FT₄ value based on the post-PEG FT₄ value, and this correlated well with results obtained on other analyzers (Table 1). In this case, it was possible to demonstrate thyroxine-autoantibody interference in the Advia Centaur FT₄ immunoassay by PEG precipitation of serum and subsequent comparison of the difference between pre- and post-PEG FT₄ results with those of controls.

The data suggest the presence of thyroid hormone autoantibodies. The patient was not treated for thyroidal illness and remained clinically euthyroid 6 months later.

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References

A Girl with Goiter and Inappropriate Thyroid-Stimulating Hormone Secretion

Mark D. Kellogg,1* Terence C. Law,1 Stephen Huang,2 and Nader Rifai1

CASE
A 15-year-old white girl presented with neck tenderness. On examination, a nodule was palpated in the right thyroid lobe. The neck was supple without abnormal lymphadenopathy. Eye findings related to Graves orbitopathy were absent. Weight, height, and blood pressure were unremarkable, but the heart rate was high at 104–114 bpm. The patient had a history of attention deficit hyperactivity disorder and was taking atomoxetine and fluoxetine. There was no history of childhood neck irradiation or family history of thyroid cancer. Several maternal relatives have acquired thyroid dysfunction.

Sonography showed a 2-cm nodule in the right thyroid lobe. Fine-needle aspiration showed benign cytology, but the family requested right thyroid lobe-
ectomy for persistent neck tenderness. Preoperative laboratory data revealed a total thyroxine (T4) concentration of 170 nmol/L (reference interval (RI) 67–138 nmol/L) (13.2 μg/dL, RI 5.2–10.7), total triiodothyronine (T3) concentration of 3.2 nmol/L (RI 1.3–2.4 nmol/L) (206 ng/dL, RI 86–153), a thyroid-stimulating hormone (TSH) concentration of 0.5 mIU/L (RI 0.3–5.0 mIU/L), and a thyroid hormone binding ratio (1/T-uptake) of 1.72 (RI 0.77–1.16) (Table 1). Analyses were conducted by chemiluminescent immunoassay on the Roche Elecsys 2010 platform. Free T3 and free T4 indices as calculated by the clinicians were 5.5 nmol/L (RI 1.3–2.4 nmol/L) and 292 nmol/L (RI 67–138 nmol/L), respectively, and, in the context of the patient’s normal TSH concentration, suggested the possibility of inappropriate TSH secretion due to resistance to thyroid hormone or a TSH-secreting pituitary adenoma. Analyses for serum free T4 measured by direct dialysis and RIA were conducted at Mayo Medical Laboratories and revealed a normal free T4 of 16.8 pmol/L (RI 10.3–25.8 pmol/L) (1.3 ng/dL, RI 1–2 ng/dL). Although certain of the patient’s features, including her tachycardia (1, 2), were consistent with the syndrome of inappropriate TSH secretion, this syndrome is extremely rare and the recommended standard of care is to repeat thyroid function tests after ≥1 week (2) to exclude the effects of nonthyroidal illness and to assess the possibility of laboratory artifact (3). Accordingly, the patient’s scheduled surgery was postponed to accurately assess her thyroid function status.

DISCUSSION

This case presents an interesting combination of thyroid function testing results with increased thyroid hormone concentrations and normal TSH concentrations in a clinically euthyroid patient. An evaluation for laboratory artifact is recommended whenever initial results suggest the diagnosis of inappropriate TSH secretion, because of the extreme rarity of this condition and the risk of iatrogenesis in patients with falsely abnormal thyroid function tests (3).

Data from a repeat analysis of thyroid function tests are presented in Table 1. Results were similar to previous analyses (Table 1). To investigate possible antibody interference, thyroid testing using an alternative chemiluminescent immunoassay was conducted at 2 laboratories using the Advia Centaur platform (Siemens Healthcare Diagnostics) (Table 1).

Samples treated using the Heterophilic Blocking Tube (Scantibodies Laboratory) confirmed that Roche Elecsys methods were affected by antibody interference (Table 1).

