

Comparison Between Thyroid Stimulating Immunoglobulin and TSH-Receptor Antibodies in the Management of Graves' Orbitopathy



Authors

Selwan Khamisi^{1,2}, Martin Lundqvist^{1,2}, Britt Edén Engström^{1,2}, Anders Larsson², F Anders Karlsson^{1,2}, Östen Ljunggren^{1,2}

Affiliations

- 1 Department of Endocrinology and Diabetes, Uppsala University Hospital, Uppsala, Sweden
- 2 Department of Medical Sciences, Uppsala University, Uppsala, Sweden

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Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence

Selwan Khamisi, MD
Department of Endocrinology
Uppsala University Hospital
751 85 Uppsala
Sweden
Tel.: +46/186110000
selwan.khamisi@medsci.uu.se

ABSTRACT

Objectives TSH-receptor antibodies (TRAb) targeting the TSH receptor (TSH-R) induce hyperthyroidism in Graves' disease (GD). Graves' orbitopathy (GO) is influenced by stimulation of the TSH-R in the orbita. GO has been, among other factors, linked to high TRAb levels. Thyroid stimulating immunoglobulins (TSI) is a relatively new method for assessing TSH-receptor antibodies. The aim of this study was to investigate the role of TSI in the management of GO.

Methods Patients with newly diagnosed GD (n = 30, median age 55 years (range 35–72), 29 women) received pharmacological therapy (methimazole + thyroxine) for up to 24 months. GO was identified by clinical signs and symptoms. Eleven patients had GO at diagnosis, and another six developed GO during treatment. Blood samples for TSI and other thyroidal biomarkers were obtained at baseline and on five occasions during the 24-month follow-up. Twenty-two subjects completed the drug regimen without surgery or radioiodine treatment.

Results At baseline, TSI was highly correlated with TRAb ($r_s = 0.64$, $p < 0.001$), and both assays similarly correlated to fT3 values. TSI and TRAb did not differ significantly between GO and non-GO patients for visit v1 (n = 30, 17 GO during the whole study) or at follow-up (n = 22, 12 GO during the whole study). During follow-up, levels of TSI and TRAb decreased and normalized in both groups.

Conclusion The present study does not support any added benefit of TSI compared to TRAb for the prediction and management of GO.

Introduction

Graves' disease (GD) is an autoimmune disease accompanied by the production of thyroid stimulating hormone (TSH)-receptor antibodies (TRAb) that bind to the TSH-receptor (TSH-R) and activate the cyclic adenosine monophosphate (cAMP) signal transduction

pathway [1, 2]. TSH-receptor-stimulating immunoglobulin (TSI) is a subtype of TRAb that causes the stimulation of thyroid hormone production [3, 4]. Other types of antibodies block the action of TSH-R and TSH-R-binding inhibitory immunoglobulins, yet others are neutral without any functional effect [5, 6]. Graves' orbitopa-

thy (GO) is clinically relevant in approximately 50% of patients with GD, with severe forms affecting 3–5% of patients with GD [7]. The risk of GO has been linked to TSH receptor stimulation [8–10], high TRAb levels [11], smoking [12], low levels of thyroid peroxidase antibodies [13–17], and thyroglobulin antibodies [14, 17]. Measurement of TRAb is a standard method for confirmation of diagnosis, monitoring of therapy, and prediction of remission or relapse in patients with GD [18–21]. A potential pitfall of using TRAb is that the method will also measure blocking and neutral antibodies, if present.

At present, there are two methods for analyzing antibodies against TSH-R. The third-generation thyrotropin-binding inhibitor immunoglobulin (TBII) assay and the functional thyroid-stimulating immunoglobulin bioassay. The TBII assay uses the ability of TRAb to inhibit the binding of radiolabeled TSH to TSH receptors. In contrast, the bioassay measures cAMP production after TSH-R antibodies bind to the TSH receptor. This method makes it possible to identify the functional properties of TSH-R antibodies.

The IMMULITE 2000/2000 XPI TSI assay uses recombinant thyroid stimulating hormone receptor (TSHR) chimeras constructed from the N-terminal domain of the TSHR, which contains the TSI epitopes, and the luteinizing hormone (LH) receptor from which the blocking epitopes are absent and used as capture antigens. This assay is suggested to be more specific for activating antibodies that cause hyperthyroidism and is less influenced by blocking antibodies [22]. However, there is a lack of clinical studies that demonstrate a clear benefit of using TSI versus TRAb.

