Clinical Experience with Sensitive Thyrotropin Measurements: Diagnostic and Therapeutic Implications

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A two-site immunoradiometric assay for serum thyrotropin (TSH) was modified to improve the analytical sensitivity. The sensitivity achieved (detection limit, ~0.1 μU/ml; lower limit of quantitative measurement, ~0.4 μU/ml) was comparable to that of the best competitive binding research assays, yet this assay can be performed routinely. Serum TSH was 1.82 ±0.69 (mean ± s.d.) (range 0.4–3.4 μU/ml) in healthy individuals and 1.83 ±0.90 μU/ml (range 0.7–3.7 μU/ml) in patients with nonthyroidal disorders. By contrast, 97% of clinically hyperthyroid patients (Graves' disease, toxic nodular goiter) with high serum free T_4 (FT_4) and T_3 had suppressed serum TSH values, i.e., <0.3 μU/ml. Among patients with euthyroid Graves' ophthalmopathy or nontoxic goiter those clinically suspected of mild hyperthyroidism had TSH values <0.3 μU/ml, while those judged euthyroid had normal values. A large proportion of thyroid patients on antithyroid drugs (poorly to well-controlled) had suppressed TSH. Of Graves' patients in remission (normal FT_4 and T_3), 75% had normal TSH, but individual levels changed significantly over time, suggesting that a progressive decline in TSH may be useful in predicting recurrences. In hypothyroid patients taking L-T_4, serum TSH was subnormal in patients with elevated FT_4, but TSH was also low in six patients clinically suspected to be thyrotoxic despite normal FT_4 and T_3 and in 32% of asymptomatic patients with normal thyroid hormone levels. Conversely, 23% of thyroid cancer patients who had undergone thyroidectomy were taking insufficient L-T_4 to completely suppress TSH secretion. In 25 individuals who underwent thyrotropin releasing hormone (TRH) stimulation tests, the baseline serum TSH value correlated well with the peak serum TSH value post-TRH (r = 0.85). We conclude that sensitive TSH measurements could establish or confirm the diagnosis of hyperthyroidism in equivocal cases, replace most TRH-stimulation tests and be of value in optimizing L-T_4 suppression therapy for thyroid cancer patients post-thyroidectomy.

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Conventional competitive binding radioimmunoassays (RIAs) for h-TSH (*1*) in clinical use today have a maximal analytical sensitivity of ~1 μU/ml serum, close to the mean serum thyrotropin (TSH) concentration encountered in healthy individuals. They differentiate well between euthyroid patients and those with primary hypothyroidism and are unquestionably of value in the diagnosis of hypothyroidism, but they lack the sensitivity to measure TSH concentrations at the lower end of the normal range precisely enough to distinguish them from the suppressed TSH levels expected in hyperthyroidism. Wehmann et al. (*2*) and Spencer and Nicoloff (*3*) have reported "highly sensitive, competitive binding, research RIAs" for TSH which could delineate the entire normal range and identify subnormal TSH concentrations. Unfortunately, both these assays have features that make them impractical for routine clinical laboratories. For instance, both assays employ the same antiserum, raised by Parlow in 1975 (*4*), which has a uniquely high avidity (K_a 10^{11} l/

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mole), but which is distributed only for research purposes by the National Pituitary Agency. Also, these assays require either extraction of TSH with concanavalin A-Sepharose from a large sample volume (*5*) or sophisticated purification procedures for the iodine-125 (^{125}I) TSH before each assay, as well as extremely long incubation times ($\sim$ 10 days).

Recently, several immunoradiometric assays (IRMA) have been developed for TSH in which TSH is bound by two or more specific monoclonal antibodies in a sandwich-like fashion. The significance of this new approach is that higher assay sensitivities can be attained, because the antibody affinities are a less limiting factor in this type of assay as compared to competitive binding assays.

After preliminary testing and comparison of six commercial procedures for TSH a two-site IRMA* was optimized for the low concentration range and met best our two criteria, high sensitivity plus suitability for routine clinical performance.

The following report deals with serum TSH determinations by this method in over 200 thyroid patients, their clinical relevance, and the potential utility of such TSH measurements.

