Thyroglobulin measurements in washout of fine needle aspirates in cervical lymph nodes for detection of papillary thyroid cancer metastases

Determinação da tireoglobulina no lavado da agulha da punção aspirativa de linfonodos cervicais para detecção de metástases do câncer papilar de tireoide

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ABSTRACT

Objective: The aim of this study was to evaluate the accuracy of the measurement of thyroglobulin in washout needle aspiration biopsy (FNAB-Tg) to detect papillary thyroid cancer (PTC) metastases. **Subjects and methods:** Forty-three patients (51.4 ± 14.6 years) with PTC diagnosis and evidence of enlarged cervical lymph nodes (LN) were included. An ultrasound-guided fine-needle aspiration of suspicious LN was performed, for both cytological examination and measurement of FNAB-Tg. **Results:** The median values of FNAB-Tg in patients with metastatic LN (n = 5) was 3,419 ng/mL (11.1-25,538), while patients without LN metastasis (n = 38) showed levels of 3.7 ng/mL (0.8-7.4). Considering a 10 ng/mL cutoff value for FNAB-Tg, the sensitivity and specificity was 100%. There were no differences on the median of FNAB-Tg measurements between those on (TSH 0.07 mUI/mL) or off levothyroxine (TSH 97.4 mUI/mL) therapy (3.3 *vs.* 3.8 ng/mL, respectively; P = 0.2). **Conclusion:** The results show that evaluation of FNAB-Tg in cervical LN is a valuable diagnostic tool for PTC metastases that can be used independent of the thyroid status. Arq Bras Endocrinol Metab. 2010;54(6):550-4

Keywords
Papillary thyroid carcinoma; metastases in cervical lymph nodes; thyroglobulin; TSH

RESUMO

Objetivo: O objetivo deste estudo foi avaliar a acurácia da dosagem de tireoglobulina no lavado da agulha da punção aspirativa (PAAF-Tg) de linfonodos (LN) cervicais para detecção de metástases do câncer papilar de tireoide (CPT). **Sujeitos e métodos:** Foram incluídos 43 pacientes (51,4 ± 14,6 anos) com diagnóstico de CPT e evidência de LN cervicais aumentados. Os LN suspeitos foram submetidos à punção aspiração com agulha fina guiada por ecografia para análise citológica e dosagem de tireoglobulina (PAAF-Tg). **Resultados:** A mediana dos valores de PAAF-Tg nos LN metastáticos (n = 5) foi 3.419,0 ng/mL (11,1-25.538), enquanto nos LN não metastáticos (n= 38) a mediana foi de 3,7 ng/mL (0,8-7,4). Utilizando-se o nível de 10 ng/mL como ponto de corte, observaram-se sensibilidade e especificidade de 100%. Os níveis de TSH sérico não interferiram na dosagem de PAAF-Tg (3,3 e 3,8 ng/mL nos grupos com TSH supresso (TSH 0,07 mUI/mL) e hipotireoidismo (TSH 97,4 mUI/mL), respectivamente, P = 0,2). **Conclusão:** Os resultados demonstram que a dosagem de PAAF-Tg é uma ferramenta importante no diagnóstico de metástases do CPT, podendo ser utilizada independente do "*status*" tireoidiano. Arq Bras Endocrinol Metab. 2010;54(6):550-4

Descritores
Câncer papilar de tireoide; metástases em linfonodos cervicais; tireoglobulina; TSH

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INTRODUCTION

Thyroid cancer is the most common malignant neoplasm of the endocrine system, mostly affecting women and individuals between 25 to 65 years (1). Differentiated thyroid cancer (DTC) is responsible for 90% of the malignant thyroid gland neoplasias (2). According to current guidelines, the initial therapeutic approach to DTC consists of total thyroidectomy and cervical lymph node (LN) dissection for the papillary type (3,4). The treatment is completed with a therapeutic dose of radioactive iodine and TSH suppression by the use of levothyroxine (3).

The disease has a generally good prognosis, especially among patients younger than 45 years of age. However, recurrence of the tumor in cervical LN occurs in up to 15% of patients after initial therapy (5). Therefore, monitoring of recurrence is mandatory. Three main diagnostic tools can be used in detecting residual or recurrent disease: serum thyroglobulin (sTg) basal and stimulated by endogenous or recombinant TSH, cervical ultrasound (US) and whole body scan with radioactive iodine in selected cases (6). Currently, it is a general consensus that the most sensitive method to detect DTC recurrence is the sTg measurement (7). Nevertheless, this method is not entirely accurate because 20% of patients with metastatic disease have undetectable sTg when in use of levothyroxine. In addition, sTg can suffer interference with the presence of antithyroglobulin antibodies, present in 25% of the patients with DTC (7).

