Undetectable Sensitive Serum Thyroglobulin (<0.1 ng/ml) in 163 Patients with Follicular Cell-Derived Thyroid Cancer: Results of rhTSH Stimulation and Neck Ultrasonography and Long-Term Biochemical and Clinical Follow-Up

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Context: Surveillance of patients with differentiated thyroid cancer (DTC) is achieved using serum thyroglobulin (Tg), neck ultrasonography (US), and recombinant human TSH (rhTSH)-stimulated Tg (Tg-stim).

Objective: Our primary aim was to assess the utility of rhTSH Tg-stim in patients with suppressed Tg (Tg-supp) below 0.1 ng/ml using a sensitive assay. Our secondary aims were to assess the utility of US and to summarize the profile of subsequent Tg-supp measures.

Design: This is a retrospective study conducted at two sites of an academic institution.

Patients: A total of 163 patients status after thyroidectomy and radioactive iodine treatment who had Tg-supp below 0.1 ng/ml and rhTSH Tg-stim within 60 d of each other were included.

Results: After rhTSH stimulation, Tg remained below 0.1 ng/ml in 94 (58%) and increased to 0.1–0.5 in 56 (34%), more than 0.5–2.0 in nine (6%), and above 2.0 ng/ml in four (2%) patients. Serial Tg-supp levels were obtained in 138 patients followed over a median of 3.6 yr. Neck US were performed on 153 patients; suspicious exams had fine-needle aspiration (FNA). All positive FNA were identified around the time of the initial rhTSH test. Six of seven recurrences were detected by US (Tg-stim >2.0 ng/ml in one, 0.8 in one and ≤ 0.5 in four). One stage IV patient had undetectable Tg-stim.

Conclusion: In patients with DTC whose T_4-suppressed serum Tg is below 0.1 ng/ml, long-term monitoring with annual Tg-supp and periodic neck US are adequate to detect recurrences. In our experience, rhTSH testing does not change management and is not needed in this group of patients. (***J Clin Endocrinol Metab*** **97: 2714–2723, 2012)**

Thyroid cancer is increasing in incidence, only partly due to early detection from imaging procedures performed for unrelated reasons (1–3). Given the overall favorable prognosis for patients with differentiated follicular cell-derived thyroid cancer, there are many long-term survivors. Current surveillance techniques for the majority of cases include serum thyroglobulin (Tg), which may indicate residual/recurrent disease or thyroid bed remnant, and neck ultrasonography (US) to detect cervical lymph nodes, the most likely location for recurrences.

Using assays with functional sensitivities of 0.5–1.0 ng/ml, it was recognized that a sizable minority of patients

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Abbreviations: DTC, Differentiated thyroid cancer; FTC, follicular thyroid cancer; HCC, Hürthle cell carcinoma; NPV, negative predictive values; PPV, positive predictive values; PTC, papillary thyroid cancer; RAI, radioactive iodine; rhTSH, recombinant human TSH; Tg, thyroglobulin; Tg-stim, stimulated Tg; Tg-supp, suppressed Tg; TNM, tumor node metastasis; US, ultrasonography.

who had previous radioactive iodine (RAI) remnant ablation with undetectable Tg levels on L-T_4 suppressive therapy [suppressed Tg (Tg-supp)] had residual cancer. To increase the sensitivity of detection, recombinant human TSH (rhTSH) stimulated Tg (Tg-stim) values have been used for more than a decade (4). A consensus conference concluded that if a Tg-stim level was below 2 ng/ml, the likelihood of detecting disease was low (5). Kloos and Mazzaferri (6) proposed that patients with a single Tg-stim value below 0.5 ng/ml indicated patients were free of tumor and concluded that these patients needed less frequent US and Tg-stim testing. The revised American Thyroid Association clinical guidelines felt that "there is good evidence that a Tg cutoff level above 2 ng/ml after rhTSH stimulation is highly sensitive in identifying patients with persistent tumor" (7).

We previously reported in 80 patients with follicular cell-derived thyroid cancer with serum Tg-supp below 0.1 ng/ml using a sensitive chemiluminometric assay that patients rarely had a Tg-stim value over 2 ng/ml and that concomitant neck US, but not rhTSH testing, was sufficient to monitor such patients (8). We now extend our observations with up to 9.6 yr of follow-up on a larger group of 163 patients with Tg-supp levels below 0.1 ng/ml who had rhTSH tests. Our primary objective was to assess the utility of rhTSH Tg-stim in patients with suppressed thyroglobulin (Tg-supp) below 0.1 ng/ml using a sensitive assay. Our secondary objectives were to assess the utility of US in the same scenario and to summarize the profile of subsequent Tg-supp measures.

Patients and Methods

Patients

The electronic medical record was reviewed for all thyroid cancer patients at Mayo Clinic in Jacksonville, FL, and Roches-

TABLE 1. Clinical and tumor characteristics in 163 patients with Tg-supp below 0.1 ng/ml

