

Diagnostic value of thyroglobulin measurement in fine-needle aspiration biopsy for detecting metastatic lymph nodes in patients with papillary thyroid carcinoma

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Abstract

Purpose We aimed to compare the diagnostic value of fine-needle aspiration cytology (FNAC) and fine-needle aspiration thyroglobulin measurements (FNA-Tg) for detecting cervical lymph node metastases from differentiated thyroid carcinomas. **Methods** This prospective study included 225 patients with neck node metastases or recurrences of papillary thyroid carcinoma. From the 225 patients, 255 lymph nodes were evaluated by FNAC and FNA-Tg. Final diagnoses confirmed by histological examination were compared to preoperative FNAC and FNA-Tg results. **Results** FNAC correctly diagnosed 212 metastatic lymph nodes but failed to diagnose 43 of them. FNA-Tg correctly diagnosed 253 metastatic lymph nodes but failed to diagnose two of them. FNA-Tg levels showed 100% sensitivity, 96%

specificity, 99% diagnostic accuracy, a 99% positive predictive value (PPV) and a 100% negative predictive value (NPV) with a threshold level of FNA-Tg with a diagnostic accuracy 28.5 ng/ml. The specificity, diagnostic accuracy, PPV and NPV of FNA-Tg were significantly higher than those of FNAC. **Conclusion** FNA-Tg measurement can be performed safely for the detection of lymph node metastasis in patients with differentiated thyroid carcinomas.

Keywords Differentiated thyroid carcinoma · Fine-needle aspiration cytology · Fine-needle aspiration thyroglobulin measurement

Introduction

Papillary thyroid carcinoma is the most common malignant thyroid tumour. Although the chances of long-term survival are usually favourable, many patients have metastatic lymph nodes that are found before or after the initial surgery [1, 2]. Data from centres where routine central lymph node dissection or sentinel lymph node biopsy is practised showed that occult metastases may be observed in up to 90% of patients. About 5–20% of patients develop local or regional recurrences after initial surgery [3, 4].

Because lymph node metastasis 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, measurement of basal and stimulated serum thyroglobulin levels, neck ultrasonography (US), fine-needle aspiration biopsy (FNAB) with cytological analysis and fine-needle aspiration thyroglobulin measurements (FNA-Tg) are commonly

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used for the early diagnosis of differentiated thyroid carcinoma neck metastases [1–4].

The FNA-Tg was initially proposed in 1992 by Pacini et al. [5] for the early detection of neck lymph node metastases in patients with papillary thyroid carcinoma (PTC). They found that FNA-Tg was 100% sensitive in detecting lymph node metastasis in patients who had been treated previously, whereas cytology alone was only 85% sensitive. Several studies have reported that FNA-Tg is more sensitive than fine-needle aspiration cytology (FNAC) for detecting metastasis and that the sensitivity of FNAC is increased when combined with FNA-Tg [6–12]. However, the diagnostic FNA-Tg threshold value has not yet been well established. The aim of this prospective clinical study was to compare the diagnostic value of FNAC and FNA-Tg for detecting cervical lymph node metastases from differentiated thyroid carcinomas.

Materials and methods

Patients

Two hundred fifty-four consecutive patients with suspicious cervical lymph nodes were evaluated at the Department of General Surgery, Istanbul Medical Faculty, between April 2007 and April 2010. The diagnosis of the central or lateral lymph node metastasis was confirmed preoperatively by fine-needle aspiration cytology or FNA-Tg under ultrasound guidance. FNA-Tg levels greater than 1 ng/ml were considered positive, and surgical treatment was performed (nine). Twenty-nine lymph nodes in 29 patients with FNA-Tg levels less than 1 ng/ml were excluded because there was no subsequent surgical excision. Thus, in all, 225 patients and 255 lymph nodes were evaluated in this prospective study. Of the 225 patients, 44 (19.5%) had previously undergone total thyroidectomy, and 36 (16%) had previously undergone total thyroidectomy and central or lateral neck dissection. One hundred forty-five patients (64.5%) had a suspicious or positive (papillary carcinoma) result from FNAC. All patients had papillary carcinoma, and other types of carcinoma (e.g. follicular, anaplastic and poorly differentiated) were excluded from this study. The study plan was reviewed and approved by our institutional ethics committee, and informed consent was obtained from all patients.

