LETTERS TO THE EDITOR

Classification of Liver Tumors by Radionuclide Imaging

The treatment of a liver tumor depends on its morphology: a nongrowing hemangioma or follicular nodular hyperplasia (FNH) without mechanical problems will need no treatment, whereas adenomas must, and single metastases should be, excised. The pretreatment diagnosis therefore must be accurate and should be noninvasive. Several diagnostic tools have been emphasized: nuclear magnetic resonance (*1*) as well as computerized tomography (*2*). We propose the use of radionuclide techniques as an accurate, noninvasive, and inexpensive diagnostic procedure for the classification of liver tumors as "benign" or "possibly malignant" and report here our initial experience.

Twenty-four of 26 FNH patients were correctly classified by three-step cholescintigraphy (*3*). The characteristic signs are: (a) hyperperfusion (focus of increased uptake in the inflow images) (b) normal uptake during the parenchymal phase, and (c) delayed excretion of labeled bile (area of increased uptake in the outflow images). Hemangiomas, on the contrary, are seldom hyperfused (three of 29 cases) and had no uptake in the parenchymal phase (photon-deficient area). The same findings are seen in cases of metastases; they can be differentiated by a blood-pool image (*4*). Hemangiomas had a high uptake 2-4 hr after application of labeled erythrocytes (Table 1). The classification of "hemangioma" was correct in 17 of our 19 patients with this disease. Adequate techniques, however, are necessary for a correct diagnosis (*5*).

If there are neither cholescintigraphic signs of a FNH nor a hemangioma-like focus increased uptake in the blood-pool image, the character of the tumor must be classified as "questionable." Up to now all 48 malignant or semimalignant liver tumors have been classified correctly, while four of 55 benign tumors were classified false-positive as "possibly malignant."

Sonographically proven liver tumors should be differentiated by radionuclide techniques. The procedure is accurate and inexpensive, without risk to the patient. Other, more invasive or expensive techniques should be used only if there is a doubtful outcome from scintigraphy (*6*).

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Re: Clinical Assessment of a Radioimmunoassay for Free Thyroxine Using a Modified Tracer

The *Journal*'s report by Chan et al. (*1*) on free T_4 (FT_4) measurements by the Amerlex RIA concluded that "either the free-T_4 index (FTI), the product of total T_4 and T_3 uptake, or the Amerlex FT_4 RIA were suitable for routine measurement of thyroid function in a variety of patients." Although the correlation coefficient between these two methods in patients with nonthyroidal illnesses (NTI) was only 0.675, the authors suggested that the "FT_4 measurements may constitute a saving in time and resources to the use of the FTI."

This current trend towards the faster and easier-to-use one-step FT_4 RIAs is also reflected in the 1982 Basic Ligand Assay Survey conducted by the College of American Pathologists (*2*). Of all participating laboratories that measured total T_4 routinely, approximately one in five performed FT_4 assays. At the beginning of 1982, 49% of the laboratories involved in FT_4 measurements used one-step FT_4 kits based on T_4 derivatives as tracer (Amerlex by Amersham, GammaCoat one-step by Clinical Assays, and Coat A Count by Diagnostic Products Corp.), whereas 18% used a two-step FT_4 procedure (GammaCoat two-step by Clinical As-

TABLE 1. SCINTIGRAPHIC FINDINGS IN LIVER TUMORS [adapted from Ref. (*6*)]

	Cholescintigraphy			
	Inflow	Parenchyma	Outflow	Blood-pool image
FNH	+++	N	++	N
Hemangioma	N	—	—	+++
Metastasis	N	—	—	N
Adenoma/Ca	N	—	++	N

++ Focus of increased uptake.
— Focus of decreased uptake.
N = Similar to normal liver tissue.

