0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(4):1364–1369

doi: 10.1210/jc.2005-1705

The Diagnostic Value for Differentiated Thyroid Carcinoma Metastases of Thyroglobulin (Tg) Measurement in Washout Fluid from Fine-Needle Aspiration Biopsy of Neck Lymph Nodes Is Maintained in the Presence of Circulating Anti-Tg Antibodies

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Objective: Serum thyroglobulin (Tg) is the marker of differentiated thyroid carcinoma (DTC) after total thyroidectomy, but its value is limited by the interference of anti-Tg antibodies (TgAb). Detection of Tg in fine-needle aspiration biopsy (Tg-FNAB) washout fluid is used to identify neck DTC recurrences/metastases, but the interference of serum TgAb in this procedure is unknown.

Patients and Methods: Seventy-three patients (41 after surgery for thyroid cancer and 32 with thyroid nodules) evaluated for suspicious cervical lymph nodes were retrospectively reviewed. Tg was assayed by immunoradiometric assay or chemiluminescent assay in ultrasound-guided FNAB used for cytology. Serum TgAb were detected by passive agglutination or chemiluminescent assay. On the basis of preliminary data obtained in lymphadenitis, Tg-FNAB more than 36 ng/ml and more than 1.7 ng/ml (in the presence or absence of thyroid gland, respectively) was considered as indicative of metastasis.

Results: In 51 TgAb-negative patients, Tg-FNAB was positive in 15 (12 with malignant and three with nondiagnostic cytology), all with histologically confirmed DTC metastases. Of the remaining 36 patients with negative Tg-FNAB, 30 had nonsuspicious and six had suspicious cytology. Histology of the latter showed four undifferentiated thyroid cancer metastases and two lymphadenitis. In 22 TgAb-positive patients, Tg-FNAB was positive in 14 (12 with malignant and two with nondiagnostic cytology), all with histologically confirmed DTC metastases.

Conclusions: Clinical performance of Tg-FNAB appears to be not substantially affected by TgAb, and this procedure remains superior to cytology in the identification of DTC neck metastases. However, cytology should always be performed because, irrespective of TgAb, Tg is undetectable in FNAB from undifferentiated metastases. **(*J Clin Endocrinol Metab* 91: 1364–1369, 2006)**

THE FOLLOW-UP OF differentiated thyroid carcinoma (DTC) after total thyroidectomy and radioiodine ablation is based on three main diagnostic tools: basal and TSH-stimulated serum thyroglobulin (Tg) measurement, ^{131}I whole-body scan, and neck ultrasound (US). Serum Tg measurement is the most sensitive and specific marker of DTC (1). Elevated concentrations of serum Tg are associated with the presence of residual or metastatic thyroid tissue. Neck represents the main site of DTC recurrences, ranging from neck lymph nodes to thyroidal bed. Demonstration of metastatic neck lymph nodes is mandatory to submit to more radical surgery patients with proven metastatic DTC before thyroidectomy (2) and to optimize the surgical excision in those displaying cancer recurrence or metastasis. The diagnosis of nodal metastasis from DTC is often complex, because inflammatory lymphadenopathies are extremely frequent; furthermore, neck lymph nodes metastases from nonthyroidal cancers are a relatively common finding. Although US-guided fine-needle aspiration biopsy (FNAB) performed for cytological examination represents an essential tool in detecting cervical DTC metastases, this technique is limited by a 6–8% of false-negative results (3–7). Measurement of Tg in the washout of the needle used in FNAB of cervical lymph nodes (Tg-FNAB) has been proposed for the early detection of neck metastases after total thyroidectomy and radioiodine ablation for DTC (8) and, more recently, also to detect DTC lymph node metastases before thyroid surgery (9). It is well known that serum Tg is seriously affected by the presence of serum anti-Tg antibodies (TgAb), which may produce a complex interference in the serum Tg assay, ranging from false-positive to false-negative results (10–16), and this problem was not resolved even using immunoradiometric assays (IRMAs) using epitope-specific monoclonal anti-TgAb (14). The interference of TgAb in serum Tg assay is a common problem, because positive TgAb are detected with different techniques in up to 25–30% of patients with DTC (15, 17), and this interference has the potential to mask the presence of a recurrent or persistent thyroid carcinoma (18).

