Clinical Evaluation of Three Free-Thyroxine Assays

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The free thyroxine (FT_4) tests of Clinical Assays, Corning Medical, and Damon Diagnostics were evaluated in 245 patients. Although all three assays generally correlated well with the clinical functional status, there were apparent discrepancies in the clinically euthyroid population. All patients with discordant values had either relatively high or low triiodothyronine (T_3) uptakes. In these instances, the free-thyroxine index gave a more accurate assessment of the clinical thyroid status than did the FT_4. In our opinion, these findings place a critical limitation on these assays as a replacement for the free-thyroxine index.

J Nucl Med 21: 576–578, 1980

Since unbound or free thyroxine (FT_4) is assumed to be the physiologically active species of circulating thyroid hormone (*1–3*), measurement of FT_4 would seem to be the ideal way to evaluate thyroid function. Dialysis has been used to measure FT_4 but is cumbersome and beyond the scope of ordinary clinical laboratories. Most laboratories currently use a free-thyroxine index (FTI), which is calculated from a triiodothyronine (T_3) uptake determination and a total thyroxine (T_4) assay. Alternatively an assay for thyroxine-binding globulin (TBG) can be substituted for the T_3 uptake in this FTI calculation. Although the FTI correlates well with FT_4 by dialysis, it is still an indirect method of estimating FT_4.

The recent introduction of commercial kits for FT_4 determination has made this measurement available to the average clinical laboratory. We examined FT_4 kits from three manufacturers: Clinical Assays (Cambridge, MA), Corning Medical (Medfield, MA), and Damon Diagnostics (Needham Heights, MA). Serum FT_4 was measured in three groups of 59 patients each: clinically hypothyroid, euthyroid, and hyperthyroid. We also examined sera from 68 clinically euthyroid patients with relatively high or low T_3 uptake.

METHODS

The three types of kits were used according to the protocols in effect at the time of the assays. The linear regression curves (Figs. 1-3) were obtained from the initial population (n=176) of clinically hypothyroid, euthyroid, and hyperthyroid patients between January and April, 1979. Since this population contained patients with normal and abnormal thyroid values, the alternate FTI using TBG was compared to this group alone. The assays on the 68 clinically euthyroid patients with a relatively high or low T_3 uptake were done in August and September, 1979.

For the total-thyroxine assay, antibody* and I-125-tagged T_4† were procured commercially. The FTI ($T_4 \times T_3$ uptake) was calculated using the T_3 uptake from Diagnostic Corporation of America. The alternate FTI (T_4/TBG) was calculated from the TBG assay of Corning Medical. Thyroid-stimulating hormone (TSH) was assayed by a commercial method.‡

All patients underwent physical examination and other thyroid studies (serum T_3RIA, antibodies, TSH) as indicated. The thyroid functional status was, therefore, a result of both clinical and laboratory assessment.

RESULTS AND DISCUSSION

Our FTI using a T_3 uptake correlated well with the FTI using TBG (n = 176, r = 0.969). This additional FTI was used as a precaution against erroneous patient classification. The linear

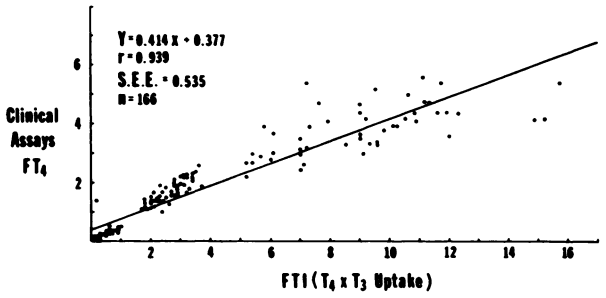

FIG. 1. Linear regression curve for Clinical Assays FT_4 vs. FTI (r = 0.939).

Received Sept. 25, 1979; revision accepted Dec. 10, 1979.

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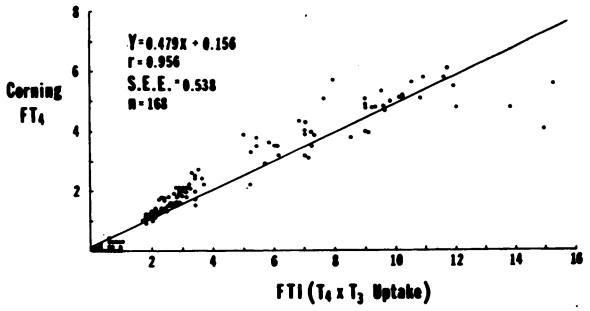

FIG. 2. Linear regression curve for Corning Medical FT_4 vs. FTI (r = 0.956).

