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Lymph node thyroglobulin in the diagnosis of metastases of thyroid carcinoma with the thyroid in situ: A prospective intraoperative study

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Abstract

Background

The use of thyroglobulin concentration in washout fluid of fine-needle aspiration (FNA-Tg) is a procedure advocated by international guidelines to diagnose metastatic LN in papillary thyroid cancer. With the increasing use of active follow-up or lobectomy alone for low-risk thyroid cancers, the determination of the diagnostic performance of FNA-Tg in the detection of metastatic PTC when the thyroid is in situ is paramount.

Materials and methods

Prospective study with measurement of Tg in washout fluid obtained from intraoperative fine needle aspiration (FNA) cytology in order to avoid contamination from thyroid tissue and rigorously isolated punctured nodes. Receiver-operating characteristic (ROC) curve and area under the curve (AUC), optimal threshold to discriminate benign and malignant LN, sensitivity and specificity were provided.

Results: a total of 58 lymph nodes from 32 patients were analyzed. ROC analysis defined the optimal cutoff values of FNA-Tg at 60 ng/ml for the diagnosis of malignant LNs in patients with a thyroid in situ. Sensitivity and specificity were 75% (95% confidence interval 57.89–86.75) and 87.5% (95%CI: 69–95.66), respectively.

Conclusion

Our results support the hypothesis that the Tg-FNA threshold for a safe diagnosis of LN metastasis in PTC is higher in presence of a thyroid gland in situ. The use of lower thresholds could result in false positive results and lead to unnecessary surgery.

Keywords

Thyroid cancer

1. Introduction

Papillary thyroid cancer (PTC) is the commonest form of thyroid cancer and is characterized by frequent spread to cervical lymph nodes. Persistent and recurrent lymph node (LN) disease occurs primarily in patients where the disease at presentation has been underestimated with suboptimal initial surgery or in whom extensive nodal involvement (high metastatic LN ratio and/or presence of extracapsular extension) is present. In PTC patients with elevated serum thyroglobulin (Tg) levels after initial management, neck ultrasound (US) is highly sensitive and specific for the detection of regional lymphadenopathy. It is of fundamental importance to distinguish LN metastasis from benign reactive LN enlargement in order to avoid unnecessary intervention and the associated risks of morbidity. Whilst fine-needle aspiration cytology (FNAC) accurately complements neck US, its sensitivity varies from 75 to 85% with a high rate of nondiagnostic aspirates [1,2]. The measurement of needle aspirate washout (FNA-Tg) have been reported to increase the sensitivity of FNA cytology alone in identifying LN metastases from differentiated thyroid cancer (DTC), especially for small LN [[3], [4], [5]] and it is a recommendation found in both the American and European Thyroid Association Guidelines [[6], [7], [8]]. In the 2015 ATA guidelines, a FNA-Tg concentration above 10 ng/ml is considered the threshold of highly suspicious of malignancy [6]. There are however potential confounders in the FNA-Tg, including the presence of thyroid tissue in situ at the time of the FNA [5]. With the increasing use of active follow-up or lobectomy alone for low-risk thyroid cancers, the determination of the optimal diagnostic threshold for FNA-Tg that confirms metastatic LN from PTC has assumed even greater importance. The purpose of this study is to address the potential discordance between the FNA-Tg and final pathological report in suspected metastatic cervical LN from PTC. The study took the form of a prospective analysis of intraoperative aspiration of cervical nodes for Tg and the comparison of the Tg values with the histological analysis. A head-to-head comparison between FNA-Tg and final diagnosis in each individual nodes was performed with estimation of the optimal FNA-Tg cutoff value for determining malignancy when the thyroid is still all or partially in situ.

2. Methods

2.1. Population

The inclusion criteria were: age ≥ 18 years, patients undergoing primary total thyroidectomy for papillary thyroid carcinoma (PTC) or benign disorder or reoperation for metastatic LN. All patients gave their signed informed consent for participation in the study. The exclusion criteria were presence of distant metastases.

The study was approved by the local ethics committee (Comité de Protection des Personnes Sud-Méditerranée II). The study had been registered with [ClinicalTrials.gov](https://clinicaltrials.gov) (identifier: NCT02602717).

