Lymph Node Metastases of Differentiated Thyroid Carcinoma: Does Serum Anti-Tg Antibodies or TSH Level Influence Tg measurement in Fine Needle Aspiration Washouts?

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Abstract

Introduction: Thyroglobulin evaluation in the washout of fine-needle aspiration (FNA-Tg) is an accurate diagnostic method of lymph node metastases (LNM) of differentiated thyroid carcinoma (DTC). Serum anti-thyroglobulin antibodies (AATg) may cause falsely low serum Tg values, but their effect on FNA-Tg has not been well established. There are also concerns about the possibility that suppressed TSH results in false-negative FNA-Tg. Our objectives were to evaluate the effect of serum AATg and TSH level on FNA-Tg of LNM of DTC and to determine the presence of AATg on the washout of fine needle aspiration (FNA-AATg).

Methods: Retrospective analysis of patients who underwent FNA-Tg assay in LNM of DTC. The sample was divided in two groups according to the presence of serum AATg at the time of FNA-Tg evaluation (Group 1: positive AATg, n=47; Group 2: negative AATg, n=50).

Results: There was no significant difference in the FNA-Tg between the two groups (p=0.066), although it was lower in Group 1 (1428 ng/mL) than in Group 2 (14842 ng/mL). FNA-Tg was able to identify 10.3% LNM of DTC that would not be diagnosed based solely on cytology. FNA-AATg evaluation was positive in 12.8% of the Group 1 patients and did not seem to interfere
with FNA-Tg value ($p=0.732$). There were no differences in the median FNA-Tg measurements between those on levothyroxine suppressive therapy and those on substitutive therapy ($p=0.800$).

Conclusion: FNA-Tg assay appears to be a good diagnostic tool even in patients with positive serum AATg and those under suppressive levothyroxine therapy.

**Keywords:** Thyroglobulin; Antibodies; Thyroid Cancer; TSH (Thyroid Stimulating Hormone)

**Introduction**

Differentiated thyroid cancer (DTC) is the most common endocrine cancer, accounting for approximately 90% of the thyroid gland malignancies [1-3]. The incidence of DTC is increasing worldwide [2, 3].

The prognosis of DTC is generally good, especially among low-risk patients [1]. However, cervical lymph node (LN) metastases (LNM) occur in 20-50% of the cases [2, 3] and are also the main cause for recurrence, which occurs in 5-20% of patients after initial therapy [1, 3-5].

The ultrasonographic diagnosis of cervical LN metastases from DTC is challenging due to its similarity with inflammatory adenopathies or nonthyroidal cancer metastases [1, 3, 6]. The diagnosis of suspicious LNs is confirmed through fine needle aspiration (FNA) cytology guided by ultrasound (US) and/or measurement of thyroglobulin (Tg) in the washout fluid of the fine needle aspiration (FNA-Tg) [1, 2]. Since inadequate cellularity or unsatisfying sampling precludes diagnosis in up to 20% of specimens with FNA [1, 3-5], measurement of FNA-Tg has been used as a powerful tool for the diagnosis of LNM, especially in cystic LNM, inadequate cytological evaluation, and divergent cytology and US findings [1, 2, 4].

Serum anti-Tg antibodies (AATg) are present in about 25% of the patients with thyroid cancer and may lower serum Tg levels and even make them undetectable (5). Nevertheless, there is still some debate on the role of AATg in lowering FNA-Tg levels and its influence in the diagnostic accuracy of FNA-Tg in suspicious LNs [1-3, 5-8]. Only a few studies evaluated the presence of AATg in the washout of the fine needle aspiration (FNA-AATg) so, there is little information about there real presence in LNM [1, 9].

In addition, there are concerns about the possibility that suppressed thyrotropin (TSH) might result in false-negative FNA-Tg in LNM [1].

Our objectives were to evaluate the influence of serum AATg on FNA-Tg of LNM of DTC, the effect of levothyroxine suppressive treatment in FNA-Tg values and to determine whether AATg can be detected in the washout fluid of fine needle aspiration of LNM of DTC.
Material and Methods:

Patients

The medical records of patients who underwent FNA-Tg measurements of suspicious cervical LNMs between December 2012 and July 2019 at a tertiary oncology center were retrospectively reviewed. Patients who had cervical LNMs with FNA-Tg evaluation were included. Those with positive serum AATg values at the time of FNA were considered in Group 1 ($n=47$) and patients with negative AATg at time of FNA were included in Group 2 ($n=50$). Patients in whom it was not possible to confirm the presence of LNMs or with incomplete clinical data were excluded.

This study was conducted in accordance with the Declaration of Helsinki. Confidentiality was kept throughout the study and the authors have followed the protocols of their center on the publication of data.