For the potential interference of serum TgAb, patients with positive TgAb have been systematically excluded from most of the previous studies using Tg-FNAB for the evaluation of DTC recurrences (9, 19). Quite recently, Baskin (20) did not find any difference between Tg-FNAB from DTC metastatic

First Published Online January 24, 2006

Abbreviations: CFDS, Color flow Doppler sonography; DTC, differentiated thyroid carcinoma; FNAB, fine-needle aspiration biopsy; IRMA, immunoradiometric assay; PTC, papillary thyroid carcinoma; Tg, thyroglobulin; TgAb, Tg antibodies; TgAb$^-$, TgAb negative; TgAb$^+$, TgAb positive; US, ultrasound.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

cervical lymph node of five TgAb-negative ($TgAb^-$) and two TgAb-positive ($TgAb^+$) patients, but the limited number of cases reported in this study prevents any general conclusion.

To evaluate the usefulness of Tg-FNAB in relation to the presence of serum TgAb, we retrospectively reviewed cytological diagnoses, serum Tg, serum TgAb, and Tg-FNAB obtained in a large number of $TgAb^+$ and $TgAb^-$ consecutive patients with cervical lymph nodes suspicious for DTC recurrences studied before and after thyroidectomy.

Patients and Methods

Patients studied

During a 4-yr period (from 2000 to 2004) a total of 73 consecutive patients with single or multiple suspicious cervical lymph nodes (41 of them on L-T_4 TSH-suppressive therapy after surgery for thyroid cancer and 32 with suspicious thyroid nodules before surgery) were referred to our outpatient clinic to exclude DTC neck metastasis. All patients (60 females, 13 males; female/male ratio, 4.6:1) were submitted to an accurate physical examination, neck US, and US-guided FNAB (for cytology and Tg-FNAB) of suspicious cervical lymph nodes. In all cases, serum Tg and TgAb were assayed using the procedures detailed below. Patients were then subdivided in two subgroups: 22 with detectable ($TgAb^+$) and 51 with undetectable ($TgAb^-$) serum TgAb.

Conventional and color flow Doppler sonography

US and color flow Doppler sonography (CFDS) of the neck was performed using an Acuson (Mountain View, CA) Sequoia until 2003 and subsequently a Sonoline G60S (Siemens Medical Solutions, Issaquah, WA) color Doppler system with a 8–13 mHz linear electronic transducers. The examination included first a conventional grayscale US, followed by CFDS; all cervical lumps were identified, localized, and counted, and their diameters were measured. The images were obtained by transverse and longitudinal planes scanning with hyperextended neck, allowing the visualization of the central compartment. All cervical lymph nodes submitted to US and US-guided FNAB were localized in the right and left side of the neck; in no case did we find suspicious lymph nodes of the central compartment. US-CFDS features suspicious for DTC neck metastasis included the following: round oval shape (short to long axis ratio, $\geq$0.7), hypoechoic, inhomogeneous pattern (including fluid areas), and/or intralesional punctate calcifications, with diffuse hypervascularity. Lymph node size ranged between 8 and 35 mm in maximum diameter. Patients with thyroid nodules had one or more US features suggestive for malignancy (hypoecogenicity, internal small calcifications, irregular limits, and increased blood flow) coexisting with suspicious cervical lymph nodes.

Thyroid function assays

Tg and TgAb assays were performed using commercial kits. Before 2003, Tg and TgAb were assayed by monoclonal antibody IRMA (Tg IRMA; CIS Bio International, Gif-sur-Yvette, France) and by passive agglutination (Serodia Fujiarebio, Tokyo, Japan), respectively. Minimum detectable Tg concentration by IRMA was 0.2 ng/ml, whereas TgAb were considered positive, according to the instructions of the manufacturer, when the titer was at least 1:100. After 2003, Tg and TgAb were detected by ultrasensitive chemiluminescent assay (Immulite 2000 Thyroglobulin and Immulite 2000 Anti-TgAb, respectively; Diagnostic Products Corporation, Los Angeles, CA). By these procedures, minimum detectable Tg amount was 0.5 ng/ml, and normal values for TgAb ranged between less than 20 to 40 IU/ml. As assessed by preliminary tests, TgAb interfered in a similar way in both Tg assays, producing marked underestimation of Tg concentrations.

