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Rituximab treatment of patients with severe, corticosteroid-resistant thyroid-associated ophthalmopathy

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Abstract

Purpose—To study the effectiveness of anti-CD20 (Rituximab, RTX, Rituxan®, Genentech Inc. USA) therapy in patients with severe, corticosteroid (CS)-resistant thyroid-associated ophthalmopathy (TAO).

Design—Retrospective interventional case series

Participants—Six consecutive subjects with severe, progressive TAO unresponsive to CS.

Methods—Electronic medical record review of consecutive patients receiving RTX during the previous 18 months. Responses to therapy were graded using standard clinical assessment and flow-cytometric analysis of peripheral lymphocytes.

Main outcome measures—Clinical activity score (CAS), proptosis, strabismus, treatment side-effects, and quantification of regulatory T cells.

Results—Six patients were studied. Systemic CS failed to alter clinical activity in all patients (CAS= 5.3 + 1.0 (mean + standard deviation) before vs 5.5 + 0.8 during therapy for 7.5+ 6.4 months,

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$p=1.0$). However following RTX treatment, CAS improved from 5.5 ± 0.8 to 1.3 ± 0.5 at 2 months post treatment ($p<0.03$) and remained quiescent in all patients ($CAS=0.7 \pm 0.8$; $p\leq 0.0001$) at 6.2 $\pm$ 4.5 month follow-up. Vision improved bilaterally in all 4 patients with dysthyroid optic neuropathy (DON). None of the six patients experienced disease relapse following RTX infusion and proptosis remained stable (Hertel measurement = 24 ± 3.7 mm before and 23.6 ± 3.7 mm after therapy, $p=0.17$). The abundance of T regulatory cells, assessed in one patient, increased within one week of RTX and remained elevated at 18 month follow-up.

Conclusions—In progressive, CS-resistant TAO, rapid and sustained resolution of orbital inflammation and DON followed treatment with RTX.

Keywords

thyroid eye disease; B cells; anti-CD20; Rituximab

Introduction

Graves' disease (GD), a common autoimmune thyroid disorder, also targets connective tissue of the orbit and skin.^{1,2} Hyperthyroidism occurs in a majority of patients, resulting from activating auto-antibodies directed against the thyroid stimulating hormone receptor (TSHR).³ Thyroid-associated ophthalmopathy (TAO), affecting 30–50% of patients with GD, manifests as orbital inflammation and expansion of fat and extra-ocular muscles. The etiology of TAO remains uncertain but lymphocytes and other mononuclear cells infiltrate the orbit and are thought to drive tissue remodeling possibly as a consequence of cytokine production and their actions on fibroblasts.^{4,5} Th1 or Th2 T cells predominate the cellular infiltrate, depending on the phase of the disease.⁶ B cells which are plentiful in affected tissues may produce locally the auto-antibodies against TSHR and insulin-like growth factor-1 receptor (IGF-1R).⁷ In addition, B cells efficiently present antigens and provide cognate and cytokine mediated co-stimulation to T cells.⁸ Thus both T and B cells deserve consideration as therapeutic targets in GD and TAO.

Treatment of the inflammatory component of TAO has not advanced appreciably over several decades.⁹ High-dose systemic corticosteroids (CS) alone or in combination with orbital irradiation remain imperfect options since recurrences are common following their discontinuation.¹⁰ Furthermore, neither modality alters the natural course of TAO.¹¹ An array of newly developed therapies have been utilized in autoimmune diseases allied to TAO. Among these biological agents, Rituximab (Rituxan®, RTX) represents a human/murine chimeric monoclonal antibody targeting CD20, a transmembrane protein present on immature and mature B cells, but absent on plasma cells.¹² RTX depletes B cells by enhancing apoptosis, promoting antibody-dependent cellular toxicity (ADCC) and complement-dependent cellular toxicity (CDCC).¹³ It was used initially to treat non-Hodgkin's B-cell lymphoma¹⁴ and more recently has benefited individuals with rheumatoid arthritis (RA).¹⁵ Previous studies, all preliminary in nature, have suggested that RTX might marginally improve thyroid dysfunction in GD.^{16–8} However, the drug appears to reduce the activity of TAO despite persisting or relapsing hyperthyroidism.^{16–20} Recently, in an open-label trial, Salvi and colleagues compared primary treatment with RTX to intravenous CS in patients with severe TAO.¹⁸ Patients receiving RTX improved as was evident by reduced disease activity and severity after 12 months when compared to those receiving CS.¹⁸

Here we report using RTX in patients with severe, progressive TAO and refractory dysthyroid optic neuropathy (DON) who had previously failed CS therapy or orbital decompression. To our knowledge, this is the first demonstration that B cell depletion might represent an effective strategy for salvaging these individuals by non-surgical means. Explicit in our findings is the

potential for RTX and similar B cell-depleting agents to benefit a wider spectrum of patients with TAO than those treated with CS.