Because recurrent disease usually occurs in the neck, cervical US has been advocated as an important tool in the follow-up of low risk thyroid cancer patients (8). Despite the presence of specific characteristics of malignancy, such as cystic appearance, hyperechoic punctuations, loss of hilum and peripheral vascularization (9), the diagnosis of cervical LN metastases of DTC can be frequently complex, because inflammatory lymphadenopathies are extremely frequent in this region and, furthermore, metastasis in cervical LN from non-thyroid cancers is also relatively common (10). Therefore, US alone is not enough to distinguish a metastatic nodule from reaction hyperplasia (7).

Studies have shown that adding fine-needle aspiration biopsy to US increased the sensitivity and specificity in detecting metastases of DTC up to 87% and 76%, respectively (11,12). Consequently, most suspicious LN undergo fine needle aspiration biopsy for cytological evaluation. Nevertheless, inadequate cellularity or non-representative sampling precludes diagnosis in up to 20% of specimens, depending on the cytopathologist's experience and skill (11,12).

In an attempt to improve the accuracy of detection of DTC metastases, the measurement of thyroglobulin in the wash-out (FNAB-Tg) from the same needle used in aspiration biopsy has been proposed (13). Indeed, previous studies have demonstrated a sensitivity of 84%-100% and specificity of 85%-95% for this method (13,14). However, there is still uncertainty related to the cutoff values, particularly for the latest generation of highly sensitive thyroglobulin assays. Moreover, it is not known whether changes in thyrotropin (TSH) levels interfere in FNAB-Tg levels.

Here we have evaluated the accuracy of the FNAB-Tg measurement in detecting metastases of PTC in a series of patients attending the Endocrine Outpatient Clinics at Hospital de Clínicas de Porto Alegre (HCPA).

SUBJECTS AND METHODS

Patients

Between November 2007 and May 2009, 43 consecutive patients with thyroid cancer and enlarged cervical LN (≥ 1 cm), detected by palpation or cervical US, attending the Endocrine Division at our Institution were invited to participate in the study. According to our current protocol, all patients with DTC underwent total thyroidectomy followed by a therapeutic dose of radioactive iodine and suppressive thyroxine therapy.

Patients underwent a physical examination and blood was obtained for measurement of TSH, sTg and antithyroglobulin antibodies. Neck US was performed on all patients by the same operator using a 7.5 MHz linear transducer. An ultrasound-guided fine-needle aspiration (FNA) of suspicious LN was performed, allowing for both cytological examination and FNAB-Tg. FNA was done with 22-25 G needles. The cells were spread on a glass slide and 1 mL normal saline (0.9% NaCl) was aspirated through the needle with a syringe from a test tube (2 mL-eppendorf) and the washout was stored in test tubes in -20°C until the analyses were performed. At the time of FNA, 10 patients were off thyroxine replacement therapy for sTg stimulation.

The results for cytology were classified into 3 distinct diagnostic categories: i) inadequate or non-diag-

nostic: presence of blood cells without lymphocytes, plasma cells, histiocytes and epithelial cells; ii) negative cytology: presence of lymphocytes and occasional plasma cells without malignant epithelial cells; and iii) positive cytology for DTC metastases: presence of epithelial cells with malignant cytological characteristics. According to the sTG measurements and cytological reports, patients were referred for surgery by the attending physician. Histology was considered the gold standard to determine the accuracy of FNAB-Tg. For patients who did not undergo surgery, we have used the negative cytological result and follow-up for at least one year. The following criteria were used to consider patients free of metastatic neck disease: cervical US without enlargement of LN or presence of LN without malignant characteristics (hyperechoic punctuations, loss of hilum and peripheral vascularization) and undetectable stimulated sTg. The study was approved by the Ethics Committee of the Hospital and all patients gave informed consent.

Laboratory measurements

Serum TSH, sTG and FNAB-Tg were measured by eletrochemiluminescent method, using a commercially available kit (Modular E-170 Roche). The TSH reference range was 0.4 to 4.2 mIU/L. The reference range for thyroglobulin measurements (serum and washout) was 1.4-7.8, with a sensitivity of 1 ng/mL. The antithyroglobulin antibodies were measured by the passive agglutination method (Serodia – ATG, Bayer Diagnostica).

Statistical analysis

Results are expressed as frequencies, mean ± standard deviation (SD) or median (range). Clinical and laboratory data were compared using the unpaired Student's t test, Mann-Whitney U test, or χ^2, as appropriate. A two-tailed $P < 0.05$ was considered statistically significant. All analyses were performed by Statistical Package for Social Science professional software version 15.0 (SPSS, Chicago, IL, USA).