Cytological examination and Tg assay in FNAB (Tg-FNAB)

Written informed consent was obtained from all patients before FNAB. US-guided FNAB was performed using 22- to 25-gauge needles attached to a 10-ml syringe, inserted to the lymph node under US visual control. The needle was repeatedly moved inside each lymph node, until the needle hub was filled with material. The smears (four to eight for each lymph node) were immediately fixed with Cytofix (Bioptica, Milan, Italy) and stained with hematoxylin-eosin. According to standard criteria, we subdivided our cytological results as follows: inadequate or not diagnostic, presence of blood cells without lymphocytes, plasma cells, and epithelial cells; reactive lymphadenitis, presence of lymphocytes and occasional plasma cells without epithelial cells; suspicious for DTC metastases, presence of atypical epithelial cells (with abnormal nuclear shape, nuclear enlargement, and nuclear polymorphisms) or sometimes cytological features of papillary thyroid carcinoma (PTC) (papillae and/or characteristic nuclear changes such as grooves and pseudoinclusions); and suspicious for poorly DTC metastases, presence of markedly pleomorphic atypical cells with frequent mitosis and cellular nests loosely cohesive with marked overlap.

After the smear preparation, the needle was washed out with 500 μl of the diluent provided by the Tg kit used to dilute the standard curve, and the solution was processed for Tg measurement. In 19 cases (eight $TgAb^+$ and 11 $TgAb^-$) TgAb was also assayed by chemiluminescent assay in FNAB washout fluid.

Cutoff values for Tg-FNAB

Because Tg may be detected in FNAB washout fluid from reactive nonmetastatic cervical lymph nodes in the presence of thyroid gland (8, 9), it was necessary to establish a value of Tg-FNAB above which the test was considered suspect for DTC metastases. To this purpose, we retrospectively analyzed the values of Tg-FNAB obtained from 38 reactive lymph nodes of 30 $TgAb^-$ and eight $TgAb^+$ patients with thyroid gland *in situ* (n = 20) or (n = 18) after total thyroidectomy. Data from $TgAb^+$ and $TgAb^-$ patients were analyzed together because of the low number of $TgAb^+$ patients. In 34 cases, lymph nodes were considered reactive on the basis of the cytological pattern and subsequent regression documented by ultrasonographic follow-up of 6–12 months. In addition, histological confirmation of the reactive lymph nodes was available in four patients submitted to thyroidectomy. In the group of patients with thyroid gland, we also included the results of Tg-FNAB obtained in three cases of lymphomas. Tg-FNAB in patients with thyroid gland was 5.9 $\pm$ 10.4 ng/ml (mean $\pm$ SD), with a range of less than 0.2 to 36 ng/ml. Because of the high variability and the relatively small size of the series, to reduce the probability of false-positive test, we selected to use as cutoff for positive Tg-FNAB the highest concentration of the range (>36 ng/ml). In patients without thyroid gland, Tg-FNAB was mostly undetectable, with a range of less than 0.2 to 1.7 ng/ml. Again, we considered as positive Tg-FNAB for thyroidectomized patients any Tg concentration more than 1.7 ng/ml.

Statistical analysis

The sensitivity and specificity for the correct identification of malignant lymph nodes were calculated by the Galen and Gambino formula (21).

Results

Among the 22 $TgAb^+$ patients, 12 showed cytological findings of DTC metastasis, two provided inadequate specimens, and eight displayed a pattern of reactive lymphadenitis. Among the 51 $TgAb^-$ patients, 18 showed cytological findings of metastases (14 well differentiated and four poorly differentiated), and 30 displayed a pattern of reactive lymphadenitis.

Tg-FNAB concentrations above the respective cutoffs were found in 14 of 22 $TgAb^+$ and 15 of 51 $TgAb^-$ patients. Lymphadenectomy with or without thyroidectomy was performed in a total of 35 patients showing a suspicious cytology, a Tg-FNAB greater than cutoff, or both. The main clinical and biochemical data of patients submitted to surgery for the presence of suspicious lymph nodes are listed together with cytological Tg-FNAB results in Table 1. The presence of metastatic lymph nodes was confirmed in 33 of 35 patients.