	Overall	Tg-stim <0.1	Tg-stim 0.1–0.5	Tg-stim >0.5–2.0	Tg-stim >2.0
n	163	94 (58%)	56 (34%)	9 (6%)	4 (2%)
Sex					
Female	113 (69%)	68 (72%)	38 (68%)	4 (44%)	3 (75%)
Male	50 (31%)	26 (28%)	18 (32%)	5 (56%)	1 (25%)
Age (yr)					
Initial surgery	47 (16–82)	48 (17–81)	48 (16–82)	37 (21–69)	40 (36–43)
rhTSH study	52 (20–85)	52 (20–85)	53 (22–84)	52 (25–70)	48 (41–51)
TNM classification					
T1N0M0	26 (16%)	21 (22%)	5 (9%)		
T1N0M1	1 (1%)	1 (1%)			
T1N1M0	23 (14%)	12 (13%)	8 (14%)	2 (22%)	1 (25%)
T1NxM0	18 (11%)	10 (11%)	8 (14%)		
T2N0M0	15 (9%)	10 (11%)	5 (9%)		
T2N1M0	18 (11%)	11 (12%)	5 (9%)	1 (11%)	1 (25%)
T2NxM0	11 (7%)	9 (10%)	2 (4%)		
T3N0M0	10 (6%)	4 (4%)	6 (11%)		
T3N1M0	13 (8%)	4 (4%)	4 (7%)	3 (33%)	2 (50%)
T3N1M1	1 (1%)		1 (2%)		
T3NxM0	15 (9%)	9 (10%)	5 (9%)	1 (11%)	
T4NxM0	1 (1%)	1 (1%)			
TxN0M0	2 (1%)		2 (4%)		
TxN1M0	5 (3%)		4 (7%)	1 (11%)	
TxN1M1	1 (1%)			1 (11%)	
TxNxM0	2 (1%)	1 (1%)	1 (2%)		
TxNxM1	1 (1%)	1 (1%)			
Pathology					
PTC	125 (77%)	69 (73%)	44 (79%)	8 (89%)	4 (100%)
FTC	24 (15%)	14 (15%)	9 (16%)	1 (11%)	
HCC	14 (9%)	11 (12%)	3 (5%)		
TNM staging					
Stage I	83 (51%)	51 (54%)	25 (45%)	4 (45%)	3 (75%)
Stage II	8 (5%)	7 (8%)	1 (2%)	0 (0%)	0 (0%)
Stage III	28 (17%)	12 (13%)	13 (23%)	2 (22%)	1 (25%)
Stage IVA	6 (4%)	2 (2%)	3 (5%)	1 (11%)	0 (0%)
Stage IVB	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stage IVC	4 (2%)	2 (2%)	1 (2%)	1 (11%)	0 (0%)
Unknown	34 (21%)	20 (21%)	13 (23%)	1 (11%)	0 (0%)
Total	163	94	56	9	4

Categorical variables are reported as n (percent). Continuous variables are reported as median (range).

ter, MN, who had rhTSH tests between August 2001 and June 2011. One hundred sixty-three patients (87 in Rochester and 76 in Jacksonville) were identified who after surgery and ^{131}I had a Tg-supp value below 0.1 ng/ml (and negative thyroglobulin antibody) with an rhTSH test within 60 days. This protocol was approved by the Mayo Clinic Institutional Review Board. The clinical and tumor characteristics are depicted in Table 1. All patients were staged according to the TNM Classification System for Differentiated Thyroid Carcinoma (7).

To ensure consistency in our observations, we started the follow-up with the time of the rhTSH stimulation performed within 60 d of a Tg-supp below 0.1 ng/ml.

Median follow-up interval from the time of the rhTSH testing was 3.6 yr, ranging from 2.9 months to 9.6 yr. The median interval between initial surgery and beginning of our observation period was 1.8 yr (0.5–45.8 yr). Mean interval between the last RAI dose and Tg-stim was 27.5 months (range 6–96 months, median 15.5 months). Twenty-five patients did not have a subsequent Tg-supp measured after the Tg-stim. Five patients had subsequent interventions (surgery or ethanol ablation) after the Tg-stim; therefore, subsequent Tg values in these five patients were not included in the analysis but reported separately.

Initial therapy

Patients were 16–82 yr old (median age, 47 yr) at the time of their initial surgery. Fifty patients had 59 subsequent operations after this initial procedure: 44 had two surgeries, four had three, one had four, and one had five surgeries. The second surgery was completion thyroidectomy in 28 cases, the remaining subsequent surgeries being radical neck dissection/cervical exploration for persistent/recurrent local disease, except for one patient who had right frontal craniectomy for skull metastases.

All patients received at least one dose of ^{131}I (initial dose range, 19.9–300 mCi). There were 16 patients who received two doses of RAI (six for local recurrence, five for persistent uptake in the neck area, one for persistently elevated Tg with negative US and scan, and two for uptake in the chest area. In two patients, details were not available. One patient received three doses for local recurrences, and one received four doses, the second and third for persistent uptake in the neck region and the fourth for persistently elevated Tg.

Two patients had received ethanol ablation of malignant cervical lymph nodes before their rhTSH test. One had one treatment, and unstimulated Tg at that time was 0.9. The second patient had three treatments 3 months apart, and associated Tg-supp was not available, 0.8, and 0.4, respectively.

rhTSH test

The rhTSH (0.9 mg im) was given on d 1 and 2. On d 3, either ^{131}I (Mayo Clinic in Jacksonville) or ^{123}I (most patients at Mayo Clinic in Rochester) was given, with Tg levels and whole-body scans performed at times previously described (8). Median (range) serum TSH level before rhTSH injection was 0.09 mIU/liter (<0.01–54.5). The one patient whose TSH was 54.5 before stimulation had been off L-T_4 for a few weeks. Three of the 163 patients had no TSH value measured before rhTSH administration.

Tg assay

Since August 2001, our laboratory has used an automated chemiluminometric assay (Access Tg; Beckman Coulter, Brea, CA; catalog item 33860) with an analytical sensitivity of 0.1 ng/ml and a functional sensitivity in our laboratory of below 0.1 ng/ml. Performance characteristics were described in detail previously (8).