2.2. Study design

This was a prospective single centre study with consecutive patients included based on the aforementioned inclusion and exclusion criteria. All patients were investigated for preoperative serum Tg, TSH and Tg antibody (TgAb).

Assessment of Tg in the washout fluid was obtained intraoperatively according to the follow-up protocol:

1

At the initial surgery performed for cytologically proven PTC but without suspected LN disease on neck US or surgery for benign thyroid disease, FNAC precedes thyroidectomy and is performed on an identified lateral LN (near the area of dissection of the carotid sheath for the implantation of a vagus nerve stimulator for intraoperative neuromonitoring) and if present an identifiable central LN.

2

At the initial surgery for PTC with suspected LN on neck US, FNAC precedes thyroidectomy and is performed on at least one macroscopically abnormal LN and if possible on one apparently normal LN.

3

At reoperative surgery for PTC, FNAC is performed on at least one abnormal LN and if possible one macroscopically normal LN.

Fnac was always performed by an experienced surgeon on isolated lymph node in order to avoid its contact with thyroid parenchyma and thus possible aspiration from the thyroid gland.

All aspirated LN and their respective samples were rigorously isolated in appropriately labelled boxes describing the LN and its precise location. Aspiration samples were subsequently dispersed onto slides (2–3 slides/node) and fixed by air-drying. The same needles were rinsed with 1 ml of normal saline stored at room temperature, and the washout was submitted for FNA-Tg measurement.

2.3. Gold standard

Diagnosis of malignancy and benignity was based histological examination of surgically resected LNs. The analysis was performed by a trained pathologist (WE) who was blinded to the Tg values.

2.4. Biochemical analysis

Tg was assayed with an immunoradiometric assay (RIA Tg-plus; BRAHMS GmbH, Hennigsdorf, Germany). The analytical sensitivity read at the optimal curve is 0.08 ng/mL, and the functional assay sensitivity (20% interassay coefficient of variation) is 0.2 ng/mL. For comparison with CRM-457-calibrated assay, the values should be multiplied by 1.92 (according to the manufacturer's recommendation). TSH was assayed with an immunoradiometric assay (Elecsys ECLIA assay; Roche Diagnostics). Anti-Tg antibody (TgAb) was assayed with RIA (anti-Tg_n; BRAHMS GmbH). The analytical sensitivity is 5.5 U/mL, and the functional assay sensitivity is < 20 U/mL.

2.5. Data analysis

To determine the performance of FNA-Tg for the diagnosis of LNs malignancy, Receiver-operating characteristic (ROC) curve and area under the curve (AUC) estimates. The optimal threshold to better discriminate between benign and malignant LNs was calculated. Sensitivity (Se), specificity (Sp), and accuracy were provided with their 95% confidence intervals (95%CI). Negative and positive predictive values (NPV and PPV) were only provided for information, Statistical analyses were performed with SPSS software (version 18.0; SPSS, Chicago, Illinois). Data with a P value < 0.05 were considered significant.

3. Results

3.1. Patients' characteristics

Thirty-two patients were included in the study, 27 with thyroid cancer (25 at presentation, 2 with recurrence) on pathological analysis and the remaining 5 cases had benign tumors (follicular adenoma). Table 1 summarizes the patient and primary tumors characteristics and preoperative baseline serum Tg and Tg antibodies.

Table 1. Patient characteristics according to the final diagnosis of pathology thyroid. IQR Interquartile Range.

Empty Cell	Thyroid cancers	Benign tumors
Patients, n	27	5
Sex (women)	25 (92.6%)	4(80%)
Age, years Median[IQR]	39.4 [26.7–51.1]	61.9 [54.5–69.1]
LN analyzed/patients Median	2	2
Histology of the thyroid		
Classical PTC, n	9 (33.3%)	0
Follicular PTC variant, n	16 (59.2%)	0
Follicular adenoma, n	0	5 (100%)
Other variants of PTC, n	2 (7.5%)	0
Preop serum TSH (mU/L), Median[IQR]	1.8 [1.2–2.3]	1.3 [0.6–2.5]
Preop serum Tg (ng/ml) Median[IQR]	18 [8.2–38.2]	16 [8–17]
Preop serum Tg-Ab (U/L), Median[IQR]	19.5 [14.3–225.5]	12 [9.54.5]