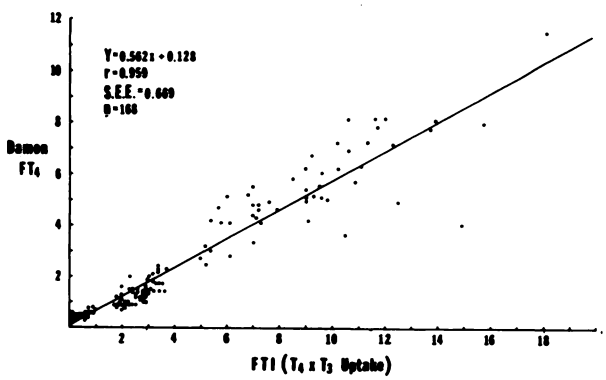

FIG. 3. Linear regression curve for Damon Diagnostics FT_4 vs. FTI (r = 0.959).

regression plots (Figs. 1-3) demonstrate good correlation with the FTI ($T_4 \times T_3$ uptake) and the various FT_4 assays. Any "off curve" results for the hyperthyroid population were not included in the regression analysis.

None of the assays produced discordant FT_4 values in the hypothyroid and hyperthyroid populations examined. From the initial group of 59 clinically euthyroid persons, one from Clinical Assays and three from Corning had borderline-hyperthyroid FT_4 values (Table 1). A borderline high T_3 uptake was the one common factor. Since the T_3 uptake test measures the unsaturated binding capacity of TBG, a high T_3 uptake is usually indicative of a relatively low TBG concentration and vice versa. Binding of T_3 to thyroxine-binding prealbumin is inhibited in this assay by use of a barbital buffer. Albumin will bind T_3, but that protein's avidity for T_3 and T_4 is much less than that of TBG. Consequently, the relatively high (but normal) T_3 uptake values for these patients with discordant FT_4 values indirectly indicates a potential problem in patients with low, but not necessarily abnormal, levels of TBG. Of course, abnormal amounts of albumin and other proteins capable of binding T_4 cannot be excluded.

TABLE 1. FT_4 DEVIATIONS IN ORIGINAL EUTHYROID GROUP*

Patient	FTI	T_4	T_3U	TBG	TSH	Clinical Assays FT_4	Corning FT_4	Damon FT_4
1-M	3.3	9.3	35	16.5	1.6	1.8	2.6†	1.7
2-F	3.4	9.5	36	16.6	2.8	2.2	2.6†	1.7
3-F	3.5	10.2	34	16.4	2.6	2.4†	2.7†	1.7

* Normal ranges: FTI 1.4–4.0, T_4 5.5–11.5 μg/dl, T_3 uptake 25–35%, Clinical Assays FT_4 0.8–2.3 ng/dl, Corning FT_4 1.0–2.5 ng/dl, Damon FT_4 0.8–2.4 ng/dl, Corning TBG 12.0–30.0 μg/ml, Pantex TSH < 10 μIU/ml. F = female M = male. Total population: 11 males, 48 females.

† High values.

TABLE 2. FT_4 DEVIATIONS IN GROUP WITH BORDERLINE OR ABNORMAL T_3 UPTAKE VALUES*

Patient	FTI	T_4	T_3U	TSH	Clinical Assays FT_4	Corning FT_4	Damon FT_4
1-F	3.9	11.6	34	2.8	2.6†	2.7†	2.2
2-M	3.9	11.6	34	2.5	2.2	2.8†	2.7†
3-F	3.7	10.0	37	3.1	1.9	2.7†	3.0†
4-F	3.4	15.6	22	2.6	2.6†	2.6†	2.4
5-F	3.6	15.0	24	3.3	2.5†	2.8†	2.3
6-F	3.7	19.3	19	2.7	2.7†	2.9†	2.3
7-M	1.9	5.7	34	3.0	0.9‡	1.6	1.6
8-F	1.8	4.9	37	1.8	0.9‡	1.6	1.5
9-F	3.5	10.4	34	1.9	1.9	2.6†	2.4
10-F	3.1	9.1	34	1.5	2.2	2.6†	2.5†
11-F	2.9	13.4	22	2.9	1.9	2.2	2.7†
12-F	3.4	10.1	34	2.0	2.5†	2.2	2.7†

