Anomalous Serum Thyroxin Measurements with the Abbott TDx Procedure

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We describe a patient whose serum gave consistently low results for thyroxin as measured with the Abbott TDx but normal results by radioimmunoassay. Further clinical and laboratory studies did not support the low TDx results. Anti-thyroxin antibodies did not explain the discordant results, and we found no evidence of heterophilic antibodies in the patient's serum. We were unable to identify the cause of the problem.

Additional Keyphrases: fluorescence polarization immunoassay · radioimmunoassay compared · analytical error

In recent years, the method involving the Abbott TDx analyzer has become popular for measuring serum thyroxin (T4) in those laboratories reporting survey results to the College of American Pathologists. Undoubtedly, this is because the TDx procedure is an automated, nonisotopic immunoassay method that is rapid, simply performed, and yields good overall agreement with results obtained by standard radioimmunoassay (RIA) procedures. However, we report here the finding of an anomalous Abbott TDx result for T4 that led to an extensive and expensive patient workup, which would have been avoided if the initial testing had been done with RIA procedures.

Patient and Methods
The Patient, an unmarried 37-year-old male laborer, had a bipolar affective disorder that had been treated since 1985 with lithium carbonate, 300 mg every 8 h. Routine screening of his serum for T4 by the Abbott TDx yielded a value <10 μg/L (reference interval 45–120). On repeat testing two months later, his serum T4 as measured by the Abbott TDx was again <10 μg/L. The concentrations of thyrotropin in these two specimens were, respectively, 1.1 and 0.8 milli-int. unit/L (reference interval 0.45–6.2). Resin uptake of triiodothyronine was 36.1% (reference interval 35–45%) for the first specimen and serum thyroxin uptake (T4-uptake) was 0.94 (reference interval 0.72–1.24) for the second specimen. Previous determinations of serum T4 by RIA for this patient, including values before and after starting lithium, had consistently been normal.

Except for occasional migraine headaches for years, the patient had been in good health. He denied symptoms of thyroid or pituitary disease. His weight had been stable. Psychological testing in 1985 indicated borderline mental retardation with a full-scale intelligence quotient of 73. An only child, neither he nor his parents reported any history of thyroid or other endocrine disease. Other medications were taking at the time of evaluation were lorazepam 1 mg b.i.d, propranolol 40 mg, and thiothixene 5 mg.

Physical examination showed that he had a pulse rate 76/min and a blood pressure of 110/68 mmHg. He was 1.8 m tall and weighed 79 kg. His thyroid was normal in size and consistency, his skin was normal, and the relaxant phase of his deep-tendon reflexes was normal.

Because of the abnormal thyroid-function test result suggestive of secondary hypothyroidism, he underwent further testing. Results of routine complete blood-cell count and serum chemical tests, including bilirubin and other liver-function tests, were all normal; his serum cholesterol concentration was 2040 mg/L and his serum triglyceride concentration was 740 mg/L. Results of chest roentgenogram, electrocardiogram, and urinalysis were also normal.

Intravenous administration of 500 μg of thyrotropin (thyrotropin-releasing hormone) increased serum thyrotropin from a baseline of 1.3 to 27.1 milli-int. units/L at 20 min, 1 milli-int. units/L at 45 min, and 10 milli-int. units/L at 1 h. The corresponding serum prolactin values were 3, 64, 52, and 44 μg/L, respectively (reference baseline interval 2–15 μg/L). The mild increase of the baseline prolactin value was attributed to the effect of the phenothiazine medication or the stress of starting the intravenous infusion. Serum somatotropin concentration was <1.0 μg/L (reference interval 0–8 μg/L). Plasma cortisone, measured 0 h, was 212 μg/L (reference interval 50–180 μg/L). Oral administration of 2.5 g of metyrapone at 2300 h the preceding day resulted in an 0600 h plasma cortisone value of 52 μg/L and a deoxycortic (Compound S) value 58 μg/L (a normal response, according to the analysis Nichols Institute, San Juan Capistrano, CA). In addition, thyroid-function tests with the Abbott TDx were performed on both parents, whose results were found to be within normal limits.

