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# TIREOGLOBULINA OBTIDA DO LAVADO DA AGULHA DE PUNÇÃO DE LINFONODO CERVICAL PARA DIAGNÓSTICO DE CÂNCER METASTÁTICO DE TIREOIDE

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# TIREOGLOBULINA OBTIDA DO LAVADO DA AGULHA DE PUNÇÃO DE LINFONODO CERVICAL PARA DIAGNÓSTICO DE CÂNCER METASTÁTICO DE TIREOIDE

Tese apresentada ao Programa de Pós-graduação em Medicina e Saúde, da Faculdade de Medicina da Bahia, Universidade Federal da Bahia, como requisito para a obtenção do grau de Doutor em Medicina e Saúde.

Orientador: Prof. Dr.Thomaz Rodrigues Porto da Cruz

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Prof. Dr. Thomaz Rodrigues Porto da Cruz
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*Aos meus pais, Rosalvo e Marineusa, a quem devo tudo o que sou.*

*A Raniere, meu companheiro e parceiro na vida, pelo amor renovado a cada dia e pelo apoio incondicional a todos os meus sonhos.*

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**SUMÁRIO**

## LISTA DE TABELAS E FIGURAS

## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| EUA | Estados Unidos da América |
| CDT | Carcinoma diferenciado de tireóide |
| CP | Carcinoma papilífero |
| CPT | Carcinoma papilífero de tireóide |
| $I^{131}$ | Iodo$^{131}$ |
| LN | Linfonodo |
| PAAF | Punção aspirativa por agulha fina |
| FNAB | Fine-needle aspiration biopsy |
| PAAF-C | Citologia do aspirado de linfonodos cervicais |
| FNAB-C | Fine-needle aspiration biopsy cytology |
| PCI | Pesquisa de corpo inteiro |
| rhTSH | Hormônio estimulante da tireoide recombinante humano |
| SPSS | Statistical package for social sciences |
| sTg | Tireoglobulina sérica |
| Tg | Tireoglobulina |
| TgAb | Anticorpo antitireoglobulina |
| Tg-PAAF | Tireoglobulina medida no aspirado de linfonodos cervicais |
| TSH | Hormônio estimulante da tireóide |
| USG | Ultrassonografia |
| VPN | Valor preditivo negativo |
| VPP | Valor preditivo positivo |

## 1 RESUMO


### TIREOGLOBULINA OBTIDA DO LAVADO DA AGULHA DE PUNÇÃO DE LINFONODO CERVICAL PARA DIAGNÓSTICO DE CÂNCER METASTÁTICO DE TIREOIDE

O carcinoma papilífero de tireóide (CPT), apesar do prognóstico favorável, apresenta frequentemente metástases linfonodais e a citologia do aspirado de linfonodos cervicais (PAAF-C) é considerada a abordagem mais importante no rastreio dessas metástases. O objetivo desse estudo foi verificar a acurácia da tireoglobulina medida no aspirado de linfonodos cervicais (Tg-PAAF) no diagnóstico de metástases de pacientes com CPT e a importância da sua associação com a PAAF-C. Foram avaliados 195 linfonodos (LNs) de 160 pacientes com CPT após tireoidectomia total e ablação com $I^{131}$. Os LN foram classificados à ultrassonografia (USG), em reacionais e suspeitos de metástase de CPT. A PAAF-C foi realizada pelas técnicas aspirativa e não-aspirativa e os esfregaços foram classificados em não diagnósticos, LN reacionais e suspeitos de metástase. A agulha da PAAF-C foi lavada com 1,0mL de soro fisiológico a 0,9% e a solução, processada para medir a tireoglobulina. O ponto de corte considerado foi de 10 ng/ml. Foram encaminhados à cirurgia os pacientes com LNs suspeitos com PAAF-C suspeita de malignidade e Tg-PAAF elevada ou não, com LNs reacionais com PAAF-C suspeita e Tg-PAAF elevada e com LNs suspeitos com PAAF-C insatisfatória e Tg-PAAF elevada. A PAAF-C falhou em diagnosticar cinco dos 52 LNs metastáticos ao histopatológico, enquanto a Tg-PAAF falhou em apenas um. A sensibilidade, a especificidade, o valor preditivo positivo (VPP), o valor preditivo negativo (VPN) e a acurácia foram respectivamente, de 96,1%, 96,5%, 90,9%, 98,6%, 96,4% para a USG, 90,4% 100%, 100%, 96,6% e 97,4% para a PAAF-C e 98,1%, 99,3%, 98,1% 99,3% e 98,9%, para a Tg-PAAF. O histopatológico confirmou metástase em 50 dos 55 LNs suspeitos à USG. A associação da USG com PAAF-C e Tg-PAAF elevou a especificidade e o VPP para 100%.O grau de concordância entre PAAF-C e Tg-PAAF foi elevado (K=0,9). Em conclusão, a Tg-PAAF é um procedimento útil na detecção de metástase de CPT, complementa a PAAF-C e pode substituí-la.