Ultrasonography

Ultrasound images were obtained using a high-frequency linear-array transducer (8–12 MHz). Per protocol, transverse and longitudinal views of the right and left neck were obtained inferiorly from the level of the clavicles to the angle of the jaw superiorly. Longitudinal views were obtained from the midline to the lateral neck.

Statistical analysis

Cox proportional hazards models were used to assess the increased risk of having a subsequent Tg-supp of at least 0.3 ng/ml among those who had an initial Tg-supp of below 0.1 ng/ml along with a Tg-stim of at least 0.3 *vs.* below 0.3 ng/ml. We estimated sensitivity, specificity, negative predictive values (NPV), and positive predictive values (PPV) for Tg-stim higher than 2.0 and at least 1.4, for US, and ^{131} I scans among patients with a Tg-supp below 0.1 ng/ml. It was feasible to estimate only NPV for Tg-stim below 0.1 ng/ml due to the choice of inclusion criteria. We used biopsy-proven disease as our gold standard for recurrence.

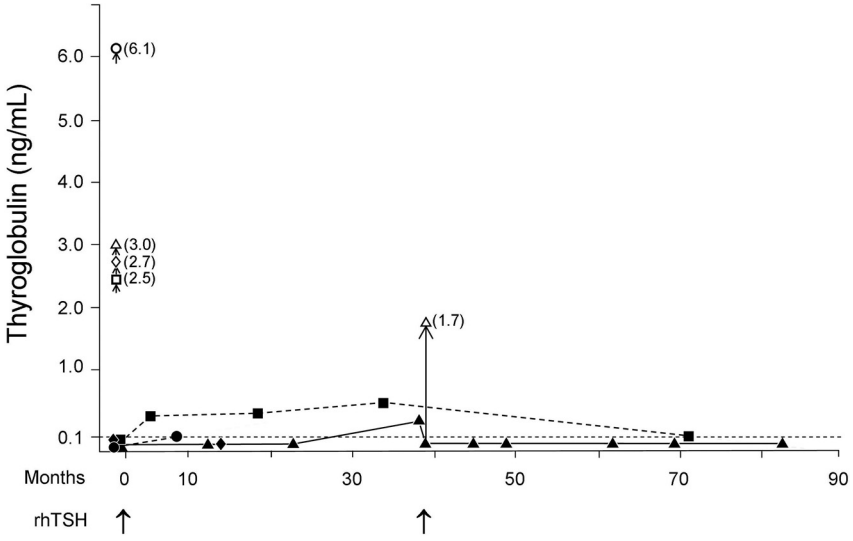

FIG. 1. Longitudinal follow-up on four patients with initial Tg stim over 2.0 ng/ml. One patient (*triangle*, no. 23) had two Tg-stim tests. One patient (*circle*, no. 24) had recurrence detected by ultrasound and subsequent surgery. In one patient (*diamond*, no. 27, TNM classification T2NxM0), Tg-supp remained below 0.1 ng/ml after initial stimulation test. One patient (*square*, no. 28, T1N1M0) had a rise in Tg-supp over the first 34 months of follow-up, followed by a Tg-supp of 0.1 more than 70 months after the initial testing. *Solid symbols* represent Tg-supp; *open symbols* represent Tg-stim.

Results

Tg levels: rhTSH testing

A total of 163 patients had a Tg-supp below 0.1 ng/ml within 0–60 d

before a rhTSH stimulation test. After rhTSH, Tg remained below 0.1 ng/ml in 94 (58%), increased to 0.1–0.5 in 56 (34%), more than 0.5–2.0 in nine (6%), and more than 2.0 ng/ml in four (2%) (Table 1). The latter four patients had individual stimulated values of 2.5, 2.7, 3.0, and 6.1 ng/ml (Fig. 1).

Tg-supp levels in follow-up

Serial Tg-supp levels (n = 757) were obtained in 138 patients followed up to 9.6 yr. Five patients had an intervention after the rhTSH test; therefore, subsequent Tg values were not included in the analysis but reported separately below. Twelve patients had a total of 20 subsequent Tg-supp values of at least 0.3 ng/ml. In 18 occurrences, an US was performed within 6 months of the result. All but one were negative. The likelihood of having a Tg-supp of at least 0.3 ng/ml was higher if the initial Tg-stim was at least 0.3 ng/ml with an estimated hazard ratio of 3.83 (95% confidence interval = 1.21–12.08; $P = 0.022$).

Of the 12 patients who had at least one Tg-supp value of 0.3 ng/ml or higher after their initial rhTSH test, no further follow-up was available in two. The Tg-supp results after rhTSH in these patients are depicted in Table 2 with a follow-up of 6 months to 7¾ yr. Tg-supp levels of 0.3 ng/ml occurred on only a single occasion in six of the 10 patients. One patient (no. 4, Fig. 2A and Table 2) whose Tg-stim was below 0.1 ng/ml had a Tg-supp of 0.3 mg/ml 5¾ yr later and then had persistent detectable Tg but no evidence of detectable disease by US or rhTSH testing with 4 yr of additional follow-up. One patient (no. 30, Table 2) had FNA-positive persistent nodal disease at the time of rhTSH stimulation, was followed for 3½ years, became Tg antibody positive, and was followed for 5 additional years with stable US. Seven of 10 patients had Tg-supp of 0.1 ng/ml or below at the last follow-up and no detectable disease.

