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Immunoradiometric Assay of Thyrotropin as a “First-Line” Thyroid-Function Test in the Routine Laboratory

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We compared the utility of a sensitive immunoradiometric assay for serum thyrotropin as a “first-line” thyroid-function test with a strategy based on first measuring total thyroxin in serum. The immunoradiometric assay appears to distinguish primary hypothyroidism and hyperthyroidism from euthyroidism in “new” patients. The role of this test in monitoring antithyroid treatment or thyroxin-replacement therapy is not yet established, there being particular difficulty in interpreting low thyrotropin concentrations in such patients. Nevertheless, because a normal thyrotropin concentration in most, if not all, situations signifies the euthyroid state, thyrotropin determination by immunoradiometric assay merits consideration as an initial test by laboratories performing thyroid-function tests.

Additional Keyphrases: thyroid status · screening

The insensitivity of most RIAs for thyrotropin (TSH, thyroid-stimulating hormone) precludes accurate measurement of TSH in all euthyroid subjects and in hyperthyroid patients, whose TSH concentrations are low.1 Given the increased sensitivity of immunoradiometric assays (IRMA) for TSH (1, 2), or of carefully optimized RIA techniques for it (3, 4), some have suggested that this single laboratory test distinguishes hyperthyroid and primary hypothyroid states from euthyroidism. Such reports have concentrated mainly on patients referred to specialist endocrine clinics. We have compared, in a routine laboratory setting, the Boott-Celltech TSH-IRMA as a “first-line” test of thyroid function against our existing repertoire of thyroid tests.

Patients and Methods

We studied 320 consecutive requests for thyroid assessment of patients in the following categories: (a) 238 “new” patients, who were not receiving treatment for thyroid disease; (b) 57 patients receiving thyroxin for primary hypothyroidism; (c) 25 patients being treated medically for hyperthyroidism. Together, these categories compose 91% of the total thyroid workload.

Present test “strategy”. Currently, we first measure the concentration of total thyroxin (TT4) in serum. If TT4 is <70 nmol/L, we then measure TSH (by RIA); if TT4 is >120 nmol/L, we measure thyroxin-binding globulin (and when indicated, total triiodothyronine). This “strategy range” (5) for TT4 represents the mean ±1.4 SD of the normal reference interval (see Figure 1).

Analytical methods. Our existing set of thyroid tests comprises radioimmunoassay of TT4, total triiodothyronine, and TSH in serum and measurement of thyroxin-binding globulin by immunoelectrophoresis. The between-batch CV over the working ranges of these assays is <8%.

For this study we also measured serum TSH by IRMA (“Sucrosep” TSH; Boots-Celltech Diagnostics Ltd., Slough, Berkshire SL1 4ET, U.K.). In this two-site IRMA, two monoclonal antibodies are used, one labeled with 125I and the other covalently bound to a solid phase (Sephacryl S300, Pharmacia). Separation of bound from unbound labeled monoclonal antibody involves passage through a sucrose solution, the bound antibody settling closer to the bottom of the tube. Assay sensitivity, derived from 20 replicate analyses of TSH-free serum, is typically <0.08 milli-int. unit/L (95% confidence limits). Between-batch CVs at mean concentrations of TSH of 0.65, 1.1, 11.0, and 28.1 milli-int. units/L were 7.1%, 5.1%, 3.7%, and 6.2%, respectively (n = 20 each).

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1 Nonstandard abbreviations: TSH, thyrotropin; IRMA, immunoradiometric assay; TT4, total thyroxin.

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CLINICAL CHEMISTRY, Vol. 32, No. 4, 1986 691
Results

New patients. Of the 238 patients not receiving treatment, those considered euthyroid, hyperthyroid, or euthyroid by our present strategy numbered 14, four, and 220, respectively. Results of the TSH-IRMA identified the hypothyroid and hyperthyroid patients. Of the 220 euthyroid patients, 198 (90%) had TSH-IRMA values within the normal reference interval (0.51 to 5.5 milli-int. units/L). Of the remaining 22 patients, six had mildly increased TSH (5.8-7.7 milli-int. units/L) and TT₄ > 70 nmol/L; one of these patients, in whom the serum TT₄ concentration was 135 nmol/L, was taking the oral contraceptive pill. Of the 16 patients with subnormal TSH-IRMA concentrations (<0.5 milli-int. unit/L), three had undetectable TSH, but normal triiodothyronine concentrations. TSH (RIA) concentrations were normal in two patients with TT₄ < 70 nmol/L and TSH-IRMA between 0.12 and 5.0 milli-int. units/L.

