by human T lymphocytes (CONFIRM anti-CD3, clone 2GV6; Ventana). CD20, a pan B-lymphocyte marker, was detected using a mouse monoclonal antibody (IgG2a κ) (CONFIRM anti-CD20, clone L26; Ventana). After presseressedasthesumofthecellsinall4fields.Morethan300lym-phoidcells, and 8 of 20 patients (40%) had more than 200 CD20-positive cells, and 8 of 20 patients (40%) had more than 200 CD20-positive cells. These cutoff values were obtained using the finite mixture models, assuming the sum of 2 gaussian curves as a model. Thus, the finite mixture model is a convex combination of 2 or more probability density functions, and mixture models are capable of approximating value distributions by combining the properties of the individual probability density functions.12 Once the parameters of the 2 curves (mean [SD]) were established, cutoff levels were calculated as the values on which the probability of the 2 curves was equal. Thus, the reported cutoff values were those that best discriminated the 2 normal distributions identified with the finite mixture model analysis. The pathologists (L.T. and F.B.) who examined the tissue sections and performed the cell counting were masked (ie, they were not aware of the clinical features of the patients).
Statistical Analysis

Data were summarized as mean (SD) or median and interquartile range. Lymphocytes were expressed in a logarithmic scale because their distribution was not gaussian. Groups were compared by simple or multiple linear regression, χ² test, t test, or Mann-Whitney test, as appropriate. Level of significance was set at P ≤ .05; testing was 2-tailed and unpaired. SPSS, version 25 (IBM Corp) was used for analysis.

Results

Clinical Features of Patients

The patient population included 8 men and 12 women, all of white race, with a mean (SD) age of 46 (13) years. Most of the 20 patients were nonsmokers (Table 1). Graves orbitopathy was associated with Graves hyperthyroidism in 19 patients and occurred alone (euthyroid GO) in the remaining patient. All of the patients with Graves hyperthyroidism had received a form of treatment (radioiodine in 10, thyroidectomy in 6, and methimazole in 3) and were euthyroid at the time of orbital decompensation. This surgery was performed for severe, disfiguring proptosis in 17 patients or for optic neuropathy in 3 patients. Four patients with GO were untreated, whereas the remaining patients had received glucocorticoids alone or with orbital radiotherapy, the latter being performed in 5 patients. The median time elapsed since the last glucocorticoid administration was 4 months. Three patients had been treated with glucocorticoids in the 3 months preceding orbital decompensation. Most patients had moderately severe GO, and the eye disease was variably active, as shown by CAS values reported in Table 1.

Infiltrating Lymphocytes and Their Correlation With CAS

Nine patients (45%) had a total number of orbital infiltrating lymphocytes above the 300 cutoff value. Two representative cases, 1 with a scarce and 1 with a marked infiltrate, are shown in Figure 1. Lymphocytes comprised a mixture of both CD3-positive T cells and CD20-positive B cells, without an overall predominance of 1 subset over another (Figure 1). Lymphocytes that stained positive for either CD3 or CD20 were also detected in thyroid tissue samples from patients with Graves hyperthyroidism, used as positive controls (not shown). In contrast, no lymphocytes and, consequently, no CD3 and CD20 staining were observed in archival orbital and abdominal fibroadipose tissue samples from patients without Graves hyperthyroidism or GO, used as negative controls.

In a simple linear regression model that assessed the overall effect of orbital lymphocytes on CAS, lymphocytes positively correlated with CAS (Table 2 and Figure 2A). For every 10-fold increase in the total number of orbital lymphocytes, CAS increased by 1.2 points (R = 0.63; 95% CI, 0.27-0.84; P = .003). The correlation was similar when lymphocytes were analyzed separately as T cells (Table 2, Figure 2B) or B cells (Table 2, Figure 2C). For every 10-fold increase in CD3-positive cells, CAS increased by 1.68 points (R = 0.59; 95% CI, 0.20-0.82; P = .006). For every 10-fold increase in CD20-positive cells, CAS increased by 1.2 points (R = 0.65; 95% CI, 0.30-0.85; P = .002). Lymphocytes did not correlate with age, sex, smoking habits, GO duration, thyroid treatment, previous GO treatments, thyroid function, levels of TRAbs, exophthalmometry, eyelid aperture, diplopia, and visual acuity, overall suggesting that their number is mainly an indicator of GO activity. Of the various patient features under examina-