Tozzoli et al. examined a cohort of 72 patients with untreated GD. The diagnostic performance of the Immulite TSI assay in GD patients was comparable to that of current TRAb assays [23]. In a study including 166 patients with GD, the TSI method showed perfect sensitivity according to the clinical diagnoses of GD [24].

GO is caused by the stimulation of TSH-R in the orbita [9]. Severe forms of GO may cause irreversible eye complications and, in some severe cases, can even threaten vision [25]. A delay in the diagnosis is associated with a worse prognosis of GO. Therefore, there is an increased need for a scoring system that includes different markers for the prediction and assessment of GO.

In a retrospective study from Korea, a total of 112 patients were investigated whether serum TSH-R antibodies in newly diagnosed, untreated GO patients were predictive of disease course beyond 1 year after initial GO diagnosis. The predictive power of the third-generation TBII assay and Thyretain TSI Stimulating Reporter Bio-Assay were similarly strong [26].

In a recent prospective study, we investigated a cohort of 30 patients with newly diagnosed GD and presented data that may suggest that evaluation of thyroglobulin levels in GD could contribute to identifying patients at increased risk of developing GO [27]. The TRAb levels were not significantly correlated to GO in that study. To investigate whether TSI is a more sensitive biomarker than TRAb for the evaluation of GO, we have now analyzed TSI with the IMMULITE 2000 Systems Analyzers using stored blood samples from the same cohort of 30 patients that participated in the previously reported study.

Materials and Methods

Study subjects

Thirty patients with *de novo* GD were recruited at the Uppsala University Hospital. GD diagnosis was based on decreased levels of TSH and sero-positivity for TRAbs. In one patient, the TRAb levels were below the reference range (1.7 IE/L, reference < 1.75). This patient otherwise had symptoms, laboratory findings, and homogeneously increased uptake on thyroid scintigraphy in line with GD. The median age of the study cohort was 55 years (range 35–72 years). Two were smokers and 29 were women.

The study consisted of six visits: at baseline, 6 weeks, 12 weeks, 6 months, 12 months, and 24 months from diagnosis. At the first visit (baseline), all patients underwent an examination, including a recording of demographic characteristics (sex and age) and medical and family history. At each visit, blood was sampled to measure TSH, fT4, fT3, TRAb, and TSI.

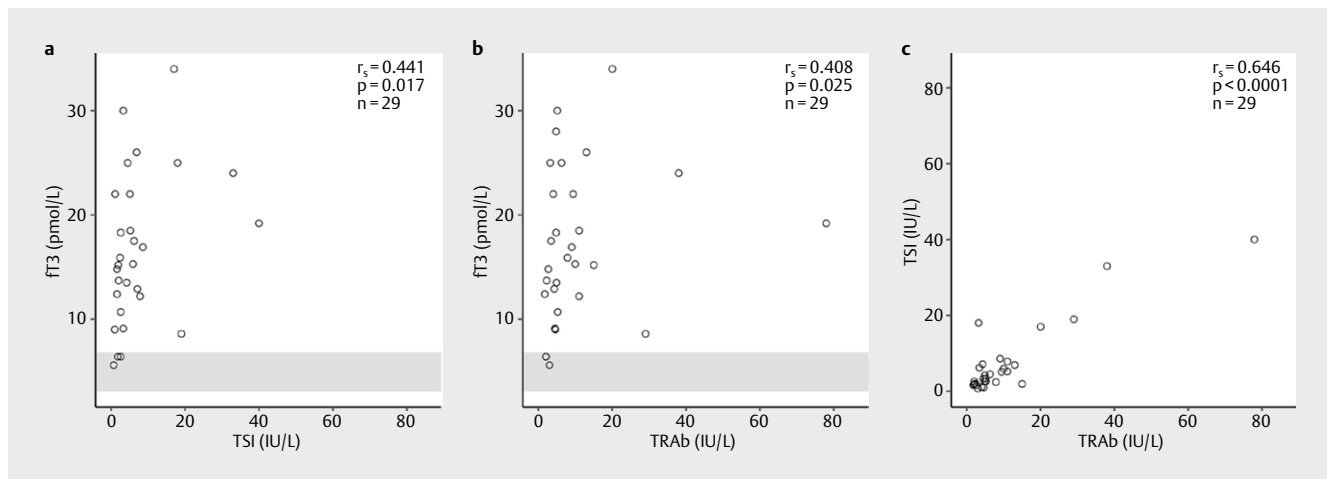
All 30 patients received the conventional block and replace treatment (tiamazol and levothyroxine). Three patients underwent total thyroidectomy, and five received radioiodine treatment during the study period. These patients were not included in the longitudinal analyses described below. Out of the remaining 22 subjects, 21 received antithyroid drug (ATD) for 18–24 months until negative TRAb. One patient had a spontaneous recovery before treatment initiation but was included in the longitudinal analyses. GO was identified by clinical signs and symptoms, and the severity of GO was assessed according to The European Group on Graves' Orbitopathy guidelines [28]. All patients were examined by a doctor and a nurse at each visit, and the patients received clear information about eye symptoms. GO was classified as 'mild' with symptoms such as gritty sensation and tearing due to dry eyes, caruncle swelling and/or redness, upper eyelid retraction, or as 'moderate to severe' in the instance of redness and/or swelling of the eyelids, chemosis, pressure or pain in the eyes, exophthalmos, diplopia or signs of optic nerve compression. The eye signs and symptoms were detailed in the medical records by the attending endocrinologist as well as by the research nurse at every visit [27].

