Evaluation of a New Strategy for Detection of Thyroid Dysfunction in the Routine Laboratory

Rhys John, Robert Henley, Geoffrey Lloyd, and George H. Elder

We assessed the use of a new strategy for detecting thyroid disorders, utilizing a sensitive assay for concentrations of thyrotropin (TSH) and free thyroid hormone in serum as a follow-up test. Of 1279 patients who were not on thyroid (T4) replacement treatment, 82% could be classified as euthyroid and would require no further tests. In patients who were on T4 replacement, 41% fell into the euthyroid category and would require no further tests. Using this strategy to replace our existing strategy of free thyroxin as a "first-line" test would reduce the proportion of patients who would require one or more follow-up tests from 49% to 24%.

Additional Keyphrases: thyrotropin • thyroxin • thyroid status • diagnostic efficiency

Recent developments in production of monoclonal antibodies have brought about a rapid change from competitive-binding assays for thyrotropin (TSH) to immunometric assays. This has produced many advantages, in that immunometric assays have short incubation times and are more robust. The labeling of antibodies with a nonradioactive substance such as europium (1), an enzyme (2), or an acridinium ester (3) has also made it possible for TSH assays to be performed in laboratories previously not licensed for work with radioactive materials. The increased sensitivity inherent in immunometric assays has allowed TSH concentrations in normal subjects to be distinguished from those in clinically thyrotoxic patients, so that interest has been aroused in the possible use of TSH as a "front-line" screen for thyroid disease, as proposed by Caldwell et al. (4). By this procedure, patients would be classified in one of five groups, depending on their basal concentrations of TSH and free thyroid hormones. If their TSH concentration in serum was within the reference interval, they would be classified as being euthyroid and have no further estimations performed. With an increased concentration of TSH, FT4 would be quantified to distinguish between overt and subclinical forms of hypothyroidism. With an undetectable concentration of TSH, either an FT3 test alone or an FT4 and an FT3 assay would be performed to distinguish hyperthyroidism from subclinical hyperthyroidism. Using this procedure, Caldwell et al. showed that they could reduce by about 50% the number of analyses needed to arrive at a diagnosis with no decrease in diagnostic accuracy and without additional delay.

In the present study we have evaluated this new strategy in patients' samples referred to a routine clinical chemistry department. We defined the biochemical thyroidal status of our patients on the basis of their TSH concentration, followed by determinations of FT3 and FT4 concentrations. The TSH assay we used is a new two-site immunoenzymometric assay in which enhanced chemiluminescence is the detection system used for quantification. We previously reported a clinical evaluation of this assay (5) and have now extended it to a much larger number of patients' samples referred for thyroid-function testing.

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Materials and Methods

Immunoenzymometric assay. The Amerlite TSH assay (Amersham International plc., White Lion Road, Bucks., U.K.) utilizes two mouse monoclonal antibodies to TSH. One antibody, directed against the β-subunit, is attached to the inside well of a 96-well microtiter plate; another antibody, which recognizes intact TSH, is conjugated to horseradish peroxidase (EC 1.11.1.7). After 100 µL aliquots of standards, quality-control materials, or samples are incubated with 100 µL of reagent buffer at 37 °C for 2 h, the wells are washed with borate buffer in the Amerlite washer, and 200 µL of conjugate reagent is added to each well and incubated at 37 °C for 1 h. After a further washing of the wells, 250 µL of signal reagent—hydrogen peroxide, luminol, and an enhancer in barbital buffer—is added to the wells without delay and the amount of light generated after 10 min at room temperature is read in the Amerlite analyzer. After 2 min of data processing the TSH concentrations in the unknown samples are printed out. The detection limit of the assay, defined as the mean +3 SD of zero standard (n = 20), was 0.02 milli-int. unit/L, with between-assay precision (CV) of 10.8% and 5.7% at 4.8 and 11.9 milli-int. units/L, respectively. The concentrations of TSH in serum from 132 euthyroid subjects ranged from 0.06 to 4.13 (mean 1.52) milli-int. units/L; TSH concentrations below 0.02 milli-int. unit/L were classified as undetectable; concentrations above 4.13 milli-int. units/L were classified as increased.

