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Case Report

Usefulness of Functional Thyroid-Stimulating and Thyroid-Blocking Immunoglobulin Bioassays in an Atypical Presentation of Graves' Disease

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ABSTRACT

Background/Objective: Thyroid-stimulating hormone (TSH) receptor antibody (TRAb) is well recognized as the pathogenic antibody that causes the clinical manifestation of Graves' disease (GD). Although the majority of TRAb measured in GD is due to thyroid-stimulating immunoglobulin (TSI), there are other functional classes of TRAb, ie, thyroid-blocking immunoglobulin (TBI) and neutral antibodies, which can alter the clinical course of the disease. We present a case of a patient who demonstrated interesting coexistence of both the forms using Thyretain TSI and TBI Reporter BioAssays.
Case Report: A 38-year-old woman presented with thyrotoxicosis (TSH level, 0.01 mIU/L, free thyroxine level, >7.8 ng/mL [>100 pmol/L], and free triiodothyronine level, >32.6 pg/mL [>50 pmol/L]) to her general practitioner. She was treated with 15-mg carbimazole twice daily before the dose was reduced to 10 mg. Four weeks later, she developed severe hypothyroidism, with a TSH level of 57.5 mIU/L, free thyroxine level of 0.5 ng/mL (6.7 pmol/L), and free triiodothyronine level of 2.6 pg/mL (4.0 pmol/L). Carbimazole was ceased; however, she remained severely hypothyroid, with a TRAb level of 35 IU/L. Both TSI (304% signal-to-reference ratio) and TBI (56% inhibition) were present, with predominance of the blocking form of thyroid receptor antibodies (54% inhibition). Thyroxine was commenced, and her thyroid functions remained steady and TSI became undetectable.
Discussion: The results of the bioassays confirmed that both TSI and TBI can coexist in a patient and that its action changes within a short period of time.
Conclusion: Clinicians and laboratory scientists should be aware of the usefulness of TSI and TBI bioassays in the interpretation of atypical presentations of GD.

Introduction

Graves' disease (GD) is an autoimmune condition that arises from the presence of antibodies directed to the thyroid-stimulating hormone (TSH) receptor,[1] commonly known as thyroid-stimulating hormone receptor antibody (TRAb). Binding of the antibody to the receptor causes activation of the intracellular cyclic adenosine monophosphate (cAMP) second messenger cascade and production of thyroid hormone, leading to hyperthyroidism.[2] However, there are different functional classes of TRAbs, including thyroid-stimulating immunoglobulin (TSI), neutral antibody, and thyroid-blocking immunoglobulin (TBI). The commercially available TRAb assay indiscriminately binds to all classes of TRAb. This is generally not a clinical issue because the predominantly functional class of TRAb in GD is TSI. However, when the predominant TRAb changes its functional class to the blocking form, it can lead to hypothyroidism. This should not be confused with Hashitoxicosis, wherein patients present with phenotypic and biochemical manifestations of GD but spontaneously develop hypothyroidism.[3,4] This mechanism is due to the destructive nature of the inflammatory process and not due

Abbreviations: cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; fT3, free triiodothyronine; fT4, free thyroxine; GD, Graves' disease; RLU, relative light unit; TBI, thyroid-blocking immunoglobulin; TRAb, thyroid-stimulating hormone receptor antibody; TSH, thyroid-stimulating hormone; TSHr, thyroid-stimulating hormone receptor; TSI, thyroid-stimulating immunoglobulin.

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to TBI. With the availability of the TSI or TBI bioassay, we present a case of a patient with GD in whom overt hypothyroidism developed because of the coexistence of both stimulating and blocking forms of TRAb.

Case Report

A 38-year-old Sudanese woman presented to her general practitioner with a 4-week history of feeling unwell with the symptoms of sore throat, heat intolerance, palpitations, loss of appetite, and a 6-kg weight loss. Her other medical problems included endometriosis and chronic back pain due to L5-S1 disk prolapse. She was not taking any regular medications or herbal supplements, and there was no history of recent exposure to iodinated contrast. Of note, there was no family history of thyroidism or any other autoimmune disorders. Her initial examination revealed a sinus tachycardia of 120 beats/min; fine hand tremor; and diffusely enlarged, nontender thyroid gland. Based on her symptomatology, the differential diagnosis of thyrotoxicosis related to either thyroiditis or GD was considered.