Assays

Plasma TSH (reference interval 0.4–4.0 mIU/L), free T4 (reference interval 12–22 pmol/L), free T3 (reference interval 3.1–6.8 pmol/L), TRAb (reference < 1.75 IE/L), were all measured on Cobas immuno-analyzers (e 801, Roche Diagnostics, Rotkreuz, Switzerland) at the Department of Clinical Chemistry of the Uppsala University Hospital. The laboratory is accredited according to SS-EN ISO 15189:2012 by Swedac (Borås, Sweden). The total coefficient of variation (CV) for the methods was TSH: 5% at 0.1 mIU/L, free T4: 4% at 16 pmol/L, free T3: 4% at 15 pmol/L, Tg: 4% at 23 µg/L, and TRAb: 6% at 17.4 IE/L. TSI assay was conducted on the IMMULITE 2000 and IMMULITE 2000 XPI Immunoassay Systems (Siemens Healthineers, Erlangen, Germany). The total CV was 5% at 0.7 IU/L. All laboratory testing was performed blinded to clinical information.

Statistical analysis

All statistical analyses were performed using R 4.0.2. Data are presented as median (range) unless otherwise indicated. *P* values < 0.05 were considered significant. Spearman's Rank correla-



► **Fig. 1** Correlation between ft3 vs. TSI (a), ft3 vs. TRAb (b), and TSI vs. TRAb (c) at baseline. Missing TSI and TRAb value for one subject. r_s = Spearman's Rho coefficient. Grey areas in A and B represent the normal range of ft3. TSI: thyroid stimulating immunoglobulins; TRAb: TSH-receptor antibodies.

tions between TSI, TRAb, and ft3 were performed at baseline and every follow-up visit. At v1, TSI and TRAb values for GO ($n = 17$) and non-GO ($n = 13$) patients were tested for the significant difference using the Mann-Whitney-U test.

For all subjects who received ATD and did not undergo surgery or radioiodine treatment ($n = 22$), the area under the curves (AUC) of TSI and TRAb after treatment (v2–6) were calculated with the trapezoid method and compared between GO ($n = 12$) and non-GO ($n = 10$) with Mann-Whitney-U tests.

Results

For all subjects, ft3 but not ft4 correlated to both TSI ($r_s = 0.44$, $p = 0.017$, ► **Fig. 1a**) and TRAb ($r_s = 0.41$, $p = 0.025$; ► **Fig. 1b**) at baseline ($n = 30$). During the remainder of the visits ($n = 22$), no significant correlations were observed between ft3 and TSI or TRAb (data not shown), whereas ft4 correlated to TRAb at v6 only ($r_s = 0.53$, $p = 0.012$) and did not correlate to TSI at any visit. TSI was highly correlated with TRAb at baseline ($r_s = 0.64$, $p < 0.001$; ► **Fig. 1c**) and across all visits and treatments ($r_s = 0.60$, $p < 0.001$ for all visits, data not shown). There were no correlations between the levels of TSI or TRAb in relation to the Tg levels (data not shown).