## ABSTRACT

## THYROGLOBULIN OBTAINED FROM THE FINE-NEEDLE WASHOUT FLUIDS FROM CERVICAL LYMPH NODES FOR THE DIAGNOSIS OF METASTATIC THYROID CANCER

Papillary thyroid carcinoma (PTC), despite a favorable prognosis, often presents lymph node (LN) metastases, and cytology of cervical lymph node aspirate (FNA-C) is considered the most important approach to screen for these metastases. The objective of this study was to determine the accuracy of the thyroglobulin measured in the fine-needle cervical aspirate (FNA-Tg) of LNs in the diagnosis of metastases in patients with PTC, and the importance of its association with FNA-C.We evaluated 195 LNs from 160 patients with PTC after total thyroidectomy and ablation with $I^{131}$. The LNs were classified by ultrasonography (USG), into reactive and suspicious of metastatic PTC. The FNA-C were performed by aspiration and non-aspiration techniques, and the smears were classified as nondiagnostic, reactive LNs, and suspicious for metastasis.The FNA-C needle was washed with 1.0 ml saline solution at 0.9%, and then the solution was processed to measure the thyroglobulin. The cutoff point was considered 10 ng/ml.The suspicious LNs with FNA-C with suspected malignancy and high or not high Tg-FNAB, reactional LNs with suspicious FNA-C and high Tg-FNAB, and suspicious LNs with unsatisfactory FNA-C and high Tg-FNAB were referred to surgery.FNA-C failed to diagnose five of the 52 metastatic LNs to the histopathological, while Tg-FNA failed in only one. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were respectively 96,1%, 96.5%, 90,9%, 98,6%, 96,4% for USG; 90,4%, 100%, 100%, 96,6% and 97,4% for C-FNA; and 98.1%, 99,3%, 98,1%, 99.3% and 98,9% for Tg-FNAB. Histopathological examination confirmed metastases in 50 of the 55 LNs that were suspicious in the USG. The association of USG with FNA-C and FNA-Tg increased sensitivity and NPV to 100%. The degree of concordance between FNA-C and Tg-FNAB was high (K = 0.9). In conclusion, Tg-FNAB is a useful procedure in the detection of metastatic PTC, complements FNA-C, and can replace it.

## 2 INTRODUÇÃO

O câncer de tireoide representa aproximadamente 1% de todos os cânceres, é a neoplasia endócrina mais freqüente e, dentre os tumores malignos é dos que apresentam melhor taxa de cura. Nos últimos anos, um aumento relevante na incidência de câncer de tireoide tem sido documentado em todo o mundo. Dados do National Cancer Institute mostram que 60.200 homens e mulheres (14.910 homens e 45.310 mulheres) receberão o diagnóstico de câncer de tireoide em 2013 e destes, 1850 homens e mulheres morrerão neste ano, devido à doença. Felizmente esse aumento se deve especialmente ao histotipo papilífero que é geralmente bem diferenciado e curável. No Brasil, há registros de 66 novos casos em cada 100.000 habitantes por ano.

Aproximadamente 80% de todos os carcinomas tireoideanos corresponde ao CP, e seu diagnóstico geralmente é estabelecido pelo exame citológico por meio de material aspirado por agulha fina. Ocorre em qualquer faixa etária, porém predomina em indivíduos mais jovens (média de 50 anos ao diagnóstico). Seu crescimento é lento e apresenta baixo grau de progressão. Sua disseminação dá-se por meio dos linfáticos intraglandulares, evoluindo do foco inicial para as outras partes da tireoide e para os LNs pericapsulares e cervicais.

