

## A Hyperthyroid Patient with Measurable Thyroid-stimulating Hormone Concentration – A Trap for the Unwary

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### Abstract

**Introduction:** In a patient with hyperthyroidism, the detection of elevated thyroid hormone concentration with measurable thyroid-stimulating hormone (TSH) value poses considerable diagnostic difficulties. **Clinical Picture:** This 38-year-old lady presented with clinical features of thyrotoxicosis. Her serum free thyroxine concentrations were unequivocally elevated [45 to 82 pmol/L (reference interval, 10 to 20 pmol/L)] but the serum TSH values were persistently within the reference interval [0.49 to 2.48 mIU/L (reference interval, 0.45 to 4.5 mIU/L)]. **Treatment:** Investigations excluded a TSH-secreting pituitary adenoma and a thyroid hormone resistance state and confirmed false elevation in serum TSH concentration due to assay interference from heterophile antibodies. The patient was treated with carbimazole for 18 months. **Outcome:** The heterophile antibody-mediated assay interference disappeared 10 months following the initiation of treatment with carbimazole, but returned when the patient relapsed. It disappeared again 2 months after the initiation of treatment. **Conclusions:** Clinicians should be aware of the potential for interference in immunoassays, and suspect it whenever the test results seem inappropriate to the patient's clinical state. Misinterpretation of test values, arising as a result of assay interference, may lead to misdiagnosis, unnecessary and at times expensive investigations, delay in initiation of treatment and worst of all, the initiation of inappropriate treatment.

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**Key words:** Heterophile antibodies, Immunoradiometric assay, Thyroid function tests, Thyrotoxicosis

### Introduction

Free thyroxine (FT4), total tri-iodothyronine (T3) and thyrotropin (TSH) are the commonly measured biochemical indices in the assessment of thyroid function in a patient with suspected thyrotoxicosis. These indices give sufficient information regarding the functional status of the thyroid gland under most circumstances. However, technical problems with immunoassays can occasionally result in falsely elevated or decreased serum hormonal values, which might either lead to unnecessary investigations or inappropriate treatment. We describe a patient who presented with clinical features of thyrotoxicosis and underwent extensive investigations because of a false elevation in serum TSH concentration due to assay interference, and discuss the approach towards a patient with elevated thyroid hormone concentration with detectable or elevated serum TSH value.

### Case Report

A 38-year-old lady was referred to the surgical clinic with an enlarged thyroid gland, which she had incidentally noticed 3 months ago. She was considered clinically euthyroid. There was a palpable nodule in the left lobe of the thyroid gland. Ultrasound of the neck revealed a 1.8-cm nodule located in the lower pole of the left lobe. Fine-needle aspiration cytology confirmed a colloid nodule. Thyroid function test results, available subsequently, revealed an elevated FT4 concentration at 45 pmol/L (reference interval, 10 to 20 pmol/L) whilst the serum TSH value of 0.7 mIU/L was within the reference interval (0.45 to 4.5 mIU/L).

She was referred to an endocrinologist for further assessment in view of the abnormal thyroid function test results. On examination, her resting pulse rate was 96/minute, regular in rhythm. There was an audible bruit over

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the thyroid gland. Investigations revealed persistently elevated FT4 values ranging from 45 to 82 pmol/L (reference interval, 10 to 20 pmol/L) but serum TSH concentrations, measured from 3 different laboratories, were persistently measurable, ranging from 0.49 to 2.48 mIU/L (reference interval, 0.45 to 4.5 mIU/L). Thyroid antibody concentrations were mildly elevated [TSH receptor antibody (TRab) at 2.7 IU/L (reference interval, 0 to 1 IU/L), anti-thyroid peroxidase antibody (anti-TPO) at 218 IU/mL (reference interval, 0 to 50 IU/mL) and anti-thyroglobulin antibody (anti-TG) at 89 IU/mL (reference interval, 0 to 40 IU/mL)].

Investigations were initiated to exclude a TSH-secreting pituitary adenoma or a thyroid hormone resistance state in view of the TSH values being persistently within the reference range, measured from 3 different laboratories, amidst unequivocally elevated thyroid hormone concentrations. Magnetic resonance imaging (MRI) of the pituitary gland revealed a 3-mm pituitary adenoma. Anterior pituitary hormone levels, including serum prolactin, were normal. During a thyrotrophin-releasing hormone (TRH) stimulation test, performed using 200 mcg of TRH administered intravenously, serum TSH response was blunted, the TSH values being 2.8, 2.68, and 3.04 mIU/L at 0, 20, and 60 minutes respectively (normal response: TSH rises by 2 mIU/L to greater than 3.4 mIU/L, and the 20-minute value is higher than the 60-minute value).<sup>1</sup> A radioactive iodine scan, obtained subsequently, revealed uniformly elevated uptake at 79.5% and 70% at 4 hours and 24 hours respectively, strongly suggestive of hyperthyroidism. Sex hormone-binding globulin (SHBG) concentration, which is a good marker for peripheral thyroid hormone action,<sup>2</sup> was elevated at 194 nmol/L (reference interval, 18 to 114 nmol/L). The blunted TSH response to TRH stimulation,<sup>3</sup> and an elevated SHBG level,<sup>2</sup> effectively ruled out a thyroid hormone resistance state.