In total, 17 patients were diagnosed with GO at baseline ($n = 11$) or during the study follow-up period ($n = 6$). TSI and TRAb did not differ significantly between GO and non-GO patients at baseline (GO:non-GO = 17:13), while ft3 and ft4 were higher in the GO group than the non-GO group at baseline (► **Table 1**). In the ATD group ($n = 22$), TSI levels appeared to be higher in GO ($n = 12$) vs. non-GO ($n = 10$) during the first three visits, but this difference was not significant when comparing the AUC during the whole follow-up period ($p = 0.49$) (► **Fig. 2a**), and TRAb levels (► **Fig. 2b**) were similarly reduced and normalized in both groups.

► **Table 1** TSI, TRAb, ft3, and ft4 at baseline presented as median and range in GO (during the whole study) vs non-GO, compared using Mann-Whitney-U-test.

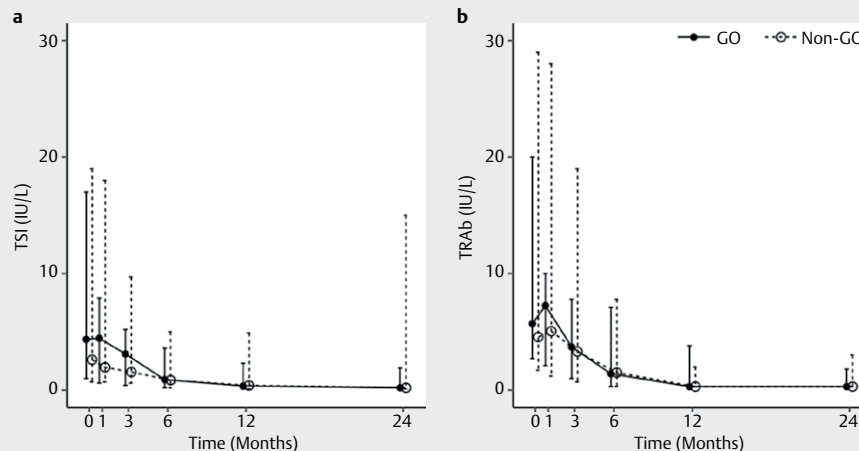
	GO (n = 17)	Non-GO (n = 13)	p-value
TSI	4.5 (1–40)	2.6 (0.7–19)	0.535
TRAb	6.3 (2.7–78)	4.3 (1.7–29)	0.053
ft3	18.3 (9–34)	12.4 (5.6–28)	0.026
ft4	48 (25–100)	35 (17.6–60)	0.008

GO: Graves' orbitopathy; TSI: thyroid stimulating immunoglobulins; TRAb: TSH-receptor antibodies; ft3: Free triiodothyronine; ft4: free thyroxine.

Discussion

GO is a common complication that is clinically relevant in about 50% of patients with GD. The symptoms are often mild, but severe forms of GO occur in 3–5% of patients [7]. GO may begin early in the course of the disease, but it is not uncommon that GO occurs later, during the follow-up period, despite ongoing treatment with ATD [9]. There are difficulties in identifying patients with a risk of developing GO and those who will develop severe forms of eye complications. There is, therefore, a need for better markers that can be useful for the management of GO. Previous studies have suggested the benefits of using TSH receptor-stimulating antibodies for the management of GO [29]. In this study, we made a comparison between TSI and TRAb in the management of GO but could not detect any superiority of TSI compared to TRAb in the prediction of GO.