Dessa forma, lesões multicêntricas na tireoide são comuns e cerca de 5-20% dos pacientes com CP tem persistência ou recorrência da doença em LNs cervicais. Dados de centros onde biópsia de LN sentinela ou dissecação de LNs centrais são praticadas de rotina, mostram que metástases ocultas podem ser observadas em até 90% dos pacientes.

O seguimento dos pacientes portadores de CP após tireoidectomia total e radioablação com Iodo$^{131}$ ($I^{131}$) é baseado na dosagem de tireoglobulina sérica basal (sTg) e estimulada pelo hormônio estimulante da tireóide (TSH) endógeno ou recombinante (rhTSH), pesquisa de corpo inteiro (PCI) com $I^{131}$ e realização de ultra-sonografia (USG) cervical. A determinação da sTg tem uma especificidade muito elevada para a detecção de recorrências, mas a sua sensibilidade na vigência de terapia supressiva com levotiroxina não é ideal para a detecção de recorrências precoces do CP e metástases em pequenos LNs. Além disso, é seriamente afetada pela presença de anticorpos anti-tireoglobulina séricos (TgAb), identificados em 25–

30% dos pacientes com CP. Tal medida, quando realizada após uso de TSH recombinante humano (rhTSH), apesar de melhorar a sensibilidade, deixa de ser custo-efetiva para o rastreamento de grande número de pacientes.

O custo da PCI com $I^{131}$ é aproximadamente cinco vezes o da USG e o uso do rhTSH eleva significativamente este valor. Além disso, os seus resultados podem ser afetados pelo tamanho do tumor, a dose de iodo radioativo e a capacidade de captação de radiotraçadores. Metástases linfonodais, por exemplo, podem não captar iodo em 20–40% dos casos, mesmo quando o exame é realizado após altas doses de $I^{131}$.

O exame ultrassonográfico do pescoço, apesar de alta sensibilidade para detecção de LNs cervicais, mesmo quando os níveis de sTg permanecem indetectáveis e a PCI é negativa, não apresenta sensibilidade necessária para diferenciação entre LNs benignos e malignos. Assim, quando detectada linfadenomegalia cervical, esta deve ser investigada através de USG cervical e, caso a suspeita seja de LN metastático, a PAAF deve ser realizada com exame citológico do material obtido e Tg-PAAF.

A PAAF-C guiada por ultra-sonografia, representa um método essencial na detecção de metástases cervicais de CP, principalmente nos pacientes com sTg indetectável e TSH suprimido e naqueles com metástases não captantes de iodo. Contudo, sua sensibilidade está longe de ser excelente, variando de 75% a 85% e é limitada por apresentar 6% a 8% de resultados falsos negativos e até 20% de amostras não representativas ou com celularidade inadequada, dependendo da experiência e habilidade do citopatologista.

Para melhorar o rendimento diagnóstico da PAAF-C, vários autores têm proposto a dosagem da tireoglobulina obtida no lavado da agulha utilizada na punção (PAAF-Tg). Este método foi proposto inicialmente em 1992 por Pacini[1] e colaboradores que demonstraram que níveis elevados de PAAF-Tg em LNs de indivíduos tiroidectomizados e submetidos à radioablação, eram decorrentes de metástases de carcinoma de tireoide, enquanto que valores indetectáveis indicavam linfadenopatia inflamatória ou de origem não tireoidiana. Nesses pacientes, a

[1] Pacini F, Fugazzola L, Lippi F, Ceccarelli C, Centoni R, Miccoli P, et al. Detection of thyroglobulin in fine needle aspirates of nonthyroidal neck masses: a clue to the diagnosis of metastatic differentiated thyroid cancer. J Clin Endocrinol Metab 1992;74:1401-4.

sensibilidade da Tg- PAAF foi 100%, enquanto que a sensibilidade da citologia foi 85,7%.