Although the diagnosis of a TSH-secreting pituitary adenoma (TSH-oma) was still a possibility, the small size of the pituitary adenoma led us to exclude false elevation in TSH concentration due to assay interference. More

detailed investigations revealed that all 3 laboratories that had earlier reported TSH values within the reference interval were using the same assay technique and system, the Ortho-Clinical Diagnostics ECi. Serum TSH values were found to be appropriately suppressed at 0.0072 mIU/L when measured on a different assay system, the Abbott Architect (Table 1). Possible assay interference from rheumatoid factor was excluded, as the concentration of rheumatoid factor was not raised. Serum TSH concentrations on the ECi assay system were unchanged following treatment with mouse serum. However, following a single treatment for heterophile antibodies using Scantibodies HBT tubes, the TSH value, measured using the Ortho-Clinical Diagnostics ECi, dropped from 0.856 mIU/L to 0.246 mIU/L, suggesting heterophilic antibody interference (Table 1). A diagnosis of thyrotoxicosis due to Graves' disease, with a false elevation of TSH concentration due to heterophilic antibody interference, was made and the patient was treated with carbimazole for 18 months. The heterophilic antibody effect disappeared 10 months after antithyroid treatment was commenced.

Six months after the completion of therapy, she presented with a clinical relapse of her hyperthyroidism. Examination of the neck revealed the earlier noted nodule, unchanged in size, in the left lobe of the thyroid. Free thyroxine concentration was elevated at 31.9 pmol/L but the TSH value was once again noted to be within the reference range at 0.91 mIU/L using the Ortho-Clinical Diagnostic ECi assay method. Thyroid antibody concentrations were elevated [TRab 25.5 IU/L (reference interval, 0 to 1 IU/L), anti-TPO >1000 IU/mL (reference interval, 0 to 50 IU/mL) and anti-TG 862 IU/mL (reference interval, 0 to 40 IU/mL)]. When measured using the Abbott Architect assay system, the serum TSH concentration was 0.004 mIU/L (Table 1). Following single and double treatments for heterophilic antibodies using the Scantibodies HBT tubes, the TSH concentration, measured using the Ortho-Clinical Diagnostics ECi assay system, was found appropriately suppressed at 0.045 mIU/L and 0.001 mIU/L respectively, suggesting heterophilic antibody interference (Table 1).

Table 1. TSH Values (mIU/L) as Assayed on the Ortho-Clinical Diagnostics ECi (ECi) and Abbott Architect TSH Assay Systems at Presentation and Following Relapse of Hyperthyroidism, and Following Treatment for Heterophilic Antibodies Using Scantibodies HBT Tube

Time	ECi	ECi + Scantibodies HBT tube	Abbott Architect
At presentation	0.856	0.246 (single treatment)	0.0072
Ten months after treatment	1.75	1.82 (double treatment)	1.748
At relapse	0.91	0.045 (single treatment) 0.001 (double treatment)	0.004
Two months after treatment	1.87	0.947	0.9262

Carbimazole was re-commenced. The heterophilic antibody interference disappeared 2 months following the initiation of treatment (Table 1). She is currently euthyroid on carbimazole 5 mg once daily and has elected to continue on carbimazole therapy for a period of 18 to 24 months instead of undergoing ablative therapy with radioactive iodine.

## Discussion

Thyrotoxicosis with elevated thyroid hormone concentration but detectable or elevated serum TSH value may be due to a TSH-secreting pituitary tumour, resistance to thyroid hormone (RTH) or assay interference. TSH values being persistently within the reference range, when measured from not 1 but 3 different laboratories, in the presence of elevated thyroid hormone concentrations in a thyrotoxic patient, misled us to initially consider the first 2 possibilities in our patient.

RTH is a syndrome of reduced responsiveness of target tissues to thyroid hormones. It is characterised by persistent elevation of thyroid hormone concentrations with normal or slightly elevated TSH levels in the absence of intercurrent acute illness, drugs or alteration of thyroid hormone binding to serum proteins.<sup>3</sup> Patients are usually euthyroid despite elevated hormone concentrations and the TSH response to TRH stimulation is normal.<sup>3</sup> Presence of clinical signs of thyrotoxicosis, blunted TSH response to TRH stimulation, elevated SHBG level and the absence of family history excluded this diagnosis in our patient.

