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ORIGINAL ARTICLE

Putative protective role of autoantibodies against the insulin-like growth factor-1 receptor in Graves' Disease: results of a pilot study

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Abstract

Background The insulin-like growth factor-1 receptor (IGF-1R) is a key element in the pathogenesis of Graves' Orbitopathy (GO), but the role of IGF-1R autoantibodies (IGF-1RAbs) has not been established.

Methods We designed a cross-sectional investigation to measure IGF-1RAbs in patients with Graves' disease (GD), with or without GO, who underwent radioiodine therapy followed by glucocorticoids (GC). Twenty-nine patients were included, 15 of which with GO. Patients were evaluated at baseline and three and 6 months after radioiodine. The primary objective was the prevalence of positive tests for IGF-1RAbs. The secondary objectives were: (1) IGF-1RAbs concentrations and their variations; (2) relationship between IGF-1RAbs and the features of GO; (3) relationship between IGF-1RAbs and anti-thyroid autoantibodies.

Results IGF-1RAbs above the cut-off value were found only in one patient with GD without GO. IGF-1RAb levels were greater in patients with GD without GO, at baseline ($P<0.0001$), and after three ($P<0.0001$) and six ($P=0.0001$) months. No correlations were observed between IGF-1RAbs and the features of GO, nor between IGF-1RAbs and anti-thyroglobulin or anti-thyroperoxidase autoantibodies. There was an inverse correlation between anti-TSH receptor autoantibodies (TRAbs) and IGF-1RAb levels in GD patients with GO at 6 months ($P=0.03$).

Conclusions IGF-1RAbs appear to be greater in patients with GD without GO compared with those with GO, suggesting a putative protective role of IGF-1RAbs on the development of GO, in line with the beneficial effects of Teprotumumab on GO. The inverse correlation between IGF-1RAbs and TRAbs 6 months after radioiodine may reflect antigen spreading and/or GC treatment.

Keywords Thyroid · Graves' disease · Graves' orbitopathy · Graves' ophthalmopathy · Thyroid eye disease · Thyroid autoimmunity · Autoimmune thyroiditis · Insulin-like growth factor-1 receptor

Introduction

Graves' orbitopathy (GO) is a disfiguring and disabling disease which affects orbital tissues, observed in ~ 25–30% of patients with Graves' Disease (GD), thereby being the most common extrathyroidal manifestation of GD [1–3]. GO is rarely associated also with hypothyroid autoimmune thyroiditis and a very small proportion of GO patients do not have a clinically overt thyroid disfunction [1–4]. According to the most widely accepted model, GO is due to an autoimmune reaction against autoantigens expressed by thyroid epithelial cells and the orbital fibroadipose tissue, either constitutively or released from the thyroid together with autoreactive T-lymphocytes to reach orbital tissues [1–4]. The major autoantigen involved in the pathogenesis

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of GD and GO is the thyrotropic hormone (TSH) receptor (TSHR) [5–7]. However, the ultimate cause of GO is still to be defined. A number of studies have proposed a role of the insulin-like growth factor-1 receptor (IGF-1R), but whether IGF-1R is an autoantigen as well as the significance of autoantibodies against it (IGF-1RAbs) have been largely questioned [8–11].

The initial observations suggesting an involvement of IGF-1R in GO pathogenesis was based on the finding, in patients with GD, of serum immunoglubulins (IgGs) binding and activating the IGF-1R in orbital fibroblasts (OFs) [12]. Subsequent studies reported that IGF-1R seems to play a role also at the immune system level, being expressed in B and T cells as well as in monocytes [8]. Moreover, IGF-1RAbs from GD patients were found to elicit the release of T-cell attracting cytokines in fibroblasts derived from the thyroid as well as from orbital tissues [13, 14]. Strong support for a role of IGF-1R in GO is provided by two multicenter, randomized, double blind, drug vs placebo, clinical trials [15, 16], which demonstrated an impressive, beneficial effects on GO exerted by Teprotumumab, an anti-IGF-1R monoclonal, blocking antibody.

Several studies investigated the presence of IGF-1RAbs in GO patients, as well as their potential pathogenetic effects on IGF-1R signaling, leading to somehow conflicting results [17–23]. Thus, IGF-1RAbs in sera from patients with GO seem to have an inhibitory, rather than stimulatory, effect on IGF-1R signaling. In line with these observations, it has been reported that a direct activation of IGF-1R by an antibody is not required to explain the involvement of IGF-1R in GO pathogenesis, which rather reflects a structural and functional relationship between IGF-1R and TSHR signaling, as observed in thyroid cells and in OFs of patients with GO [24]. Actually, the beneficial effect of Teprotumumab in clinical trials may reflect an interference of the monoclonal antibody in the TSHR/IGF-1R cross talk, rather than a direct action on IGF-1R signaling.