Desde então, vários outros autores têm realizado este teste, alcançando sensibilidade de 81,4%-100% e especificidade de 85%-100%, embora algumas controvérsias ainda persistam sobre certas questões como a escassez de estudos para validar o benefício da PAAF-Tg sobre a PAAF-C isolada.

Assim, sendo o carcinoma diferenciado de tireóide uma doença que acomete especialmente pessoas jovens e que apresentam uma sobrevida prolongada, fazendo com que estes sejam acompanhados para rastreio de metástases por longo período, este estudo se propôs a avaliar a utilidade da PAAF-Tg na detecção de LNs metastáticos e o valor da sua associação com a PAAF-C em pacientes com CP.

## 3 OBJETIVOS

### 3.1 Principal

Verificar a acurácia da dosagem da tireoglobulina obtida do lavado da agulha da punção aspirativa de LNs cervicais no diagnóstico de metástases em pacientes com CP e seu valor nos casos em que a citologia for não diagnóstica.

### 3.2 Secundário

Verificar o valor da associação da citologia e da dosagem da tireoglobulina obtida do lavado da agulha da punção de LNs cervicais no diagnóstico de metástases em pacientes com CP.

# 4 METODOLOGIA

## 4.1 Desenho do estudo

Estudo de Avaliação de Testes Diagnósticos.

## 4.2 Período

Junho de 2009 a junho de 2010.

## 4.3 Local do Estudo

Clínica Radiológica Dr. Wanderley – Campina Grande – PB.

## 4.4 População do estudo

Pacientes portadores de CPT com linfonodos cervicais vistos à USG, submetidos à tireoidectomia total e ablação com I131 encaminhados para investigar metástases cervicais.

## 4.5 Critério de inclusão

Pacientes submetidos à tireoidectomia total CPT e ablação com $I^{131}$ portadores de LN encaminhados para investigação de metástase cervical.

## 4.6 Critérios de exclusão

Pacientes submetidos à tiroidectomia parcial por CPT e pacientes tireoidectomizados por carcinoma não diferenciado de tireóide.

# 5 ARTIGOS

## 5.1 Artigo 1

**TÍTULO:** Thyroglobulin in the washout fluid of lymph node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma? - revisão da literatura

**PERIÓDICO:** Thyroid

**SITUAÇÃO:** Aceito

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Date: 2013/8/31
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To: rosetorres.maria@gmail.com

31-Aug-2013

Dear Dr. Torres:

It is a pleasure to accept your manuscript entitled "Thyroglobulin in the washout fluid of lymph node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma?" for publication in Thyroid.

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Thank you for your fine contribution. On behalf of the Editors of Thyroid, we look forward to your continued contributions to the Journal.

Sincerely,

Peter Kopp, MD
Editor-in-Chief
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# Thyroglobulin in the washout fluid of lymph node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma?

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Title: Thyroglobulin in the washout fluid of lymph node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma?

Keywords: Thyroglobulin, Thyroid Cancer-General, Endocrinology-Adult

## ABSTRACT

**Background:** The clinical evaluation of enlarged local lymph nodes (LNs) is difficult at the beginning and throughout the follow-up of differentiated thyroid carcinoma (DTC). Although the examination of samples collected from LNs by fine-needle aspiration biopsy cytology (FNAB-C) is extremely specific for the diagnosis of metastases, its sensitivity is low, especially in paucicellular samples. **Abstract**: Measurement of thyroglobulin (Tg) in the FNAB washout fluid (FNAB-Tg) increases the diagnostic performance of cytology to up to 100% sensitivity and specificity. However, the application of FNAB is currently hindered by the absence of methodological standardization, a lack of definite cutoff points, and the ongoing debate regarding its accuracy in non-thyroidectomized patients, those with elevated serum Tg, and those with circulating anti-Tg antibodies. **Conclusion**: FNAB-Tg improves the diagnostic performance of FNAB-C in LN metastases, even when the latter is unable to diagnose the metastases. For that reason, FNAB-Tg must be included in the monitoring of DTC.

## INTRODUCTION

The incidence of thyroid cancer has increased worldwide in the past few years (1-5). Epidemiological studies conducted in the United States of America (USA) show that among all tumors affecting males and females, the incidence of thyroid cancer has increased the most (6). Approximately 56,460 new cases were diagnosed in 2012 in the USA, and three out of four were diagnosed in females, an increase that is due largely to the papillary histotype (1-3, 7).