RESULTS

Table 1 shows the clinical and laboratory characteristics of the studied patients. Forty-three patients with suspect LN were included (30 women and 13 men) with a mean age of 51.4 ± 14.6 years. According to TNM staging for DTC (15), patients were distributed as follows: stage I (48.8%), II (18.6%), III (27.9%) and IV (4.7%). Thirty-three patients were receiving levothyroxine TSH-suppressive therapy, while 10 patients were in hypothyroidism (levothyroxine withdrawn). Mean size of LN was 1.7 ± 0.4 cm and mean follow-up was 22.8 ± 8.2 months. The median (range) values of sTg and FNAB-Tg in patients on T4 and off T4 were as follows: 1.0 ng/mL (1.0-4,283.0) and 4.0 ng/mL (0.9-3,742.0); 2.3 ng/mL (1.0-191.8) and 3.9 ng/mL (0.8-25,538), respectively. Five patients presented positive antithyroglobulin antibodies.

Table 1. Clinical and laboratory characteristics of the 43 patients with papillary thyroid cancer and enlarged cervical lymph nodes

Age (years)	51.4 ± 14.6
Sex (F/M)	30/13
TNM Stage*	
I	21 (48.8%)
II	8 (18.6%)
III	12 (27.9%)
IV	2 (4.7%)
Follow-up (months)	22.8 ± 8.2
Lymph node size (cm)	1.7 ± 0.4
TSH (mUI/mL)**	
On T4 (n = 33)	0.09 (0.01-12.60)
Off T4 (n = 10)	82.2 (50.3-308.0)

* TNM staging for differentiated thyroid cancer; ** Median (range).

Four patients presented positive cytological results and were referred for surgery. Three of them had their results confirmed by histology. The FNAB-Tg values of these patients were 3,419 (TSH 0.49 mUI/mL; sTg 732.6 ng/mL), 25,538 (TSH 62.08 mUI/mL; sTg 191.8 ng/mL) and 3,742 (TSH 0.1 mUI/mL; sTg 4,283 ng/mL). For the patient who presented a negative histology, the FNAB-Tg value was 3.1ng/mL.

Thirty patients presented negative cytology and 9 showed unsatisfactory samples. Two patients who had an unsatisfactory cytological result underwent surgery by decision of his attending physician. PTC metastasis in LN was confirmed by histology in both cases and the FNAB-Tg value was 11.1 ng/mL (TSH of 0.02 mUI/mL and sTg of 8.9 ng/mL) and 13.5 ng/mL (TSH of 0.29 mUI/mL; sTg of 10.9 ng/mL). All other patients were followed for 22.7 ± 7.8 months, without clinical evidence of metastatic disease by cervical US and undetectable stimulated sTg. One patient presented sTg of 26 ng/mL with pulmonary metastasis and was excluded from the analyses. The values of FNAB-Tg did not

differ between patients with negative (3.6 ng/mL) or unsatisfactory cytology (6.2 ng/mL; P = 0.07).

Considering a cutoff value of 10 ng/mL, based on previous reports (7,16), all metastatic LN (n = 5) had higher FNAB-Tg values (median of 3,419.0 ng/mL, range 11.1-25,538.0). In all patients without LN disease (n = 38), confirmed by negative cytology and at least 1-year follow-up, the FNAB-Tg was below 10 ng/mL with a median of 3.7 ng/mL (0.8-7.4) (Table 2).

Table 2. FNAB-Tg* values and cytological results according to the lymph node histological examination

	Benign (n = 38)	Metastatic (n = 5)
FNAB-Tg* (ng/ml)	3.7 (0.8-7.4)	3,419.0 (11.1-25,538)
Cytology		
Positive	1 (2%)	3 (60%)
Negative	30 (79%)	
Unsatisfactory	7 (19%)	2 (40%)

* Thyroglobulin in washout of fine-needle aspiration.

TSH effect on FNAB-Tg

Next we evaluated the role of serum TSH on FNAB-Tg levels in patients without evidence of LN metastases by cytology. Twenty-eight patients were receiving levothyroxine suppressive therapy (TSH = 0.07 mUI/mL) and 10 patients were on hypothyroidism for a stimulated sTg measurement (TSH = 82.2 mUI/mL). Interestingly, we found no significant difference in the median values of FNAB-Tg between the groups 3.3 ng/mL (0.9-6.8) *vs.* 3.8 ng/mL (0.8-7.4; P = 0.2), indicating that serum TSH has no effect on FNAB-Tg levels (Figure 1).