Table 1. Demographic and Clinical Features of the Patient Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Age, mean (SD) [range], y</td>
<td>46 (13) [16-67]</td>
</tr>
<tr>
<td>Smoking habits, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>13 (65)</td>
</tr>
<tr>
<td>Thyroid treatment, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Methimazole</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Radiiodine</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6 (3)</td>
</tr>
<tr>
<td>None</td>
<td>1 (5)</td>
</tr>
<tr>
<td>FT3+, mean (SD) [range], pg/dL</td>
<td>0.38 (0.04) [0.32-0.47]</td>
</tr>
<tr>
<td>TSH, median (IQR) [range], μU/mL</td>
<td>0.6 (0.1-1.9) [0.004-4.1]</td>
</tr>
<tr>
<td>TRAbs, median (IQR) [range], U/L</td>
<td>3.9 (2.3-5.7) [0.3-9.8]</td>
</tr>
<tr>
<td>GO duration, median (IQR) [range], mo</td>
<td>34 (18-51) [2-85]</td>
</tr>
<tr>
<td>Indication for OD, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Optic neuropathy</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Severe proptosis</td>
<td>17 (85)</td>
</tr>
<tr>
<td>GO treatment, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Intravenous GC and radiotherapy</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Intravenous GC alone</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Oral GC and radiotherapy</td>
<td>1 (5)</td>
</tr>
<tr>
<td>None</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Months since last GC administration, median (IQR) [range]</td>
<td>4 (3-18.5) [0-36]</td>
</tr>
<tr>
<td>Exophthalmometry, mean (SD) [range], mm</td>
<td>24.6 (2.7) [17-28]</td>
</tr>
<tr>
<td>Clinical activity score, median (IQR) [range]*</td>
<td>4 (3.5) [1-7]</td>
</tr>
<tr>
<td>Diplopia (Gorman score), No. (%) of patients</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Intermittent</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Inconstant</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Constant</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Visual acuity (Snellen), mean (SD) [range]</td>
<td>18.6/20 (2.4/20) [12/20-20/20]</td>
</tr>
</tbody>
</table>

Abbreviations: FT3+, free triiodothyronine; GC, glucocorticoid; GO, Graves orbitopathy; IQR, interquartile range; TRAbs, anti-thyrotropin (TSH) receptor antibodies.

* SI conversion factor: To convert FT3+ to picomoles per liter, multiply by 0.0154.

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tion, apart from orbital lymphocytes, smoking and GO duration correlated with CAS (Table 2).

As reported in Table 2, in a multiple linear regression model that analyzed the effect on CAS not only of the orbital lymphocytes but also of smoking and GO duration, orbital lymphocytes maintained their action on CAS when adjusted for the other covariates, highlighting even more the important role of orbital lymphocytes in affecting CAS.

**Features of Patients According to the Orbital Lymphocytic Infiltrate**

In confirmation of the findings reported above, when patients were grouped based on the total number of orbital infiltrating lymphocytes, those with counts above the cutoff value had a greater CAS than those below the cutoff value (Figure 3D). In addition, patients with counts above the cutoff value were more often smokers (4 smokers, 1 ex-smoker, and 4 nonsmokers vs 1 smoker, 1 ex-smoker, and 9 nonsmokers in patients with a number of lymphocytes below the cutoff level; \( P = .007 \)), which was in line with the correlation between smoking and CAS. The remaining features (age, sex, GO duration, thyroid treatment, previous GO treatments, thyroid function, levels of TRAbs, exophthalmometry, eyelid aperture, diplopia, and visual acuity) did not differ significantly between the 2 groups.