* Normal ranges listed under Table 1. Total population: 60 females, 8 males. High T_3 uptake population: n = 27, $\bar{x} \pm$ s.d. = 35.9 ± 3.7, range = 33–52%. Low T_3 uptake population: n = 41, $\bar{x} \pm$ s.d. = 22.7 ± 1.8, range = 18–25%.

† High values.

‡ Borderline.

After this initial population was examined, we discovered that the three companies were making various changes in their procedures. These included a longer incubation period (Clinical Assays), a different method of data reduction (Corning), and a different wash solution (Damon). After these changes were instituted, we examined a separate group of 68 clinically euthyroid patients with relatively high (>32%) or low (<26%) T_3 uptake. These patients were picked solely on the basis of their normal clinical status and T_3 uptake values. Five patients from Clinical Assays, eight from Corning, and five from Damon had borderline or elevated FT_4 values (Table 2). In addition, two patients from Clinical Assays had borderline-low values. In these cases, the FTI gave a more accurate assessment of clinical thyroid status than did the FT_4.

The interassay mean of a euthyroid pool was determined by assaying the pool six times for 4 consecutive days. The means (±s.d.) obtained by Clinical Assays and Corning were 1.88 ± 0.38 (CV 20.1%) and 1.69 ± 0.12 (CV 7.2%), respectively. The mean obtained by Damon was 1.67 ± 0.25 (CV 14.9%).

We conclude that all three assays produce FT_4 values that generally agree with the clinical evaluations. However, discordant results are observed in clinically euthyroid patients with low-normal and high-normal T_3 uptake values. This is significant since it was hoped that the FT_4 would be especially useful in patients with marked TBG alterations. In these instances, the FTI does not always completely compensate due to the limited sensitivity of the T_3 uptake test.

Since high or low T_3 uptake values are involved in all of the discordant patients, the FT_4 values obtained may be due to the relative amounts of TBG present (*4, 5*). Other thyroxine-binding proteins, such as albumin, as well as total T_4, may be involved (*6*). Or perhaps the circulating FT_4 is being accurately measured, but does not reflect the tissue concentration, and consequently the patient's clinical status. The FTI correctly reflected the clinical status of every patient examined in this study. The same cannot be said for these three FT_4 assays. It is our opinion, therefore, that this problem limits the usefulness of these current FT_4 assays as replacements for the FTI in routine clinical use. At the present time, the FTI is still the preferred "single" method for thyroid screening.

FOOTNOTES

* Antibodies Inc., Davis, CA.
† Diagnostic Corp. of America, Arlington, TX.
‡ Pantex, Santa Monica, CA.

ACKNOWLEDGMENTS

We thank Clinical Assays, Corning Medical, and Damon Diagnostics for the use of their material. Also, our thanks to Nuclear Diagnostics, Inc., Troy, Michigan, for supplying the Corning Medical TBG assay.

REFERENCES

1. ROBBINS J, RALL JE: The interaction of thyroid hormones and proteins in biological fluids. *Recent Progr Horm Res* 13: 161-208, 1957
2. INGBAR SH, FREINKEL N: Regulation of the peripheral metabolism of the thyroid hormones. *Recent Progr Horm Res* 16:353-403, 1960
3. INGBAR SH, BRAVERMAN LF, DAWBER NA, et al: A new method for measuring the free thyroid hormone in human serum and an analysis of the factors that influence its concentration. *J Clin Invest* 44:1679-1689, 1965
4. WITHERSPOON LR, GARCIA MM: Free T_4 problem: clarifying and resolving a controversy. *Ligand Review* 1:11, 1979
5. EKINS R: Free T_4 problem: clarifying and resolving a controversy. *Ligand Review* 1:14, 1979
6. PERISTEIN MT, HERNER AE: Free T_4 problem: clarifying and resolving a controversy. *Ligand Review* 1:10, 1979