SUBJECTS AND METHODS

Patients

Nearly all patients were seen and evaluated as outpatients in our institution's thyroid clinic by two of us (J.P.K. and I.R.M). Only in a few overtly hyperthyroid patients had the diagnosis been made by other physicians at the institution's medical center, and in these cases all pertinent data were retrieved from the patients' charts. All patients had a serum specimen sent to our laboratory for routine thyroid function tests. The selection of patients for the "sensitive TSH test" was random, except for perhaps preferentially selecting borderline hyperthyroid and difficult-to-diagnose patients, or an attempt to keep the size of various patient groups similar. Based on the standard clinical criteria, history, physical examination, serum FT_4, T_3 (Table 2), and TSH levels (by a conventional assay, data not given and used only for classification), patients were assigned to one of eight groups, four groups of hyperthyroid patients and four groups of hypothyroid patients and/or patients on L-T_4 therapy:

Control 1. Forty-one healthy laboratory volunteers.
Control 2. Twenty-one ambulatory patients with exclusively nonthyroidal disorders including cancer, arteriosclerosis or hypertension.
Group 1. Twenty-nine hyperthyroid patients (Graves' disease, toxic multinodular goiter, toxic nodule, or unknown etiology) who, with a few exceptions of prior radioiodine treatment, were untreated.
Group 2. Ten clinically and biochemically euthyroid (or at most borderline hyperthyroid) patients with various diagnoses (Graves' ophthalmopathy, simple or multinodular nontoxic goiter, autonomous nodule).
Group 3. Thirteen hyperthyroid patients under treatment with propylthiouracil; their clinical status at the time of the TSH measurement ranged from euthyroid to hyperthyroid.
Group 4. Seven hyperthyroid patients in clinical remission after therapy, all judged clinically euthyroid, taking no medications for at least several months (five patients had received antithyroid drugs, one had had thyroid surgery and one had received radioiodine treatment).
Group 5. Sixteen overtly hypothyroid patients with subnormal thyroid hormone levels.
Group 6. Forty-six patients with a previous diagnosis of hypothyroidism (status post-treatment for Graves' disease, Hashimoto's thyroiditis or unknown etiology) who were taking L-T_4 (levothyroxine sodium) at the time of evaluation. This group was further subdivided, depending on the patients' clinical assessment and their thyroid hormone test results; *6a*: 15 patients whose clinical status ranged from borderline to clearly hyperthyroid and who also had abnormally high serum free T_4 (FT_4) and T_3 levels, confirming the clinical impression; *6b*: 25 patients who were asymptomatic, had no clinical findings that could be related to overtreatment and had normal FT_4 and T_3 levels; *6c*: six patients who had subtle clinical signs of hyperthyroidism, such as onycholysis, quadriceps weakness or rest tachycardia, suggesting that the dose of L-T_4 was excessive, but who had serum FT_4 and T_3 levels repeatedly within the normal range.
Group 7. Thirty-five patients who had undergone thyroidectomy and/or iodine-131 (^{131}I) ablation therapy for papillary and/or follicular thyroid cancer, all taking Synthroid at the time of this study.
Group 8. Ten patients on L-T_4 suppression therapy for benign thyroid nodules.

A TRH-stimulation test was performed on 21 apparently healthy individuals and on four patients with equivocal thyroid disease in the course of their clinical evaluation.

Procedures

Sensitive TSH measurements were carried out using a two-site solid-phase, sequential IRMA*, which we modified for optimal sensitivity. The assay principle is as follows: standards or sera are first incubated with a β-subunit specific monoclonal anti-TSH antibody immobilized on large polystyrene beads (one bead/tube). After decanting the assay mix, the beads are reincubated with a second, iodine-125-labeled, α-subunit specific monoclonal anti-TSH antibody. The binding of the latter is proportional to the amount of TSH bound to the bead during the first incubation and, in turn, to the TSH concentration in standard or sample. (The specificity of the two antibodies

TABLE 1
TSH Assay Performance Characteristics

Representative standard curves (linear up to 2.0 μU/ml)

y = 1,230 + 1,044* (1st incubation: 4 hr)
y = 995 + 1,018 (1st incubation: 2 hr)

Nonspecific binding B_0 (TSH-free serum, nine assays, mean ± s.d.: 1,244 ± 229 counts/min)

Precision

TSH concentration (mean, μUl/ml)	Intra-assay coefficient of variation (%, ten replicates)	Interassay coefficient of variation (%, nine assays) Triplicates	Duplicates
0.46	8.9	10.7	(15.8)
0.82	5.5	7.0	(11.4)
1.19	4.2	5.6	(10.8)
1.59	4.0	6.4	(7.5)
≥5.0	2.0-3.5	2.0-3.6	(1.9-3.9)

Sensitivity

Detection limit: ~0.1 μU/ml
Lower limit of quantitative determination: ~ 0.4 μU/ml

Specificity

Kit TSH standards and serial dilutions of sera from hypothyroid patients gave parallel curves.

Hormone	% Cross reactivity‡
LH	5×10^{-6}
hCG	3×10^{-8}
FSH	1×10^{-5}

*Expressed as counts/min bound versus TSH concentration (μU/ml).
†For comparison, only the first two tubes of each set of triplicates were analyzed.
‡Defined as TSH result found (μU/ml) divided by concentration of respective, potentially cross-reacting hormone assayed (μU/ml) × 100. Human thyrotropin research standard 68/38, Internatl. Lab. for Biolog. Stds., London, England was used for calibration.

for α, or β subunit, respectively, were communicated to us by the manufacturer.)