The absence of a correlation between the lymphocytic infiltrate in orbital tissues and TRAbs was surprising in view of the knowledge that TRAbs are correlated with GO activity and severity. Individual levels of TRAbs according to the presence of a relevant orbital lymphocytic infiltrate are shown in

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**Table 2. Correlation Between Clinical Activity Score of Patients With GO and the Indicated Features**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Linear Regression</th>
<th>Multiple Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of lymphocytes</td>
<td>0.63 (0.27 to 0.84)</td>
<td>0.58 (0.18 to 0.82)</td>
</tr>
<tr>
<td>CD3-positive cells</td>
<td>0.59 (0.20 to 0.82)</td>
<td>0.58 (0.17 to 0.82)</td>
</tr>
<tr>
<td>CD20-positive cells</td>
<td>0.65 (0.30 to 0.85)</td>
<td>0.59 (0.19 to 0.83)</td>
</tr>
<tr>
<td>Smoking habits</td>
<td>−0.63 (−0.84 to −0.25)</td>
<td>NA</td>
</tr>
<tr>
<td>vs Total No./GO duration</td>
<td>NA</td>
<td>−0.43 (−0.73 to 0.01)</td>
</tr>
<tr>
<td>vs CD3/GO duration</td>
<td>NA</td>
<td>−0.43 (−0.73 to 0.01)</td>
</tr>
<tr>
<td>vs CD20/GO duration</td>
<td>NA</td>
<td>−0.44 (−0.74 to 0.003)</td>
</tr>
<tr>
<td>GO duration</td>
<td>−0.70 (−0.88 to −0.36)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>vs Total No./smoking</td>
<td>NA</td>
<td>0.68 (−0.86 to −0.34)</td>
</tr>
<tr>
<td>vs CD3/smoking</td>
<td>NA</td>
<td>−0.69 (−0.87 to −0.36)</td>
</tr>
<tr>
<td>vs CD20/smoking</td>
<td>NA</td>
<td>−0.68 (−0.86 to −0.34)</td>
</tr>
</tbody>
</table>

Abbreviations: GO, Graves orbitopathy; NA, not applicable.

* Population included 20 white patients (8 men, 12 women; mean [SD] age, 46 [13] years).

* Multiple regressions were performed individually.

* For lymphocytes, log10 values were used.

* Smoking habits were converted into continuous values.
Figure 3. The findings probably reflect the relatively long GO duration (median, 34 months) and especially the fact that most patients had undergone an ablative thyroid treatment that, with the exception of a known transient increase in TRAbs after radioiodine,14 is generally followed in the long term by a reduction of these autoantibodies.15 This reduction may explain the relatively low levels and, consequently, the lack of correlation with orbitallymphocytes. Overall, our findings do not exclude with certainty a general correlation between orbitallymphocytic infiltrate and antibodies to the TSH receptor; some studies have shown that thyroid-stimulating immunoglobulins may be more sensitive than TRAbs in correlating with GO features.16

Discussion

In the present study, we show that the lymphocytic infiltrate of orbital tissues in patients with GO correlates with GO activity, thereby shedding light on an association between GO immunologic features and clinical expression and possibly pos-
themonthprecedingtissueharvesting.21

ment (glucocorticoids and, in 1 patient, also azathioprine) in
2 of these 5 patients had undergone immunosuppressivetreat-

ingsmayreflectthefact,inthepreviousinvestigation,
number(5)of

studyonthesameissuewasconductedinasmallernumber
inculturewasinvestigated,19,20 toourknowledge,1previous

earlyandlateGO.21 Thisapparentdiscrepancywithourfind-

lymphocytic infiltrate were more often smokers, thereby in-

established using a finite mixture model, compared with pa-

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inthatpreviousinvestigation,patientswere selected and notconsecutive,thenuumber(5)
of