Tg, AATg and TSH methods

Serum Tg and FNA-Tg were measured using a chemiluminescent immunometric assay (IMMULITE® 2000, Siemens Healthcare Diagnostics) with a functional sensitivity of 0.9 ng/mL and an analytical sensitivity of 0.2 ng/mL. Serum AATg and FNA-AATg were measured by an immunofluorimetric assay (UNICAP® 100 Phadia AB, Thermo Fisher Scientific) with an analytical sensitivity of 244 U/mL. Serum TSH was measured by an electrochemiluminescence immunoassay (COBAS® e411, Roche Diagnostics) with a functional sensitivity of 0.005 µIU/mL.

Ultrasonography and FNA

US-guided FNA was performed by experienced radiologists with high-resolution US guidance on suspicious or indeterminate neck LNMs with a largest diameter in the transverse plane equal to or greater than 5 mm. Five hundred microliters of saline solution were used to wash the needle and syringe used for FNA to perform Tg and AATg measurements. The LNMs volume was calculated by the formula $\frac{4}{3} \pi abc$ (ovoid) when all three axes were provided and by the formula $\frac{4}{3} \pi a^2b$ (sphere) when just two axes were available.

Cytological Evaluation

Cytological evaluation was performed by experienced pathologists. They were categorized accordingly to the Bethesda System for Reporting Thyroid Cytopathology [10].
Definitions

FNA-Tg was considered positive and indicative of LNM if higher than 10 ng/mL [11], antibody positivity was defined as a AATg exceeding 280 U/mL and levothyroxine suppressive therapy when serum TSH was below 0.5 µIU/mL.

The US features suggestive of metastatic LNs included a round shape, the absence of an echogenic hilum, microcalcifications, cystic change, and peripheral blood flow on the color Doppler image.

LNs were diagnosed as metastatic only if there was histological or cytological confirmation of metastasis and/or a positive FNA-Tg in the context of a clear clinical and imaging evidence of metastasis, such as suspicious US features.

Statistical Analysis

Continuous variables are presented as the medians with range or means ± standard deviation (SD). Categorical variables are shown numerically with percentages. T-Test, the Mann-Whitney U test, and the X² test were used to determine whether there was a statistically significant differences between groups. To evaluate the correlation between variables the Pearson’s correlation coefficient was used. Statistical analysis was performed using Jamovi 1.0.7.0® software and p <0.05 was considered statistically significant.

Results:

Clinical Characteristics

Data from 176 patients were reviewed. After initial evaluation, patients in whom LNM were not clearly due to DTC origin (n = 57) and those with incomplete data in their medical records (n = 22) were excluded. Thereafter, we remained with 97 LN metastases. The mean age was 54.4 years (± 19.3 years) with 82.5% (n = 66) were females. Total thyroidectomy with or without central/lateral neck department dissection was performed in 97.9% (n = 95) of the cases previously to the diagnosis of LNM. Papillary carcinoma was found in 94.8% (n = 92) of the cases, 2.1% (n = 2) of the cases had papillary carcinoma with poorly differentiated areas and 1.0% (n = 1) with anaplastic areas. One patient (1.0% of the cases) had a thyroid follicular carcinoma.
The median serum Tg level was significantly different (p < 0.001) between Group 1 (<0.2 ng/mL, interquartile range 0.5 ng/mL) and Group 2 (2.3 ng/mL, interquartile range 16213 ng/mL). The median serum AATg level for Group 1 was 1429 U/mL (interquartile range 2340 U/mL).

**Lymph Node Metastases Characteristics**

Neck LNM from DTC were located in the central compartment in 28.9% (n = 28) of the cases and in the lateral compartments in 71.1% (n = 69).

Lymphadenectomy with histological confirmation of LN metastases was present on 78.7% (n = 37) of the patients of Group 1 and in 48.0% (n = 24) of the patients of Group 2.

There was no significant difference between biopsed LN volumes in both groups (Group 1: median 440 mm$^3$, interquartile range 1807 mm$^3$; Group 2: median 754 mm$^3$, interquartile range 2300 mm$^3$; p = 0.311).

The clinical, biochemical and US characteristics of the patients of both groups are presented in Table 1.

**Thyroglobulin Evaluation on FNA Washout Fluid**

There was no significant difference in the Tg value in the FNA washout fluid between the two groups (p = 0.069), although it was lower in Group 1 (median 1428 ng/mL; interquartile range 11696 ng/mL) than in Group 2 (median 4842 ng/mL; interquartile range 29799 ng/mL).

Figure 1 presents the distribution of FNA-Tg between groups.