In a previous study, we found that serum autoantibodies against the IFG-1R are present in ~ 25% of patients with GD, regardless of the presence of GO [25]. Interestingly, we found a significantly inverse correlation between serum levels of IGF-1RAbs and the activity of GO, evaluated by the Clinical Activity Score (CAS), suggesting that IGF-1RAbs may play a protective role on GO [25].

To investigate this issue further, the present cross-sectional study was undertaken to determine the presence and the variations of serum IGF-1RAbs in consecutive patients with Graves' disease (GD) with or without GO following radioiodine and glucocorticoid (GC) treatment.

Methods

Study design

The study was aimed at assessing IGF-1RAbs in patients with GD and GO compared with patients with GD without GO, in a cross-sectional investigation. The research design entailed the inclusion of all consecutive patients who came to our observation over a period of 2 months to receive radioiodine followed by oral low-dose or intravenous high-dose (ivGC) GC treatment, according to the European Group On Graves Orbitopathy (EUGOGO) guidelines [1, 26].

Setting

The study was carried out in the GO Clinic of the Endocrinology Units I and II and of the Ophthalmopathy Unit I of the University Hospital of Pisa, a tertiary referral center. Patients were included by consecutive sampling and the inclusion criteria adopted are reported below. The study was not blinded. The validation procedures employed for database data collection were: allowed character checks, batch totals, missing records check, cardinality check, digits check, consistency check, control totals, cross-system consistency check, data type check, hash totals, limit check, logic check, presence check, range check, spelling and grammar check, and uniqueness check.

Participants

Inclusion criteria were: (1) both groups: a diagnosis of GD, based on a history of hyperthyroidism, associated with previous or present detectable serum autoantibodies against the TSH-R (TRAbs); (2) GO group: a diagnosis of GO, based on the presence of at least one of the following eye features [27]: (a) exophthalmometry ≥ 17 mm in women and ≥ 19 mm in men, based on measurements in the normal population [27–32]; (b) eyelid aperture ≥ 9 mm [28, 32]; (c) clinical activity score (CAS) ≥ 1/7; (d) presence of diplopia; (e) presence of GO-related corneal alterations; (f) presence of GO-related alterations of the fundi; and (g) reduction in visual acuity that could be attributed to GO; (3) male and female patients aged 18–85 years; (4) written, signed informed consent to data use. Exclusion criteria were: (1) earlier treatment of hyperthyroidism by methods other than anti-thyroid medications; (2) any previous treatments for GO other than eye lubricants; (3) treatment with GC or any

immunosuppressive medication in the preceding 3 months; (4) lack of informed consent. A total of 29 subjects who satisfied the inclusion criteria and evaded the exclusion criteria were recruited, among which 15 had GO. They were enrolled in the study and evaluated at baseline and three and 6 months after radioiodine treatment. Being an observational study, no ethical approval was required.

Outcomes

The primary outcome of the study was the prevalence of positive serum IGF-1RAbs in patients with GO, compared with patients with GD without a clinically relevant GO.

The secondary outcomes were: (1) the serum concentrations of IGF-1RAbs and their variations after therapy in patients with GO, compared with patients with GD without GO; (2) the relationship between serum IGF-1RAbs (both levels and prevalence) and the clinical features of GO; (3) the relationship of serum IGF-1RAbs (both levels and prevalence) with anti-thyroglobulin autoantibodies (TgAbs), anti-thyroid peroxidase autoantibodies (TPOAbs) and TRAbs.

Sources of data and measurements

An ophthalmological evaluation was performed in all patients, including (1) exophthalmometry; (2) measurement of eyelid aperture; (3) evaluation of CAS; (4) assessment of diplopia; (5) assessment of the corneal status; (6) examination of the fundi; and (7) measurement of visual acuity.

The following blood tests were performed in all subjects: (1) FT_4 and FT_3 (Vitros Immunodiagnostics, Raritan, NJ); TSH (Immulite 2000, Siemens Healthcare, Gwynedd, UK); (2) TgAbs and TPOAbs (AIA-Pack TgAbs, Tosoh Bioscience, San Francisco, CA); and (3) TRAbs (Brahms, Berlin, Germany).

IGF-1RAbs were measured using a commercial ELISA (MyBioSource, San Diego, CA). Serum samples were incubated in microtiter plates coated with human recombinant IGF-1R and then with horseradish peroxidase-labeled goat anti-human IgG. In a previous study, we evaluated the inter- and intra-assay variations as well as the assay specificity [25]. The inter-assay variation was 4.3% and the intra-assay variation was 3.6%. We established a cut-off value for positive tests at 55.2 ng/mL, corresponding to the 97th percentile of the normal population. However, because data available on the significance of lower levels of IGF-1RAbs are lacking, IGF-1RAb concentrations below 55.2 ng/mL may still be somewhat meaningful. The specificity of the assay used was established by inhibition experiments using recombinant human IGF-1R [25].