TABLE 1. Individual data of $TgAb^+$ and $TgAb^-$ patients with neck lymph nodes submitted to surgery

Patients (n)	Gender	Age (yr)	Thyroid presence	Serum TgAb (titer or IU/ml)	Serum Tg (ng/ml)	US size (mm)	Cytology	Tg-FNAB (ng/ml)	Histology
1	F	27	+	1,248		10	Pos	954	PTC
2	M	07	+	492	15.2	30	Pos	>3,000	PTC
3	F	56	+	264	102	15	Inad	256	PTC
4	F	26	−	1:25,600	0.2	12	Pos	767	PTC
5	F	43	−	1:400	0.09	10	Inad	326	PTC
6	F	35	−	1:6,400	02	8	Pos	566	PTC
7	F	62	−	1:102,400	0.02	11	Pos	785	PTC
8	F	69	−	214	1.35	18	Pos	>3,000	PTC
9	F	61	−	1:102,400	0.02	11	Pos	55	PTC
10	F	62	−	565	<0.5	13	Pos	112	PTC
11	F	34	−	1:1,600	0.01	16	Pos	385	PTC
12	F	63	−	534	<0.5	15	Pos	117	PTC
13	F	60	−	1,079	<0.5	20	Pos	>30,000	PTC
14	F	25	−	276	<0.5	18	Pos	>30,000	PTC
15	M	18	+	Neg		18	Pos	1,149	PTC
16	F	28	+	Neg	1.83	33	Pos	293	PTC
17	F	42	+	Neg		12	Pos	941	PTC
18	M	71	+	Neg		16	Pos	893	PTC
19	M	34	+	Neg		8	Inad	1,215	PTC
20	F	30	+	<20		15	Inad	1,294	PTC
21	F	35	+	<20		12	Pos	>3,000	PTC
22	F	30	−	Neg	1.5	15	Pos	1,204	PTC
23	F	22	−	Neg	0.31	12	Pos	806	PTC
24	F	23	−	<20	0.5	12	Pos	886	PTC
25	F	48	−	<20	16.8	16	Inad	>3,000	PTC
26	F	39	−	<20	1.99	8	Pos	49	PTC
27	F	60	−	<20	1.84	12	Pos	1195	PTC
28	M	23	−	<20	1.1	15	Pos	>30,000	PTC
29	F	28	−	<20	<0.5	12	Pos	6031	PTC
30	F	32	+	<20		8	Pos	0.08	PDTC
31	F	79	+	<20		17	Pos	0.62	AC
32	F	62	−	<20	30[a]	16	Pos	0.4	AC
33	M	65	−	<20	23[a]	18	Pos	<0.5	PDTC
34	F	50	−	Neg		6	Pos	0.12	Reactive
35	F	30	−	<20	0.01	15	Pos	0.17	Reactive

F, Female; M, male; Neg, negative; Pos, positive; Inad, inadequate; PDTC, metastases of poorly differentiated PTC; AC, metastases of anaplastic thyroid carcinoma.

[a] Test performed 2 wk after thyroidectomy before radioiodine ablation of residual tissue.

The only two cases showing benign lymph nodes had a cytological pattern suspicious for DTC metastases but undetectable Tg-FNAB (Table 1, patients 34 and 35). Tg-FNAB was found remarkably increased in all DTC metastases, independent of the presence of detectable TgAb. In particular, Tg-FNAB concentrations from metastatic lymph nodes in $TgAb^-$ patients ranged from 49 to more than 3000 ng/ml in thyroidectomized patients and from 293 to more than 3000 ng/ml in patients before thyroidectomy. Similar Tg-FNAB concentrations (55 to >3000 ng/ml in thyroidectomized patients and 256 to >3000 ng/ml in patients before thyroidectomy) were found in $TgAb^+$ patients. It should be noted that, when Tg-FNAB levels exceeded the highest measurable concentration for the assay, an additional dilution of the sample to calculate the precise Tg concentration was performed only in a minority of cases. Because of the retrospective nature of this study, we could not therefore perform any reliable comparison between the mean Tg-FNAB found in $TgAb^+$ and $TgAb^-$ patients. Tg-FNAB was undetectable in four lymph nodes (Table 1, patients 30–33) with clear metastatic cytology, and TgAb was undetectable by chemiluminescent assay. Histological examination displayed metastases from anaplastic thyroid carcinoma (2) and from poorly differentiated PTC (2), with Tg undetectable by immunohistochemistry in all cases.