Materials and Methods

The study was approved by the Institutional Review Board of the Center for Health Sciences at University of California at Los Angeles (UCLA) and was conducted according to the tenets of the Declaration of Helsinki. A retrospective review of electronic medical records (McCann Medical Matrix, Salt Lake City, UT, U.S.A.) identified patients in the database of the Orbital and Oculoplastic Clinic, Jules Stein Eye Institute, who had received RTX treatment over the period extending from October 1, 2007 to February 1, 2009. GD was diagnosed using standard clinical parameters such as signs and symptoms of thyrotoxicosis and diffuse goiter, elevated free thyroxine (FT₄), low or undetectable serum TSH, and presence of thyroid stimulating immunoglobulins.³ TAO was identified by the presence of proptosis, lid retraction, restrictive strabismus, conjunctival injection, chemosis, periocular erythema, and swelling.²¹ It was graded with the 7-item clinical activity score (CAS),²² using the un-operated orbit to avoid bias imposed by surgery. Patient 6 underwent bilateral decompression surgery 2 months prior to RTX treatment and CAS was measured in the more affected orbit. All patients were analyzed with orbital computerized topography or magnetic resonance imaging.²¹

Inclusion Criteria

Subjects were considered for RTX infusion therapy if they met one of the following criteria:

1. poor response to CS as defined by a decrease in the CAS of < 2 , or
2. recurrence of TAO with an increase in CAS ≥ 2 following the taper of CS, or
3. development of DON during the course of CS.²³ or
4. persistence of DON after medial wall orbital decompression and CS therapy²⁴

CS was administered orally at a dose of 1mg/kg/day for at least four weeks either alone or combined with intravenous pulse-methylprednisolone (n=1), or with an immunosuppressant such as CellCept® (mycophenolate mofetil, Roche, Nutley, NJ, USA; n=1). All patients were receiving CS at the initiation of RTX treatment. An orbital surgeon (RSD), a rheumatologist (DK), and endocrinologists (CD and TJS) managed all patients throughout the study. Pre-treatment laboratory investigations included complete blood count, assessment of liver and kidney function, serum immunoglobulin levels, and hepatitis B and C serology. Prior immunizations for tetanus, pneumococcus, and diphtheria were verified and updated if necessary. Two infusions of RTX, 1 gram each, were administered two weeks apart; in accordance with the U.S. Food and Drug Administration protocol approved for the treatment of RA.¹⁵ Pre-medications included 100mg intravenous methylprednisolone, 1 gram oral acetaminophen, and 50mg diphenhydramine, given 30 minutes prior to RTX infusion.¹⁵ Patients were assessed at 2, 4, 6, 8, and more than 12 weeks following RTX administration. Assessment included laboratory testings and standardized ophthalmic examination of CAS, visual acuity, color vision, pupillary response, funduscopy, and Goldmann applanation tonometry. External photographic documentation was performed and evaluated by two independent observers (RSD and KC).

Immunohistochemistry of orbital tissues

Patient 1 underwent orbital decompression for DON 12 days after RTX treatment and orbital tissues were processed for immunohistochemical studies. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin and cut in thin sections (4µm). The sections were deparaffinized with xylene and rehydrated through graded ethanol. Endogenous peroxidase

activity was blocked with 3% hydrogen peroxide in methanol for 10 min. Heat-induced antigen retrieval was carried out for all sections in 0.01M sodium citrate buffer, pH = 6.00 using a vegetable steamer at 95°C for 25 minutes. Mouse monoclonal antibody to CD20 (Dako Corporation, Carpinteria, CA) and rabbit polyclonal antibody to human CD3 (Dako Corporation) were applied at a dilution of 1:1000 and 1:100, respectively, for 45 minutes at room temperature. The signal was detected using the DAKO horseradish peroxidase EnVision kits (DAKO), and visualized with the diaminobenzidine reaction. The sections were counterstained with hematoxylin and coverslipped.