Total T4 and T3 assays on the Roche Elecsys platform use sheep-derived antibodies and are based on competitive test principles. The T-uptake assay also uses sheep antibodies and follows a modified competitive principle. In contrast, the Siemens Advia Centaur platform uses mouse antibodies. The Roche TSH assay uses mouse antibody and a human/mouse chimera antibody and is noncompetitive in principle.

The Scantibodies Heterophilic Blocking Tube data strongly suggested interference by heterophile antibodies (HABs), which are endogenous antibodies that bind to immunoglobulins of other species (4). Typically, HABs bind to the constant portion of antibody and in noncompetitive assay formats create a bridge between capture and detection antibodies leading to falsely increased results (5). In competitive assay formats, this Fc binding can prevent or interfere with antigen binding, leading to a false increase (5). Our observation that the heterophile blocking tube caused an increase (not decrease) in the noncompetitive assay (TSH) did not support typical HAB interference via binding the Fc portion. A false-negative value can be seen, however, when the interfering antibody binds to 1 of the 2 antibodies in a noncompetitive assay (6), as in the Roche Elecsys TSH assay.

Heterophile antibody binding to the variable region of the assay antibody (idiotypic interaction) is less common (5). Idiotypic interactions occur mainly in patients who have received treatment with animal immunoglobulins (5). In light of the rarity of idiotypic interactions, the interferences observed in samples from this patient with assay antibodies against 3 differing antigens, coupled with no known history of treatment of the patient with animal immunoglobulins, practically excluded the possibility of an idiotypic interaction from a human antianimal antibody.

Rheumatoid factor (RF) can also exhibit nonspecific binding and typically binds to the Fc portion of assay antibodies, inhibiting or preventing antigen binding (5). In the noncompetitive format of the Roche total TSH assay, this would typically result in a signal increase, opposite of our findings.

As such, we began to look for a common factor in all the assays. According to the manufacturer, all the assays used a streptavidin-labeled magnetic particle to which biotin (attached to exogenous hormone or a TSH-specific antibody) would bind. Additionally, all had a ruthenium complex linked to the detection antibody. Therefore, antibodies against streptavidin, biotin, and the ruthenium–complex label were considered (7).

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3 Nonstandard abbreviations: T4, thyroxine; RI, reference interval; T3, triiodothyronine; TSH, thyroid-stimulating hormone; HAB, heterophile antibody.
An aliquot sent to Roche Diagnostics was tested with the addition of “Ru-interference blocking agent” above that normally found in the assay. Results were not suggestive of interference against the ruthenium complex [free T4 without blocker 14.7 pmol/L (1.1 ng/dL) and with blocker 14.6 pmol/L (1.1 ng/dL)]. However, because free T4 by the Roche method did not appear to exhibit interference as seen with the other Roche methods, the workup conducted by Roche Diagnostics to investigate possible Ru-interference may not be sufficient to address the question. A more appropriate investigation would have used total T4 (with demonstrated interference) and addition of the specific blocker to see if the interference is removed.

To investigate streptavidin and biotin interference, patient sample was applied to a streptavidin agarose column (Pierce Biotechnology). Untreated sample results were 0.51 mIU/L, 165 nmol/L (13 μg/dL) and 1.72 for TSH, total T4, and T-uptake, respectively. After treatment, the values were 0.48 mIU/L, 154 nmol/L (12 μg/dL), and 1.77. The results indicate that interference due to endogenous biotin, or to antibodies against streptavidin or other molecules interacting with streptavidin, was unlikely. To date, the exact nature of the antibody interference is unknown.

Like all immunoassays, methods for thyroid hormones can be affected by unpredictable and sometimes transient antibody interferences (5, 8). Because these cases are rare and patient specific, it is not possible to detect them using routine quality control practices. Assay reagents typically contain blocking agents (nonimmune sera, antibody fragments, immobilized IgG) to reduce the incidence of antibody-based interference, but it has been estimated that 0.1% of samples have titers high enough to overcome these blockers (8). Estimates of the rate of interference are typically 1% but range from approximately 0.4% to 4% (5, 9).