Four of 20 patients (not included in Table 1) with both reactive cytology and Tg-FNAB lower than cutoffs (range of <0.5 to 22.9 ng/ml) were submitted to thyroidectomy and lymphadenectomy on the basis of the clinical picture and/or the results of thyroid cytology. Histological examination of the thyroid nodule revealed two cases of PTC, an atypical microfollicular adenoma, and an atypical hyperplastic nodular Hashimoto's thyroitis associated in all cases with a pattern of reactive lymph nodes. The remaining lymph nodes with negative Tg-FNAB/cytology (16 from patients with thyroid nodules and 18 from patients with DTC after total thyroidectomy) were followed by periodic US examination. In all cases, a progressive reduction of the lymph nodes was documented, further supporting their reactive nature.

In a limited number (n = 19) of recently performed FNAB, TgAb were also assayed together with Tg in FNAB washout fluid. TgAb was undetectable in 11 cases with negative serum TgAb. The results obtained in the eight patients with positive serum TgAb showed that TgAb were detected in FNAB washout fluid of two of eight (25%) patients (Table 2). As also shown in Table 2, Tg-FNAB concentrations in $TgAb^+$

TABLE 2. Tg and TgAb detected in serum and FNAB fluid from metastatic and reactive lymph nodes of $TgAb^+$ patients

Cases (n)[a]	Serum		FNAB fluid		Cytology	Histology
	Tg (ng/ml)	TgAb (IU/ml)	Tg (ng/ml)	TgAb (IU/ml)		
9	<0.1	1:102,400[c]	55	722	Pos	PTC
10	<0.5	565	112	162	Pos	PTC
12	<0.5	534	117	<20	Pos	PTC
2	15.2	492	>30,000	<20	Pos	PTC
13	<0.5	1,079	>30,000	<20	Pos	PTC
14	<0.5	276	>30,000	<20	Pos	PTC
[b]	<0.5	96	<0.5	<20	Neg	Reactive
[b]	<0.5	146	<0.5	<20	Neg	

Pos, Positive; Neg, negative for DTC metastasis.
[a] The numbers reported corresponding to those in Table 1.
[b] Patients not included in Table 1.
[c] Assay performed with passive agglutination.

FNAB washout fluids from metastatic lymph nodes were lower than those found in $TgAb^-$ FNAB washout fluids, suggesting an interference of TgAb in Tg determination. However, the concentration of Tg-FNAB remained clearly above the cutoffs even in the presence of detectable TgAb in FNAB washout fluids.

The comparison of cytological and histological results in all $TgAb^+$ and $TgAb^-$ patients included in this study is reported in Table 3. These data were used to calculate the sensitivity and specificity of Tg-FNAB and cytology in $TgAb^+$ and $TgAb^-$ patients. To this purpose, we considered true positive as any cytology or Tg-FNAB greater than cutoff and false negative as any inadequate cytology or Tg-FNAB lower than cutoff in histologically proven metastatic lymph nodes. As shown in Fig. 1A, Tg-FNAB reached 100% sensitivity and specificity in DTC metastases, irrespective of the presence or absence of serum TgAb, whereas cytology displayed a lower sensitivity. Somewhat surprisingly, serum Tg was slightly increased in two patients with anaplastic thyroid carcinoma (Table 1, patients 32 and 33). It should be noted, however, that the assay was performed shortly after (<2 wk) thyroidectomy and before radioiodine ablation. Serum Tg could therefore be expression of incomplete clearance of postoperative Tg peak (22, 23).

When all histological types of thyroid cancer metastases were considered, sensitivity of Tg-FNAB was lower (Fig. 1B), but this phenomenon was unrelated to serum TgAb and was exclusively a result of the undetectable Tg-FNAB in the four lymph nodes with anaplastic or poorly differentiated metastases.

Because TgAb were detected in our retrospective series by two methods that may substantially differ in sensitivity and specificity (17), we also separately calculated the clinical performance of Tg-FNAB in lymph nodes from patients with TgAb detected by passive agglutination (n = 37) or by chemiluminescent assay (n = 36). The results obtained showed no difference between the two groups, confirming a sensitivity and specificity of 100% for DTC metastases. In particular, TgAb were undetectable in all of the four cases with negative Tg-FNAB and positive cytology, excluding false-negative results attributable to the low level of TgAb possibly missed by passive agglutination.