FNAC and FNA-Tg

Neck US examinations were performed by the same skilled operator with a digital US scanner equipped with an 11.4-MHz linear transducer (Sonoline Antares, Siemens, Erlangen, Germany) for evaluation of the lymph nodes.

FNAB was also performed for suspected lymph nodes when US indicated suspicious findings (i.e. an axial diameter more than 6 mm, irregular borders and microcalcifications). During the procedure, the patient was kept in the supine position with a slight hyperextension of the neck. Local anaesthetic was routinely applied. Once the needle was introduced into the lymph node, 3–5 ml of negative syringe pressure was applied. After aspiration, the smears were placed on slides and dried. One to three slides from each patient were stained with haematoxylin and eosin. The same needle and syringe were rinsed with 3 ml of normal saline, and the washout was submitted for Tg analysis (FNA-Tg; Figs. 1, 2 and 3). The FNAC results were interpreted by one cytopathologist, who specialised in thyroid cytology.

Biochemical analysis

Tg was assayed with a monoclonal antibody immunoradiometric assay (IRMA; CIS Bio International, Gif-sur-Yvette, France).

Evaluation of the diagnostic value

The numbers of true-positive (TP), true-negative (TN), false-positive (FP) and false-negative (FN) results were calculated. The suspicious/malignant FNAC and positive FNA-Tg results were considered to be TP in cases where histological examination revealed a malignancy, and the results were considered to be FP when no malignancy was found. The benign FNAC and negative FNA-Tg results were considered to be TN if the histological findings were benign and were considered to be FN for cases of histologically proven malignancy. The sensitivity, specificity,

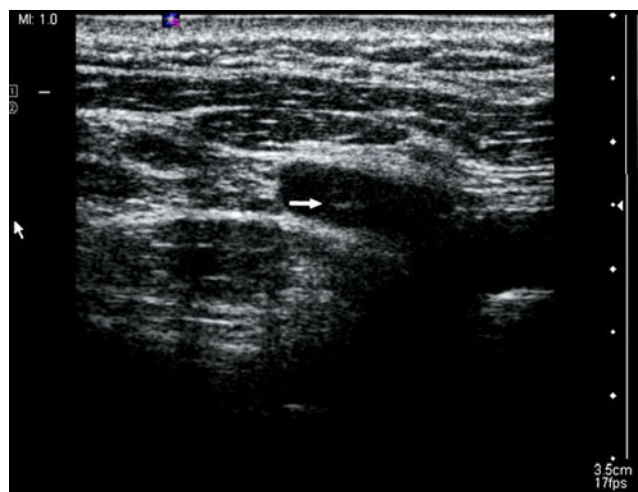


Fig. 1 Thyroglobulin washout procedure. A 22-G needle (*arrow*) is inserted inside an ovoid, hypoechoic lymph node and it is being aspirated to obtain a small sample



Fig. 2 A suspicious lymph node in a patient with papillary thyroid carcinoma. The node is almost completely cystic with few septations and microcalcific foci

diagnostic accuracy, positive predictive value (PPV) and negative predictive value (NPV) of FNAC and FNA-Tg were calculated. Sensitivity, specificity and accuracy were determined according to the following formulas:

- $PPV (\%) = TP / TP + FP \times 100$
- $NPV (\%) = TN / TN + FN \times 100$
- $Sensitivity (\%) = TP / TP + FN \times 100$
- $Specificity (\%) = TN / TN + FP \times 100$
- $Accuracy (\%) = TP + TN / TP + TN + FP + FN \times 100$

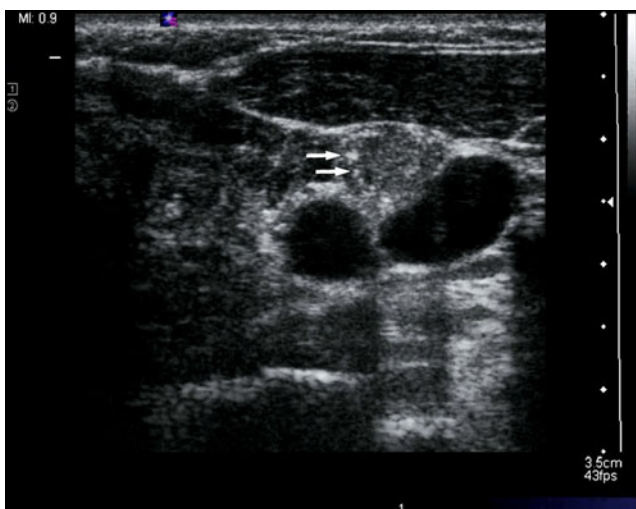


Fig. 3 A suspicious solid lymph node with microcalcifications (arrows) in a patient with papillary thyroid carcinoma

Statistical analysis

Data were analysed using SPSS 11.0 for Windows. The results were expressed as the mean \pm SD. Comparisons of the data were carried out using chi-square tests. ROC analysis was used to identify the cut-off value for the FNA-Tg level. The results were considered statistically significant when the two-tailed *p* value was less than 0.05.