TABLE 1. ABNORMAL FT_4 RESULTS IN EUTHYROID PATIENTS WITH NTI

FT_4 RIA	Number of patients	% Patients with abnormal FT_4		Reference
		Low values	High values	
Amerlex	14	[a]p <0.001		9
(Amersham Corp)	25	40		10
	80	21	1	1
	33	45	6	11
GammaCoat 1-step	25	28		10
(Clinical Assays)	12	[a]p <0.001		12
	33	48	9	11
Coat-A-Count	24	a		13
(Diagnostic Products Corp)				
Immophase Single-Step	29	41	3	14
(Corning Medical)				
GammaCoat 2-step	16	none		6
(Clinical Assays)	51	2	8	15
	85	5	~40[b]	16
	26	8	42[b]	4
	89	c		17
	71	4	3	7
	59	10	10	5
	12	d		18
Immophase two-tube assay	35	26		19
(Corning Medical)	47	2		20
	85	~16	2	16
	89	c		17
	51	4	18	15
	45	22	7	21
	26	73		4
	59	81		5
	33	39	12	11
Liquisol	85	2	~23[b]	16
(Damon)	26	50		4
	59	29	2	5
	33	30	7	11

[a] Mean FT_4 significantly lower than in normals.
[b] Normal range in this report is narrower than that usually adopted.
[c] Not significantly different from control but there is overlap of hypothyroid NTI with euthyroid NTI.
[d] Not significantly different from normals.

says). Miscellaneous other kits in use included those by Corning and Damon. At the end of 1983, even more laboratories (59%) performed one-step procedures and fewer (15%) the two-step procedure.

This trend, in our judgment, is not consistent with the results of clinical investigations. In general, these commercial FT_4 assays will identify most hyperthyroid and most hypothyroid patients, provided they are not suffering from severe systemic illnesses, and FT_4 results tend to correlate well with clinical findings in patients with abnormal levels of thyroxine-binding globulin (TBG) (*3*), even though all these assays quantify portions of total T_4 significantly larger than the actual FT_4 concentrations.

FT_4 results in patients with severe systemic NTI vary considerably, depending not only on the method but also on the illnesses of the patients studied. However, several reports (*4–7*) suggest that GammaCoat two-step FT_4 RIA is clinically more valuable in patients with NTI than the various one-step procedures.

This fact should probably not be overlooked, since current estimates are that 2–5% of all hospitalized patients may have thyroid disorders that are difficult to diagnose. Total thyroid-hormone concentrations are often abnormal in euthyroid sick patients (euthyroid sick syndrome) and results of total T_4, T_3, or FTI measurements can be misleading (*8*). Therefore, in order to be diagnostically useful and to avoid large numbers of faulty FT_4 results in hospitalized patients, FT_4 results should be normal in euthyroid patients with NTI and should be able to differentiate between euthyroid and hypothyroid sick patients to ensure that only the latter receive thyroid replacement therapy.

A review of the literature indicates that the various one-step FT_4 RIAs that use a labeled T_4 derivative as tracer produce a high percentage of abnormally low FT_4 results in euthyroid NTI patients, and thus cannot distinguish between patients with NTI and hypothyroid patients (Table 1).

There is evidence that the Amerlex tracer interacts significantly with serum albumin (*22,23*) or measures albumin-bound T_4 (*24*). This binding to albumin violates the essential premise of the

method, and makes FT_4 results dependent on the serum albumin concentrations as well as on the concentrations of various anions that can compete with the I-125 T_4 derivative for albumin-binding sites. For instance, the spuriously low postheparin Amerlex FT_4 values (*25*) are most likely artifacts of the method caused by high fatty-acid concentrations inhibiting tracer binding to albumin.

The compositions of the T_4 derivatives used as tracers in the various one-step FT_4 RIAs have not yet been disclosed, but one can speculate that the primary amino and carboxyl groups in the alanine moiety of T_4 are coupled to molecules carrying NH_2- or SH- groups, whereas the other portion of the molecule with the phenolic hydroxyl group remains unsubstituted (for antibody recognition and iodination) and may be responsible for the binding to albumin. Since coupling to T_4 of molecules as large as enzymes (horseradish peroxidase) did not abolish the binding to albumin (*26*), it is doubtful whether the concept of these one-step FT_4 methods can be applied to sera of patients with NTI, where albumin, metabolites, and drug concentrations can vary enormously.