Initial investigations (Table) showed a suppressed TSH level of <0.01 mIU/L (reference intervals, 0.4-4.8 mIU/L), an elevated free thyroxine (fT4) of >7.8 ng/mL (reference intervals, 0.6-1.2 ng/mL) (>100 pmol/L [reference intervals, 11-22 pmol/L]), and a free triiodothyronine (fT3) level of >32.6 pg/mL (reference intervals, 2.1-4.0 pg/mL) (>50 pmol/L [reference intervals, 3.1-6.4 pmol/L]). The thyroid antibody panel showed elevated levels of TRAb of 41 IU/L (reference cut-off, <1.8 IU/L), thyroid peroxidase antibody of 438 IU/mL (reference cut-off, <35 IU/mL), and thyroglobulin antibody of 864 IU/mL (reference cut-off, <5 IU/mL). She also had deranged liver function test results, with elevated levels of alanine aminotransferase of 108 U/L (reference intervals, 5-30 U/L), aspartate aminotransferase of 89 U/L (reference intervals, 10-35 U/L), and γ-glutamyl transferase of 186 U/L (reference range, 5-35 U/L) and a normal level of alkaline phosphatase of 89 U/L (reference intervals, 20-105 U/L). The full blood count, kidney function, electrolyte levels, and inflammatory markers were within normal limits. Thyroid scintigraphy showed a diffusely increased uptake of pertechnetate of 14% (no reference range provided) throughout both lobes, consistent with GD.

Carbimazole at 15 mg was commenced twice daily to treat the patient's GD, which was subsequently reduced to 10 mg twice daily after 3 weeks following improvement of her clinical symptoms and thyroid hormone levels, with normal-range fT4 and fT3 levels of 0.93 ng/mL (12 pmol/L) and 2.2 pg/mL (3.4 pmol/L), respectively, whereas her TSH level remained suppressed. Her liver function test results also returned to within normal limits.

Four weeks later, the patient developed significant hypothyroidism, with an elevated TSH level of 57.5 mIU/L, low fT4 level of 0.5 ng/mL (6.7 pmol/L), and normal fT3 level of 2.6 pg/mL (4.0 pmol/L). She was advised to cease carbimazole therapy. Despite this, her thyroid function continued to deteriorate, and repeat testing after 6 weeks showed a TSH level of 82.6 mIU/L, fT4 level of 0.5 ng/mL (6.5 pmol/L), and fT3 level of 2.7 pg/mL (4.1 pmol/L). At this point, the possibility of thyroid receptor-blocking antibodies was considered, and her blood sample was sent to a tertiary reference laboratory for the measurement of TSI and TBI (Thyretain TSI and TBI Reporter BioAssays, Quidel Corporation).

These bioassays use genetically engineered Chinese hamster ovary (CHO) cells that have been transfected with a chimeric human TSH-rat luteinizing hormone/chorionic gonadotrophin receptor (referred to as the Mc4 chimera)[5] and a cAMP-induced luciferase reporter gene.

Highlights

- Hypothyroidism can present in Hashimoto's thyroiditis and Graves' disease
- Conventional assays cannot distinguish between types of thyroid receptor antibodies
- Bioassays are useful in distinguishing the activity of thyroid receptor antibodies

Clinical Relevance

Conventional thyroid receptor binding assays (commonly known as thyroid-stimulating hormone receptor antibody assays) detect all forms of the thyroid receptor antibodies. This case demonstrates the clinical utility of functional, cell-based assays using the Thyretain TSI and TBI Reporter Bioassays to assist clinicians with the interpretation of atypical presentations of Graves' disease. This case also encourages the interaction between clinicians and laboratory scientists in the management of patients.