3.2. Optimal cutoff values of FNA-Tg in determining LNs malignancy

A total of 58 lymph nodes (LN) were analyzed with comparisons of FNA-Tg and pathological analysis for each individual nodes. For 54 LN, FNA was performed at initial surgery prior to thyroidectomy: 46 in patients operated for thyroid cancer group and the latter 8 nodes in patients operated for benign disorder. In malignant (n = 34) and benign (n = 24) LNs, median [Interquartile, IQR] FNA-Tg were 629.5 [47.5–1503.5] and 1.5[0–28], respectively (p=<0.001). The optimal cutoff values of FNA-Tg in determining malignant LNs from ROC analysis was 60 ng/ml with sensitivity and specificity of 75% (95%CI: 57.89–86.75) and 87.5% (95%CI: 69–95.66), respectively. When evaluating Tg-FNA in the thyroid cancer group only, the cutoff value of FNA-Tg was 34 ng/ml with sensitivity and specificity of

78.1% (95%CI: 61.24, 88.98) and 87.5% (95%CI: 64.15, 93.32), respectively (Fig. 1). All the details are provided in Table 2.

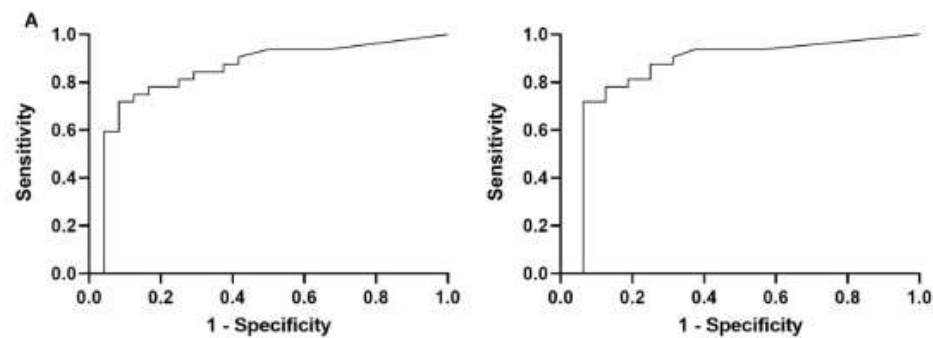


Fig. 1. ROC curves for FNA-Tg in the diagnosis of malignant LN
A. All cases. B. Thyroid cancer patients only.

Table 2. FNA-Tg in determining LNs malignancy
AUC area under curve; Se sensitivity; Sp specificity; PPV positive predictive value; NPV negative predictive value; 95% CI 95% confidence interval.

Empty Cell	All cases	Thyroid cancers
AUC (95% CI)	0.85 (0.74–0.96)	0.86 (0.72–0.99)
Se (95% CI)	0.75 (0.58–0.87)	0.78 (0.61–0.88)
Sp (95% CI)	0.88 (0.70–0.96)	0.83 (0.64–0.93)
PPV (95% CI)	0.89 (0.72–0.96)	0.86 (0.69–0.94)
NPV (95% CI)	0.72 (0.54–0.85)	0.74 (0.55–0.86)
Accuracy (95% CI)	0.80 (0.68–0.89)	0.80 (0.68–0.88)

4. Discussion

The use of Tg concentration in the washout fluid from fine-needle aspiration (FNA-Tg) of cervical LN in thyroid cancer is an established technique of diagnosing LN metastases. However, there is much heterogeneity across studies that may influence the values of FNA-Tg found (e.g., serum TSH and Tg, presence of a thyroid gland in situ, gold standard, sampling bias, Tg assays etc.) [5]. In the largest study from Moon et al. which included 528 LNs (190 metastatic, 338 benign), the optimal cutoff value of FNA-Tg in distinguishing malignant LNs from benign LNs was 1.0 ng/mL with high sensitivity and specificity, 93.2% and 95.9% respectively [9]. The cutoff value of FNA-Tg was 2.24 ng/mL in the subgroup (n = 87) of patients who did not undergo thyroidectomy (sensitivity = 95.0%, specificity = 96.3%). In this study, the gold standard comparator was adopted (i.e. histological examination of surgically resected LNs or cytological evidence of recurrence at follow-up or negative histological examination of surgically resected LNs; or in the case of a benign diagnosis imaging showing disappearance or absence of evolution without cytological evidence of malignancy at 12 months or more follow-up). However, as in other studies, there is still uncertainty regarding the concordance between the biopsied node for FNA-Tg assessment and the final pathological report. For example, a low preoperative FNA-Tg can be obtained in a LN with inconclusive cytology (possibly benign) and final report can describe a metastatic LN found

in one of multiple resected lymph nodes, leading to a potential misleading interpretation of the result. It should be emphasized that in PTC, where lymph node metastasis may predict recurrence but not patient survival the priority is to minimise false positive results in order to avoid unnecessary surgery.