On the other hand, two-step FT_4 RIAs—where FT_4 is first bound to solid-phase antibodies, and the tracer, I-125 T_4, is added in a separate step after removal of the remaining serum—are considerably less prone to interference by abnormal serum constituents, and may in general differentiate better between hypo- and euthyroid sick patients. As illustrated in Table 1, in NTI the GammaCoat two-step RIA tends to give normal-to-elevated FT_4 results that are in better agreement with those by equilibrium dialysis. The relatively large portion of abnormally high FT_4 values reported by Kaptein et al. (*4*) and Slag et al. (*16*), but not observed by others, are probably due to the use of very narrow reference ranges, 0.7-1.2 and 0.8-1.7, respectively, derived from a small number of normals, instead of 0.8-2.3 as suggested by the manufacturer.

In summary, contrary to the suggestions by Chan et al. (*1*) the GammaCoat two-step FT_4 RIA may be a better alternative for hospital laboratories than either the Amerlex FT_4 RIA or the FTI. For best patient management and to avoid wrong signals to industry, new tests should be introduced primarily on the basis of improved clinical accuracy rather than speed and ease of use.

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Reply

Our report on the "Clinical Assessment of A Radioimmunoassay For Free Thyroxine Using A Modified Tracer" (*1*) was based on the comparison of the Amerlex free-T_4 RIA with traditional free-T_4 (FT_4) methods—i.e., free-thyroxine index (FTI) and equilibrium dialysis. The conclusion for its acceptability was reached on both clinical and analytical performances. Clinically, the Amerlex FT_4 assay correctly classified 98% euthyroid patients, 92% hypothyroid, 100% euthyroid, 100% euthyroid patients with elevated TBG and 87% of phenytoin. Analytically, the precisions were between 3-7% for the useful free-T_4 concentrations, and the correlation coefficients were 0.911 for FTI and 0.871 for the dialysis method. In addition to the clinical and analytical accuracy, one should consider the economic issue in designing an assay that is easy to use and time saving, especially in this era of cost containment and the Diagnosis Related Groups (DRG) reimbursement system.

We agreed that free-T_4 assays should be studied in patients with nonthyroidal illnesses (NTI). In this report, we found 21% of the 80 NTI patients to have free T_4 in the hypothyroid range, 78% in the euthyroid range and 1% in the hyperthyroid range. Bayer and McDougall correctly state in their letter that "FT_4 results in patients with severe systemic NTI vary considerably, depending not only on the method but also on the illnesses of the patients studied." On our subsequent report (*2*) with a different group of NTI patients, we found the Amerlex FT_4 assay gave 56% hypothyroid, 44% euthyroid, and no hyperthyroid results (Table 1). With the controversy surrounding the issue of NTI (*3,4,5*) we were unable to verify the usefulness of this free-T_4 assay in NTI patients. This is particularly true for patients with borderline hypothyroid results. TSH measurement remains one of the most helpful tests in distinguishing the true hypothyroid patient.

This report examines only the Amerlex free T_4. Bayer and McDougall's suggestion that the Gamma Coat two-step FT_4 RIA may be a better alternative than Amerlex FT_4-RIA or the FTI was outside the scope of our report. However, documentation such as Table 1 of their letter is useful. This table agreed with some of our findings (Table 1, below) as reported elsewhere (*2*). It is apparent that in NTI patients, the one-step FT_4 RIA gave lower results whereas two-step FT_4 RIA tended to give higher results. The Gamma Coat 2-step assay showed a large percentage in the hyperthyroid range in at least two reports, as pointed out by Bayer in Table 1 of the letter. We also found this assay to be less precise (*2*). It is dangerous to pick an overall "winner" for FT_4 assays, whether it is a one-step or two-step RIA. We have not found a single FT_4 RIA that is both analytically and clinically superior in the testing of thyroidal and nonthyroidal illnesses.

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TABLE 1. CLINICAL ANALYSIS OF NTI PATIENTS

	Laboratory results in the range of			Total
	Hypothyroid	Euthyroid	Hyperthyroid	no.
FTI-RIA	55%	45%	0%	40
FTI-IIA (Abbott)	50%	48%	2%	40
FTI-TBG (Clinical Assays)	27%	68%	5%	40
FT_4-Dialysis (BioScience)	16%	76%	8%	25
FT_4-Amerlex (Amersham)	56%	44%	0%	39
FT_4-Gamma Coat 1 (Direct) (Clinical Assays)	60%	40%	0%	40
FT_4-Gamma Coat II (Two Steps) (Clinical Assays)	20%	65%	15%	40