Flow cytometry

Peripheral blood (approximately 5 ml) was stored in tubes containing ethylenediaminetetraacetic acid (EDTA). Staining buffer (SB) was prepared in phosphate-buffered saline containing 4% fetal bovine serum and 0.1% sodium azide (Sigma Aldrich). Cell staining was performed within 24 hours of blood collection, according to the manufacturer's instructions (BD Biosciences, San Jose). Briefly, 100 μ l whole blood was placed in 12 $\times$ 75 mm polypropylene tubes and fluorochrome-conjugated monoclonal antibodies were added (1 μ g/10⁶ cells). These were then incubated in the dark for 20 minutes at room temperature. FACSlyse solution (2 ml) was added for 10 minutes at room temperature to lyse red blood cells. Cells were washed twice with SB, re-suspended in Cytotfix (BD Biosciences) and kept in the dark at 4°C until analysis performed on a FACS Calibur flow cytometer (BD Biosciences). CD25⁺ T cells, B cells, and those lymphocytes displaying IGF-1R were identified as those with increased fluorescent intensity compared to isotype controls. FACSlyse buffer, Cytotfix, and antibodies to CD3, CD4, CD8, CD19, CD25, IGF-1R α subunit (clone 1H7), and control mouse IgG1 were purchased from BD Biosciences (San Jose, CA).

Statistical analysis was performed with commercially available software (Microsoft® Office Excel 2007, SPSS version 13.0, SPSS Inc, Chicago, IL and GraphPad Prism 5, GraphPad Software). Non-parametric Wilcoxon signed rank and Friedman tests were used to compare between treatment groups. Data were expressed as the mean + standard deviation (SD). Differences were considered significant at the level of $p \leq 0.05$.

Results

Clinical improvement of TAO after RTX

Demographic and clinical data from the 6 patients are shown in Table 1 (available at <http://aaojournal.org>). Their age ranged from 43 to 68 years (54.3 $\pm$ 9.1; mean $\pm$ SD). Four patients were female and 3 smoked tobacco at the time they developed TAO. Two patients exhibited orbital manifestations prior to hyperthyroidism (patient 3, one month and patient 5, five months). Two patients developed thyroid dysfunction and TAO simultaneously, and 2 were hyperthyroid before the onset of TAO. Patients 1 and 2 underwent radioactive iodine ablation followed by thyroxine supplementation while patients 3, 4, 5, and 6 were treated with anti-thyroid medications. All patients were euthyroid at the time of RTX infusion and had previously received 1 to 3 courses of CS for active TAO. Duration of TAO prior to CS treatment was 5.0 $\pm$ 2.8 months (range 2 to 10 months). CS was administered for 7.5 $\pm$ 6.4 months (range 1 to 16 months) before RTX and all patients were receiving CS at the time of RTX treatment. Duration of TAO prior to initiation of RTX therapy was 12.5 $\pm$ 5.2 months (range 6–18 months).

CAS did not improve in any of the 6 patients following treatment with CS (CAS: 5.3 $\pm$ 1.0 prior to CS vs. 5.5 $\pm$ 0.8 during CS, $p=1.0$) (Figure 1A and 1B). Conversely, improvement was observed as early as 4 weeks following the first infusion of RTX (CAS 5.3 $\pm$ 1.0 prior-RTX vs. 1.3 $\pm$ 0.5, 8 weeks post series of 2 infusions, $p<0.03$) and this improvement was sustained

(CAS $0.7 + 0.8$ at $6.2 + 4.5$ month follow up, $p \leq 0.0001$) (Figure 1B and Figure 2). CAS was measured in the unoperated orbit for Patients 1 to 5 and the worse orbit for Patient 6. Figure 2 demonstrates clinical photos of Patients 1 and 4. Despite improvement in orbital inflammatory signs, Patient 4 experienced progressive strabismus after RTX treatment (Figure 2B).

Patients 1, 3, 4, and 6 developed early signs of DON during CS therapy. Patient 1, who had bilateral asymmetric disease, underwent unilateral, transcaruncular medial wall orbital decompression on day 12 after her first RTX infusion due to continued DON.²³ Patient 6 presented initially with unilateral DON but subsequently developed bilateral involvement and was sequentially decompressed after failing CS treatment. Despite high dose CS postoperatively, the DON persisted for 2 months. Alternative causes such as ischemic optic neuropathy were considered unlikely based on clinical evidence and repeated MRI scans of the brain and both orbits. RTX infusions were administered 2 months after surgery in combination with 20 Gy fractionated orbital irradiation divided into 10 doses. The patient subsequently demonstrated improved CAS and visual acuity at 2 weeks post series of 2 infusions, which were sustained (Figure 1A). Patient 3 developed unilateral DON, failed to respond to CS therapy and underwent surgical decompression with improvement of visual acuity. Orbital inflammation was exacerbated during steroid taper and he again developed DON in the same eye and underwent RTX treatment 9 months after surgical decompression. DON improved within 4 weeks of RTX treatment (Figure 1A).