Follow-up rhTSH tests

A second stimulation test was performed in 21 patients, whereas four had three tests and one had four tests. Figure 2, A and B, depicts the Tg-supp and Tg-stim values during a median follow-up of 31 months (11–66 months). Of 13 patients whose initial Tg-stim was below 0.1 ng/ml, nine (69%) had undetectable Tg-stim, and 11 (85%) had Tg-stim no higher than 0.1 ng/ml at last follow-up (median 24 months; range, 11–66 months). In these patients, time interval between last RAI dose and initial Tg-stim ranged between 6 and 60 months, with a median of 7 months. One patient (no. 13, Fig. 2A and Table 3) had metastatic Hürthle cell carcinoma (HCC) and died 18 months after the second stimulation test.

Of the 13 patients whose initial Tg-stim value was 0.1 ng/ml or higher, only five (38%) had follow-up tests with Tg-stim no higher than 0.1 ng/ml, nine had their most recent Tg-stim the same or less than the first study, and four had their most recent Tg-stim value greater than the initial study (Fig. 2B). One patient (no. 24, Fig. 2B), whose Tg-stim was 6.1 ng/ml had a recurrence, and modified radical neck dissection was done before the second rhTSH test. In this group of patients, the interval between last dose of RAI and initial Tg-stim ranged between 6 and 96 months (median 18 months).

Tg-stim levels over 2.0 ng/ml

Four patients had Tg-stim levels over 2 ng/ml (Fig. 1). One had recurrent disease detected by US and subsequent surgery (no. 24, Fig. 2B). One patient (no. 28, Fig. 1 and Table 2) had four negative US performed over 5 yr, and one (no. 23, Fig. 1 and Table 2) had seven negative US performed yearly. A fourth patient (no. 27, Fig. 1) had one follow up Tg-supp value but no US. The last three patients had follow-up of 1–7 yr with low to undetectable Tg-supp values and no evidence of recurrent disease.

TABLE 2. Longitudinal Tg-supp follow-up on 12 patients who had at least one Tg-supp value of at least 0.3 ng/ml after their initial rhTSH test

Patient	TNM stage	Months RAI to TG-stim	Tg-stim	yr 1 Q1	yr 1 Q2	yr 1 Q3	yr 1 Q4	yr 2 Q1	yr 2 Q2	yr 2 Q3	yr 2 Q4
4	T3NXM0	6	<0.1							<0.1 (neg)	
5	T3N0M0	7	<0.1				0.3 (neg)				0.1 (neg)
10	T2N1M0	6	<0.1		0.2 (neg)		0.3 (neg)			0.1 (neg)	<0.1 (n/a)
18	T2N0M0	51	0.1		0.3 (n/a)		0.1 (neg)		0.2 (n/a)		0.3 (n/a)
23	T3N1M0	96	3				<0.1 (neg)				<0.1 (neg)
28	T1N1M0	12	2.5		0.3 (neg)				0.3 (neg)		
29	T3N1M0	18	<0.1				0.1 (neg)				0.2 (neg)
30	T1N1M0	13	0.8	0.1 (pos)		0.4 (pos)					
31	T1NXM0	58	0.1				0.3 (neg)				<0.1 (neg)
32	T1N0M0	21	0.3						0.3 (n/a)	<0.1 (n/a)	
33	T1N1M0	30	0.2				0.2 (neg)				
34	T3N0M0	12	0.1		0.8 (susp)						

(*Continued*)

US findings are reported as negative (neg), positive (pos), not performed (n/a), and suspicious (susp). Two patients did not have follow-up after their initial increase in Tg (nos. 33 and 34). Suspicious US in patient 34 was followed by two negative FNA. Patient 30 has FNA-proven recurrence followed with serial imaging; additional information is detailed in Table 3. Q, Quarter.

^{131}I uptake and scans

One hundred thirty-eight of the 163 patients had isotopic whole-body imaging at the time of rhTSH testing. The median (range) of neck bed uptake was 0.07% (0–0.9%), with visible uptake seen in 11 patients. In eight of the 11 patients, neck US performed within 60 d from the rhTSH-stimulated scan did not confirm recurrence. In one patient (no. 33, Table 2), US described a suspicious area too small to be amenable to FNA, and a repeat US 4 months later was negative. Tg-stim and Tg-supp at the time of the abnormal US was 8.6 and 0.5 ng/ml, respectively. A second patient (no. 35, Table 3) whose rhTSH scan described a "faint focus, reduced compared with prior imaging" had an ultrasound "worrisome for recurrence" with subsequent positive FNA followed by alcohol ablation. At that time his Tg-stim was 0.2 ng/ml. A third patient (no. 30, Tables 2 and 3) with biopsy-proven persistent neck disease had Tg-stim of 0.8 ng/ml. Tg-stim in the remaining eight cases was 0.1 ng/ml (one), below 0.1 ng/ml (six), and not available (one).

Uptake outside the neck was observed in 2 patients. In both cases, additional imaging, CT of the abdomen in one case and thoracic MRI in the other, did not confirm the rhTSH scan findings. Tg-supp at the time of these findings was below 0.1ng/ml and 0.1ng/ml, respectively. Associated Tg-stim were 0.1 ng/ml and below 0.1 ng/ml, respectively.

US imaging

Serial neck US, usually done annually, were performed on 153 patients. When suspicious sonographic features were observed (microcalcifications, hypoechogenicity, increased vascular flow, or abnormal shape of lymph nodes), fine-needle aspirates were done for cytological examination. A total of 597 US exams were done, with a median (range) follow-up of 3.0 yr (0 months to 9.6 yr).