FNA-Tg was able to identify more 10.3% (n = 10) LN DTC metastases, nine of them in the Group 1 patients, than cytological evaluation, either because cytology was negative, inconclusive, or insufficient sample. From this group (n=10), 7 patients were submitted to cervical lymphadenectomy and 2 were selected for active surveillance, presenting stable disease.

There were 7.2% of the patients (n = 7), with a negative FNA-Tg, despite having a cytology suggestive of LN DTC metastasis, five of them from Group 1. From these, 3 were submitted to surgery that confirmed metastases and 4 were selected for active surveillance, presenting stable disease.

The percentages of cases with true positive FNA-Tg (Group 1: 85.1%; Group 2: 86.0%) or false negative FNA-Tg (Group 1: 14.9%; Group 2: 14.0%) did not differ significantly between groups ($X^2 = 0.9$).
There was no correlation between FNA-Tg and serum AATg values among patients (Pearson’s $r = 0.106; \ p = 0.300$).

**Anti-Thyroglobulin Antibodies Measurement in FNA Washout Fluid**

FNA-AATg measurement was performed in 83.0% of the Group 1 patients ($n = 39$) with positive results in 5 of the cases (Table 2).

Serum AATg was not significantly different between patients with positive and negative FNA-AATg (median of 1395 U/mL vs 6228 U/mL; $p = 0.175$).

FNA-Tg did not seem to differ significantly between patients with positive or negative FNA-AATg (median of 8059 ng/mL vs 6199 ng/mL, $p = 0.735$).

In Table 2, we present the characteristics of the FNA washout fluid of patients with positive FNA-AATg. All, but one patient, had possible blood contamination.

**TSH Interference on FNA-Tg Evaluation**

There were no significant differences in the median FNA-Tg measurements between patients on levothyroxine suppressive therapy (median TSH <0.02 µIU/mL) and those on substitutive levothyroxine (median TSH 2.97 µIU/mL) (median Tg 2563 ng/mL, interquartile range 29789 ng/mL vs median Tg 4534 ng/mL, interquartile range 27046 ng/mL; $p = 0.820$), although there was a tendency for a lower FNA-Tg in the group with suppressive therapy.

**Discussion**

As previously suggested by various authors [1, 6] our study confirms that FNA-Tg is an important tool for the diagnosis of LN DTC metastases. It allows the identification of approximately more 10.3% LN DTC metastases than cytological evaluation.

The optimal cutoff point for the FNA-Tg value has not yet been definitively established and different authors use distinct values [3, 4, 9, 11]. We considered >10 ng/mL as a positive value for FNA-Tg as there is evidence that this cutoff has the highest sensitivity and accuracy for the diagnosis of LN DTC metastases in several studies and is used in the majority of published reports [7, 9]. The interpretation of any slightly to moderately elevated FNA-Tg level should also take in consideration the serum Tg level because it may represent blood contamination. In the majority of cases of DTC-LNN metastases, FNA-Tg is usually several times higher than serum Tg [12].
Since serum AATg may interfere with the evaluation of serum Tg by immunometric assays, it is still a matter of debate whether it also interferes with FNA-Tg levels and decreases the ability to diagnose LN DTC metastases [1, 3, 5-8]. In our work, the presence of serum AATg seemed to reduce the FNA-Tg value, but not to affect its discriminatory capacity.

Also, the volume of punctured adenopathies does not seem to be influencing the outcome of FNA-Tg.

We have shown that the presence of FNA-AATg is rare and might be the result of blood contamination rather than active production of AATg in the LN [9].

Since, levothyroxine suppressive therapy lowers serum Tg, the concern that it could also reduce the FNA-Tg has been raised. In our study, as well as in others previously reported, levothyroxine suppressive therapy does not seem to reduce the diagnostic accuracy of FNA-Tg [1, 9].

Our study has some limitations such as its retrospective nature, the fact that best FNA-Tg cutoff value is not currently well established, the absence of histological confirmation of some DTC metastases, the absence of FNA-Tg dilution on the highest values and the absence of analytical confirmation of blood contamination.

In conclusion, Tg assay in FNA washout fluid is an excellent diagnostic tool of DTC LNM, which is not affected by positive serum AATg or by levothyroxine suppressive therapy.

**Declarations**

**Funding:** There were no funding sources to this study.

**Conflicts of interest:** The authors declare that they have no conflicts of interest.

**Data availability:** The data that support the findings of this study is available upon request from the corresponding author. Due to privacy restrictions it is not publicly available.

**Statement of Ethics:** This study was conducted in accordance with the Declaration of Helsinki. Confidentiality was kept throughout the study and the authors have followed the protocols of their center on the publication of data.

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**Figure Legends**

Figure 1: FNA-Tg distribution between groups. Group 1: AATg+; Group 2: AATg-. FNA-Tg in ng/mL.