Immunoradiometric assay, in house. This assay, which is automated in the Kemtekt 3000 radioimmunoassay system (Kemble Instruments Ltd., Burgess Hill, Sussex, U.K.), is a modification of our previously described method (6), as follows: The mixture of two monoclonal antibodies labeled with 125I has been replaced by a different mouse monoclonal antibody, also obtained commercially (Serono Diagnostics, Surrey, U.K.). The labeling of the monoclonal antibody has been modified to increase the incorporation of 125I onto the antibody. The labeled antibody is dissolved in 15 mL of phosphate buffer (50 mmol/L, pH 7.4) containing, per liter, 5 g of bovine serum albumin, 2 mL of normal sheep serum, 2 g of sodium azide, and diluted (10 mL/L) mouse serum to give a concentration of 1.47 mCi/L. The labeled antibody is then diluted fivefold in assay buffer just before use.

Thyrotropin standard. The TSH standard was obtained from Serono Diagnostics. Six liquid standards (2 mL) containing 50, 15, 5, 1.5, 0.5, and 0.15 milli-int. units of TSH of human pituitary origin per liter are dissolved in TSH-free bovine serum; the zero standard (3 mL) is bovine serum. All standards are stable at 4 °C for eight weeks. The manufacturer calibrated the standards against the Medical Research Council human pituitary thyrotropin, 2nd IRP 80/558 (National Institute of Biological Standards and Control, Holly Hill, Hampstead, London, U.K.), and we checked this calibration with 80/558 in our assay system.

Solid-phase antibody. Solid-phase antibody was prepared as previously (6), except that we included 10 mL of newborn-calf serum per liter in the assay buffer. The solid-phase antibody is then diluted 40-fold in this buffer.

Mix 100 µL of labeled antibody (70 100 counts/min) with 100 µL of standards or samples in duplicate and allow to react for 2 h at room temperature. Incubate for another 2 h at room temperature with 100 µL of solid-phase antibody, then filter the contents of the tubes. Wash the antibody-bound fraction three times with 1.2-mL portions of wash buffer. Count the radioactivity in the antibody-bound fraction for 60 s. The functional sensitivity, defined as the concentration of TSH at which the interassay precision is <10%, was from 0.5 to 50 milli-int. units/L.

Other thyroid hormones. We measured FT4 and FT3 concentrations in serum with an analog-based technique (Amersham International). The between-batch precision of the FT4 assay was 4.5% at 12.7 pmol/L and 5.8% at 42 pmol/L; that of the FT3 assay was 3.3% at 3.4 pmol/L and 5.7% at 11 pmol/L. The reference interval was 8 to 26 pmol/L for FT4 and 3 to 9 pmol/L for FT3.

Immunoradiometric assay—Amerwell. For some patients we measured serum TSH concentration by Amerwell (Amersham International).

Radioimmunoassay. For some patients we measured FT4 and TSH concentrations in serum by a simultaneous radioimmunoassay (SimulTRAC; Becton Dickinson U.K. Ltd., Between Towns Road, Cowley, Oxford, Ox4 3LY), according to the manufacturer’s instructions.

Blood samples. The patients’ samples consisted of all those received during a four-week period in this department, which receives samples from all hospitals in this Health Authority and from general practitioners, for which thyroid-function tests were requested. The blood samples had been taken at any time of the day, and most samples had been centrifuged within 2 h; however, a small proportion of the samples, from general practitioners, were unseparated for up to 16 h. Serum was separated from the cells and stored at 4 °C until all assays were completed, usually within 24 h, and then stored at −20 °C. Samples were assayed in batches

<table>
<thead>
<tr>
<th>TSH, milli-int. units/L</th>
<th>FT4 pmol/L</th>
<th>FT3 pmol/L</th>
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<tr>
<td>&lt;0.02 (undetectable)</td>
<td>&gt;26</td>
<td>&gt;9</td>
</tr>
<tr>
<td>0.03–0.05 (low)</td>
<td>8–26</td>
<td>3–9</td>
</tr>
<tr>
<td>0.06–4.13 (normal)</td>
<td>8–26</td>
<td>&lt;3</td>
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<tr>
<td>&gt;4.13 (increased)</td>
<td>&lt;8</td>
<td>&lt;3</td>
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*Not classified (see text).