Procedures
Ethanol extraction was performed essentially as previously described (1), with de-ionized water as the blank. T4 buffer was used to reconstitute the ethanol extracts, and the contents of all tubes were analyzed for T4 in the TDx. All TDx T4 results in this report were performed by using Abbott's "T4-Plus" procedure.

Incubation of sera with radiolabeled T4 and isolation the immunoglobulin fraction with polyethylene glycol (Carbowax 6000, PEG) were performed as follows: 50 μL of sera was incubated overnight at 4 °C in a final volume 1.0 mL of phosphate-buffered isotonic saline containing about 22 000 counts/min of 125I-T4 (specific activity ~3 Ci/g). An equal volume of 250 g/L PEG solution was added after incubation for 15 min at 4 °C, the precipitated T4 fraction was isolated by centrifugation at 1500 × g for

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min and the radioactivity was counted.

Electrophoresis of thyroxin-binding proteins (2) was done at the Mayo Clinic Laboratories. In this procedure, 0.8 μCi of \([^{125}\text{I}]\text{T}_4\) (specific activity ~2200 Ci/g) and 0.1 μg of unlabeled \(\text{T}_4\) were incubated with 100 μL of serum. The mixture was then electrophoresed on polyacrylamide gel. \(\text{T}_4\)-binding proteins were quantified by counting the radioactivity in slices of the gel and multiplying the fraction of counts in each peak times 1000 μg of \(\text{T}_4\) per liter (0.1 μg of \(\text{T}_4\) per 100 μL). Binding was expressed as \(\text{T}_4\)-binding capacity at 1000 μg of \(\text{T}_4\) per liter. The concentration of total \(\text{T}_4\) added to the serum as indicated above was sufficient to saturate thyroxin-binding globulin but not the other normally occurring thyroxin binders, albumin and prealbumin. The reference intervals for \(\text{T}_4\)-binding capacity by this procedure are as follows: prealbumin, 488–704 μg/L; albumin, 115–341 μg/L; thyroxin-binding globulin, 103–249 μg/L.

Results and Discussion

The clinical and laboratory data presented for our patient are not consistent with hypothyroidism despite the low \(\text{T}_4\) measured by the Abbott TDx. Similarly, RIA measurements did not confirm the low serum \(\text{T}_4\) measured by the TDx method. Table 1 shows that \(\text{T}_4\) results between the TDx and the RIA were discordant during an 18-month period. Values for \(\text{T}_4\)-uptake by the Abbott TDx assay were also within normal limits during this period, as were those for thyroid-binding globulin, triiodothyronine-resin uptake, and thyrotropin as determined in other laboratories.

On the premise that the TDx might not be measuring all the \(\text{T}_4\) actually present, we extracted the serum of the patient and controls with ethanol (950 mL/L) as described above. Table 2 shows the TDx results and the \(\text{T}_4\) recovered after extraction and correction for an extraction blank. Utilizing an average control-patient recovery of 41%, we estimated the patient’s serum to have 76 μg/L (31 μg/L divided by 0.41) of \(\text{T}_4\) actually present and not 19 μg/L as measured by the TDx for the unextracted sample.

What might be causing the low \(\text{T}_4\) value seen with the TDx method but not with the RIAs? A brief review of the theory of measurement of the TDx (3, 4) is in order. The TDx method is an automated homogeneous fluorescence polarization immunoassay procedure for the measurement of \(\text{T}_4\). The amount of polarized emitted light measured by the TDx increases when \(\text{T}_4\)-fluorescein tracer becomes bound to antibody. The higher the concentration of endogenous \(\text{T}_4\) in a patient’s serum, the more competition there is for tracer binding to antibody. Consequently, less tracer is bound to antibody and therefore less polarized emitted light is measured. In the TDx procedure a \(\text{T}_4\) value is assigned to the unknown specimen by comparing the observed polarization value with a stored standard curve.