Sample size

To our knowledge, no previous studies were conducted on the behavior of IGF-1RAbs in GD patients over time. Therefore, a sample size could not be established. We arbitrarily chose to include in the study all consecutive patients with GD scheduled to undergo radioiodine treatment over a period of 2 months.

Quantitative variables

Numerical data with a normal distribution, which was assessed using the Shapiro-Wilks test, are presented as mean ± SD. The remaining numerical data are presented as median and IQR.

Statistical analyses

Statistical analyses were performed using StatPlus (AnalystSoft Inc., Walnut, CA). When appropriate, the following tests were performed: (1) ANOVA with Bonferroni's correction; (2) Wilcoxon-Kruskal Wallis; (3) Mann–Whitney; (4) Chi-square; and (5) linear regression.

Results

Participants

We measured IGF-1RAbs in 29 patients with GD, of which 15 had GO. Demographical, clinical and biochemical data on the patient population are reported in Table 1. The two groups had a significantly different age ($P=0.05$) and thyroid disease duration ($P<0.0001$), but no relationship was observed between these two variables and the prevalence or levels of IGF-1RAbs (not shown). At baseline, all patients were on methimazole (MMI) and were scheduled to radioiodine therapy. Thus, the two groups were homogeneous, not having received earlier treatments for hyperthyroidism other than MMI and having stopped MMI 3 days before the baseline observation. Accordingly, there were no significant differences regarding FT_3, FT_4 and TSH values between the two groups (Table 2).

Of the 15 patients with GO, following radioiodine, 11 were treated with ivGC and four with oral, low dose GC. The latter was also given to GD patients without GO. At three and 6 months, patients were euthyroid on levothyroxine for radioiodine-induced hypothyroidism, without a significant difference between the two groups (Table 2).

TgAbs and TPOAbs were positive in all patients in both groups at baseline, as well as at three and 6 months, but their

Table 1 Features of 29 consecutive subjects tested for serum IgF1R-Abs

Feature	Graves' disease	Graves' disease and GO	*P*
Number of subjects	14	15	N/A
Age (yr.)	45.5 ± 12.6 (range: 25–62)	51.6 ± 14.4 (range: 25–76)	0.05*
Sex	11 women, 3 men	11 women, 4 men	NS**
Smoking	Smokers 4 Ex-Smokers 2 Nonsmokers 8	Smokers: 5 Ex-Smokers: 4 Nonsmokers: 6	NS**
Thyroid disease duration (mo.)	34 (IQR 11.7–78)	12 (IQR: 8–27)	< 0.0001***
GO duration (mo.)	N/A	33 (IQR: 26–39.5)	N/A
Thyroid treatment	MMI: 14	MMI: 15	NS**
GO treatment	N/A	None: 15	N/A

Numerical values are reported as mean ± SD or median and IQR, as appropriate

GO Graves' orbitopathy, *MMI* methimazole

P values were obtained by *ANOVA with Bonferroni's correction, **Chi-square test, or ***Mann Whitney

levels had a significantly different distribution across the study population. As shown in Table 2, there was a significant difference in the levels of TgAbs (greater in GD patients) and TPOAbs (greater in GO patients) between the two groups at baseline as well as at three and 6 months after radioiodine. These findings were somehow unexpected, as, to our knowledge, were not reported previously. Further investigations are needed to elucidate their significance, which however was not among the objectives of the present study. In addition to the latter observations, TgAbs and TPOAbs levels varied significantly within groups over time ($P < 0.0001$), presumably reflecting antigen spreading following radioiodine.

In patients with GD without GO, TRAbs were detectable in 10/14 patients (71.4%) at baseline, in 11/14 (78.5%) at 3 months, and in 12/14 (85.7%) at 6 months. Similar findings were obtained in patients with GO, namely 12/15 (80%) at baseline, three and 6 months, with no differences between the two groups. However, the serum levels of TRAbs were significantly different between the two groups, reflecting higher levels in GO patients, both before and after treatment (Table 2). As reported for TgAbs and TPOAbs, also TRAb varied significantly within groups over time ($P < 0.0001$).

As shown in Table 3, on average, the 15 patients with GO had a moderately severe, active eye disease, as defined by the guidelines of the EUGOGO [1], namely having two or more of the following: (1) lid retraction ≥ 2 mm; (2) moderate or severe soft-tissue involvement or exophthalmos ≥ 3 mm above normal for race and gender; and (3) inconstant or constant diplopia.