Suspicious US exams were followed by FNA in 18 patients, and seven FNA in five patients had cytological findings suspicious for persistent/recurrent disease. Two of these patients with minimal disease are being followed up with serial US. A sixth patient had ethanol ablation without an FNA based on suspicious US features.

Table 3 provides more detail on these patients. Recurrent/persistent disease was detected not only in one patient with Tg-stim over 2.0 ng/ml but also in four whose Tg-stim was no higher than 0.5 ng/ml (0.1, 0.2, 0.3, and 0.5 ng/ml) and one who had Tg-stim of 0.8 ng/ml. All local recurrences were detected in the neck by US, and all but one patient had tumor node metastasis (TNM) classification N1 disease at the time of their initial diagnosis. One high-risk stage IV patient with Hürthle cell carcinoma with distant disease detected by chest x-ray had an undetectable stimulated Tg (no. 13). Detection of recurrence led to additional therapies including surgery and/or ethanol ablation in five of these patients.

No patient whose US imaging was unremarkable at the time of rhTSH testing developed an abnormality leading to a suspicious FNA subsequently.

Other imaging

Chest CT was the most common imaging besides US and rhTSH-stimulated scan. Pulmonary micronodules were detected on 23 CT scans performed on 16 patients; 11 with papillary thyroid cancer (PTC) and five with follicular thyroid cancer (FTC). Tg-stim was below 0.1 ng/ml in 11 patients (six patients with PTC and the five patients with FTC), 0.1–0.5 ng/ml in four patients, and over 0.5 ng/ml in one patient. Tg-supp was measured within 60 d of the chest CT in 11 instances. In 10 measurements (nine patients), Tg-supp was below 0.1 ng/ml, and one patient had a Tg-supp of 0.1 ng/ml. In 13

TABLE 2. Continued

yr 3				yr 4				yr 5			
Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
		0.1 (n/a)				0.2 (neg)					0.3 (neg)
	<0.1 (neg)					0.1 (neg)				0.1 (neg)	
<0.1 (neg)				0.2 (neg)				0.1 (neg)			
			0.3 (neg)	<0.1 (n/a)	<0.1 (n/a)		<0.1 (neg)				<0.1 (neg)
		0.4 (neg)									
				0.3 (neg)							
							0.1 (pos)				
			0.2 (neg)			<0.1 (neg)		<0.1 (n/a)			
	0.2 (neg)				<0.1 (neg)				0.1 (neg)		
	0.5 (neg)										

(*Continued*)

instances, there was no Tg measurement around the time of the CT scan.

One patient, whose Tg-stim was 0.1 ng/ml, had a single focus of uptake on rhTSH scan but not in the area of the micronodules. This patient also had calcified granulomas. In all other cases, there was no visible uptake on the rhTSH scan.

Six patients had a second chest CT study performed at a 6- to 18-month interval from the first one (median, 9 months), and of these, two had a third study, 5 months and 34 months after the second one, respectively. All subsequent studies reported stable pulmonary micronodules.

Chest x-ray was the method of detection of rib metastases in the one high-risk patient with HCC (Fig. 2A and Table 3, no. 13).

Follow-up Tg-supp and US in patients with recurrence after subsequent treatment

Recurrence was detected in seven patients (Table 3). Two are followed clinically, and five had subsequent treatments. One patient had neck surgery (Fig. 2B, no. 24) followed by six Tg-supp values measured over 4 subsequent years, all no higher than 0.1 ng/ml, and three negative US. One had thoracic surgery for distant metastases (Fig. 2A, no. 13) and had three subsequent Tg-supp values, all below 0.1 ng/ml, measured over 2 additional years of follow-up and one negative US. He died from metastatic disease to the liver. A third patient (no. 37) had ethanol ablation of cervical lymph nodes, followed by additional neck surgery 10 months later, and then he had nine additional Tg-supp values no higher than 0.1 ng/ml and nine negative US tests over 7 yr. In this case, Tg-supp at the time of ethanol ablation was below 0.1 ng/ml. Two patients (nos. 35 and 36) had no follow-up Tg after ethanol ablation. In one of these cases (no. 36), Tg-supp at the time of ethanol ablation was below 0.1 ng/ml, and in the other it was not available.

Diagnostic utility of Tg-stim and US after Tg-supp below 0.1 ng/ml

Because seven of the 163 patients studied here with Tg-supp below 0.1 ng/ml had recurrence, the estimated NPV from this set of patients was 156 of 163 (96%). Table 4 depicts estimates of sensitivity, specificity, PPV, and NPV for Tg-stim cutoffs of over 2.0 and over 1.4 ng/ml, neck US, and ^{131}I scan among this set of patients with Tg-supp below 0.1 ng/ml. Tg-stim cutoff levels of 1.4 and 2.0 ng/ml had similar high degrees of specificity and NPV for disease recurrence, but a low PPV and the lowest sensitivity for disease detection of all tests examined. ^{131}I imaging yielded similar results. In contrast, US had by far the greatest sensitivity with little reduction in specificity, a modest improvement in PPV, and the highest NPV.

Discussion

This longitudinal study of 163 patients with undetectable Tg-supp (<0.1 ng/ml) in a sensitive Tg assay provides several important observations that should assist clinicians in monitoring their patients with differentiated thyroid cancer (DTC).