The assay protocol was modified as follows: TSH-free serum was prepared either by stripping human serum (TSH <2 μU/ml) with a solid-phase anti-h-TSH antiserum or by pooling sera from extremely thyrotoxic patients (T_3 >500 ng/dl, FT_4 >5.0 ng/dl). An additional standard solution of TSH-1.0 μU/ml) was made from one of the higher kit standards by dilution with TSH -free serum and stored frozen in aliquots. One aliquot of this standard, the in-house TSH-free serum and at least two serum pools with TSH concentrations in the range of primary interest (see below) were included in each assay to monitor nonspecific binding, variations between batches of kit standards, and to control assay performance in general. In a few assays where our standard S_0 and $S_{1.0}$ deviated significantly from the curve obtained with the kit standards, the standard curve was adjusted between the differing data points such that it produced values for the control pools which agreed best with prior determinations. All standards, samples, and controls were assayed in triplicate. The order of serum and bead addition recommended by the manufacturer was reversed to reduce the risk of front-to-back variations within assays which may arise if there is, e.g., a 30-min difference between the pipetting of the first and the last sample and, thus, in the length of the first incubation. The first incubation was extended to 4 hr at 37°C. All tubes were counted for at least 5 min, and borderline results of 0.3–0.5 μU/ml were accepted only when the triplicate counts varied by ≤4%; otherwise the determinations were repeated (<10% of cases).

For confirmation of some of these TSH estimates we used two other commercial procedures†,‡, (*6,7*) both slightly modified in our laboratory for optimal sensitivity.

Serum FT_4, T_3, and TSH (by a conventional assay) were measured as reported previously (*8*). The normal range for T_3 was 70–200 ng/dl, for FT_4 0.8–2.3 ng/dl. Tests for autoantibodies directed against T_4 or TSH were carried out according to a previously described method (*9*).

Intra-assay precision and interassay reproducibility in the very low assay range were determined by analyzing four different pools of human serum (TSH 0.5–2.0 μU/ml) prepared from hypothyroid sera by appropriate dilution with TSH-free serum. The detection limit defined as the TSH concentration corresponding to $B = B_{zeroTSH}$

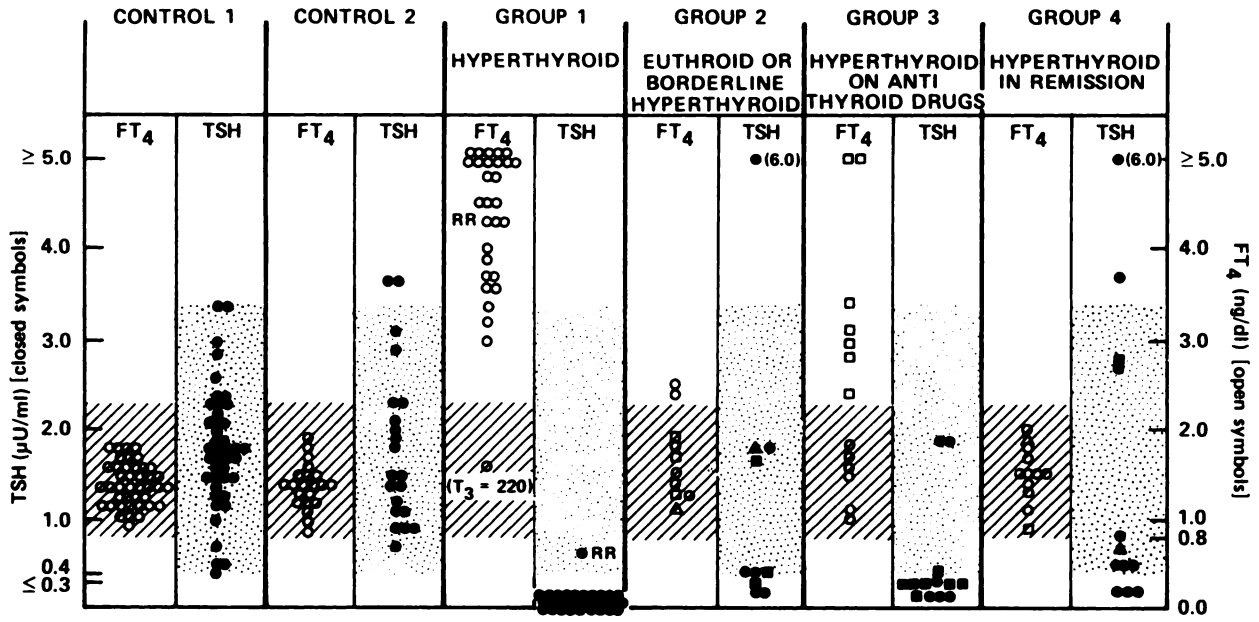

FIGURE 1
Distribution of serum TSH (closed symbols) and FT_4 (open symbols) in healthy individuals (Control 1), patients with nonthyroidal diseases (Control 2) and four groups of hyperthyroid patients. Group 2: (●,○)Graves' ophthalmopathy; (■,□) Simple/multinodular goiter; (▲,△) Hot nodule. Group 3: (■,□) Elevated FT_4; (●,○) Normal FT_4. Group 4 (seven patients, 12 studies): (●,○) Postprophylthiouracil (■,□) Postsurgery; (▲,△) Post ^{131}I