Primary outcome data: prevalence of serum IGF-1RAbs in the study population

Using the cut-off value of 55.2 ng/mL (see "Methods"), only one patient with GD was found to have a positive test for serum IGF-1RAbs (Fig. 1a), at baseline, 3 and 6 months. No patients with GD and GO were found to test positive (Fig. 1b).

Secondary outcome data

Levels of serum IGF-1RAbs in the study population

As shown in Table 2 and in Fig. 2, serum concentrations of IGF-1RAbs were significantly different between the study groups at baseline ($P < 0.0001$), reflecting higher concentrations in patients with GD without a clinically relevant GO. This difference was confirmed also three and 6 months after radioiodine and GC treatment. Moreover, similarly to TgAbs, TPOAbs and TRAbs, there was a significant variation in the concentrations of IGF-1RAbs within groups over time (Table 2).

Relationship between serum IGF-1RAbs, clinical features and treatment of GO

Within patients with GO, there was no relationship of the serum concentrations of IGF-1RAbs with GO duration, as well as with the clinical features of the eye disease, namely exophthalmometry, eyelid aperture, CAS, degree of diplopia, and visual acuity (not shown).

Relationship between serum IGF-1RAbs and TgAbs, TPOAbs and TRAbs

There was no relationship between the serum concentrations of TgAbs and TPOAbs with the levels of IGF-1RAbs (not shown). Interestingly, there was an inverse correlation between TRAb and IGF-1RAb concentrations in GO patients 6 months after radioiodine ($P = 0.03$), and a trend to a similar correlation at 3 months (Fig. 3). The finding is in line with the observation that serum IGF-1RAbs were higher in patients with GD

Table 2 Laboratory values of the patient population

Laboratory test	GD patients	GO patients	*P*
FT4 (ng/dL); NV: 0.7–1.7			
Baseline	1.2 ± 0.5 (range 0.8–3.1)	0.9 ± 0.2 (range 0.3–1.3)	NS*
3 months after I^{131}	1.0 ± 0.3 (range 0.2–1.7)	1.1 ± 0.5 (range 0.2–2.2)	NS*
6 months after I^{131}	1.2 ± 0.2 (range 0.7–1.8)	1.2 ± 0.4 (range0.1–1.8)	NS*
FT3 (pg/mL); NV 2.7–5.7			
Baseline	5.0 ± 2.6 (range 1.2–13.3)	4.1 ± 1.0 (range 2.6–6.6)	NS*
3 months after I^{131}	3.3 ± 0.7 (range 1.5–4.7)	3.3 ± 1.7 (range 1.6–8.5)	NS*
6 months after I^{131}	3.4 ± 0.5 (range 2.2–4.1)	3.4 ± 1.1 (range 1.3–5.4)	NS*
TSH (μU/mL); NV 0.4–4			
Baseline	0.45 (IQR: 0.02–1.45)	1.12 (IQR: 0.17–2.34)	NS**
3 months after I^{131}	5.2 (IQR: 2.2–22.1)	7.0 (IQR: 2.5–27.8)	NS**
6 months after I^{131}	2.1 (IQR: 1.1–3.7)	0.9 (IQR: 0.04–4.6)	NS**
TgAbs (IU/mL); NV ≤ 30			
Baseline	181.5 (IQR: 103.5–463.2)	164.9 (IQR: 131.5–667.5)	< 0.0001**
3 months after I^{131}	652.0 (IQR: 407.2–1970.5)	397.0 (IQR: 227.0–1035.5)	< 0.0001**
6 months after I^{131}	539.0 (IQR: 294.0–1318.5)	516.0 (IQR: 228.5–1647.5)	< 0.0001**
P within group‡	0.006*	0.0003*	
TPOAbs (IU/mL); NV ≤ 10			
Baseline	262.0 (IQR: 140.0–916.5)	294.0 (IQR: 144.0–948.5)	< 0.0001**
3 months after I^{131}	937.5 (IQR: 576.0–1000.0)	1000.0 (IQR: 485.5–1000.0)	< 0.0001**
6 months after I^{131}	754.0 (IQR: 618.5–1000.0)	898.5 (IQR: 373.2–1000.0)	< 0.0001**
P within group‡	< 0.0001*	< 0.0001*	
TRAbs (IU/L); NV ≤ 1.5			
Baseline	3.3 (IQR: 1.4–6.2)	6.9 (IQR: 3.5–11.6)	< 0.0001**
3 months after I^{131}	6.8 (IQR: 2.2–9.1)	7.9 (IQR: 4.1–23.6)	< 0.0001**
6 months after I^{131}	3.0 (IQR: 1–12.3)	9.1 (IQR: 3.8–37.2)	< 0.0001**
P within group‡	< 0.0001*	< 0.0001*	
IGF-1RAbs (ng/mL); NV 55.2			
Baseline	10.1(IQR: 8.7–13.8)	2.9 (IQR: 0.0–10.4)	< 0.0001**
3 months after I^{131}	8.4 (IQR: 4.3–25.2)	5 (IQR: 4.3–7.8)	< 0.0001**
6 months after I^{131}	7.6 (IQR: 3.2–14.9)	3 (IQR: 0.25–11.7)	0.0001**
P within group‡	< 0.0001*	< 0.0001*	