It is generally accepted that interference investigations should include: (a) repeat analysis; (b) dilutional analysis; (c) addition of immunoglobulins to block interfering antibodies; and (d) use of an alternative immunoassay (5, 10). Several studies have shown that the investigation must use multiple approaches (8, 10). It is important to keep in mind that negative results do not exclude the presence of an interfering substance. Detailed studies, typically

<table>
<thead>
<tr>
<th>Table 1. Laboratory data.a</th>
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<tr>
<td>March 1, 2007</td>
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<td>Roche Elecsys</td>
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<td>March 12, 2007</td>
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<tr>
<td>Roche Elecsys pre-HBT</td>
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a Unless noted otherwise, reference intervals for total T₄, 67–138 nmol/L (5.2–10.7 μg/dL); total T₃, 1.3–2.4 nmol/L (86–154 ng/dL); TSH, 0.3–5.0 mIU/L; and thyroid hormone binding ratio (1/T-uptake), 0.77–1.16. HBT, Heterophilic Blocking Tube (Scantibodies Laboratory).

b Reference interval 64–126 nmol/L (5.0–9.8 μg/dL).

c Reference interval 1.5–2.8 nmol/L (97–186 ng/dL).

d Reference interval 0.7–6.4 mIU/L.

e Reference interval 0.79–1.16.
However, as Ismail and colleagues point out, within 10% of the original undiluted sample result, of thumb such as that linear results should agree. Assessment of linearity is subjective, based on rules of thumb such as that linear results should agree within 10% of the original undiluted sample result. However, as Ismail and colleagues point out (8, 9), this is not appropriate and will provide poor detection of interference. Additionally, laboratories should use only diluents validated by the manufacturer, and be aware that some immunoassays do not support sample dilution.

Although immunoglobulins from the animal species used to generate assay antibodies are added to bind with potential interferents, and mitigate their impact, it has also been recommended to add immunoglobulin from a second species (11). This makes for a complex investigation if multiple species are involved. A commercially available product (Heterophilic Blocking Tube; Scantibodies Laboratories) adds proprietary binders to the sample and simplifies the conduct of blocking studies.

**Points to Remember**

- Investigation of immunoassay interference should include repeat analysis, determination of nonlinearity with dilution, testing by alternative method, and removal of interfering antibodies or blocking studies.
- Changes in measured concentration after removal of immunoglobulins or addition of blocking agents are indicative of antibody interference, but no effect does not rule out interference.
- Results obtained after dilution, immunoglobulin removal, or blocking studies should not be reported, as they may not reflect true concentrations.
- Clear communications between clinicians and the laboratory are required to minimize the impact of assay interference on clinical decision making.

Removal of interfering antibodies can be accomplished with commercially available protein G, protein A affinity columns, or polyethylene glycol (PEG). It is important to control for the effect of the treatment by measuring another analyte and correct for recovery. Results obtained from antibody-removal studies are not reportable, and indicate only that the original values are questionable.

The requirements for proper assessment of dilution and blocking studies are time consuming and costly. Thus, retesting with an alternative method is the easiest and quickest means to assess interference. It is important to select methods that use antibodies from different animal species than those in the original assay. Laboratory scientists should keep in mind that using alternative method evaluation does not indicate which method is correct.

In the case of this specific patient, the incorrect diagnosis of inappropriate TSH secretion would have obligated expensive tests such as brain MRI and, if a pituitary incidentaloma was present, could have led to inappropriate treatment with medications or even surgery. Clinicians must recognize that symptoms of thyroid dysfunction can be variable and nonspecific and consider the possibility of laboratory artifact. In summary, antibody interference in immunoassays remains a problem that requires constant vigilance and good communication between laboratory scientists and clinicians. Both need to maintain awareness of the problem and review data against clinical findings before initiating interventions that may be unnecessary.

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**References**