TSH-omas are rare, accounting for 0.5% to 2% of all pituitary adenomas.<sup>2,4</sup> TSH-omas are usually large at presentation, with 88% being macroadenomas.<sup>4</sup> Goitre is observed in 94% of patients, visual field disturbances are seen in 42%, and 30% of female patients present with menstrual disorders and galactorrhea.<sup>2</sup> A TRH stimulation test distinguishes between a TSH-oma and RTH, with the former demonstrating a blunted TSH response whilst a normal or exaggerated response is seen in the latter.<sup>2</sup> The blunted response to the TRH-stimulation test in our patient led to the MRI of the pituitary gland. However, the small tumour size of 3 mm and the presence of normal anterior pituitary function tests made the diagnosis of a TSH-oma less likely in our patient, and led us to look for false elevation in TSH concentration due to assay interference. When retested using a different assay method to rule out heterophile antibody interference, TSH concentration was found to be appropriately suppressed at 0.0072 mU/L, thereby conclusively excluding secondary hyperthyroidism due to a TSH-oma, and confirming the presence of primary hyperthyroidism.

The 3 major sources of antibody interference in thyroid hormone immunoassays are autoantibodies, heterophile antibodies and rheumatoid factor. Of these, heterophile antibodies and rheumatoid factor are known to interfere

with TSH assays. Possible interference from rheumatoid factor was excluded in our patient.

Heterophile antibodies are antibodies against immunoglobulins of various animal species that cross-react with the monoclonal animal antibodies used in immunometric assays. The best-known heterophile antibodies are the human anti-mouse antibodies (HAMA). Prevalence of heterophile antibodies in the general population has been reported to range from 0.2% to 15%.<sup>5</sup> They are found more commonly in patients who have received infusions of murine monoclonal antibodies as chemotherapy, vaccines that contain animal immunoglobulin, or have contacts with animals such as in animal breeders and veterinary workers, none of which was evident in our patient's history. Heterophile antibodies cause interference through immunoglobulin aggregation by binding of the capture antibody, which can lead to overestimation of TSH in immunometric ("sandwich") assays. Methods that use 2 site immunometric assays and those using more than 1 mouse monoclonal antibodies are more prone to interferences. Despres and Grant<sup>5</sup> have reported on a few patients, similar to the case presented here, with false elevations in serum TSH values in the setting of elevated T4 concentrations which, when remeasured with a different assay technique or after addition of blocking reagents, showed appropriately suppressed TSH levels. Interestingly, in our patient, the heterophile antibody interference was seen only with the Ortho-Clinical Diagnostics ECi and not the Abbott Architect TSH assay system despite both being immunometric ("sandwich") assays. Despres and Grant<sup>5</sup> have also reported on a few patients with assay interference seen with 1 immunometric assay system that disappeared when a different immunometric assay system was used. Since both these assay systems use mouse monoclonal antibodies from different manufacturers, the resulting slight difference in the antibody binding sites was presumably the source of the different assay response seen in our case.

Heterophile antibodies can be blocked by the addition of non-immune serum from the same animal species used to raise the antibody reagent or after treatment with heterophilic blocking agents.<sup>5</sup> In our patient, the serum TSH values failed to normalise after treatment of the patient's serum with mouse serum. However, a significant drop in TSH concentrations was observed when the heterophilic blocking agents were added, which confirmed the presence of assay interference. Recent work has demonstrated the unreliability of any single approach in ruling out heterophilic antibody interference and a 3-pronged approach of performing dilution studies, heterophilic antibodies blocking studies and reanalysis via an alternative method seems the best pragmatic approach.<sup>6</sup>

The other interesting feature in our patient was the disappearance of heterophile antibody interference in the Ortho-Clinical Diagnostics ECI assay system 10 months after the initiation of treatment during her first presentation and 2 months after the initiation of treatment during the relapse of her thyrotoxicosis. Although such transient heterophilic antibody interference has been described previously,<sup>7,8</sup> the mechanism of this phenomenon remains unknown.

### Conclusion

Thyrotoxicosis with elevated thyroid hormone concentration but measurable serum TSH value presents the clinician with a diagnostic challenge. Clinicians should be aware of the potential for interference with immunoassays, and suspect it whenever the test results seem inappropriate to the patient's clinical state. Communication between laboratory professionals and clinicians is critical in these situations because one should *first* exclude assay interference as the possible reason for a measurable serum TSH value in a thyrotoxic patient with elevated thyroid hormone concentration before embarking on the more detailed and expensive biochemical and imaging studies to exclude either a TSH-secreting pituitary tumour or resistance to thyroid hormone. Misinterpretation of test values, arising as a result of assay interference, might lead to misdiagnosis, unnecessary and at times expensive investigations, delay in

initiation of treatment and worst of all, the initiation of inappropriate treatment.

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