Patients taking T4 (n = 219)

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</tr>
<tr>
<td>10 Hyperthyroid</td>
<td>9 Hyperthyroid</td>
</tr>
<tr>
<td>51 Subclin. hyperthyroid</td>
<td>34 Subclin. hyperthyroid</td>
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<td>1 —</td>
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<td>2 —</td>
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<tr>
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<td>19 Hypothyroid</td>
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<td>19 Hypothyroid</td>
<td>7 Hypothyroid</td>
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Table 1. Numbers and Classification of Patients’ Thyroid Status, Based on Amerlite TSH, FT4, and FT3 Results

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of 80, and all assays were performed in duplicate. For this study we determined FT₄, FT₃, Amerlite TSH, and in-house TSH for all samples.

**Diagnostic strategy.** The current strategy in this laboratory is to use an FT₄ assay as the screening assay; we then assay TSH in samples with an FT₄ concentration <14 pmol/L and FT₃ in samples with an FT₄ concentration >22 pmol/L. For patients receiving T₄ replacement, the cutoff values of FT₄ for performing a TSH or FT₃ estimation are <18 and >26 pmol/L, respectively. Patients whose FT₄ concentrations are normal do not warrant further tests unless the clinical information suggests otherwise. For the purpose of this evaluation, we quantified FT₄, FT₃, and TSH for all samples.

**Results**

Of 1585 patients' samples received during this study, 87 were either insufficient for complete testing or had no clinical information on the request form and were not included further in the evaluation. The study group of 1498 patients was divided into two groups: those taking T₄ replacement (219 patients) and those who were not (1279 patients). We then classified the patients according to the strategy proposed by Caldwell et al. (4), but added an extra category for patients who could not be classified by their strategy (Table 1).

**Patients Not Taking T₄**

**Euthyroid group.** Of the 1279 patients not taking T₄, 81.9% had a normal serum TSH concentration by Amerlite and would not have merited further tests. Not all of these patients, however, had normal concentrations of free thyroid hormones. We found three patients who had increased concentrations of FT₄ and FT₃ with a normal TSH concentration. In the two samples that were available for repeat assay, the TSH concentration of one remained the same and within the reference interval; the second sample had undetectable TSH. A further 14 patients had increased FT₄ and normal FT₃ concentrations; eight had normal FT₄ with increased FT₃ concentrations; and 16 patients had low FT₄ and normal or low FT₃ concentrations.

**Hyperthyroid group.** There were 37 patients for whom TSH was undetectable and an FT₄ concentration was increased, all but eight of whom also had an increased FT₃ concentration. Of the patients with an undetectable TSH concentration, the largest category (50.4%) had FT₄ and FT₃ concentrations within their reference intervals; by this strategy, they would be classified as having subclinical hyperthyroidism. Interestingly, almost two-thirds of these subclinical hyperthyroid patients also had decreased concentrations of TSH (<0.15 milli-int. unit/L) as evaluated by our in-house assay.

**Hypothyroid group.** The largest group of patients among those with increased TSH concentrations were subjects who had normal concentrations of FT₄ (81.8%) and so would be considered as having subclinical hypothyroidism.