Up to 30% of the patients with papillary thyroid carcinoma (PTC) exhibit recurrent or persistent metastasis to the neck lymph nodes (LNs) (8,9). Data from centers where sentinel LN biopsy or central LN dissection are routinely performed show that occult metastases occur in up to 90% of PTC patients (10, 11).

The clinical evaluation of enlarged local LNs is difficult at the beginning and throughout the follow-up of patients with differentiated thyroid carcinoma (DTC) because inflammatory lymphadenopathies are extremely frequent and metastases of non-thyroid cancers occur quite commonly in the neck LNs. Therefore, several tools have been suggested to facilitate the accurate identification of metastatic LNs before and after surgery. Although not exempt from some shortcomings, the measurement of serum basal and thyrotropin (thyroid-stimulating hormone, TSH)-stimulated thyroglobulin (Tg) levels, whole-body scan (WBS) using radioactive iodine (RAI), neck ultrasound (US), and fine-needle aspiration biopsy (FNAB) with cytological analysis of the LNs are widely used in clinical practice.

Although the specificity of serum Tg (sTg) to detect recurrent disease is high, it is not the ideal measurement for metastases affecting small LNs. In addition, it is not able to establish the location of the neoplastic focus and is highly affected by the presence of serum anti-thyroglobulin antibodies (TgAb), which are found in 25-30% of patients with DTC (12, 13). In turn, the results of WBS might be affected by the tumor size, RAI dose, and radiotracer uptake. For instance, LN metastases might not take up iodine in 20-40% of cases, even when high $^{131}$I doses are applied (14, 15).

The role of US has been increasingly important in the follow-up of patients with DTC after initial treatment (16). The sensitivity of US to detect occult masses and non-palpable neck LNs is well documented. However, despite the establishment of

findings predictive of malignancy, its ability to distinguish between “benign” (inflammatory or reactive) and “malignant” (metastatic) LNs is still low (17).

Cytological analysis of FNAB samples (FNAB-C) from LNs has been widely used to confirm suspect findings on US, mainly in patients with undetectable sTg due to suppressed TSH or with a non-iodine-concentrating metastasis (16). Nevertheless, the sensitivity of this method is far from ideal, as it varies from 75 to 85% (18, 19), with a rate of false-negative results of 6 to 8% (18, 20) and a rate of up to 20% of non-representative samples or samples with inadequate cellularity, which are a function of cytopathologists’ skill and experience (21, 22).

To improve the diagnostic performance of FNAB-C, several authors have suggested measuring Tg in the needle washout fluid (FNAB-Tg). This method was initially suggested in 1992 by Pacini and colleagues (18), who showed that high FNAB-Tg in the LNs of individuals subjected to thyroidectomy and radioablation was due to thyroid carcinoma metastasis, whereas undetectable FNAB-Tg indicated inflammatory lymphadenopathy or lymphadenopathy of non-thyroid origin. In that study, the sensitivity of FNAB-Tg was 100%, whereas the sensitivity of cytology was only 85.7%. In 1993, Lee et al. (23) reported that the combination of FNAB-C and FNAB-Tg detected a larger number of metastatic LNs than either technique alone and reached 100% sensitivity and specificity.

Several authors have performed FNAB-Tg and found that its sensitivity varied from 81.4% to 100% and its specificity from 85% to 100% (18, 19, 24-35). In spite of those results, controversy remains on some issues, including the scarcity of studies validating the alleged benefit of FNAB-Tg over FNAB-C alone and the variation of the cutoff points used, which range from 0.9 to 39 ng/mL as a function of the method used in the measurement of Tg (19, 25-27), in addition to divergences related to its diagnostic performance in patients not yet subjected to thyroidectomy. Moreover, the best method of FNAB-Tg has not yet been standardized, and thus, all published studies on this topic exhibit significant differences in the techniques used to wash needles and to measure Tg, as well as the units used (ng/FNAB, ng/mL) and the interpretation of the FNAB-Tg values. In consequence, the proper role of FNAB-Tg in the follow-up of patients with DTC is still a subject of debate.