Results

The mean age of the patients was 44.8 ± 11 years (range, 21–72 years). The female/male ratio was 2.7/1 ($n=165/60$). There were no complications related to the FNAC or the FNA-Tg measurement. Of the 225 patients, 145 underwent total thyroidectomy and central and/or lateral neck dissection, and 80 (44%) underwent metastatic lymph node(s) excision.

Fine-needle aspiration cytology vs. final pathology

Based on preoperative evaluation with FNAC, 223 lymph nodes were positive, and the remaining 32 were negative for metastasis. Based on the final pathology, 200 lymph nodes were positive, and the remaining 55 were negative for metastasis. FNAC correctly diagnosed 212 metastatic lymph nodes but failed to diagnose 43 of them. The cell blocks corresponding to false-negative and false-positive FNAC results were re-evaluated, but the results did not change, and malignant cells were not seen. The sensitivity, specificity and diagnostic accuracy values of FNAC for the preoperative diagnosis of thyroid carcinoma were 95%, 40% and 83%, respectively. The PPV and the NPV were found to be 85% and 68%, respectively (Table 1).

Fine-needle aspiration-Tg measurement vs. final pathology

The value of FNA-Tg ranged from 450 to 5,000 ng/ml in metastatic lymph nodes (mean $3,606 \pm 1,854$ ng/ml) and from 2 to 32 ng/ml in nonmetastatic lymph nodes (mean 9.9 ± 7.8 ng/ml). When the cut-off value of FNA-Tg was 1 ng/ml, FNA-Tg correctly diagnosed 200 metastatic lymph nodes but FNA-Tg failed to diagnose 55 of them. The sensitivity, specificity and diagnostic accuracy values of FNA-Tg (cut-off=1 ng/ml) for the preoperative diagnosis of thyroid carcinoma were 100%, 0% and 78%, respectively. The PPV and the NPV were found to be 78% and 0%, respectively.

The cut-off value derived from the ROC curves for the best sensitivity and specificity was 28.5 ng/ml for the FNA-Tg level. FNA-Tg correctly diagnosed 253 metastatic lymph nodes but failed to diagnose two of them. The

Table 1 Evaluation of metastatic lymph nodes according to the diagnostic modality

	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
FNAC	95	40	85	68	83
FNA-Tg (1 ng/ml)	100	0*	78*	0*	78*
FNA-Tg (28.5 ng/ml)	100	96*	99*	100*	99*

* $p < 0.05$ compared with FNAC

sensitivity, specificity and diagnostic accuracy values of FNA-Tg (cut-off=28.5 ng/ml) for the preoperative diagnosis of thyroid carcinoma were 100%, 96% and 99%, respectively. The PPV and the NPV were found to be 99% and 100%, respectively.

Discussion

We compared the diagnostic values of fine-needle aspiration cytology and fine-needle aspiration thyroglobulin measurement. Seventeen percent of metastatic lymph nodes were not diagnosed by FNAC, whereas only 1% of metastatic lymph nodes were not diagnosed by FNA-Tg (cut-off value=28.5 ng/ml). The specificity, diagnostic accuracy, PPV and NPV of FNA-Tg were significantly higher than of the corresponding values for FNAC.

Lymph node metastases are common in patients with PTC, with an incidence as high as 50%. Many patients having clinically negative nodes on preoperative evaluation are later found to have central lymph node metastases [1, 2]. Clinical examination may detect lymph node involvement in 15–30% of patients. However, data from centres where routine central lymph node dissection or sentinel lymph node biopsy are practised showed that occult metastases may be observed in up to 90% of patients [3, 4]. Although the prognosis of PTC is usually favourable, patients are at risk of lymph node metastases during the follow-up period. About 5–20% of patients develop local or regional recurrences after the initial surgery. Approximately two-thirds of PTC recurrences occur in the cervical region, with most of them in the loco-regional lymph nodes [1–4].