In our study, the higher Tg-FNA threshold can be attributed to the large proportion of patients with a thyroid in situ.

Very few studies have investigated the difference in the cutoff values for LN aspirates between patients with a thyroid in situ and those where the thyroid has already been removed [[9], [10], [11]]. In one large study that has specifically addressed this issue in the Chinese population operated with various surgical procedures, the cutoff value for Tg-FNA was higher in patients with thyroid tissue (before initial surgery or after lobectomy) compared to those without thyroid (55.99 vs 9.7 ng/mL, respectively) [12]. One of the hypotheses is that it could be related to secretion and circulation of small amounts of Tg in the lymphatic vessels and nodes. The influence of intact thyroid tissue should be also considered for example for thyroid cancer patients who have been treated by lobectomy alone, a situation that may become more frequent for low-risk thyroid cancers. One scenario which still remains unresolved is the value of Tg in washout fluid from LN in the presence of contralateral thyroid tissue. Pak et al. also recommended to raise the Tg cutoff value to 32.04 ng/mL for preoperative patients [11]. Finally, another study has evaluated the value of intraoperative measurement of thyroglobulin in lymph node aspirates for guiding the proper surgical procedure. In this study, only one patient had previous history of thyroidectomy. Although the purpose of this study was different, the optimal Tg-FNA cutoff value for malignancy was high (147.5 ng/mL, sensitivity of 81.1% and specificity of 100%, 95%CI: 0.863–0.99) [13].

We could also argue whether the manipulation of the thyroid gland during surgery might induce Tg release. Of note, LN aspiration was performed prior thyroidectomy. A recent study by Senese et al. has analyzed Tg preoperatively (T₀), intraoperatively (T₁), and postoperatively (T₂) in a series of 67 patients. The authors found that Tg levels increased significantly from T₀ to T₁ and decreased significantly from T₁ to T₂. These results support that manipulation of the thyroid leads to changes in thyroid hormone levels but that study only analyzed changes in serum Tg and did not analyse Tg in washout fluid [14].

It is well known that serum Tg concentrations can be affected by the presence of TgAb but this seems to not interfere with Tg in washout fluid [15].

In our study, the optimal cutoff value of FNA-Tg in determining malignant LNs is 60 ng/ml in all enrolled cases and 34 ng/ml in the thyroid cancer group only. We acknowledge several limitations of the present study such as limited number of cases mainly due to the design of the study.

In conclusion, our results support the hypothesis that presence of thyroid increases the Tg-FNA level. In this setting, the use of lower thresholds could result in false positive results and lead to unnecessary surgical intervention. Our results would need to be evaluated in patients under active surveillance or being managed after thyroid lobectomy.

Conflict of interest

None.

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CRediT authorship contribution statement

Santa D'angeli: Formal analysis, Data curation, Data analysis and interpretation, Manuscript preparation, Writing – review & editing, Manuscript editing. **Nunzia Cinzia Paladino:** Funding acquisition, Data curation, control of data and algorithms, Writing – review & editing, Manuscript editing. **Christelle Fargette:** Funding acquisition. **Cendrine Archange:** Study design, Funding acquisition. **Wassim Essamet:** Funding acquisition, Formal analysis, Data curation, Data analysis and interpretation. **Zeinab Hamidou:** Formal analysis, Statistical analysis. **Fausto Palazzo:** Data curation, Quality control of data and algorithms. **Karine Baumstarck:** Formal analysis, Statistical analysis. **Frédéric Sebag:** Data curation, Quality control of data and algorithms, Manuscript review. **David Taïeb:** Study concepts, Study design, Data curation, Quality control of data and algorithms, Formal analysis, Data curation, Data analysis and interpretation, Writing – review & editing, Manuscript editing, Manuscript review.

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