Numerical values are reported as mean ± SD or median and IQR, as appropriate

GD Graves'Disease, *GO* Graves' orbitopathy, *NV* normal values, *TgAbs* anti-thyroglobulin autoantibodies, *TPOAbs* anti-thyroperoxidase autoantibodies, *TRAbs* anti-TSH receptor autoantibodies

P values were obtained by *ANOVA with Bonferroni's correction (using Log10 values for data without normal distribution, as indicated by ‡) and ** Mann–Whitney

without GO, both before and after therapy, whereas TRAb levels had an opposite trend, being higher in GO patients.

Discussion

The objectives of the present study were to determine whether there is a difference in the prevalence and/or the levels of IGF-1RAbs between patients with GD without GO compared with patients with GD and clinically relevant GO, as well as in the behavior of these autoantibodies after treatment. We measured Igs recognizing the IGF-1R in the two study groups, using a commercial ELISA, as reported in a previous study [22]. The assay was previously shown to be precise (very low inter- and intra-assay variations) and specific, as demonstrated by the inhibitory effect exerted by recombinant soluble IGF-1R on the binding of human Igs to IGF-1R-coated ELISA plates. The prevalence of serum samples with IGF-1RAb concentrations equal or above a previously established cut-off value of 55.2 ng/mL did not differ between the two study groups. Thus, only one patient with GD had detectable serum IGF-1RAbs, whereas none of the patients with GO tested positive. The clinical significance of detectable serum IGF-1RAbs below the cut-off value is

Table 3 Features of 15 patients with Graves' orbitopathy at ophthalmological examination

Feature	Baseline	3 months	6 months
Exophthalmometry (mm)	20 (IQR 18.5–22.5)	20 (IQR 19–22)	20 (IQR 19–21.5)
Exophthalmometry ≥ 3 mm above normal for race and gender	13/15 (86.6%)	13/15 (86.6%)	13/15 (86.6%)
Eyelid aperture (mm)	11 (IQR: 10–12)	10 (IQR: 9.5–12)	10 (IQR: 9–12)
Eyelid retraction ≥ 2 mm	11/15 (73.3%)	10/15 (66.6%)	9/15 (60%)
Clinical activity score	4 (IQR: 2–5)	3 (IQR: 2–4)	2 (IQR: 0.5–3)
Clinical activity score ≥ 3 points	11/15 (73.3%)	8/15 (53.3%)	5/15 (33.3%)
Diplopia	Absent: 5 Intermittent: 1 Inconstant: 6 Constant: 3	Absent: 5 Intermittent: 1 Inconstant: 6 Constant: 3	Absent: 6 Intermittent: 1 Inconstant: 6 Constant: 2
Visual acuity (decimals)	0.1 (IQR 0.09–0.1)	0.1 (IQR 0.09–0.1)	0.1 (IQR 0.09–0.1)
GO degree according to EUGOGO guidelines [1]	Mild: 4 (26.6%) Moderate-to-severe 11 (73.3%) Sight threatening 0 (0%)	Mild: 5 (33.3%) Moderate-to-severe 10 (66.6%) Sight threatening 0 (0%)	Mild: 6 (40%) Moderate-to-severe 9 (60%) Sight threatening 0 (0%)

Continuous variables refer to the most affected eye. Numerical values are reported as mean ± SD or median and IQR, as appropriate

not established. Taken this into account, here we found that IGF-1RAb concentrations are higher in patients with GD without GO compared with patients with GD and GO, both before and after treatment. In addition, we found a significant difference in the concentrations of IGF-1RAbs between baseline and follow-up examinations within groups, possibly indicating that radioiodine and GC treatment may influence IGF-1RAbs over time. In patients with GO, there was no relationship between the clinical features of GO and the serum levels of IGF-1RAbs. Likewise, no correlations were observed between IGF-1RAbs and TgAbs or TPOAbs. Interestingly, there was an inverse correlation between TRAb and IGF-1RAb serum levels in patients with GO 6 months after radioiodine. In the latter group, TRAbs increased over time, presumably reflecting antigen spreading following radioiodine, whereas IgF-1RAbs did not, which may explain the correlation. A similar correlation, the significance of which remains to establish, was not observed in patients with GD without GO. In this regard, it should be considered that both groups underwent GC treatment after radioiodine, although at different dosages, which may have affected serum autoantibodies. Additional studies in a larger number of patients are required to investigate the correlation between IGF-1RAbs and TRAbs further.