First, if a Tg-supp is below 0.1 ng/ml, the likelihood of rhTSH Tg-stim being over 2 ng/ml is small. We previously found that only 2.5% of patients (two of 80) had such a response (8), and this was confirmed in our current study (four of 163, 2.5%). Using the same Tg assay, Schlumberger *et al.* (9) found a Tg-stim over 2 in 1% of 521 patients, Spencer *et al.* (10) in only two of 655 patients (0.3%), and Malandrino *et al.* (11) in three of 331 (0.9%) if Tg-supp was no higher than 0.1 ng/ml. Iervasi *et al.* (12) had eight of 160 patients whose Tg-stim was over 2 ng/ml, but none had Tg-supp below 0.1 ng/ml. The NPV for recurrence using this assay ranged from 90–99.2% (12, 13) and was 96% using an assay with sensitivity of 0.2 ng/ml

TABLE 2. Continued

yr 6				yr 7				yr 8				yr 9		
Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
	1 (n/a)				0.4 (neg)				0.6 (neg)				0.7 (neg)	
		<0.1 (n/a)				<0.1 (neg)				<0.1 (neg)				
		<0.1 (n/a)												
<0.1 (neg)	<0.1 (n/a)				<0.1 (neg)				0.1 (neg)				<0.1 (neg)	
		<0.1 (neg)				<0.1 (neg)								
		0.1 (neg)												
			0.4 (neg)											
	0.1 (neg)							<0.1 (n/a)						
	0.1 (neg)	<0.1 (n/a)		0.2 (neg)				<0.1 (neg)				0.1 (neg)		

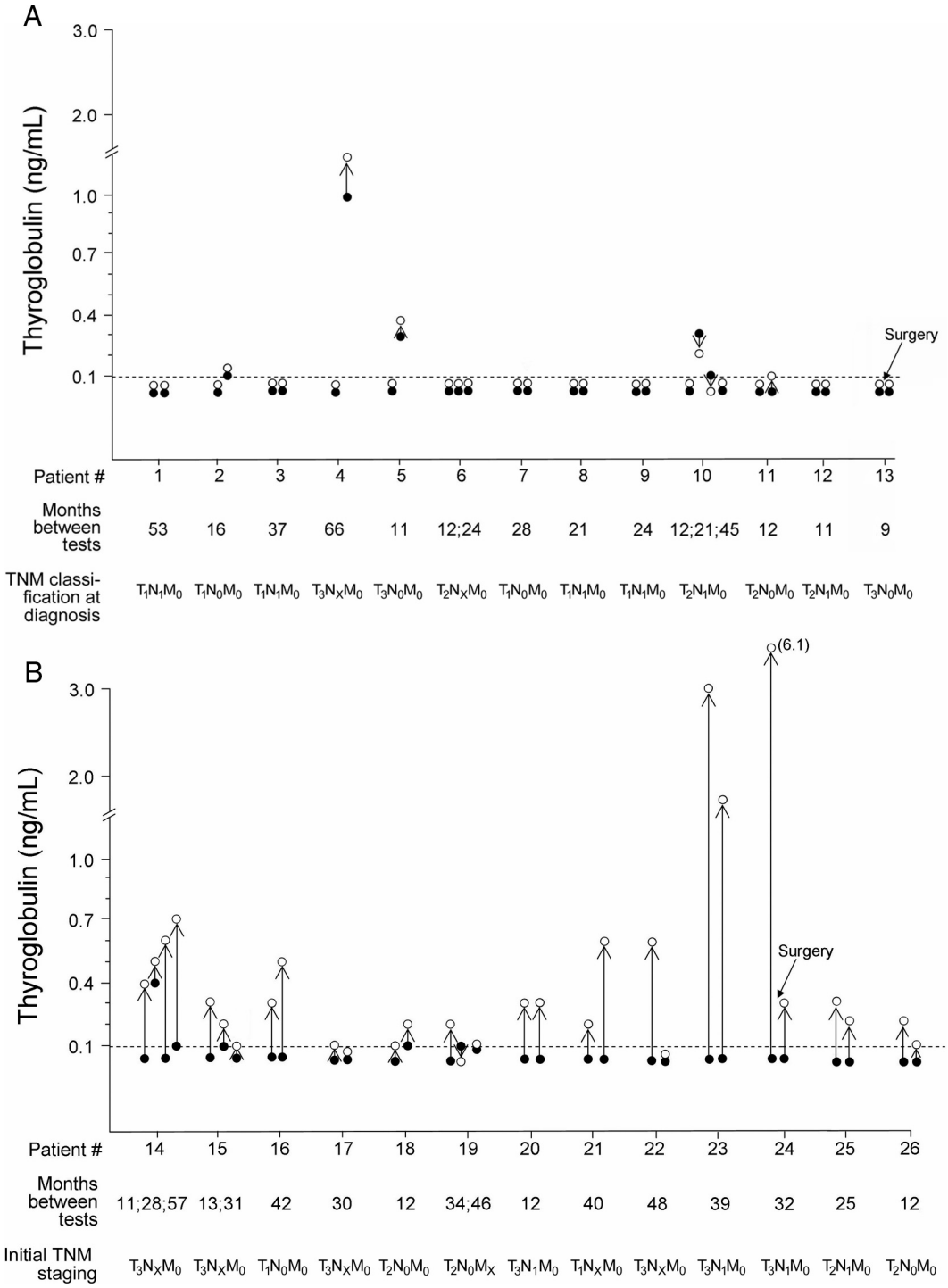

FIG. 2. A, Sequential rhTSH stimulation tests in 13 patients with initial Tg-stim below 0.1 ng/ml followed up to 66 months. In patient no. 10, the second and third Tg-stim values were less than the preceding Tg-supp. Patient no. 13 died from metastatic disease 18 months after the second Tg-stim. B, Sequential rhTSH stimulation tests in 13 patients with initial Tg-stim of at least 0.1 ng/ml followed up to 57 months. Patient no. 24 had recurrent disease and surgery before the second Tg-stim. *Solid symbols* represent Tg-supp; *open symbols* represent Tg-stim.