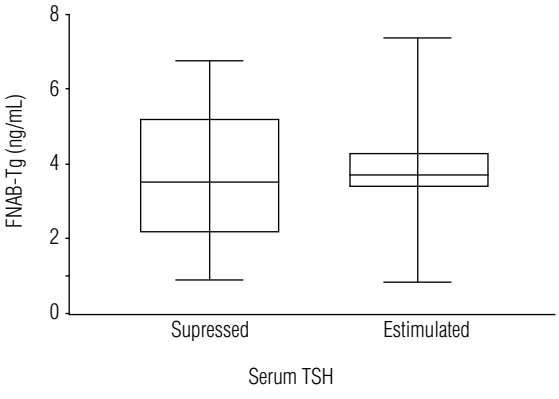

Figure 1. Thyroglobulin measurements in washout needle aspiration biopsy (FNAB-Tg) in cervical lymph nodes in papillary thyroid cancer patients under suppressive thyroxine therapy (TSH 0.07 mUI/mL; n = 28) or thyroxine withdrawal (TSH 97.4 mUI/mL; n =10) for serum thyroglobulin stimulation (P = 0.21).

DISCUSSION

In the present work we have further demonstrated that the measurement of FNAB-Tg is an excellent tool for detecting PTC recurrence, improving the evaluation of suspicious LN in these patients. Furthermore, we showed that the serum TSH does not seem to interfere in the detectable FNAB-Tg levels observed in negative cases.

Previous studies have already demonstrated the accuracy of FNAB-Tg in suspicious LN metastases of DTC (14,17,18), with a sensitivity and specificity ranging from 84% to 100% and 85% to 95.4%, respectively. Cutoffs used in previous studies have varied widely, without a consensus about the most appropriate level (6,19,20). However, a recent study with 168 patients that analyzed 4 different levels of cutoffs (1, 10, 100 ng/mL and FNAB-Tg mean ± SD) indicated that the best level for diagnosis of DTC persistence/recurrence was < 10 ng/mL (16). Here, considering this cutoff, the sensitivity and specificity of FNAB-Tg was 100% and all cases were distinguishable as positive or negative based on FNAB-Tg. In two patients with LN metastasis showed FNAB-Tg levels close to the cutoff value (11.1 and 13.5 ng/mL). In these cases, the likely explanation for the low levels of FNAB-Tg might be the small amount of cells (unsatisfactory sample) since the histology had confirmed classical PTC. Nevertheless, low FNAB-Tg levels may also occur in LN metastases of poorly differentiated PTC that are unable to synthesize/secrete quantifiable amounts of thyroglobulin. It is interesting to note that the diagnostic performance of FNAB-Tg compared favorably with cytology, allowing the diagnosis in 3 patients in whom cytological results were false-positive or nondiagnostic. These cases further illustrate the importance of this method to improve the accuracy of detection of DTC metastases.

In agreement with previous studies (7,13,16), we observed that some patients without LN metastasis presented detectable FNAB-Tg levels. In these patients, the levels of FNAB-Tg ranged between the method detection limit (< 1 ng/mL) and the cut-off value (10 ng/mL) and, in some cases were even superior to sTg. The reasons for these FNAB-Tg values are unclear. A possible contamination by circulating sTg can be theoretically ruled out based on the results of Borel and cols. (21) that demonstrated that sTg interferes with FNAB-Tg measurement only when high sTg levels are present. These authors, based on recent studies (19,20), suggest that in all patients who do not have high levels of sTg, a detectable level of FNAB-Tg

in a cervical mass is related to the presence of thyroid cells. Nevertheless, low levels of FNAB-Tg have been observed in control patients (7,13) and can be explained by presence of 'matrix effects' artifacts depending on the antibodies and medium used (21). To further support this hypothesis, we demonstrated here that the FNAB-Tg level does not change according to the serum TSH in patients without LN disease.

A possible limitation of our study is that none of the 30 patients with a benign cytology underwent LN surgery. In these patients, we cannot provide evidence of benign histology. Consequently, false negatives in cytology associated with negative FNAB-Tg cannot be excluded. To overcome this limitation, we have used stimulated sTg measurement after 1 year follow-up considering sTg values < 1 ng/mL as indicative of disease-free. The small number of patients with lymph node metastases could also be a limiting factor to the conclusions of this study.

In conclusion, our results showed that US-guided FNAB-Tg should be performed adjunct to cytology in patients with suspicious cervical LN. This method proved to be a useful exam in the follow-up of patients with PTC and can contribute to diminish the number of unnecessary surgeries, reducing costs and patient morbidity.

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