Different tools have been proposed to identify metastatic lymph nodes at the onset or during the follow-up of thyroid cancer. Among these, whole body scanning with radioiodine; measurement of basal and stimulated serum thyroglobulin levels and high-resolution US, FNAC and FNA-Tg are the most efficient. However, none of these tools are perfect, and all of them have limitations [1, 2, 12, 13].

Tg is a glycoprotein produced only by thyroid follicular cells, and Tg is produced by almost all differentiated thyroid cancer tissues. Serum thyroglobulin determination plays a central role, in combination with neck US, in the follow-up of thyroid cancer patients. Following total thyroidectomy for thyroid cancer, serum Tg should be undetectable, and any detectable serum Tg indicates neoplastic thyroid foci [14,

15]. The highest sensitivity is attained following thyroid hormone withdrawal or stimulation with recombinant human thyroid-stimulating hormone. Tg is undetectable in 20% of patients with isolated lymph node metastases during thyroid hormone treatment and in 5% after thyroid hormone withdrawal. The presence of anti-thyroglobulin antibodies, which occur in approximately 25% of thyroid cancer patients, will induce false-negative results. The measurement of Tg does not localise neoplastic foci [14, 15].

Some authors advocate monitoring stimulated Tg and whole body scanning for the detection of metastatic lymph nodes. However, the results of these tests can be affected by a number of variables, including the dose and capability of trapping radiotracers and tumour size. For example, additional metastatic foci have been reported in 10–26% of patients scanned following high-dose radioiodine treatment when no foci were detected by the diagnostic scan [16].

US and US-guided fine-needle aspiration cytology are the most useful techniques for diagnosing nodal metastases. Sonographic features suggestive of abnormal metastatic lymph nodes include the loss of the fatty hilus, a rounded rather than oval shape, hypoechogenicity, cystic changes, calcifications and peripheral vascularity. No single sonographic feature is adequately sensitive for the detection of metastatic lymph nodes [1, 2, 17, 18]. Confirmation of malignancy in lymph nodes with a suspicious sonographic appearance is achieved by FNAC. False-negative FNAC results are unavoidable and occur in 6–8% of cases [6, 19]. These false negatives could be due to the absence of tumour cells in the FNAB specimen, cystic changes in metastatic lymph nodes or partial or focal involvement of the lymph node.

In 1992, Pacini et al. [5] first reported the use of Tg measurements in FNAB specimens. These authors concluded that FNA-Tg had a better negative predictive value than cytology alone. Several studies have reported that FNA-Tg identifies PTC metastases of the neck with higher sensitivity and specificity than FNAC. FNA measurement of Tg is valid even in patients with circulating anti-thyroglobulin antibodies. In our study, the specificity, PPV, NPV and diagnostic accuracy values of FNA-Tg were significantly higher than those of FNAC.

However, there is no established diagnostic cut-off value. Most past studies used different threshold values. Some studies used the highest Tg concentration measured in patients with reactive lymph nodes, the functional sensitivity of the assay or the serum Tg level [6–10]. In

some studies, cut-off values derived from the original study itself were used [11, 12]. In our study, we arbitrarily selected 1 ng/mL as a cut-off value, and levels greater than 1 ng/mL were accepted as an indication for surgery. When we used FNA-Tg was 1 ng/ml, the NPV was 0%. The range values of nonmetastatic lymph nodes were 2–32 ng/ml. The specificity, PPV, NPV and diagnostic accuracy values of FNA-Tg were significantly lower than those of the FNAC. The best sensitivity and specificity was observed when a cut-off value of 28.5 ng/ml was used for FNA-Tg. Our study-derived cut-off value showed higher sensitivity, specificity, diagnostic accuracy, PPV and NPV than previous studies. Further studies are needed to determine the optimal cut-off value in patients with or without previous thyroidectomy.

In conclusion, there is no established diagnostic cut-off value of FNA-Tg, and most past studies used different threshold values. After an exact diagnostic cut-off value has been determined, FNA-Tg may be considered as an alternative to FNAC. FNA-Tg is the best available technique for the early diagnosis of differentiated thyroid carcinoma metastases because it is inexpensive, easy to perform, not influenced by problems related to FNAC, reliable even in very small lesions, not affected by Tg antibodies and has a high diagnostic accuracy.

Conflicts of interest None.

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