Discussion

Because neck recurrence is a frequent finding at the onset or during the follow-up of thyroid cancer, many tools have been proposed to identify metastatic lymph nodes before or after surgery. Among these, US-guided FNAB, performed to obtain both cytology and Tg determination, is commonly considered the best available technique for the early diagnosis of DTC neck metastases (2, 8, 9, 19). As discussed in the introductory section, the interference of serum TgAb is undoubtedly the most serious technical problem that currently compromises the use of serum Tg as a marker for DTC recurrence (15, 17, 18, 24–26). The interference of TgAb has been considered previously as a potential limit of Tg-FNAB, and, accordingly, $TgAb^+$ patients have been excluded from recent series evaluating the clinical performance of this technique (9, 19). Surprisingly, however, scanty, if any, data have been reported so far on the value of Tg-FNAB of cervical lymph nodes in the presence of positive serum TgAb. Very preliminary data collected in a few patients suggest that Tg-FNAB is not affected by serum TgAb (20), but, to our knowledge, no extensive study on this problem was performed until now. In the present paper, we retrospectively

TABLE 3. Tg-FNAB results compared with cytological and histological data in $TgAb^+$ and $TgAb^-$ patients

Groups	Patients (n)			
	Tg-FNAB > cutoff (14)		Tg-FNAB < cutoff (8)	
$TgAb^+$ (22)	$Cyto^+$ (12) $Histo^+$ (12)	$Cyto^-$ (2) $Histo^+$ (2)	$Cyto^+$ (0)	$Cyto^-$ (8)
	Tg-FNAB > cutoff (15)		Tg-FNAB < cutoff (36)	
$TgAb^-$ (51)	$Cyto^+$ (12) $Histo^+$ (12)	$Cyto^-$ (3) $Histo^+$ (3)	$Cyto^+$ (6) $Histo^+$ (4)[a]; $Histo^-$ (2)	$Cyto^-$ (30)

Cyto, Cytology; Histo, Histology; +, positive for malignancy; −, negative for malignancy.
[a] Anaplastic metastases or poorly differentiated PTC metastases.

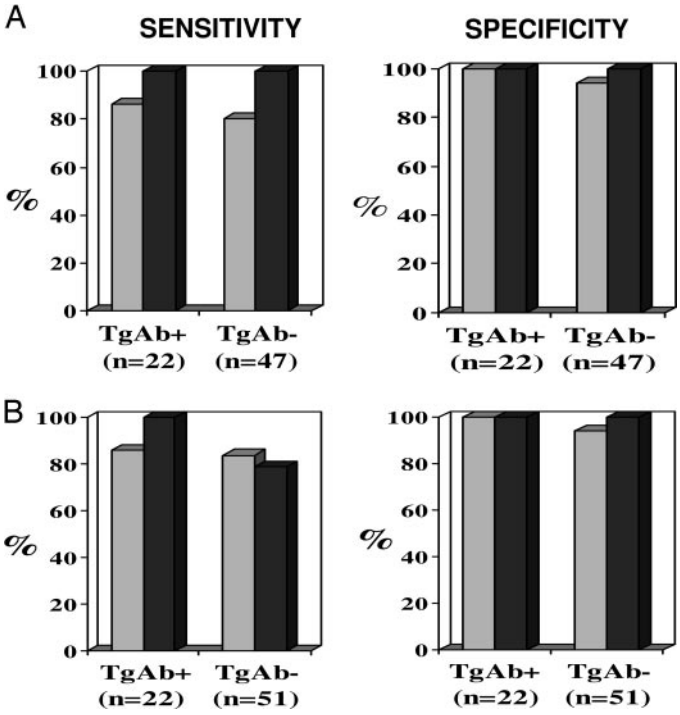

FIG. 1. Sensitivity and specificity of Tg-FNAB (*black squares*) and cytology (*gray squares*) in identifying thyroid cancer metastases in cervical lymph nodes in $TgAb^+$ and $TgAb^-$ patients. A, Analysis performed only in DTC metastases. B, Analysis performed in all histological types.

