Influence of Thyroid Hormone Autoantibodies on 7 Thyroid Hormone Assays

To the Editor:

Spurious results of thyroid function tests (TFT) can be recognized when they do not reflect the clinical status of the patient or are not internally consistent [e.g., increased FT4 with nonsuppressed thyroid-stimulating hormone (TSH)]. Potential causes of spurious TFT results include nonspecific binding of endogenous circulating factors, such as heterophilic antibodies, with assay reagents (1), the presence of albumin variants found in familial dysalbuminemic hyperthyroxinaemia (2), and thyroid hormone autoantibodies (THAA) (3). We describe a patient who had discordant TFTs due to circulating THAA and report differences in TFT results obtained on a variety of automated immunoassay platforms.

A 19-year-old man presented with tiredness spanning a 12-month period following a prolonged viral illness. A family history of hypothyroidism prompted a request for TFTs. These showed increased FT4 of 60 pmol/L (reference interval 10–25 pmol/L) and FT3 of 8.1 pmol/L (reference interval 2.5–6.5 pmol/L), but an nonsuppressed TSH of 0.9 mU/L (reference interval 2.5–6.5 pmol/L), which was clinically euthyroid. Use of residual samples was in accordance with the Human Tissue Act of 2004 and the guidelines of the Royal College of Pathologists. The terms of the Declaration of Helsinki were also fully observed. Test results for heterophilic antibodies using heterophile blocking tubes (Scantibodies) were found to be negative. A screening test for familial dysalbuminemic hyperthyroxinaemia (4) was also negative. Incubation of the patient’s serum with radiolabeled T3 resulted in the presence of 8-anilino-1-naphthalene sulfonic acid, followed by precipitation with polyethylene glycol (5), indicated the presence of T3 autoantibodies as the cause of the erroneously high FT4 result. A similar screen for T4 autoantibodies (5), using a similar method but with radiolabeled T3, was negative for this patient.

Serum FT4 measured by equilibrium dialysis (EDFT4) was within the reference interval (Table 1). Although the general effects of THAA on assay methods have been previously reviewed (3), the specific effects on currently used assays methods are not precisely known. We compared our TFT results for this patient with results obtained with different analytical platforms: Advia Centaur (Siemens Medical Solutions Diagnostics), Elecsys E170 (Roche Diagnostics), TOSOH AIA 1800 (Tohoh Bioscience), Architect c800 (Abbott Limited Diagnostics), UniCel Dxi 800 (Beckman Coulter UK), and Wallac Delfia (PerkinElmer UK).

Measurements of TSH obtained with the different systems did not show marked assay-dependent variation (Table 1). The Immulite 2500 was the only assay that gave an increased FT4 result of 9.1 pmol/L (reference interval 2.5–6.5 pmol/L). Although no T4 autoantibodies were detected, endogenous antibodies to T4 may possibly cross-react with the T3 label used in the Immulite assay. FT4 results varied from within the reference range with Wallac Delfia to >200% of the upper reference limit with the Advia Centaur, suggesting that observed differences in FT4 results are likely due to differences in assay design. One-step procedures showed the greatest positive interference; high results were observed with both the Advia Centaur and Immulite 2500. The TOSOH AIA 1800, another 1-step assay, appeared less affected, a finding that may be attributable to a different tracer.
used for signal detection. Both the Advia Centaur and Immulite 2500 use chemiluminescent substrates, whereas the TOSOH uses a fluorogenic tracer. The different molecular sizes of the labels may influence whether they are recognized by the THAA.

Two-step methods appear not to be susceptible to THAA interference, because the procedure ensures that there is no contact between serum components and analog tracer (3). This characteristic was evident in the Wallac Delfia, which gave results comparable to the EDT4. Conversely, the other 2-step assays, Abbott Architect and Beckman DXI 800, gave borderline low results, which may have been related to the nature of the tracer used in these assays. Both the Abbott and Beckman assays use T₃ acridinium–labeled tracer in the second step of the reaction, when the tracer binds to unbound sites of the capture antibody. Any THAA left after the washing step will bind only the analyte and not the tracer, and hence increase the signal and decrease the FT₄ concentration detected. The Elecsys E170 is a competitive 1-step assay that differs in that it involves 2 incubations steps, but without a washing step in between. First the serum and a ruthenium–labeled capture antibody are mixed, then the biotin–labeled T₄ analog that binds the remaining free sites on the capture antibody is added. This assay design may account for the borderline positive interference observed (Table 1).

Our data demonstrate that the presence of THAA can lead to both positive and negative interference in FT₄ assays, and an increased FT₃ in only 1 assay. Serum TSH provides the most reliable assessment of thyroid function for patients found to have such antibodies.

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References


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Table 1. TFT Measurements by 7 Different Immunoassays and Equilibrium Dialysis.a

<table>
<thead>
<tr>
<th>Immunoassay system</th>
<th>Principle</th>
<th>TSH, mU/L</th>
<th>FT₄, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immulite 2500</td>
<td>1-Step chemiluminescent assay</td>
<td>0.93 (0.3–5.5)</td>
<td>50.8 (10–25)</td>
</tr>
<tr>
<td>Advia Centaur</td>
<td>1-Step chemiluminescent assay</td>
<td>1.10 (0.4–5.5)</td>
<td>55.3 (9–20)</td>
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<tr>
<td>TOSOH AIA 1800</td>
<td>1-Step fluoroimmunoassay</td>
<td>1.08 (0.4–4.0)</td>
<td>27.3 (10.6–21)</td>
</tr>
<tr>
<td>Elecsys E170</td>
<td>1-Step (2-step incubation) Chemiluminescent assay</td>
<td>1.04 (0.3–4.5)</td>
<td>24.9 (10–22)</td>
</tr>
<tr>
<td>Architect</td>
<td>2-Step chemiluminescent assay</td>
<td>0.93 (0.2–5.0)</td>
<td>9.0 (9–19)</td>
</tr>
<tr>
<td>Beckman DXI 800</td>
<td>2-Step chemiluminescent assay</td>
<td>0.96 (0.4–4.5)</td>
<td>7.3 (7–17)</td>
</tr>
<tr>
<td>Wallac Delfia</td>
<td>2-step assay</td>
<td>0.97 (0.4–4.0)</td>
<td>14.1 (9–20)</td>
</tr>
<tr>
<td>Nichol FT₄ Equilibrium dialysis</td>
<td>Physical separation of free hormone, then immunoassay</td>
<td>NA</td>
<td>16.1 (10–36)</td>
</tr>
</tbody>
</table>

* Patient results outside reference intervals are in bold type. NA indicates not measured.