Thus, the aims of the present article are to summarize the published evidence on the use of FNAB-Tg in the follow-up of individuals with DTC and to discuss the following topics: 1) the accuracy of FNAB-Tg in the detection of neck metastases in

individuals subjected to or awaiting total thyroidectomy; 2) the diagnostic benefit of FNAB-Tg compared to FNAB-C alone in DTC recurrence in cervical LNs; 3) the methods reported in the literature to establish the cutoff points of FNAB-Tg, including a comparison of their diagnostic performance to detect malignancy; and 4) the differences among the techniques used to measure FNAB-Tg, including an assessment of the possible influence of technical variations on its diagnostic accuracy.

**REVIEW**

The European consensus for the management of patients with DTC recommends screening the patients six to 12 months after the initial treatment by means of neck US and recombinant TSH-stimulated Tg (10).

The selection of patients for FNAB must be grounded in the architecture of the investigated LN on US. The most reliable indicators of malignancy are the presence of heterogeneous hyperechogenicity compatible with micro-calcifications, loss of hilum, cystic alterations, and peripheral vascularization (36). All such ultrasonographic findings exhibit strong correlations with malignant findings on postoperative histological analysis (16).

Some authors (10, 36, 37) suggest that FNAB should be performed only in cases when the smallest diameter of the LN is greater than 5 mm, as this criterion is associated with a satisfactory balance between the sensitivity and specificity of cytology alone for detection of metastatic PTC (61% and 96%, respectively) (16).

Since Pacini et al. (18) reported approximately 20 years ago that high Tg concentrations are detectable in LNs with thyroid cancer metastases, several studies have shown that the sensitivity of FNAB-Tg to detect DTC neck LN metastases is higher compared to that of FNAB-C (18, 19, 24-35). Frasoldati et al. (19) assessed the role of FNAB-Tg in the detection of LN metastases in patients with DTC before thyroidectomy and found a sensitivity and specificity of 78.6% and 100%, respectively. In addition, the sensitivity of combined FNAB-C and FNAB-Tg to assess neck LNs was better compared to that WBS and sTg measurement. In a later study on patients with neck-recurrent DTC, those same authors showed that the diagnostic sensitivity increased from 84.8% to 95.6% when FNAB-Tg was added to cytology (38).

In 2003, Cignarelli et al. (24) showed that FNAB-Tg might also contribute to diagnosis in paucicellular samples from cystic metastases. The diagnosis in two out of the six patients with PTC cystic metastases in the neck LNs included in the study was established by FNAB-Tg but not by cytology alone.

Bournaud et al. (34) assessed 114 consecutive patients with thyroid cancer and 16 controls. The sensitivity of FNAB-Tg was 94.2% and its specificity 97.8%. As for FNAB-C, although its specificity was 100%, its sensitivity was 71%, mostly due to inadequate samples.

Salmaslhoğlu et al. (33) compared the final histological diagnosis of 255 LNs with the results of preoperative FNAB-C and FNAB-Tg. FNAB-C failed to detect 17% of the metastatic LNs, versus 1% by FNAB-Tg. The specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of FNAB-Tg were significantly higher compared to FNAB-C.

The cystic degeneration of PTC metastatic deposits is not uncommon (39, 40), and FNAB of LNs in those cases, even with ultrasonographic guidance, might only show colloidal material, cell remnants, and macrophages, but no identifiable tumor cells. Such samples are usually classified as “nondiagnostic” or “inadequate” (41-43). In turn, FNAB-Tg has shown high sensitivity in the detection of cystic metastases when cytology is inconclusive. For instance, Cunha et al. (27) assessed 83 enlarged neck LNs from 67 patients with DTC and found that the sensitivity of FNAB-Tg was 100%, whereas cytology alone failed to diagnose nine out of the 17 patients with recurrent disease. FNAB-C alone identified only one out of six metastatic LNs with a cystic component (16.7% sensitivity).

Uruno et al. (29) studied 129 enlarged LNs from 111 patients with DTC and found that the sensitivity of FNAB-Tg and cytology was 81.4 and 78%, respectively, and that the sensitivity of FNAB-Tg for detection of cystic or mixed PTC metastases was 100%. On the whole, 36.4% of the cases classified as benign and 37.5% of the cases classified as inadequate on cytological examination were classified as malignant based on FNAB-Tg.