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articular glucocorticoids, GO activity, and immunohistologic

tweenresponse to immunosuppressive treatments, in par-

tinthisregard,itiswidelyusedinthisman-

levels of orbital infiltrating lymphocytes equal
to or below the cutoff value. Patients with a relevant orbital

were detected to a greater extent in patients with GO of recent onset compared with those with long-standing GO, which, in view

GO was more active in its early phases,23

ourfindingshereandinagreementwiththenotitionthatsmok-

Age, 46 [13] Years)

Age, 46 [13] Years)

Antibodies (TRAbs) in White Patients (8 Men, 12 Women; Mean [SD]

TRAbs, U/L

Figure 3. Individual Levels of Serum Anti–Thyrotropin Receptor

Orbital Lymphocytes, No. of Cells per Tissue Sample

Levels of serum TRAbs according to presence of a relevant (>300 cells

in 4 fields) orbital lymphocytic infiltrate.

Cytes above a cutoff level of 300 in 4 microscopic fields, estab-

inada mixture model, compared with pa-

etiology of GO, as well as for future studies aimed at

ory of GO, although data are not sufficient to prove it, which is a

remaindersto beestablishedwhetherlymphocytesaresomewhat “trapped” in

the variable presence of orbital infiltrating lymphocytes

and, in particular, CD20-positive cells explains the discrep-

Thus, rituximab was shown to determine an improvement of

inpatients with an eye disease of recent onset but not in

trast GO.21 This apparent discrepancy with our find-

patients were selected and not consecutive, the number (5) of

of subjects with early and presumably active GO was limited, and

2 of these 5 patients had undergone immunosuppressive treat-

and in agreement with the notion that smoking is a GO risk factor.11

Apart from studies in which the phenotype of orbital T cells

culture was investigated,19,20 to our knowledge, 1 previous

study on the same issue was conducted in a smaller number

patients (n = 14).21 In that study, orbital T cells were de-

studies are needed. Another limitation of our study is

that CAS was not originally intended as a long-term factor

In this regard, we considered the possibility that the variable

in orbital tissues or whether they continue to marginate; fur-

On the contrary, the correlation between response to

in GO activity, and immunohistologic features of GO.8 In this regard, we considered the possibility that the variable presence of orbital infiltrating lymphocytes

and, in particular, CD20-positive cells explains the discrep-

ating this observation is unknown. It remains to be estab-

thisobservationisunknown.Itremainstobeestablishedwhetherlymphocytesaresomehow “trapped” in

and B cell inactivity of GO and establishes a basis for under-

latingtheassociationbetweentheimmunesystemand

16 [80%]) from whom tissues were taken had been

of glucocorticoids before orbital decompres-

and, it could be argued that this intervention may have

However, the median time that elapsed since the last glucocorticoid administration was longer than 3 months, suggesting that glucocorticoids unlikely affected the

there was no correlation between previous glucocorticoid treatment or dos-

age and the lymphocytic infiltration in orbital tissues. On the

same line, 5 patients had received orbital radiotherapy, but

again there was no correlation between orbital radiotherapy and the findings reported above. Our results may suggest a possible role of infiltrating lymphocytes in the pathogenesis

feature of GO, as well as for future studies aimed at

or bellow the cutoff value. Patients with a relevant orbital

or below the cutoff value. Patients with a relevant orbital

lymphocytic infiltrate were more often smokers, thereby indi-

directly confirming the correlation between smoking and CAS

affected the results. However, the median time that elapsed

since the last glucocorticoid administration was longer than

sition, and it could be argued that this intervention may have

received orbital radiotherapy, but

withrituximabconsideringthatmostofourpatientshadlong-

positive lymphocytes and response to rituximab may exist. In

any case, our investigation may not be relevant to treatment

with rituximab considering that most of our patients had long-

standing GO, which may not be the proper population to be

with the anti-CD20 monoclonal antibody.