+2s.d. was determined according to Rodbard (*10*). The lower limit of quantitative determination defined by the expression s.d. (x) ≤10% of × (measurement error is not more than 10%) according to Kalman (*11*) was estimated graphically from the interassay coefficients of variation of the four serum pools with very low TSH concentrations.

RESULTS

The assay performance characteristics are summarized in Table 1.

The distribution of serum TSH and FT_4 results in controls and various groups of patients is illustrated in Figs. 1 and 2. Means for serum TSH and serum thyroid hormones are shown in Table 2 and individual cases that might be of particular interest are listed in Table 3.

Serum TSH concentrations measured in healthy individuals (Control 1) were 1.82±0.69 μU/ml (mean±s.d.) (range 0.4–3.4 μU/ml) and patients with miscellaneous nonthyroid disorders (Control 2) had similar TSH levels, 1.83±0.90 μU/ml (range 0.7–3.7 μU/ml).

Clinically hyperthyroid patients with Graves' disease or toxic multinodular goiter (Group 1) had subnormal serum TSH concentrations of <0.3 μU/ml, with only one exception. The one patient whose TSH was not suppressed had a serum FT_4 of 4.3 ng/dl, a T_3 of 195 ng/dl, and a TSH of 0.6 μU/ml. Anti-T_4 autoantibodies, which in rare instances can cause misleadingly high FT_4 or T_4 results, were tested for in this case, but not found.

Among untreated patients with normal to borderline high thyroid hormone levels (Group 2), four clinically euthyroid patients (two with Graves' ophthalmopathy, one with a goiter and one with a hot nodule) had normal to slightly elevated serum TSH results of 1.76–6.0 μU/ml, (FT_4 1.2–1.9 ng/dl; T_3 95–160 ng/dl), while six other patients with a similar diagnosis (four Graves' disease, two goiters), clinically suspected to be mildly hyperthyroid had significantly lower serum TSH levels of <0.3–0.4 μU/ml (FT_4 1.3–2.5 ng/dl; T_3 155-200 ng/dl). Furthermore, one of the later (TSH <0.3 μU/ml; FT_4 1.8 ng/dl; T_3 200 ng/dl) became grossly hyperthyroid within ~2 mo.

Of thyrotoxic patients who were undergoing treatment with antithyroid drugs (Group 3), approximately half were poorly controlled as judged by their elevated serum thyroid hormone levels (n=7, FT_4 2.4–5.0 ng/dl; T_3 105–350 ng/dl), and they had serum TSH of <0.3–0.4 μU/ml. Of the other patients with normal thyroid hormone levels (n=6, FT_4 1.0–1.8 ng/dl; T_3 100–200 ng/dl) four had suppressed and two had normal TSH values.

The majority (75%) of previously thyrotoxic patients in clinical remission (Group 4) had normal rather than subnormal serum TSH. Sequential studies in three of these patients indicated that their serum TSH changed significantly in all of them in the course of 3 mo; TSH increased from subnormal to normal in two patients and fell from a borderline high level to low normal in the third patient [Table 3(B)].

Clinically hypothyroid patients had TSH concentrations >25 μU/ml. In hypothyroid patients on thyroid replace-