In the TSI bioassay, TSI in the patient serum binds to the thyroid-stimulating hormone receptor (TSHr) and stimulates the cAMP second messenger pathway to produce cAMP and luciferase substrate. Cells are then lysed with luciferin, and luminescence is measured using a luminometer (Enspire Multimode Plate Reader, PerkinElmer). TSI is reported as the percentage of the relative light units (RLUs) of the sample to those of the reference control:

$$\text{TSI (\%SRR)} = \frac{\text{Patient Sample RLU}}{\text{Reference Control RLU}} \times 100$$

where SRR indicates the signal-to-reference ratio. A TSI result of >140% signal-to-reference ratio is considered positive for TSI activity.

In the TBI bioassay, however, patient samples are diluted at a ratio of 1:11 in a working solution containing bovine TSH, which competes with TBI in the patient sample for TSHr binding. TBI is calculated as follows:

$$\text{TBI (\%Inhibition)} = \frac{\text{RLU of Reference control} - \text{RLU of patient sample}}{\text{RLU of reference control}} \times 100$$

A TBI result of >34% inhibition is considered positive for TBI activity. The patient's results showed the presence of TBI (54% inhibition; normal reference interval < 34%) in the serum when the patient was off her antithyroid medication, with subclinical hypothyroidism, a TSH level of 6.48 mIU/L, an fT4 level of 0.7 ng/mL (12.1 pmol/L), and an fT3 level of 3.6 pg/mL (5.5 pmol/L). Remarkably, when the fT4 level returned to within the reference interval associated with a corresponding reduction in TSH levels, both TSI (304% sample-to-reference ratio; normal reference interval < 140%) and TBI (56% inhibition) were coexistent before reverting to predominance of TBI 5 months later (50% inhibition) (Table). During this time, the patient was commenced on thyroxine at 50 μg daily, which was subsequently increased to 100 μg daily, resulting in normalization of both TSH and fT4.

Discussion

Bioassays have been demonstrated to be useful in cases of fluctuating thyroid function in pregnancy and pediatric care[6] where thyroid scintigraphy is contraindicated or not available, in those on

Table
Laboratory Results[a]

Test	Jul 24, 2017	Feb 27, 2018	Nov 30, 2018	Dec 3, 2018		Dec 27, 2018		Jan 22, 2019		Mar 18, 2019	Apr 3, 2019		Apr 26, 2019	Sep 12, 2019	
TSH (0.4-4.80 mIU/L)	2.29	1.38	<0.01 (L)	<0.01 (L)	Carbimazole started at 15 mg twice daily	<0.01 (L)	Carbimazole reduced to 10 mg twice daily	57.5 (H)	Carbimazole stopped	82.6 (H)	47.4 (H)	Thyroxine 50 mcg commenced	6.48 (H)	9.30 (H)	Thyroxine increased to 100 mcg
FT4			>7.8 (>100)	>7.8 (>100) (H)		0.93 ng/dL (12.0)		0.5 (6.7) (L)		0.5 (6.5)	0.7 (9)		0.9 (12.1)	1.0 (12.9)	
0.6-1.2 ng/mL 8.0-16.0 pM															
FT3			>32.6 (>50)	>32.6 (>50) (H)		0.26 ng/dL (3.40)		2.6 (4.0) (L)		2.7 (4.1)	3.4 (5.2)		3.6 (5.5)	3.5 (5.3)	
2.1-4.0 pg/mL 3.2-6.1 pM															
Tg (1.6-50.0 ng/mL)				11.9											
TPOAb (<35 IU/mL)				438 (H)											
TgAb (<5.0 IU/mL)				864 (H)											
TRAb (<1.8 IU/L)				41 (H)							35 (H)			>40 (H)	
TSI (<140% SRR)											<140 (negative)		304 (positive)	<140 (negative)	
TBI (% Inhibition)											54 (positive)		56 (positive)	50 (positive)	

Abbreviations: Apr = April; Dec = December; Feb = February; FT3 = free triiodothyronine; FT4 = free thyroxine; Jan = January; Jul = July; Mar = March; Nov = November; Sep = September; SRR = signal-to-reference ratio; TBI = thyroid-blocking immunoglobulin; Tg = thyroglobulin; TgAb = thyroglobulin antibody; TPOAb = thyroid peroxidase antibody; TRAb = thyroid receptor antibody; TSH = thyroid-stimulating hormone; TSI = thyroid-stimulating immunoglobulin.