**Others.** Twenty-four of our patients did not fall into any of the categories proposed by Caldwell et al. (4). Most (20 patients) had TSH concentrations between the lower limit of sensitivity of the assay and the bottom of the reference interval. These were very much borderline results and could easily have been assigned to some category on the basis of the results of their free thyroid hormone concentrations. Fourteen of these 20 had normal FT₄ and FT₃ concentrations, another three had increased FT₄ and FT₃ concentrations, and three had either an increased FT₄ or FT₃ concentration.

**Patients Taking T₄**

**Euthyroid group.** Of the patients taking T₄, 41.1% had a normal TSH concentration by Amerlite and would not require any further testing. All these patients also had a normal TSH concentration by our in-house assay. On examination of their free thyroid hormone concentrations, only six of 90 patients with normal TSH concentrations had FT₄ concentrations exceeding the reference interval.

The proportion of patients who were on T₄ replacement and had an undetectable TSH concentration was 25.6%, compared with 7.9% of patients with an undetectable TSH concentration who were not taking T₄. Of the 18 patients with an undetectable TSH concentration and an increased FT₄ concentration, only four could be considered to be overtreated, i.e., also had an increased FT₃ concentration. The largest group of patients taking T₄ whose TSH was undetectable had FT₄ and FT₃ concentrations within their respective reference intervals. About half of these patients had suppressed concentrations of TSH by our in-house assay. One patient, who was being treated with T₃ replacement, had an undetectable TSH and an increased FT₃ concentration.

**Hypothyroid group.** A surprisingly large proportion (28.8%) of patients who were on T₄ replacement still had an increased TSH concentration, and seven of the 62 such patients had FT₃ concentrations below the euthyroid reference interval.

**Others.** As in patients who were not taking T₄, a small group of patients who were taking T₄ had TSH concentrations between the limit of detection of the assay and the bottom of the reference range (5%). Only one of these patients had above-normal FT₄ and FT₃ concentrations.

**Assay Comparison**

In most of the samples analyzed, the results for TSH by Amerlite were approximately 25% lower than results by our in-house assay. For 14 patients' samples, however, the Amerlite assay gave results that were higher than those by our in-house procedure. Except for one sample (TSH 11.2 milli-int. units/L), the TSH concentrations by our in-house assay were within the normal reference interval. We confirmed that the results with our in-house assay were accurate by measuring TSH with the SimulTRAC FT₄/TSH assay and the Amerwell TSH assay. Additionally, because the FT₄ concentrations by Amerlex and SimulTRAC were the same and within the normal range, we suspected that there was an interference in some samples with the Amerlite TSH assay (7). The manufacturer is aware of this problem, and steps have been made to correct the interference by the addition of non-immune mouse serum and anti-IgM in current batches of reagents.

**Discussion**

Because of the increasing number of the commonly available tests for assessing thyroid function, some investigators have suggested that TSH measured by a sensitive method, followed by an FT₄ assay when the TSH value is abnormal, will help minimize the number of tests required to reach a correct diagnosis.

The use of a TSH assay to distinguish hypothyroid patients is without doubt so that patients with only the mildest forms of hypothyroidism, and with FT₄ concentra-
tions within the reference range and only modest increases in TSH concentrations, can be identified. Some clinicians have not treated these patients but have reviewed them for evidence of further progression to overt disease. Others view this progressive change to overt hypothyroidism as grounds for treatment. TSH assays that distinguish the decreased TSH concentrations in thyrotoxic patients from normal circulating concentrations are a more recent addition to the laboratory assessment of thyroid dysfunction. Although these assays have been shown to predict accurately the biochemical thyroid status of clinically well-defined patients, experience with their use in the routine laboratory is more limited (9, 10).

Recently, using the Amerwell immunoradiometric assay for TSH as a front-line screen, followed by a T₄ assay if the TSH value was abnormal, Gow et al. (6) found that, in 1916 routine requests for thyroid function, 1.4% had low TSH concentrations (<0.3 milli-int. unit/L) and 0.7% had undetectable TSH concentrations with normal T₃ concentrations. However, they made no attempt to identify the patients with normal TSH concentrations who might have an abnormal thyroid function. In our own study, we have identified such patients by comparing the use of an Amerlite TSH assay as a front-line screen with the results of our in-house TSH assay and the FT₄ and FT₃ concentrations for every patient.