TSH-R is well-documented to be expressed in the orbita [30]. Stimulation of TSH-R in the orbita leads to stimulation of fibroblasts resulting in fibroblast proliferation, an increase in hyaluronic acid production, and an increased inflammatory response [9]. Higher levels of TRAb have been associated with an increase in the prevalence of GO [13], which we also noted in the present study. A Swedish report found that a TRAb level of 6.3 IU/L at diagnosis of GD was associated with an elevated risk of developing GO [16]. However,



► **Fig. 2** TSI and TRAB levels presented as median and range in all visits for the ATD group ($n = 22$) divided into GO ($n = 12$) and non-GO ($n = 10$), showing a higher but not significant course for TSI during the first 3 months (a) in GO vs. non-GO group, whereas TRAB levels (b) were similarly reduced a ATD: antithyroid drug; GO: Graves' orbitopathy; TSI: thyroid stimulating immunoglobulins; TRAB: TSH-receptor antibodies.

current standard methods, which are widely routinely used, measure all antibodies, i. e., if present, also blocking and neutralizing antibodies. Techniques that measure only the stimulating antibodies would possibly increase the precision of the diagnosis and follow-up of patients with GD, including those suffering from eye complications. Currently, only bioassays appear to be able to detect purely stimulating antibodies. Thyretain by Quidel is one such method that uses engineered CHO cells with chimeric TSH-R (MC4) to measure luciferase gene expression due to activation of the cAMP signal transduction pathway [5]. Lytton et al. showed that the clinical sensitivity and specificity of the Mc4/TSI were greater than TRAB in GO [5]. An interesting new technique, "aequorin TAb assay," has been recently developed [31]; it uses a cell line CHO transfected with human TSH receptor, cyclic nucleotide-directed calcium channel, and aequorin. These bioassays appear to be precise and might add to the prediction of the course of disease and risk of GO, but it is unlikely that they will be developed into clinical routine. However, the TBII assay has satisfactory clinical sensitivity and specificity (97.4% and 99.2%, respectively). In this setting, the bioassays should be reserved for fine and complex diagnoses and for particular clinical conditions [32, 33].

The IMMULITE 2000 TSI assay by Siemens is a bridge-binding immunoassay by which TSH-R antibodies bind to a pair of recombinant TSH-R constructs; one is a capture, and the other is signal receptor for measuring secreted alkaline phosphatase. This method is supposed to provide a more sensitive measurement of stimulating antibodies compared to the routinely used competitive-binding assay. In this prospective study of patients with newly diagnosed GD, we could, however, not show any significant difference between TSI and TRAB, neither among GD patients at diagnosis or follow-up nor between those with and without GO. The correlation between ft_3 and TSI and ft_3 and TRAB in this study is statistically significant but with low coefficients. This may be related to the bioactivity of the antibodies and their effect on the degree of hyperthyroidism. Typically, high antibody titers lead to more hyperthy-

roidism, but pronounced hyperthyroidism may also occur at lower antibody levels and vice versa. The correlation between TSI and TRAB at baseline and in the subsequent visits was good throughout, indicating that TSI is not more specific than TRAB in assessing the severity of hyperthyroidism.

Our results are in line with a newly published study from Australia [34], in which 140 participants were recruited, of which 75 (53.6%) had GO. Although Immulite TSI level was higher in the presence of GO, it showed poor diagnostic accuracy and no correlation with clinical markers of GO severity or activity. To our knowledge, only one more study has been reported to investigate TSI in GO [35]. In that study, TSH-R antibodies were measured with Immulite TSI assay and with Elecsys IMA in patients with moderate to severe GO. Both methods showed a comparable correlation with GO activity during ivGCs therapy. The data from our prospective study confirm and add to the findings from these studies showing similar performance of the two assays in clinical routine.

The weakness of our study is the relatively small sample size. However, the prospective design with continuous follow-up of the clinical and laboratory status for two years gives the present study sufficient statistical strength to rule out significant differences regarding the parameters we have measured.

In conclusion, TSI is highly correlated with TRAB in patients with GD. The tests did not differ significantly between GO and non-GO patients before the treatment of GD. After treatment, both TSI and TRAB levels were reduced and normalized in both groups. The present study does not support any added benefit of TSI compared to TRAB for the prediction and management of GO.

Patient Consent

Consent was obtained from each patient after a full explanation of the purpose and nature of all procedures used.

Ethical Approval

The study complied with the Declaration of Helsinki and was approved by the Regional Ethics Committee in Uppsala (Dnr 2015/469).

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by SK, ML, and ÖL. The first draft of the manuscript was written by SK and ÖL, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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