All 4 patients who developed DON exhibited improvement in visual acuity within 4 weeks of their first RTX infusion and improved to pre-neuropathy vision over 2 months. (Figure 1C). All patients receiving RTX were concurrently receiving CS. Within 4 weeks of the first RTX infusion, CS was tapered and none of the patients experienced recurrence during the follow up period. As shown in Figure 1D, proptosis remained unchanged. Hertel measurements were 24 ± 3.7 mm before and 23.6 ± 3.7 mm after therapy ($p=0.17$, $n=6$). None of the patients demonstrated improvement in extraocular motility (data not shown) while patient 4 demonstrated progressive strabismus (Figure 2).

Adverse events during RTX therapy included urinary tract infection in Patient 4 which resolved with oral antibiotics. Patient 5, having preexisting hypertension, developed further elevation during the first RTX infusion which was readily controlled. Patient 3 expired from sudden cardiac death 3 months after receiving the final infusion.

Absence of orbital B cells after RTX

Since the therapeutic efficacy of RTX is associated with depletion of B cells in target tissues²⁵, we assessed the presence of mononuclear cells in orbital tissue obtained during decompression surgery for DON in Patient 1. Tissue was obtained 12 days after the initial infusion of RTX. As demonstrated in Figure 3, adipose cells were heterogeneous in size with prominent fibrovascular septae. There was paucity of mononuclear infiltrates (Figure 3A). Neither B cells (Figure 3B) nor T cells (Figure 3C) were present.

Assessment of B and T cell phenotype before and after RTX

To determine potential biologic mechanism(s) of B cell depletion in TAO, we assessed the frequency of regulatory T cells (Tregs) and of IGF-1R⁺ T and IGF-1R⁺ B cells. Tregs have been shown to reflect disease severity and response to treatment in patients with rheumatoid arthritis and diabetes.²⁶ These cells may dampen autoimmune responses when present in adequate number. Their absence is associated with disease progression, while disease resolution can be accompanied by increased Treg frequency.^{27,8} Figure 4 demonstrates the frequency of CD4⁺ T cells co-expressing CD25^{Hi} (Tregs) in Patient 1. This cell population also expresses Foxp-3, a transcription factor specific to Tregs.²⁹ Prior to RTX, Tregs accounted

for less than 1% of CD4⁺ T cells. In contrast, the abundance of Tregs increased within 2 weeks of initiation of RTX (2.3% Tregs at 2 weeks) and was sustained at 18 months follow-up (3.0% Tregs).

Earlier studies demonstrate increased frequency of IGF-1R⁺ T and B cells in GD.^{5,7} We investigated the expression of IGF-1R by T and B cells from Patient 1. Table 2 (available at <http://aaojournal.org>) demonstrates increased abundance of IGF-1R⁺ T cells prior to RTX and this level was maintained after treatment. CD69, an activation associated marker expressed by T cells was also unaltered after treatment. Thus lymphocyte expression of two surface receptors associated with GD, IGF-1R and CD69; do not appear to be altered with RTX.^{5,7,30}

Discussion

In this retrospective case series we demonstrate that RTX was associated with significant improvement of the clinical manifestations of severe TAO which were refractory to CS therapy or orbital decompression. Four of the six patients developed DON prior to RTX treatment. Orbital inflammation (elevated CAS scores) and DON improved without recurrence in all patients but proptosis and strabismus were unimproved by RTX. These results must be interpreted with caution since the study was uncontrolled regarding concurrent treatment modalities including Patient 3 and 6 who underwent decompression surgery prior to RTX infusion and Patient 6 who received concurrent orbital irradiation. However, as the molecular mechanisms underlying TAO become better understood, more precise drug targets should be identified. The prominent role of B cells in autoimmune responses, including antibody and cytokine production and antigen presentation, makes them particularly attractive. The success of B cell depletion as a strategy for treating rheumatoid arthritis found unresponsive to tumor necrosis factor- α blocking agents suggests that this approach might also be well-suited for patients with TAO.³¹