We found that a correct assignment to the euthyroid group, on the basis of a normal TSH concentration and normal FT₄ and FT₃ concentrations, was possible in most patients (96.1%) who were not taking T₄. A small group of subjects (2.4%) had increased FT₄ or FT₃ concentrations without suppression of their TSH concentrations below the reference interval. A smaller percentage of patients (1.5%) with a normal TSH concentration had low concentrations of FT₄ and FT₃. A problem may arise with this protocol in identifying secondary causes of hypothyroidism if the basal TSH concentration is not decreased below the bottom of the reference interval. In patients with low FT₄ and FT₃ concentrations, the normal TSH concentration by Amerlite was confirmed by our in-house assay; thus they were not misdiagnosed cases of primary thyroid failure. More likely, their low FT₄ and FT₃ concentrations were the result of disturbances in free thyroid hormone concentrations, which can occur in nonthyroidal illness. In these patients, a single basal measurement of TSH will be more reliable than measurement of FT₄ or FT₃ in assessing their thyroid status (11).

We found that 5.3% of all patients who were not on T₄ replacement had TSH concentrations that were undetectable or were suppressed below the reference range and had no evidence of increased thyroid hormone concentrations. Of these patients, almost half (49%) had been thyrotoxic in the past and either were now, or had been, treated with carbimazole or radio-iodine. A further quarter of these patients (24%) had various illnesses that might have contributed to their low TSH concentrations. One other factor to consider in the remaining patients was the possibility that some were on T₄ replacement but this information had not been put on the requesting form. In an unselected population of patients such as ours there will be a significant proportion who, because of their pre-existing thyroid disease or present illness, do show undetectable TSH concentrations with normal free thyroid hormone concentrations. A correct interpretation of thyroid function tests in these particular clinical situations will only be possible with knowledge of their clinical state.

We are unhappy with the category of subclinical hyperthyroidism based on an undetectable TSH concentration with normal free thyroid hormone concentrations. Because of the many reasons, both methodological and physiological, that could result in an undetectable TSH concentration, we consider it unwise to label patients as hyperthyroid on the basis of an undetectable TSH concentration alone. Recently, patients with severe nonthyroidal illness were described who had low concentrations of TSH that were not associated with high concentrations of thyroid hormones but with low concentrations of T₄ (12).

In the hypothyroid group, only a small percentage of patients (17.2%) with increased TSH concentrations had FT₄ concentrations below the reference interval. Most patients who had increased TSH concentrations had FT₄ concentrations that extended well into the reference interval. To detect all these patients by using FT₄ as the screening assay would require TSH to be measured in a large proportion of patients whose FT₄ concentrations were low normal.

By our strategy of assaying TSH in those samples with an FT₄ of <14 pmol/L, we would have missed 26/1279 (2.0%) with increased TSH concentrations as measured by our in-house assay and 30/1279 (2.3%) of those measured by the Amerlite assay. All those 26 (or 30) patients had FT₄ concentrations <14 pmol/L. For those patients who were on T₄ replacement, assaying TSH only in those samples with an FT₄ of <18 pmol/L would have missed 13/219 (5.9%) samples with increased TSH concentration by either assay. Obviously, using TSH as the front-line screen has the advantage of detecting all patients who have an increased TSH concentration and a normal FT₄ concentration.