We previously reported that serum autoantibodies against the IFG-1R are present in 25% of patients with GD, regardless of the presence of GO. Moreover, we found a significant, inverse correlation between serum IGF-1RAbs and CAS [22]. The latter observation may reflect a protective role of IGF-1RAbs on GO, thereby paralleling the action of the monoclonal, blocking anti-IGF-1R antibody Teprotumumab, which was shown to exert a remarkable beneficial effect in GO patients compared with placebo [12, 13]. Here we found only one patient with GD and no GO who had IGF-1RAbs above the cut-off value, and no correlation with CAS in GO patients. In addition, we observed a significant difference in IGF-1RAb serum levels between GD and GO patients, which was not found in our previous study. These discrepancies may be explained by the different sample sizes, as well as by the greater homogeneity of the present cohort of patients, who had not received any previous treatments for their thyroid disease other than anti-thyroid drugs, and, concerning patients with GO, no treatment for their eye disease. On the contrary, in our previous study patients had been variably treated for their thyroid diseases as well as for GO, which may have affected IGF-1RAbs. Taken together, our studies suggested that IGF-1R signalling may play a role in the pathogenesis of GO and that IGF-1RAbs may have a protective role by interfering with the TSHR/IGF-1R cross talk. Given the limited number of patients studied here and the lack of functional tests (see below), this conclusion is at present preliminary.

Following the initial observation that Igs from GD patients can bind to the IGF-1R [9], several attempts have been made to confirm their presence in the bloodstream of GD patients, as well as to define their function and relation to GO. However, whether there are autoantibodies in the sera of patients with GO which directly stimulate IGF-1R remains controversial. It has been also described that IGF-1R activation by GO-Igs occurs via the TSHR/IGF-1R cross talk, rather than through a direct binding to IGF-1R [16, 21]. Cross talk between G-protein coupled receptors and receptor tyrosine kinase is well described as a signaling mechanism. The IGF-1R is known to cross-talk with a number of