It is reasonable to consider that anti-\(\text{T}_4\) antibodies explain the low TDx \(\text{T}_4\) results in the patient’s serum. Endogenous anti-\(\text{T}_4\) antibodies are well known to be a cause of erroneous results with immunoassays of \(\text{T}_4\) (5, 6), and Abbott also notes their interference with the TDx procedure for \(\text{T}_4\). The presence of endogenous anti-\(\text{T}_4\) antibodies would be expected to result in more \(\text{T}_4\)-fluorescein tracer being bound. This would produce a higher polarization value and lead to a lower result for apparent \(\text{T}_4\) detected by the TDx.

Several observations appear to reject the possibility that the low TDx results for \(\text{T}_4\) in the patient’s serum are ascribable simply to the presence of antibodies to \(\text{T}_4\). First, no evidence for the presence of anti-\(\text{T}_4\) antibody was found when we incubated his serum with radiolabeled \(\text{T}_4\) and isolated the immunoglobulin fraction with PEG as described in the methodology section of this report. The IgG fraction (precipitated with PEG) contained 4.0–4.9% of the \([^{125}\text{I}]\text{T}_4\) for normal control subjects and 4.0% for our patient.

Second, electrophoresis of the patient’s serum for thyroxin-binding proteins showed normal \(\text{T}_4\)-binding capacities for prealbumin, albumin, and thyroid-binding globulin. The \(\text{T}_4\)-binding capacity of prealbumin was 633 μg/L, albumin 182 μg/L, and thyroxin-binding globulin 185 μg/L. No evidence was observed for the presence of anti-\(\text{T}_4\) antibodies in the region of the gel where such antibodies have previously migrated (Figure 1).

Third, all the RIA methods indicated in Table 1 yielded generally similar findings. Laboratory A’s method was based on a magnetic solid-phase antibody separation; laboratory B’s method was based on an antibody-coated-tube separation. In laboratory C’s method, a pepsin digest was used to release \(\text{T}_4\) from binding proteins before RIA. Whereas methods from laboratories A and B could be expected to yield too-high results for \(\text{T}_4\) in the presence of endogenous anti-\(\text{T}_4\) antibodies, laboratory C’s method would not be expected to be affected. The results of laboratory C’s method were similar to those of the other RIAs.

### Table 1. Repetitive Discordant Serum \(\text{T}_4\) Results for the Patient as Measured by the Abbott TDx Procedure and by Radiimmunoassays

<table>
<thead>
<tr>
<th>Sample interval</th>
<th>TDx by Abbott</th>
<th>TDx by RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>18</td>
<td>63 (A); 65 (B)</td>
</tr>
<tr>
<td>3 months</td>
<td>24</td>
<td>97 (A); 83 (C)</td>
</tr>
<tr>
<td>4 months</td>
<td>19</td>
<td>99 (A)</td>
</tr>
<tr>
<td>5 months</td>
<td>19</td>
<td>66 (A)</td>
</tr>
</tbody>
</table>

* Reference interval: 45–120 μg/L. * The letters in parentheses refer to the different laboratories performing the radiimmunoassays (see text for the different methods). Reference intervals (μg/L): (A) 50–120; (B) 45–125; (C) 0–125.

### Table 2. Analytical Recovery of \(\text{T}_4\) in Ethanol Extracts of the Patient’s Serum and Control Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Before extraction</th>
<th>In ethanol extracts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDx low control</td>
<td>40</td>
<td>29 (73%)</td>
</tr>
<tr>
<td>TDx medium control</td>
<td>79</td>
<td>48 (61%)</td>
</tr>
<tr>
<td>TDx high control</td>
<td>156</td>
<td>93 (60%)</td>
</tr>
<tr>
<td>Control patient 1</td>
<td>95</td>
<td>36 (38%)</td>
</tr>
<tr>
<td>Control patient 2</td>
<td>80</td>
<td>34 (43%)</td>
</tr>
<tr>
<td>Our patient</td>
<td>19</td>
<td>31%</td>
</tr>
</tbody>
</table>

* The \(\text{T}_4\) values listed have been corrected for an extraction blank. The percentage of \(\text{T}_4\) recovered is given in parentheses.