In earlier studies, RTX appeared to reduce the need for continued thionamide therapy in GD-associated hyperthyroidism.^{16–9} Those effects were independent of detectable changes in anti-TSHR antibody levels.¹⁷ However, anti-TSHR antibodies, as measured in that study, comprise both stimulatory and blocking molecules as well as those not influencing receptor function. In subsequent studies involving these same patients, levels of stimulatory anti-TSHR antibodies were found to be reduced by RTX. This finding agrees with those in other autoimmune disorders where levels of autoreactive antibodies were reduced as a result of preferential elimination of autoreactive B cells.³³ Moreover, reduction of B cells infiltrating affected tissues appears to be an important indicator of successful RTX therapy.^{34,6} Here we demonstrate an absence of B cells infiltrating orbital tissue obtained during decompression surgery 12 days after first RTX treatment (Figure 3) which is in agreement with a recent report by Salvi et al.³⁶

The clinical benefit of B cell depletion in TAO may derive in part from the altered abundance of Tregs, unique T cells which can modulate autoimmune responses.^{35,7} Their impact on human immunity remains uncertain but they appear to act in large part through the transforming growth factor (TGF)- β pathway. Their frequency is reduced in autoimmune diseases.²⁶ RA improves with B cell depletion in patients demonstrating increasing levels of Tregs, a trend predictive of therapeutic success.^{27,8} Levels of Tregs following RTX therapy have not been previously examined in GD. Findings in the single patient reported here suggest that they may become more frequent following RTX treatment, analogous to the experience in RA.^{27,8} (Figure 4). The safety profile of RTX compares favorably to that of oral and IV steroids.¹⁸ All patients were being treated with CS at the time of RTX treatment and the infusion was well tolerated. In the current study, two well-tolerated infusions of the agent uniformly depleted B cells. The infusion frequency and dose currently recommended for autoimmune disease are

substantially lower than those used for lymphoma and are associated with fewer side-effects.
38

We report here the first case series of patients with severe, active, and CS resistant TAO treated with RTX. Substantial clinical benefit was achieved in all 6 patients receiving the drug. Our findings suggest that patients who had already failed to respond to CS represent an expanding indication of RTX as salvage therapy in refractory TAO. Prospective studies are now required to establish RTX as a therapeutic option in these clinically challenging patients.

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Figure 1A

Figure 1B

Figure 1C

Figure 1D

Figure 1. Clinical response after Rituximab (RTX) infusion

A) Clinical activity score (CAS) of thyroid associated ophthalmopathy (TAO) evaluated before and after infusion of RTX, wk = weeks.

* denotes patients who developed dysthyroid optic neuropathy (DON) during corticosteroid (CS) treatment.

B) Summary of CAS before and after CS and RTX therapy in all six patients.

* $p < 0.05$ (after CS and after RTX at 2 months), Wilcoxon signed rank test

** $p < 0.0001$ (after CS, after RTX at 2 months and at last follow-up), Friedman test

C) Change in visual acuity (reciprocal) in subjects with DON before and after RTX infusion. Abscissa represents individual eye, top of arrow indicates initial visual acuity, arrowhead points to final visual acuity.

(*) Final visual acuity assessed prior to transcaruncular medial wall orbital decompression.

(**) Initial visual acuity assessed after transcaruncular medial wall orbital decompression but prior to RTX infusion.

D) Assessment of proptosis (mean + standard deviation) before and after RTX.

Measure of proptosis was performed before and after RTX in the unoperated orbit for patients 1–5 and the worse side for patient 6. $P = 0.17$, Wilcoxon signed rank test

Figure 2.

Photographs of two patients demonstrating orbital inflammation before and after Rituximab (RTX) therapy. Patient 4 demonstrated significant periorbital inflammation (A) prior to RTX treatment which decreased dramatically after therapy (B). Strabismus and eyelid retraction increased despite improvement of inflammatory signs and symptoms. Patient 1 prior to RTX treatment (C) and approximately 18 months after treatment (D).

Figure 3. Histology and immunohistochemical staining of orbital tissues obtained during decompression surgery from patient 1

A) High-powered view demonstrating adipocytes with prominent fibrovascular septae and paucity of mononuclear inflammatory infiltrates.
 B) High-powered view demonstrating absence of CD20⁺ B cells and C) High-powered view demonstrating absence of CD3⁺ T cells
 A) Hematoxylin and Eosin. B & C) counterstained with Hematoxylin. Magnification, x400, (inset, x40).

Figure 4.

Flow cytometric analysis of regulatory T (Treg) cells in Patient 1 before the initiation of therapy, 2 weeks and 18 months after Rituximab (RTX) infusion. The data show increased Treg (CD25^{Hi}) cells among CD4⁺ T cells.