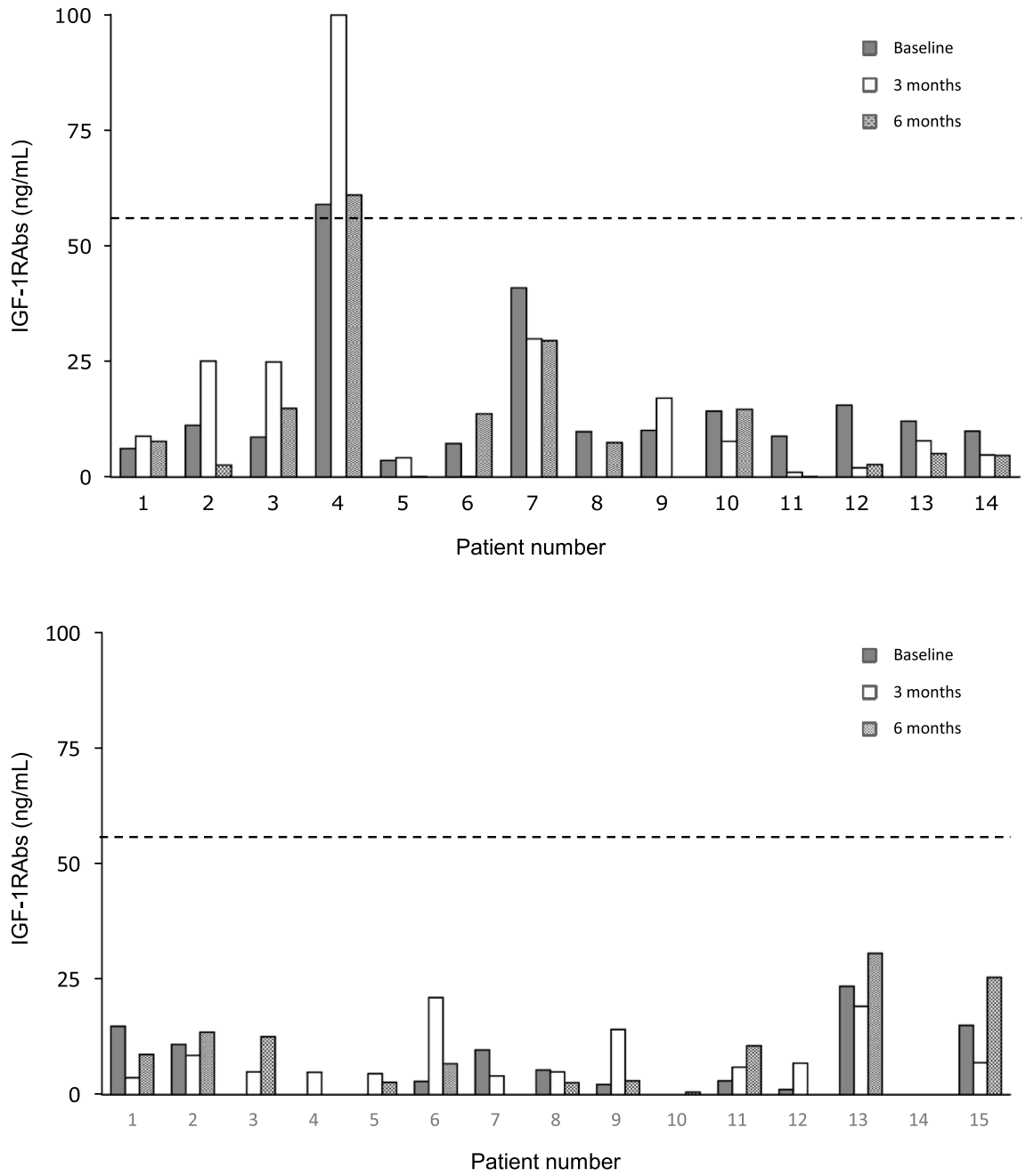

Fig. 1 Levels of serum autoantibodies against the insulin-like growth factor-1 receptor (IGF-1RAbs) in the study population. **a** Levels of serum IGF-1RAbs in patients with Graves' disease (GD) without Graves' orbitopathy (GO), at baseline and after 3 and 6 months from radioiodine treatment. Dotted line indicates the cut-off level for positivity at 55.2 ng/mL. **b** Levels of serum IGF-1RAbs in patients with GO at baseline and after 3 and 6 months from radioiodine treatment. Dotted line indicates the cut-off level for positivity at 55.2 ng/mL

G protein-coupled receptors, among which the TSHR, as reported by studies conducted in primary cultures of fibroblasts obtained from orbital tissues of patients with GO [16, 21]. It has been demonstrated that simultaneous activation by TSH and IGF-1 synergistically increases hyaluronic acid (HA) secretion and that dose-dependent HA production mediated by the anti-TSHR monoclonal antibody M22 is biphasic, with the highest potency phase influenced by IGF-1R [21]. Clearly, full HA induction may occur only in metabolically active cells requiring the use of media which contain also IGF-1. Therefore, it would not be surprising if basal IGF-1R activity is important for HA induction by TSHR. In the same study, the authors also reported that M22 did not stimulate IGF-1R phosphorylation, and that a

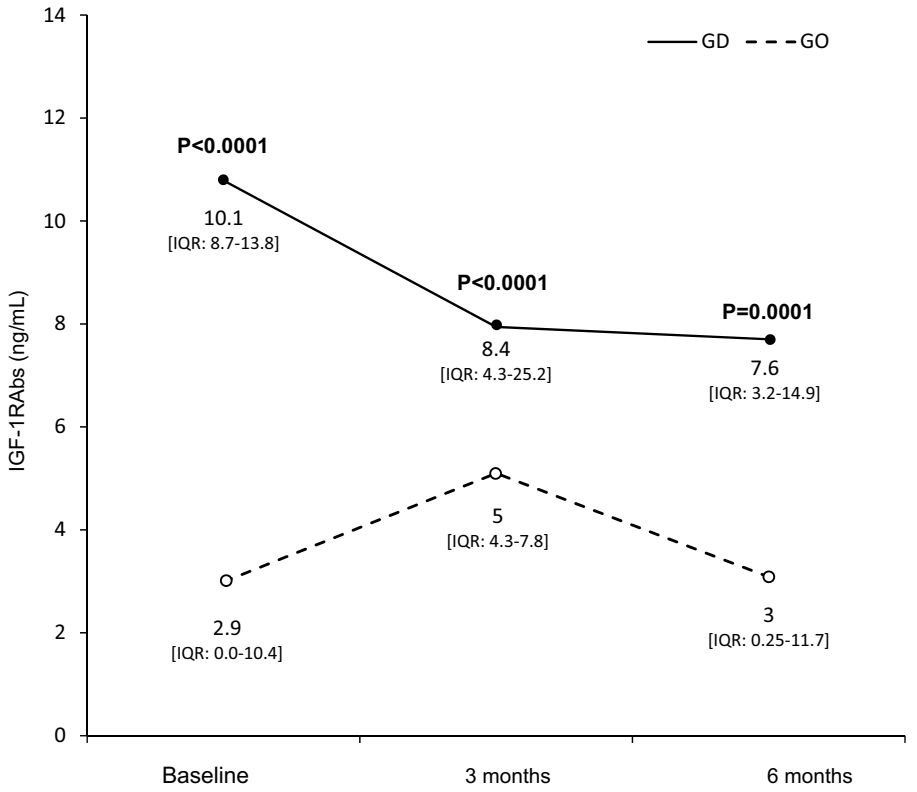

Fig. 2 Concentrations (median and IQR) of serum autoantibodies against the insulin-like growth factor-1 receptor (IGF-1RAbs) in patients with Graves' disease, with or without Graves' Orbitopathy (GO), at baseline and after three and 6 months from radioiodine treatment. *P* values were obtained by Mann Whitney