* Based on an estimated 41% recovery of \(\text{T}_4\) (average of values for control patients 1 and 2), the patient is estimated to have 76 μg of \(\text{T}_4\) present per liter (31 μg/L + 0.41).
used. These findings argue against the possibility that anti-T₄ antibodies were present in the patient's serum.

Lastly, in addition to the TDx T₄ procedure, the TDx T-uptake procedure (which also involves use of a T₄-fluorescein tracer) can be affected by the presence of anti-T₄ antibodies. The TDx T-uptake values for our patient were within the normal range and consistent with the thyroid-binding-globulin determination, triiodothyronine-resin uptakes, and T₄ binding to thyroxin-binding globulin, as estimated by thyroxin-binding protein electrophoresis. These findings also do not appear to support the presence of antibodies to T₄ in this patient's serum.

Heterophilic antibodies also are known to cause interferences with immunoassays (7). They can simulate the presence of analyte by linking immobilized and signal antibodies in two-site immunoassays or can affect analyte recognition by binding to a single reagent antibody. The TDx T₄ procedure involves the use of anti-T₄ antiserum raised in sheep, so it is reasonable to consider the possibility that the presence of anti-sheep (heterophile) antibodies in the patient's serum is the cause of the low TDx T₄ result.

The following indirect observations argue against the possibility that heterophilic antibodies are present in the patient's serum. First, mixture (1:1) of his serum (18 µg of T₄ per liter) with normal sheep serum (80 µg of T₄ per liter) resulted, if anything, in a lower recovery of T₄ than that predicted from the sum of the two (predicted, 49 µg/L; found, 37 µg/L). This recovery was comparable with that seen when a TDx high control was mixed (1:1) with the patient's serum. If heterophilic antibody accounted for the falsely low TDx T₄ result, mixing of sheep serum with the patient's serum before assay would be expected to reduce assay interference. Normal sheep serum would be expected to bind competitively to the heterophilic antibodies, reducing their binding to the TDx antisera. Second, in the TDx phenytoin procedure, antiserum from sheep also is used and therefore heterophilic antibodies might interfere with this assay as well. No such interference was observed. Because this patient was not receiving phenytoin, his TDx-measured phenytoin concentration was appropriately less than the detection limit of the assay. In addition, when serum from a patient receiving phenytoin (concentration 18.2 g/L) was mixed (1:1) with our patient's serum, we could account for 9.5 g/L in the mixture, a finding consistent with an uninfuenced dilution. Thus, neither of these observations supported the presence of interfering heterophilic antibodies in our patient's serum.

The presence of endogenous antibodies directed against the fluorescein component of the tracer or against the T₄-fluorescein complex also could explain the low serum TDx T₄ in our patient. However, if endogenous anti-fluorescein or anti-T₄-fluorescein antibodies were present in the patient's serum, one might have expected a falsely high TDx T-uptake result. This is because the TDx T-uptake assay is a direct-binding method, in which T₄-fluorescein is the tracer; such an assay might be expected to yield higher results in the presence of additional tracer-binding species. As noted before, TDx T-uptake values were within normal limits for this patient and were consistent with other findings. This fact thus does not support the presence of endogenous antibodies to fluorescein or T₄-fluorescein in this patient.

During the course of our studies the patient was receiving a drug regimen of lithium, lorazepam, benztropine, and thiouoxetine. Lithium caused no interference with the TDx T₄ procedure when added to normal serum to give a therapeutic concentration. Although we cannot at present completely rule out an analytical interference by another of these commonly administered medications or its metabolites, we are aware of no literature reference citing such an interference, and we consider it unlikely. The possibility also exists that the T₄ in our patient's serum is bound to a binding species with higher than normal affinity and thus is not released by the TDx pretreatment displacing agent (surfactant in buffer).

In summary, although our studies do not indicate the specific cause for the low T₄ results measured with the TDx, our observations appear to represent a previously unrecognized analytical error that others who report TDx T₄ results need to consider. We believe that all clearly low TDx T₄ results that are not explained by T-uptake and thyrotropin testing should be checked by RIA.

References