Conclusions

Our study provides evidence for a correlation between both T

in GO and establishes a basis for understanding the association between the immune system and clinical features of GO, as well as for future studies aimed

With the findings reported above. Our results may suggest a

possible role of infiltrating lymphocytes in the pathogenesis

of GO, although data are not sufficient to prove it, which is a

limitation of our study. In this regard, it should be taken into

account that lymphocytes likely represent only 1 of the

many causative cell elements involved, in addition to orbital

fibroblasts and fibrocytes.4 We observed a persistent

lymphocytic infiltration that correlates with CAS even in

chronic, long-standing cases of GO. The mechanism under-

lying this observation is unknown. It remains to be estab-

lished whether lymphocytes are somehow “trapped” in

orbital tissues or whether they continue to marginate; fur-

ther studies are needed. Another limitation of our study is

that CAS was not originally intended as a long-term factor

for GO evaluation (although it is widely used in this man-

ner), instead being intended as an indicator for the early

stages of the disease.11

As mentioned above, our findings may pose the basis for

future studies aimed at investigating the association be-

between response to immunosuppressive treatments, in par-

cular glucocorticoids, GO activity, and immunohistologic

features of GO.8 In this regard, we considered the possibility that the variable presence of orbital infiltrating lymphocytes

and, in particular, CD20-positive cells explains the discrep-

ant findings on the action of rituximab in patients with GO.8

Thus, rituximab was shown to determine an improvement of

in patients with an eye disease of recent onset but not in

those with long-standing GO.8 However, we did not find a

correlation between CD20-positive lymphocytes infiltrating

orbital tissue and disease duration, suggesting that the variable

response to rituximab unlikely reflects a different expression

of these cells in orbital tissues. However, Salvi et al23 showed

that CD20-positive lymphocytes infiltrating orbital tissues were

depleted after administration of rituximab in a small number

(2) of patients with active GO who responded to the treat-

ment, which suggests that an association between CD20-

positive lymphocytes and response to rituximab may exist. In

any case, our investigation may not be relevant to treatment

with rituximab considering that most of our patients had long-

standing GO, which may not be the proper population to be

with the anti-CD20 monoclonal antibody.

Limitations

Most patients (16 [80%]) from whom tissues were taken had

been treated with glucocorticoids before orbital decompres-

sion, and it could be argued that this intervention may have

affected the results. However, the median time that elapsed

since the last glucocorticoid administration was longer than

3 months, suggesting that glucocorticoids unlikely affected the

lymphocytic infiltration of orbital tissues. There was no

relation between previous glucocorticoid treatment or dos-

age and the lymphocytic infiltration in orbital tissues. On the

same line, 5 patients had received orbital radiotherapy, but

again there was no correlation between orbital radiotherapy and the findings reported above. Our results may suggest a possible role of infiltrating lymphocytes in the pathogenesis

feature of GO, as well as for future studies aimed at

comprehension of the response of GO to immunosuppres-

sive treatment based on its activity.
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**Author Contributions:** Drs Rotondo Dottore and Marinì had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Caturegli, Sframeli, Sabini, Nardi, Latrofa, Vitti, Marcocci, Marinì. Acquisition, analysis, or interpretation of data: Rotondo Dottore, Torregrossa, Ionnì, Sframeli, Sabini, Menconi, Piaggi, Sellari-Franceschini, Latrofa, Basolo, Marinì. Drafting of the manuscript: Rotondo Dottore, Torregrossa, Ionnì, Sframeli, Sabini, Piaggi, Marinì. Critical revision of the manuscript for important intellectual content: Caturegli, Sabini, Menconi, Sframeli, Sabini, Menconi, Piaggi, Sellari-Franceschini, Nardi, Latrofa, Vitti, Marcocci, Basolo, Marinì. Statistical analysis: Piaggi, Marinì. Administrative, technical, or material support: Torregrossa, Menconi, Sellari-Franceschini, Latrofa, Basolo, Marinì. Study supervision: Caturegli, Menconi, Nardi, Latrofa, Vitti, Marcocci, Marinì.

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**REFERENCES**


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