(14). Using receiver operating characteristic curves, Brassard *et al.* (15) determined that the optimized functional sensitivity for Tg-supp was 0.27 ng/ml and that the cutoff for Tg-stim was 1.4 ng/ml using the same assay we used. NPV for recurrence for this Tg-stim value was 99%. For the same Tg-stim cutoff, we obtained a NPV for recurrence of 96% (Table 4). Malandrino *et al.* (11) found that a cutoff for basal Tg of 0.15 ng/ml provided a NPV of 98.6%.

Second, we studied the durability of an undetectable sensitive Tg-supp and the variability over time. In our study, 7.4% of the patients (12 of 163) had one or more

TABLE 3. DTC recurrences in seven patients with serum Tg below 0.1 ng/ml

Patient	Age (yr)		Sex	TNM		Histology	Previous therapies	Detection method	Tumor location	Therapy at recurrence	Tg-supp at recurrence
	At diagnosis	At recurrence		At diagnosis	At recurrence						
rhTSH-Tg <0.1											
13	81	82	M	T3N0M0	T3N0M1	HCC	NTT, RAI	CXR	Pleura, rib	RT, zoledronic acid, left thoracotomy	<0.1
rhTSH-Tg 0.1–0.5											
35	61	62	M	T3N1M0	T3N1M0	PTC	TT, central compartment LN dissection	US; ^{131}I	Bed	ETOH	<0.1
36	52	52	F	TxN1M0	TxN1M0	PTC	TT, cervical reexploration	US	LN	ETOH	<0.1
37	32	33	F	TxN1M0	T3N1M0	PTC	TT, RAI	US	Bed	ETOH, surgery	<0.1
38	48	49	F	T1N1M0	T1N1M0	PTC	TT, RAI	US	LN	Serial follow-up	<0.1
rhTSH-Tg >0.5–2.0											
30	34	35	F	T1N1M0	T1N1M0	PTC	TT, RAI	US, ^{131}I	Bed	Serial follow-up	<0.1
rhTSH-Tg >2.0											
24	39	41	M	T3N1M0	T3N1M0	PTC	NTT, RAI	US	LN	Surgery	Positive Ab

Ab, Antibodies; Bed, thyroid bed; CXR, chest x-ray; ETOH, ethanol ablation; LN, lymph nodes; NTT, near total thyroidectomy; RT, external radiation therapy; TT, total thyroidectomy.

Tg-supp of at least 0.3 ng/ml during follow-up. We found that patients had a greater chance of having all subsequent Tg-supp being no higher than 0.3 ng/ml if their initial rhTSH stimulated level was no higher than 0.3 ng/ml. Subsequent Tg-supp levels were no higher than 0.2 ng/ml in all but three patients who were followed for 3, 4, and 8 yr, respectively. Concomitant serial ultrasound imaging did not show any recurrence. Most patients with a Tg-supp of at least 0.3 ng/ml had subsequent return to lower values, and recurrence was detected on imaging in only one patient. We believe that at these very low levels of Tg, serial Tg-supp measurements will be necessary before concluding there is disease recurrence. Although sensitive Tg assays may lose some specificity for recurrence (9), these findings illustrate the importance of serial supp-Tg measurements using sensitive assays and considering the overall clinical picture when assessing for persistent disease. A recent study by Castagna *et al.* (16) found that low detectable ultrasensitive Tg levels converted to undetectable (<0.1 ng/ml) in 80% of the patients and that mild elevations are not clinically relevant. Mazzaferri (17) felt that long-term follow-up was needed to assure that a serum Tg below 0.1 ng/ml remained below 0.1 ng/ml and, perhaps more importantly, that very small Tg elevations will spontaneously resolve without further treatment. We believe our results indicate this to be the case.

Third, there is no level of Tg (either during L-T_4 suppression or TSH stimulation) below which recurrent cancer can be absolutely excluded using the current sensitive assays. A consensus conference (5) felt that patients with rhTSH Tg-stim levels below 2 ng/ml were unlikely to have disease, and Kloos and Mazzaferri (6) felt patients were free of disease if Tg-stim was below 0.5 ng/ml, although with longer follow-up they reported recurrence in two of 62 such patients (18). Our results clearly indicate these conclusions are not always accurate, because only one of our seven patients with recurrence had Tg-stim over 2, one had a value of 0.8, four had levels between 0.1 and 0.5, and one had a Tg-stim below 0.1 ng/ml after rhTSH. Giovanella *et al.* (14) observed 14 recurrences in 117 patients. Their Tg assay had a functional sensitivity of 0.2 ng/ml. In four of the 14 cases, Tg-supp was below 0.2 ng/ml; stimulated Tgs remained below 0.2 in two and were 0.9 and 1.7 ng/ml in the other two. Using a similar assay, Robbins *et al.* (19) reported four cases of recurrent disease with a low Tg-stim under 2.0 ng/ml, one of four cases being a patient with HCC with negative scan, in which the disease was diagnosed by fluorodeoxyglucose positron emission tomography. In a retrospective review of 278 patients with DTC, Klubo-Gwiezdzinska *et al.* (20) report a rate of potential recurrence similar to ours. In their study, of 11 patients with potential residual/recurrent thyroid cancer, one patient with FNA-proven neck disease had repeated Tg-stim below 0.5 ng/ml. It is important to mention, however, that the definition of recurrence varies greatly across studies, making a direct comparison of the results somewhat difficult. Most reports used Tg levels and/or imaging characteristics to define recurrence and included patients