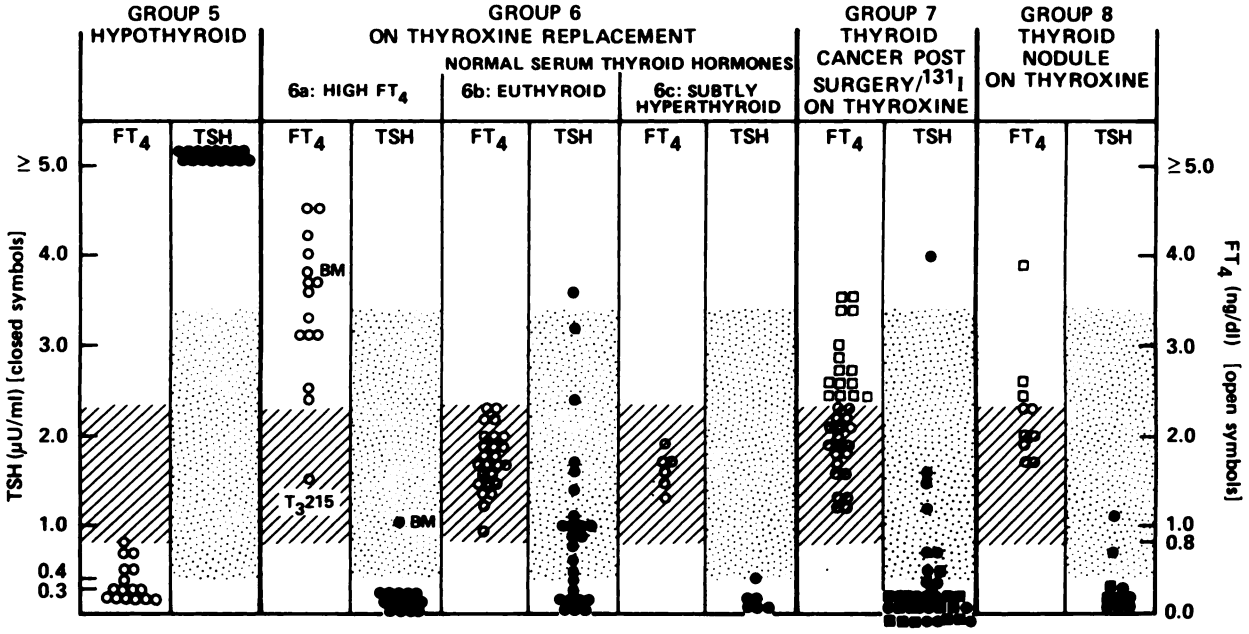

FIGURE 2
Distribution of serum TSH (closed symbols) and FT_4 (open symbols) in four groups of hypothyroid patients or patients taking L-T_4. Group 7 and 8: (●,○) Normal FT_4, (■,□) Elevated FT_4

TABLE 2
Estimates for Serum TSH, FT_4 and T_3 Concentrations in Various Patient Groups

Group	Clinical condition	No. of patients (male, female)	TSH (μU/ml) mean (s.d.)*	Range	FT_4 (ng/dl) mean (s.d.)	Range	T_3 (ng/dl) mean (s.d.)	Range
C-1	Healthy	41 (10 M, 31F)	1.82 (0.69)	0.4–3.4	1.43 (0.22)	1.0–1.8	126 (18)	90–175
C-2	Euthyroid sick	21 (5 M, 16 F)	1.83 (0.90)	0.7–3.7	1.39 (0.25)	0.9–1.9	—	—
2	Hyperthyroid	29 (5 M, 24 F)	<0.3	<0.3†	4.79 (1.08)	3.0–>5.0§	417 (164)	195-820
2	Euthyroid or borderline hyperthyroid	10 (2 M, 8 F)	see text	<0.3–6.0	1.70 (0.46)	1.2–2.5	160 (34)	95–200
3	Hyperthyroid on therapy	13 (2 M, 11 F)	see text	<0.3–1.85	2.72 (1.65)	1.0–>5.0	200 (87)	100–350
4	Hyperthyroid in remission	7 (1 M, 6 F)	see text	<0.3–6.0	1.51 (0.32)	0.9–2.0	154 (47)	85–200
5	Overt hypothyroid	16 (4 M, 12 F)	80.8 (26.9)	25–>100	0.38 (0.20)	<0.2–0.8)	—	—
6	On T_4, previously hypothyroid							
	a) high FT_4, T_3	15 (1 M, 14 F)	<0.3	<0.3¶	3.4 (0.82)	2.4–4.5§	178 (27)	150–230
	b) normal FT_4, T_3 clincally euthyroid	25 (3 M, 22 F)	0.98 (0.93)	<0.3–3.6	1.75 (0.34)	0.9–2.3	—	—
	c) normal FT_4, T_3 mildly hyperthyroid	6 (6 F)	<0.3	<0.3–0.4)	1.62 (0.20)	1.3–1.9	130 (23)	105–170
7	Thyroid cancer, postsurgery/^{131}I, on T_4	35 (8 M, 27 F)	0.45 (0.74)	<0.3–4.0	2.29 (0.67)	1.2–3.6	—	—
8	Thyroid nodule, on T_4	10 (1 M, 9 F)	0.33 (0.32)	<0.3–1.1	2.28 (0.64)	1.7–3.9	—	—

*A value of 0.15 was used instead of <0.3 for calculation of mean (s.d.).
†One exception: TSH, 0.6 μU/ml.
‡12 studies.
¶One exception: TSH, 1.0; FT_4, 3.8; T_3, 195.
§Excluding one case of T_3 toxicosis.

ment therapy (Group 6) serum TSH varied considerably: Patients taking excessive amounts of levothyroxine sodium as indicated by their elevated serum FT_4 (Group 6a) had, with one exception, subnormal TSH of <0.3 μU/ml. All six patients of Group 6c who had repeatedly normal serum thyroid hormone concentrations, but who pre-