compared clinical data from a large number of consecutive patients submitted to a diagnostic evaluation for suspicious lymph nodes in relation to the presence or absence of serum TgAb. We found high Tg-FNAB concentrations in all histologically proven DTC metastases, independent of serum TgAb (including high Ab titers). In particular, Tg-FNAB reached 100% sensitivity and specificity in both $TgAb^+$ and $TgAb^-$ when only DTC metastases were considered. Because only a minority of Tg-FNAB were appropriately diluted to calculate the precise Tg concentration, we were unable to perform a reliable comparison between the mean Tg-FNAB levels in $TgAb^+$ and $TgAb^-$ patients. Thus, it is quite possible that some degree of underestimation of Tg-FNAB concentration may occur in the presence of circulating TgAb. In keeping with this concept are the preliminary results obtained in a limited series of FNAB washout fluid in which TgAb were assayed in parallel to Tg. These results showed that TgAb were indeed detectable in FNAB washout fluids, although this phenomenon was evident only in two of eight (25%) cases, both with high serum TgAb titers. The presence of TgAb in FNAB washout fluids could be attributable to either blood contamination or active lymph node synthesis (27). Tg-FNAB concentrations were clearly lower in the two cases with TgAb detectable in FNAB washout fluid, suggesting that TgAb in FNAB may actually interfere in Tg detection. However, this interference appears to have a very small effect on the clinical performance of the test, because Tg-FNAB levels found in $TgAb^+$ washout fluids were in any case well above the cutoff. Thus, although we cannot exclude that in some cases the concentration of TgAb in FNAB washout fluid may fully prevent the detection of Tg from metastatic lymph nodes, this phenomenon, not observed in the present large series, is probably very rare. The most probable explanation is that the exceedingly elevated Tg concentration in positive Tg-FNAB is able to saturate TgAb binding sites. Finally, it should be noted that the presence of serum TgAb did not affect the sensitivity and specificity of Tg-FNAB even in patients examined with the thyroid gland *in situ*. Indeed, low Tg-FNAB concentrations are often found in nonmetastatic lymph nodes from patients with thyroid nodules and increased serum Tg as expression of Tg contamination from the peripheral blood during FNAB. This potential source of bias was solved in previous studies (8, 9, 19) by the adoption of appropriate cutoff values in relation to the presence or absence of thyroid gland in $TgAb^-$ patients; the same methodological approach was successfully used by us also in $TgAb^+$ patients. In addition, to establish a cutoff level for Tg-FNAB, it is also recommended to always assay Tg-FNAB in parallel with serum Tg. Unfortunately, in the first cases of our series, serum Tg was not always assayed in patients with thyroid gland *in situ*, and, because of the retrospective nature of the present study, serum was not more available for subsequent assay.

Together, our data extend to $TgAb^+$ patients the conclusion of previous studies performed only in $TgAb^-$ cases, *i.e.* that Tg-FNAB is more sensitive and specific than cytology in detecting neck DTC metastases (8, 9). Conversely, our study shows that the only source of false-negative Tg-FNAB is represented by anaplastic or very undifferentiated PTC metastases, unable to synthesize/release significant amounts of Tg (19). These results are apparently in contrast with those reported by Cignarelli *et al.* (28) who found increased Tg-FNAB in three lymph nodes with poorly differentiated PTC metastases. However, in our series, two of the four undetectable Tg-FNAB metastatic lymph nodes were from anaplastic thyroid tumors, and the other two were from very undifferentiated PTC (in all cases, Tg was undetectable by immunohistochemistry in the metastatic tissue). Differences in the quantitative Tg expression may explain the difference between our data and that reported by Cignarelli *et al.* (28). In any case, all anaplastic or undifferentiated PTC metastases with undetectable Tg-FNAB were correctly identified by cytology: thus, we could fully confirm the previous recommendation to use combined cytology and Tg-FNAB rather than either technique alone to detect any histological type of thyroid cancer metastases (8, 19, 29).

In conclusion, our study provides the first demonstration that the diagnostic accuracy of Tg-FNAB as a marker of DTC recurrence in cervical lymph nodes is highly reliable even in the presence of detectable serum TgAb and confirms that cytological examination associated with Tg-FNAB should be considered the most suitable method for the early diagnosis of thyroid cancer metastases.

Acknowledgments

Received July 29, 2005. Accepted January 17, 2006.

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This work was partially supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (Rome, Italy) and by funds of Regione Autonoma Sardegna to the Centro Studio per la Prevenzione e Terapia delle Malattie della Tiroide.

F.B., G.B., F.A., M.L.L., G.F., and S.M. have nothing to declare.

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