Snozek et al. (25) assessed 122 FNAB samples from 88 patients with a history of DTC and thyroidectomy. They found that Tg was 1 ng/mL or lower in 50 out of the 52 benign samples (96.2%) and higher than 1 mg/mL in all 70 malignant samples. Five (4.9%) of the 103 samples with diagnostic cytology, had discordant Tg results, in four of the these FNAB-Tg was concordant with the final diagnosis. As 18 out of 19

(94.7%) cases classified as nondiagnostic or with absent cytology were correctly diagnosed by FNAB-Tg, the authors suggested replacing FNAB-C with FNAB-Tg in the assessment of neck LN metastases.

The European consensus for the management of patients with DTC (10) currently recommends combining FNAB-Tg with the cytological assessment of FNAB samples for confirmation of suspected LN metastases. However, the American Thyroid Association (44) does not even mention this method in its guidelines for the management of DTC. This lack of agreement most likely reflects the lack of international standards for the performance and interpretation of FNAB-Tg. Consequently, the data available to assess its diagnostic value result from various types of Tg assay, variable volumes of needle washout fluid, and cutoff points establishing the negativity of test results (16).

**Factors that might affect the accuracy of Tg assays**

Measuring Tg poses one of the major challenges to laboratories due to interfering factors that might alter the test results, the lack of methodological standards, inadequate functional sensitivity, and variability in the specificity of the commercially available antibody kits. All of these factors affect the accuracy of the results and are associated with large inter-assay variation, which makes comparison among studies difficult.

The methods available to measure Tg include immunoassays, such as immunometric assay (IMA) and radioimmunoassay (RIA), in addition to enzyme-linked immunosorbent assay (ELISA), which is seldom used. The older competition-based measurement methods using radioactive iodine tracers have been progressively replaced by the more specific and sensitive IMA, which comprises immunoradiometric (IRMA) and immunochemiluminescence assays (ICMA) (43). The IMA method allows Tg levels lower than 0.1 ng/mL to be detected, and its technical advantages include shorter incubation periods and more stable reagent antibodies (45). Historically, several authors reported inadequate inter-assay precision in several Tg detection tests. Inter-assay precision must always be maximized so that the interpretation of results is not compromised by changes in reagents or equipment calibration over the course of the patients' follow-up.

Coefficients of inter-assay variation of 48% for sTg values of approximately 5.0 ng/mL and 80-120% for undetectable Tg values have been reported (46). Such discrepancies are partially accounted for by the heterogeneity of human Tg and the differences in the epitope specificity of the antibodies used as reagents in the various immunoassays (47). In particular, thyroid carcinomas might secrete one predominant or various Tg isoforms, which are detected with variable intensity as a function of the antibodies and assay methods used (48).

Various guidelines and consensuses recommend the use of kits that comply with the European standards for manufacturing Tg assays, e.g., CRM 457 (49, 50), which recommends the use of purified Tg obtained from human thyroid as antigenic stimulus (51). Unfortunately, because many assays do not comply with that consensus, although it reduces the variability in the methods available to measure Tg, it cannot eliminate it completely (48, 52).

Additionally, the functional sensitivity is of fundamental importance in the assessment of the performance of Tg measurement kits. Performance is defined as the lowest measured Tg concentration with a coefficient of inter-assay variation equal to or lower than 20% (53), and it represents the lowest clinically relevant value detected by the test. The functional sensitivity has gradually improved since the earliest methods for Tg measurement, from 5–10 ng/mL with the older RIA to 0.5–0.9 ng/mL with the first-generation IMAs and to 0.05–0.1 ng/mL with the “ultrasensitive” IMAs (54).

The functional sensitivity of a method directly influences the cutoff points. The original study by Pacini et al. (18) showed that the cutoff point, defined as the mean + two standard deviations (SD) of the FNAB-Tg value of patients with the final diagnosis of benign or malignant non-thyroid disease, was 21.7 ng/FNAB. Such a high cutoff point might be explained by the low functional sensitivity of the method used to measure Tg, which was 3 ng/mL. The greater functional sensitivity of the currently available assays accounts for the progressive reduction of the suggested diagnostic thresholds. Thus, some authors suggest that the FNAB-Tg cutoff point might be adapted to the method of Tg measurement used by each individual center (34).