TABLE 3
Serum TSH, FT_4, T_3 and T_4 Concentrations in Individual Patients

Patient	Test date	TSH (μU/ml)	FT_4 (ng/dl)	T_3 (ng/dl)	T_4 (μg/dl)	Diagnosis and prior treatment	Current treatment T_4 (mg/day)
A. Comparison of TSH levels pre- and postsurgery or radioiodine treatment							
1	1/84	<0.3*	1.5	115	8.9	Hot toxic nodule	—
	4/84	2.8	0.9	85		Postsurgery	—
2	7/84	0.4	2.5	140		Borderline toxic	—
	11/84	0.7	1.9	120		Post ^{131}I	—
B. Changing TSH concentrations during remission post antithyroid drug treatment							
3	5/84	0.3	1.5	140		Graves', PTU	—
	7/84	0.5	1.5	—			—
	11/84	2.7	1.5	—			—
4	6/84	<0.3	1.3	—	7.1	Graves', ^{131}I, PTU	—
	9/84	0.5	1.1	145	6.9		—
5	8/84	6.0	1.4	200		Graves', PTU	—
	9/84	3.1	1.7	200			—
	10/84	0.8	2.0	200			—
C. Extreme pairs of TSH/FT_4 values in hypothyroid patients on T_4							
6	7/84	<0.3	0.9	100	5.6	Graves', ^{131}I	0.1
7	5/84	1.0	2.3	130		Graves', ^{131}I, surgery	.15†
D. Results of sequential studies in hypothyroid patients on T_4 therapy							
8	4/84	<0.3	1.3	110	9.9	Unclear	0.3
	7/84	1.3	0.4	90			0.1
	8/84	0.8	0.9	90			0.15
9	6/84	<0.3	4.5	155		Hashimoto's thyroiditis	0.25
	8/84	1.4	1.6	110			0.20
10	6/84	<0.3	3.4	295		Hyperthyroid	—
	9/84	91	0.2			Post ^{131}I	—
	11/84	3.6	2.2				0.15–0.2
11	5/84	<0.3	2.1	140		Thyroid cancer, ^{131}I	0.15
	6/84	0.4	1.8	105			0.15, 5x/wk
	11/84	1.6	1.6	115			0.075
12	6/84	<0.3	2.5	90		Hürthle cell tumor	0.2
	11/84	1.5	1.8				0.15

*Peak TSH post-TRH stimulation: 2.0 μU/ml.
†Possibly noncompliance with treatment schedule.

sented with symptoms or signs suggesting L-T_4 overdosage had either suppressed TSH of ≤0.3 μU/ml or a borderline TSH of 0.4 μU/ml (one case). In addition, we found suppressed serum TSH levels of <0.3 μU/ml in a relatively large percentage (32%) of patients on L-T_4 replacement therapy who were asymptomatic and had serum thyroid hormone levels within the normal range (Group 6b). As a rule the subnormal TSH levels in these patients were associated with FT_4 levels in the upper portion of the normal range, i.e., FT_4 ≥ 1.7 ng/dl, but in the most extreme case we found a TSH of <0.3 μU/ml in the presence of a FT_4 of 0.9 ng/dl [Table 3(C)]. These suppressed serum TSH concentrations in conjunction with clinical euthyroidism and normal thyroid hormone levels were confirmed using two other sensitive TSH assays[†,‡], in order to rule out any artifacts in the TSH measurements. The majority of these sera, whenever there was sufficient specimen available, were also tested for autoantibodies against h-TSH since, in IRMA-type assays, the presence of such autoantibodies can be responsible for too low results. In none of the sera tested were anti-TSH autoantibodies detected. Follow-up studies on patients

with initially suppressed serum TSH showed that the TSH could be normalized by changing the L-T_4 treatment dosage (Table 3(D), #8–10). The observed changes in the TSH were similar to those noted in hyperthyroid patients after successful treatment by surgery or radioiodine [Table 3(A)].

Among thyroid cancer patients who had undergone thyroidectomy and/or ^{131}I-ablation and who were receiving levothyroxine sodium as replacement and suppression therapy (Group 7), one-third (33%) had serum TSH levels of <0.3 (or at most 0.4 μU/ml) paired with a normal serum FT_4 (range, 1.2–2.3 ng/dl), as is considered optimal. 43% of the patients had an elevated FT_4 (range 2.4–3.6 ng/dl) and TSH <0.3 μU/ml. The remaining 23%, however, had clearly measurable serum TSH ranging from 0.5–4.0 μU/ml (FT_4 1.2–2.5 ng/dl).