Patients receiving thyroxin. The 57 patients receiving thyroxin for primary hypothyroidism showed a statistically significant correlation (r = −0.44; p < 0.001) between TSH-IRMA and TT₄ concentrations in serum. However, the TT₄ concentration could not be predicted from the TSH concentration; the proportions of patients with TT₄ results within the normal range who had low, normal, or high values for TSH-IRMA were 24%, 28%, and 48%, respectively.

Patients being treated for hyperthyroidism. There was no correlation (r = −0.21; p > 0.1) between TT₄ concentration and TSH-IRMA in 26 hyperthyroid patients who had been treated with antithyroid drugs for periods ranging from a week to longer than 20 months. There was no significant difference in TT₄ concentrations between those with normal or low TSH-IRMA concentrations. Normal TT₄ concentrations were observed in 70% of those with reduced TSH-IRMA concentrations.

Discussion

For those "new" patients who are euthyroid by present tests, diagnosis is secured by the single finding of a normal TSH concentration by IRMA. Primary hypothyroidism is indicated by an increased TSH-IRMA concentration. Use of TSH-IRMA as a "first-line" test also identifies patients with possible mild hypothyroidism who have "normal" TT₄ with above-normal TSH. However, such results can be seen in subclinical hypothyroidism, and the benefits of identifying such patients may be uncertain (6, 7); defining the significance of this condition is not made any easier by the small component contribution of within-individual variation to the "normal range" for thyroid hormones (8).

As shown by Seth et al. (9), hyperthyroidism is accompanied by undetectable TSH concentrations in the Boots-Celltech assay. However, a significant number of our apparently euthyroid patients had decreased TSH-IRMA concentrations. Reported lower limits for this assay range from 0.3 to 0.5 milli-int. unit/L (10), and further work is required to define reference intervals in the hospital population, where drug effects and illness (11) may significantly affect TSH secretion. In patients considered hyperthyroid or primary hypothyroid from a TSH-IRMA result, measurement of serum thyroid hormone concentrations would be indicated to confirm the diagnosis and assess biochemical severity of the disease before treatment.

Discussion

While increased TSH-IRMA concentrations indicate suboptimal thyroxin replacement in treated hypothyroidism, enhanced intracellular de-iodination of thyroxin to triiodothyronine in the pituitary, as compared with other tissues, may suggest that decreased TSH concentrations would not necessarily indicate overtreatment (12). Whether this seriously compromises the use of sensitive TSH-IRMA methods as "first-line" tests for monitoring replacement therapy remains to be seen.

The lack of correlation between TT₄ and TSH-IRMA in hyperthyroid patients who are undergoing treatment possibly relates to prolonged suppression of the pituitary thyrotroph (13). Whether the appearance of detectable serum TSH by IRMA might predict therapeutic success (14), or its disappearance subsequently predict relapse (as claimed for TSH responsiveness of thyropliberin (15)), again must await further study.

The "Sucrosep" TSH-IRMA has proved to be a reliable and robust method in our laboratory. Between-batch reproducibility and sensitivity of the method are both satisfactory (16). Although use of TSH-IRMA as a "first-line" test simplifies laboratory work, the clinical acceptability of replacing the traditional thyroxin measurement by TSH-IRMA requires further discussion and investigation. At first sight, however, determination of TSH by "Sucrosep" IRMA has much to commend it in distinguishing hyperthyroid and primary hypothyroid states from euthyroidism.

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Fig. 1. Total thyroxin (TT₄) and thyrotropin (TSH) concentrations in 238 consecutive "new" patients (i.e., not being treated for thyroid disease).  
---, thyroid status by TT₄-based test strategy: euthyroid.  
X, thyroid status by TT₄-based test strategy: hyperthyroid.  
\[\text{Conversion: SI to traditional units: thyroxin 1 nmol/L = 0.07 \mu g/100 mL}\]
References


Determination of Vanillylmandelic Acid with Ion-Pair Chromatography and Fluorescence Detection

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We describe a chromatographic procedure for sensitive (2.0 \( \mu \text{mol/L} \)) specific quantification of vanillylmandelic acid (VMA) in urine with iso-VMA as an internal standard. After rapid extraction from urine, the VMA is determined by isocratic reversed-phase ion-pair chromatography on a bonded C8 column and detection of the native fluorescence on excitation at 285 nm. The fluorescence signal is quite dependent on the pH of the mobile phase. Results by the method vary linearly with VMA concentration up to 320 \( \mu \text{mol/L} \) and correlate well \(( r = 0.9880)\) with those obtained by conventional ultraviolet spectrophotometry. The mean 24-h excretion of VMA from 29 healthy volunteers was 21.4 (SD 5.4) \( \mu \text{mol} \).

Additional Keyphrases: chromatography, reversed-phase variation, source of urine, reference values, screening

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