TABLE 4. Sensitivity, specificity, PPV, and NPV values for Tg-stim cutoffs of more than 2.0 and 1.4, respectively, for US and ^{131}I using biopsy-proven disease as gold standard for recurrence among patients with Tg-supp below 0.1 ng/ml

Test	Tg stim >2.0 ng/ml (%)	Tg-stim ≥1.4 ng/ml (%)	US (%)	^{131}I scan (%)
Sensitivity	14 (1/7)	14 (1/8)	86 (6/7)	28 (2/7)
Specificity	98 (153/156)	97 (152/156)	92 (134/146)	93 (122/131)
PPV	25 (1/4)	20 (1/5)	33 (6/18)	18 (2/11)
NPV	96 (153/159)	96 (152/158)	99 (134/135)	96 (122/127)

with Tg-supp levels above 0.1 ng/ml. We required the additional evidence of a positive tissue diagnosis, because neck US, even when suspicious, resulted in a negative FNA in 12 of 18 patients, accounting for the low PPV. In addition, our results are restricted to patients with Tg-supp levels below 0.1 ng/ml.

Fourth, our study in a subset of patients who had repetitive rhTSH stimulation tests showed that if the initial Tg-stim was less than 0.1 ng/ml, there was a higher likelihood that follow-up stimulated Tg values would remain below 0.1 than if the initial Tg-stim was detectable. However, 38% of the patients whose initial Tg-stim was detectable had undetectable levels on repeat testing. In retrospect, it was felt that knowledge of the stimulated Tg value had no clinical impact in this cohort of patients whose Tg-supp is below 0.1 ng/ml.

Finally, our study emphasizes the critical importance of neck US in follow-up, carefully examining not only the thyroid bed and central compartment but lateral neck lymph node chains for evidence of recurrence. Suspicious findings should prompt an US-FNA. In seven of 163 patients (4.3%) with Tg-supp below 0.1 ng/ml, persistent disease was detected. However, in all but one, neck US readily identified abnormal tissue. The one exception was a high-risk patient with stage IV HCC. A second patient (not included in the study because he did not meet inclusion criteria) with a widely invasive HCC stage IVA had an undetectable Tg-stim and negative ^{131}I scans and CT scans postoperatively. He has recently developed biopsy-proven liver metastases and Tg-stim remains undetectable. Although our study does not address the optimal methods for high-risk patients, consideration should be given to periodic assessment with other methods in addition to serum Tg and neck US. Nevertheless, the latter two tests used together have been shown by others to confer a high NPV of 99–100% (13, 14, 16).

The significance of the small indeterminate pulmonary micronodules found on 16 of our patients is unclear. Lack of correlation with ^{131}I scan findings, and in the context of a Tg-supp less than 0.1 ng/ml, would point toward no clinical significance. Defining the best approach in follow-up of indeterminate lung nodules is the subject of several large studies in the United States and Europe currently underway. Partial data, however, suggest a high rate of false-positive results (95–98% in the North American National Lung Screening Trial) (21). Swensen *et al.* (22) found that even in patients at high risk for lung cancer (69% had indeterminate lung nodules), 99% were felt to be benign at follow-up.

A limitation to our study is represented by its retrospective design and therefore with the inherent inclusion of patients who had extensive history of disease before their evaluation here. Eighty of the 163 patients included in our study had their first endocrinology visit within 12 months from the initial surgery. This, however, reflects common clinical practice in a tertiary referral center and makes possible observing the course of the disease over a long period of time.

Our results and those of other recent reports in which sensitive Tg assays were used (10, 12–14) should be evaluated in the context of the revised American Thyroid Association management guidelines (7). Recommendation 45(a) states, "in low-risk patients . . . serum Tg should be measured after thyroxine withdrawal or rhTSH stimulation approximately 12 months after the ablation to verify absence of disease" (A rating). Although our patients were not all low risk and often tested at a more remote time from their initial therapy, we found that in the subset of patients with undetectable serum Tg (<0.1 ng/ml), an rhTSH test did not provide adequate additional information to identify or exclude disease. Recommendation 45(b) states that "low-risk patients who have had remnant ablation, negative cervical US, and undetectable TSH-stimulated Tg can be followed primarily with yearly clinical examination and Tg measurements on thyroid hormone replacement" (B rating). Although our findings do not clarify the frequency, we believe that periodic neck US (a relatively inexpensive test with no radiation exposure) remains an important tool for following patients, even when Tg-supp is below 0.1 ng/ml. As additional data emerge and Tg assays of even greater sensitivity are developed, the optimal approach to monitoring these patients should become more evident. Any type of Tg evaluation may miss recurrence in rare cases, and in low-risk patients, neck US can help detect recurrence with a reported diagnostic accuracy of 71.1% (23).

In conclusion, we believe that in almost all patients with DTC whose T_4-suppressed serum Tg is below 0.1 ng/ml, long-term monitoring with annual Tg-supp and periodic neck US are adequate. In our experience, the results of rhTSH testing do not impact or change management and therefore are not needed.

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