In one of the thyroid cancer patients, because of repeated abortions possibly due to thyrotoxicosis, the L-T_4 dose was deliberately reduced to allow the TSH to rise into the normal range (Table 3(C), No. 11). In other thyroid cancer patients an attempt to normalize serum FT_4 concentrations by adjusting the L-T_4 dosage downward also sometimes resulted in concomitant normal TSH (Table 3(C), No. 12), generally not regarded as optimal suppressive therapy.

In patients on suppression therapy for benign thyroid nodules (Group 8), eight patients (80%) had suppressed serum TSH of <0.3 μU/ml, while two patients had a normal TSH.

Data collected from 25 TRH stimulation tests indicated a good correlation between baseline serum TSH levels pre-TRH and peak serum TSH values 20–30 min after TRH injection (Fig. 3, r=0.85). A subnormal TSH of <0.3 was found in a patient with no rise in TSH, or borderline TSH values of 0.4–0.5 μU/ml in two patients with a blunted response. All individuals with a normal to slightly exaggerated TRH test (Δ TSH ≥5 μU/ml) had a normal to borderline high baseline TSH.

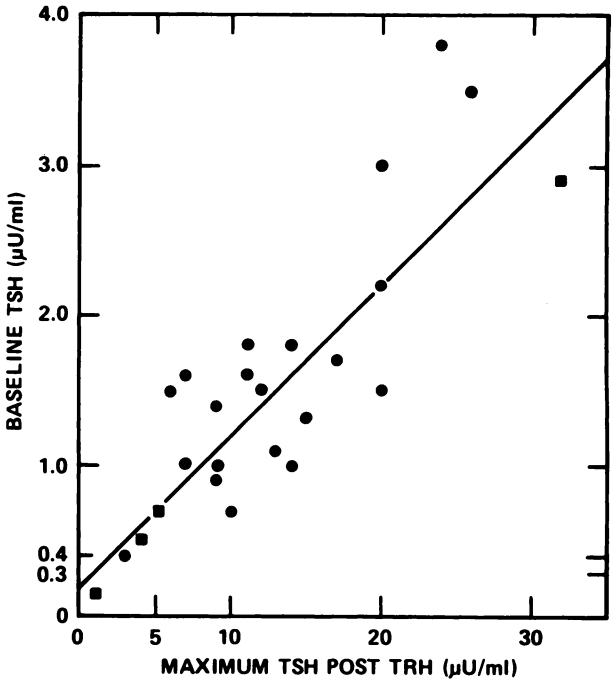

FIGURE 3
Correlation between baseline serum TSH levels measured before administration of TRH and peak TSH levels observed 20–30 min post i.v. bolus of 400 μg TRH in 21 individuals without any known organic disease (●) and four thyroid patients with equivocal diagnosis (■)
n = 25; y = 0.10x + 0.21; r = 0.85

DISCUSSION

One of the principle findings of this investigation was that IRMA-type assays which make use of two or more monoclonal anti-TSH antibodies in sequential fashion, can be optimized to achieve sensitivities comparable to the best conventional competitive binding research assays and, in contrast to the latter, can be performed routinely.

The specific TSH assay under investigation* had, after minor modifications, a detection limit of ~0.1 μU/ml, a value similar to those reported for the two most sensitive competitive binding RIAs (0.33 or 0.05–0.1 μU/ml) (*2,3*).

Although the detection limit (or detection threshold) is widely used to characterize assay sensitivity, it merely states the smallest hormone concentration that can be discriminated from "zero" hormone, and as such is probably inadequate when assay sensitivity is *the* key issue. Therefore, we have also determined the "lower limit of quantitative determination," as recently proposed (*11*), to indicate that the smallest TSH concentration which can be precisely measured (s.d. ~ 10% of mean) by the assay is ~0.4 μU/ml. Values below 0.3 μU/ml are not as reproducible and are thus stated as <0.3 μU/ml.

Serum TSH concentrations found in healthy individuals without thyroid, pituitary, or hypothalamic disorders were consistently above this lower limit of quantitative measurement, ranging from 0.4–3.4 μU/ml. In patients with miscellaneous nonthyroidal diseases the range was 0.7–3.7 μU/ml. By contrast, 97% of hyperthyroid patients had a serum TSH of <0.3 μU/ml, i.e., below the limit of quantitative determination. Thus the method provides practically a complete separation between normal serum TSH concentrations and those found in hyperthyroid patients. Moreover, the results for the normal range, or for patients with hyperthyroidism, respectively, are similar to those obtained by competitive binding assays (*3,12*).

While this work was in progress, Pekary and Hershman (*13*) reported a higher detection limit of 0.6 μU/ml, larger intra-, as well as interassay coefficients of variation (15.5 or 37.7%, respectively, at 1.5 μU TSH/ml) and a considerable overlap (20%) between TSH levels in normal and

hyperthyroid patients, using the same assay reagents as we, but the protocol supplied by the manufacturer. We believe these differences in the assay performance can be ascribed to our modifications of the procedure.