Table 1

Patients demographics and clinical characteristics

Patient number	1	2	3	4	5	6	Mean $\pm$ SD
Age	68	58	59	43	51	47	54 $\pm$ 9
Gender	F	F	M	F	M	F	
Smoking status	NS	NS	CRS	CRS	CRS	NS	
PMH	CA breast, DM, HT, RA, Sjogren's syndrome	Not significant	Splenectomy due to trauma, hemochromatosis	Not significant	Not significant	Not significant	
Endocrinologic treatment of GD	RAI, T ₄	ATD, RAI, T ₄	ATD	ATD	ATD	ATD	
Chronology of TAO	After GD	After GD	Before GD	Concurrent with GD	Before GD	Concurrent with GD	
Duration of TAO prior to any CS (m)	5	5	3	5	2	10	5 $\pm$ 2.8
Duration of CS prior to RTX (m)	10	1	13	1	16	4	7.5 $\pm$ 6.4
Duration of TAO prior to RTX (m)	15	6	16	6	18	14	12.5 $\pm$ 5.2
Clinical response to CS	Progression; DON	Progression	Progression; DON during CS taper	Progression; DON	No response	Progression to DON which persisted after bilateral decompression	
Additional immunosuppressant before RTX	PMP	None	Cellcept	None	None	Fractionated orbital irradiation (20Gy in total) 1 week after 1 st RTX tx	
# Episode of DON (side)	1 (OD)	None	2 (OD)	1 (OD)	None	2 (OU)	
Orbital decompression	1(After first RTX)	None	1 (9 month prior RTX due to DON)	None	None	2 (OU, 2 months prior)	
CAS before CS	5	6	6	5	5	4	5.3 $\pm$ 1.0 ^f
CAS after CS (before RTX)	5	7	5	5	5	6	5.5 $\pm$ 0.8 ^g
CAS at 8 weeks post RTX	1	2	1	1	1	2	1.3 $\pm$ 0.5 ^h
CAS at last FU	0	1	1	0	0	2	0.7 $\pm$ 0.8 ^e
Hertel prior RTX*	27	23	25	17	25	27	24 $\pm$ 3.7
Hertel at latest follow-up*	26	23	25	16.5	24	27	23.6 $\pm$ 3.7 ^ψ

Patient number	1	2	3	4	5	6	Mean+/-SD
Recurrence of TAO after RTX	None	None	None	None	None	None	
FU after RTX(m)	14	8	2	6	5	2	6.2+/-4.5
Adverse events	None	None	Cardiac arrest 3 months after 2 nd Ix	UTI 3 days after 2 nd Ix	Raised BP during 1 st Ix	None	

All durations in months (m) unless otherwise specified.

* Unoperated orbit measured in millimeters. Value from the more affected orbit after decompression and before RTX (patient 6)

[¶] p=1.0(before and after CS).

[§] p<0.03 (after CS and after RTX at 8 weeks).

^ϕ p=0.0001(before, after CS and after RTX at 8 weeks).

⁵ p≤0.0001(after CS, after RTX at 8 weeks and at last follow-up).

^ψ p=0.17(after CS and after RTX at 8 weeks)

Abbreviations: PMH=past medical history; RTX= Rituximab infusion; SD=standard deviation; M=males; F=female; NS=non-smoker; CRS=chronic-smoker; ATD=anti-thyroid drug; RAI=radioactive iodine; T4=L-thyroxine supplement; CA breast=carcinoma of breast; DM=diabetes mellitus; HTN=hypertension; RA=rheumatoid arthritis; TAO=thyroid associated ophthalmopathy; DON=dysthyroid optic neuropathy; Ix=infusion; CAS=7-item clinical activity scores; CS=corticosteroid; PMP=pulse methylprednisolone infusion; FU=follow-up; UTI=urinary tract infection; BP=blood pressure

Table 2

Lymphocyte phenotype of Patient 1

Percent positive expression		Pre RTX	1 week post RTX	18 months post RTX
CD3	IGF-1R	36	37	49
CD4	IGF-1R	31	27	42
CD8	IGF-1R	40	43	50
CD19	CD20	20	0	30
CD19	IGF-1R	7	NA	13
CD3	CD69	3	2	2

Details refer to *Materials and Methods: Flow cytometry*.

RTX= Rituximab infusion; NA=not applicable; IGF-1R= Insulin-like growth factor 1 receptor