selective TSHR antagonist did not inhibit the phosphorylation of IGF-1R stimulated by IGF-1, but effectively inhibited the IGF-1-mediated HA production. These findings suggest that M22 does not activate IGF-1R directly, and the cross-talk between TSHR and IGF-1R does not require the direct activation of IGF-1R.

Certain studies have suggested that anti-IGF-1R Igs exist, are specific for GD and GO, and can directly bind and activate the IGF-1R in thyroid cells and in orbital fibroblasts [7, 8, 10, 11]. Nevertheless, all of these studies used GD-Igs containing several types of antibodies, because of which the evidence that GD-Igs bound to IGF-1R could not definitively demonstrate that they are stimulatory. On the contrary, Minch [14] et al. and Weightman [9] et al. reported the presence of IGF-1RAbs which bind IGF-1R with an inhibitory effect.

Coupled with the previous observation of an inverse correlation between CAS and IGF-1RAbs [22], the results of our studies are in line with the previous reports which suggested the existence of serum antibodies against the IGF-1R with inhibiting properties. Based on the evidence that binding to IGF-1R is not necessary for its involvement in the pathogenesis of GO, which rather occurs via the TSHR/IGF-1R cross talk, a speculative hypothesis is that the higher concentrations of IGF-1RAbs in GD patients without GO may reflect a protective action through their interference, directly or sterically, with the TSHR/IGF-1R cross talk, thereby inhibiting it and consequently inhibiting the trigger for GO induction. As reported above, this may also explain the clinical effect of Teprotumumab [33, 34]. Clearly, any considerations on a real, effectively protective action of IGF-1RAbs against the development of GO in patients with

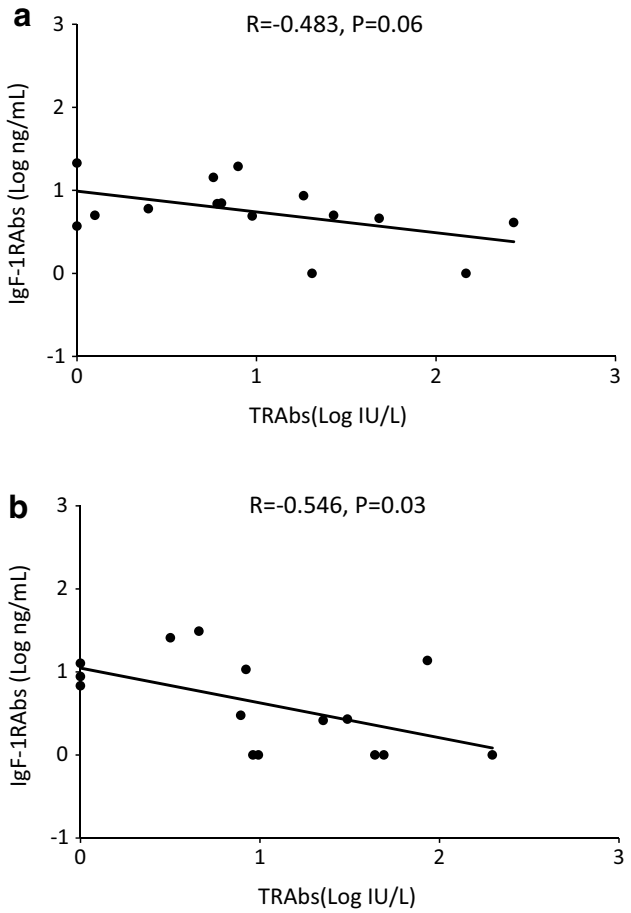

Fig. 3 Correlation between the concentrations (median and IQR) of serum IGF-1RAbs and those of anti-TSH-receptor autoantibodies (TRAbs) in patients with Graves' Orbitopathy, three (a) and six (b) months after radioiodine treatment. *P* values were obtained by linear regression of Log10 values

GD are preliminary and require confirmation by functional assays.

In conclusion, serum autoantibodies against the IFG-1R seem to be higher in patients with GD without GO, thereby suggesting a protective action on the development of GO, but further functional studies are needed to establish whether this is the case. In addition, our data await confirmation in studies performed in a larger number of patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedure performed in the study involving human participants were in accordance with the ethical standards of the Trust and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from the individual participant included in the study.

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