[a] FT4 conversion factor: pmol/L *0.077688 = ng/dL; FT3 conversion factor: pmol/L*0.651 = pg/mL.

alemtuzumab therapy for multiple sclerosis,[7] and in those who are resistant to antithyroid medications.[8]

A number of studies[8,9] have demonstrated that patients with a high variability of thyroid receptor epitopes (heterogeneous epitope distribution) achieved the highest response to antithyroid medications compared with those with homogenous thyroid receptor epitopes. It has been suggested that the measurement of TRAb epitopes prior to commencement of antithyroid medications could predict the patient's long-term response.

Unfortunately, this requires a laboratory assay that is able to distinguish between stimulating and blocking epitopes. Conventional TRAb assays based on competitive binding inhibition rely on the binding avidity of TRAb in the serum sample to the capture antibody and, therefore, detect all forms of TRAb. Third-generation TRAb assays now have the added advantage of automation and tracking systems to minimize specimen handling and reduce the turnaround time of results. These assays are also traceable to the World Health Organization 2nd International Standard 08/204 international reference standard, which is a monoclonal stimulating antibody (M22 immunoglobulin G) produced from lymphocytes of pregnant patients with GD undergoing regular plasmapheresis.[10] More recently, an automated TSI Bridge assay (Siemens Immulite) was released, which claims to detect TSI. However, a study by Diana et al[11] showed that the bridge assay cannot definitively differentiate between TSI and TBI.

The earliest report of a patient with coexisting stimulating and blocking TRAbs was published in 1988 using porcine thyroid cells.[12] The availability of functional bioassays has largely improved since then, and the use of CHO K1 cells stably transfected with chimeric human TSH receptor and a suitable reporter gene has enabled distinction between stimulating and blocking antibodies to be reliably determined and with high reproducibility. Other commercially available bioassays, such as the thyroid-stimulating antibody bioassay (RSR Pty Ltd), use similar transfected CHO cells; however, activity is determined based on the measurement of cAMP in an enzyme-linked immunosorbent assay format.

In our patient, we used Thyretain TSI and TBI Reporter BioAssays. These use the Mc4 CHO cell line that has amino acid residues 262 to 368 of the human TSH receptor replaced by amino acid residues 262 to 334 from the rat luteinizing hormone/chorionic gonadotropin hormone receptor. The cells are also transfected with a cAMP-luciferase reporter gene to generate a luminescent signal when oxidized with luciferin. The TSI bioassay has a diagnostic sensitivity of 97% and specificity of 82% in the detection of GD (in-house data, unpublished); however, another study[13] reported a sensitivity of 100% in patients with hyperthyroidism and GD and 100% specificity in healthy controls. Positive and negative predictive values were also 100% compared with 5 other thyroid-binding assays.

The results of the bioassays confirmed that both stimulating and blocking forms can coexist in a patient and that the balance of their action on TSHr can change within a short period of time and during the course of antithyroid medication. Monitoring of individual subtypes can provide insights into predominant TRAbs and their role in clinical presentation.

Clinicians and laboratory scientists should be aware of the usefulness of functional thyroid-stimulating and -blocking antibody assays in patients presenting with GD, in whom there is a swing between hyperthyroidism and hypothyroidism. This distinction could be vital to clinicians in managing their patients' care, particularly in the context of pregnancy, wherein it may also have implications for the newborn.

Disclosure

The authors have no multiplicity of interest to disclose.

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Author Contributions

All authors confirmed that they have contributed to the intellectual content of this paper and have met the following 4 requirements: (1) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting or revising the article for intellectual content; (3) final approval of the published article; and (4) agreement to be accountable for all aspects of the article, thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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