The strategy proposed by Caldwell et al. (4) was investigated only in patients suspected to have thyroid disease. Because in most laboratories a substantial proportion of samples to be tested for thyroid function come from patients who are taking T₄, any strategy for testing must include this category of patient. Sensitive TSH assays, with their ability to distinguish euthyroid subjects from thyrotoxic patients, appear to offer a reduction in the number of in vitro tests required for adequate monitoring of therapy. Of the 219 treated patients studied, fewer than half (41%) had a normal TSH concentration and were rendered biochemically euthyroid. Just over a quarter (28%) of all patients treated had TSH concentrations exceeding the normal range and so might benefit from an increase in their dose of T₄. A quarter of the patients taking T₄ had undetectable TSH concentrations, but only a very small percentage of these had increased FT₄ and FT₃ concentrations and so could be considered to be over-replaced with T₄. Of all subjects taking replacement T₄, 32.1% had increased FT₄ concentrations, which is consistent with other findings of high FT₄ concentration in subjects on T₄ replacement (13). A surprisingly high proportion (60.7%) of patients who were taking T₄ and had normal free thyroid hormone concentrations also had undetectable concentrations of TSH. This might be related to the same problem of overlap seen at the low end of the assay in patients who were not taking T₄, or it could be due to a suppression of the thyrotroph by replacement T₄ without the production of supraphysiological concentrations of free thyroid hormones. In their proposed strategy, Caldwell et al. recognized that it would be inappropriate to assume that an undetectable TSH and an increased FT₄ imply overtreatment. The value of TSH as a screening test
in patients who are taking $T_4$ is not as great as in those patients who are not on $T_3$ replacement.

If we disregard the 14 samples that gave erroneously high results with the Amerlite assay, the use of TSH as a frontline screen in our laboratory has changed the numbers of samples that require additional assays to be done after the initial screening assay. Using FT$_4$ as a screening assay and the cutoff values noted, we would do a TSH assay on 44% of samples and an FT$_3$ assay on a further 5% of patients' samples. If we used TSH as the screening assay, 24% of all patients would require an FT$_4$ assay and an additional 9% would require an FT$_3$ assay if the strategy proposed by Caldwell et al. were adopted. In the latter case, therefore, we would perform 16% fewer additional tests after the screening assay.

We conclude that it is premature to propose that TSH assays replace estimates of free thyroid hormones in the diagnosis of thyroid dysfunction, but with further careful evaluation TSH assays may be reliable as screening tests with free thyroid hormones as backup tests.

We are grateful to Amersham International for the loan of the Amerlite system to carry out this evaluation.

References

Clin. Chem. 34/6, 1114–1116 (1988)

Effect of Glucose Loading on Concentrations of Chromium in Plasma and Urine of Healthy Adults
Brian W. Morris, Huw Griffiths, and Graham J. Kemp

We report here a small study designed to identify the effect of a 75-g oral glucose load on concentrations of chromium in plasma and urine of apparently healthy volunteers. We detected a consistent and significant ($P < 0.01$) decline in plasma chromium after glucose administration, the nadir of the chromium response coinciding with the zenith of the glucose concentration.

Additional Keyphrases: trace elements · nutrition · atomic absorption spectroscopy

Since the early work in the 1950s–1970s (1–4) there has been much interest in chromium and the nutritional state of chromium, especially in diabetes and in other conditions associated with glucose intolerance.

The minimum daily human requirement for chromium necessary to maintain health is not known. Mertz (5) suggested an allowance of 50 to 200 μg/day. Schroeder et al. (6) found a mean chromium content of 72 μg in the daily diet of institutionalized subjects, and Levine et al. (7) reported a daily intake of 5 to 125 μg in the diet of elderly subjects. The requirement for chromium in the diet therefore seems to be variable, and may depend on the chemical form in which it is available.

Impaired glucose tolerance in humans was improved by the simple addition of 150 μg of inorganic chromium salt to the daily diet (7–9), and various workers (10, 11) have reported the apparently beneficial effects of an organic form of chromium, "glucose tolerance factor" (GTF).

The improvements in glucose tolerance observed on supplementing the diet with chromium salt may have resulted from the conversion of inorganic chromium to the biologically active form (GTF), which potentiates the effects of available insulin (12).

Now that more-reliable techniques for determination of chromium concentrations in plasma and urine have become available, findings reported for diabetic patients (13–15)