In accord with the negative feedback relationship between serum FT_4 concentrations and pituitary TSH secretion, we also observed subnormal serum TSH levels in patients of various other groups who had elevated serum thyroid hormones, e.g., in thyrotoxic patients not yet properly controlled by antithyroid drugs, or in patients taking excessive amounts of exogenous L-T_4

Clinically, and particularly relevant from a diagnostic point of view, was the observation that some patients whose serum FT_4 and T_3 were within the normal range determined for the general population, but who were clinically suspected of borderline hyperthyroidism, did indeed have suppressed serum TSH concentrations, thus confirming the clinical impression. In one of the "euthyroid-borderline hyperthyroid" Graves' patients studied, the detection of a suppressed TSH preceded overt hyperthyroidism by a few months. These findings provide further evidence that suppression of pituitary TSH synthesis and/or secretion could be measured routinely as the first biochemical change in the development of hyperthyroidism and underscore the clinical utility of sufficiently sensitive TSH assays in the early detection of hyperthyroidism. The good correlation found between (precisely measured) basal serum TSH and the results of TRH-stimulation tests would imply that sensitive TSH measurements could replace some, if not all, TRH-tests in the diagnosis of equivocal cases of hyperthyroidism, since TRH-tests are time consuming, more stressful to be patient and much more expensive.

Although our follow-up data on Graves' patients currently in remission [Table 3(B)] are too few to draw any definitive conclusions, they suggest that sequential serum TSH measurements may be useful in predicting recurrences, or detecting recurrences before they become clinically overt. A marked decline in serum TSH may identify patients who are at greatest risk of suffering a relapse, while a rise in TSH from subnormal to normal levels probably reflects a return to the euthyroid state.

With respect to the management of hypothyroid patients on thyroid replacement therapy, we found subnormal serum TSH in a rather large percentage (45%) of patients who appeared treated appropriately with L-T_4 based on their normal serum FT_4 and T_3 (Groups 6b and 6c). Some of these patients (19%, Group 6c) had subtle signs suspicious of slight overtreatment, but in the majority of the cases (26%) there was no clinical evidence of thyrotoxicosis medicamentosa.

It is difficult to explain all the TSH results, specifically the two most extreme sets of data listed in Table 3(C), i.e., in Patient 6 a subnormal serum TSH in the presence of a borderline low serum FT_4 while another patient (No. 7) had a normal TSH associated with a borderline high FT_4. We cannot rule out completely any hypothalamic, or pituitary abnormalities, and we do not know with certainty whether these patients complied with their prescribed treatment plan and whether, at the time of the TSH measurement, the serum FT_4 concentrations and pituitary TSH secretions had indeed reached a steady state, as would be required for any meaningful interpretation of the results. However, the fact that it was possible to normalize TSH levels through changes in the treatment regimen in most patients where serial measurements are available is consistent with the concept that TSH synthesis and secretion by the pituitary thyrotroph is tightly regulated by relatively small fluctuations in the peripheral serum FT_4 concentrations (*14*) (and intrapituitary FT_4 and T_3). The serum level of FT_4 which is associated with a normal serum TSH level, may vary considerably from patient to patient. Consequently, when only thyroid hormone levels are monitored, a very significant number of hypothyroid patients are actually receiving thyroid suppression therapy instead of thyroid hormone replacement therapy, and vice versa, some thyroid cancer patients are merely receiving replacement therapy when pituitary TSH secretion should be fully suppressed.

We routinely monitor our patients by serum FT_4 measurements, while Wehmann et al. (*12*) used serum total T_4 in the evaluation of their patients. Our finding of subnormal serum TSH levels in 45% of hypothyroid patients taking Synthroid, despite normal FT_4 and T_3 values, is not significantly different from "approximately 50%" reported by Wehmann. Thus, serum FT_4 does not seem to be a significantly better marker for the negative feedback regulation of TSH secretion, unless binding protein abnormalities are present. Rather, as suggested by Wehmann, after the serum thyroid hormone levels have been brought into the normal range, a further "fine adjustment" of the treatment dose based on serum TSH levels appears to be necessary to achieve true physiologic and biochemical euthyroidism. It remains doubtful whether such fine adjustments will always be mirrored by discernable changes in the patients' symptoms or clinical signs and whether they offer a clear benefit to the patient.

Nonetheless, we conclude that sufficiently sensitive TSH measurements are of clinical value in the diagnosis and management of many hyperthyroid patients, especially those with equivocal disease, or those presumably in remission after therapy, and also in optimization of L-T_4 suppression therapy for thyroid cancer patients.

FOOTNOTES

*"Tandem-R TSH", two- step immunoradiometric assay by Hybritech, San Diego, CA.

†TSH_3 Maiaclone" by Serono, Randolph, MA.

‡"Quantimune hTSH IRMA" by Bio-Rad Labs., Richmond, CA.

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