Several published studies focus on methodological factors that might influence the Tg values found in the FNAB washout fluid, such as the volume and type of fluid

used, procedures for sample collection, and the interference of TgAb and sTg with the FNAB-Tg values.

## Needle washing

### *Needle washout fluid*

The medium used to wash the needle used in FNAB varies among studies, from 0.9% saline solution to solutions provided with Tg measuring kits (assay buffer or Tg-free solution; zero standard provided in the assay kit). The saline solution is the most widely accessible and least expensive option and is therefore the most commonly used (25-27, 30-33). To compare the various types of washout fluid used in FNAB, Frasoldati et al. (19) used different needles to collect two consecutive samples from the same 10 metastatic and 10 non-malignant LNs; one needle was washed with 1.0 mL of saline solution, and the other was washed with 1.0 mL of Tg-free solution. The FNAB-Tg concentration did not differ between washouts as a function of the rinsing method.

However, the studies by Baskin et al. (12) and Snozek et al. (25) pointed out the possibility of a matrix effect when the saline solution is used. The matrix effect is a type of interference that alters the reactivity of Tg with the assay antibodies due to the medium components. Snozek et al. (25) performed a recovery test after exogenous Tg overload and found that the Tg values increased by approximately 25% (varying from 100 to 140% of the expected values) when the FNAB-Tg needle was washed with saline solution. In fact, the nature of the buffer used to wash the needle might influence the protein conformation, thereby affecting the binding of antibodies, which was observed in the case of Tg when saline solution was used as a buffer (25, 26, 29, 35). Such a matrix effect might account for the high Tg levels found by some authors in benign LNs (34). Nonspecific interactions might be the cause of such interference in the measurement of Tg, as observed by Baskin et al. (12).

Therefore, although the amount of solution included in the kits is limited and its cost higher, it is preferable to saline solution, as this procedure avoids creating a bias in the measurement of Tg in the FNAB washout fluid (34). Nevertheless, when saline

solution is used due to practical reasons, ruling out the presence of a matrix effect is recommended (55). For instance, to validate the use of saline solution in their study, Borel et al. (26) measured the immunoreactivity of Tg in samples of Tg-free solution, saline solution, and saline solution supplemented with 70 g/L of human serum albumin. The results showed that the immunoreactivity of Tg did not reach the assay detection threshold in any of the study samples.

### *Volume of washout fluid*

According to various studies, the volume of fluid used to rinse the FNAB needle varies from 0.5 to 3.0 mL, and 1.0 mL is the amount most widely used (19, 26, 27, 30-32, 34, 38). That is also the amount recommended in the consensus published by Leenhardt et al. (55). Some authors suggest washing the needle several times and collecting the full amount of washout fluid in a single tube. According to Borel et al. (26), three rinses adding up to 1.0 mL of fluid suffice to collect more than 97% of the Tg present in the needle. Snozek et al. (25) assert that the volume of washout fluid should not exceed 1.0 mL because larger amounts might dilute the sample and give false FNAB-Tg results.

### Sample collection

Several test tubes are commercially available, and their use in FNAB-Tg must be validated. According to Giovanella et al. (56), the different types of tubes used to store the washout fluid might be a cause of bias in the measurement of FNAB-Tg. Following US-guided FNAB of 156 LNs from 108 patients subjected to thyroidectomy and radioablation due to DTC, those authors washed the needles using 1.0 mL of saline solution, which they divided homogeneously into three different types of test tubes: simple serum, serum separator, and lithium heparin tubes. The use of serum separator or lithium heparin tubes was associated with significant reduction of FNAB-Tg compared to the simple tubes. In addition, the measurement of FNAB-Tg from the simple serum and serum separator tubes had 100% sensitivity, whereas two false-negative results occurred in the samples collected in lithium heparin tubes (98% sensitivity). On those grounds, and to avoid methodological interference, those

authors recommend collecting a standard amount of washout fluid in simple serum tubes.

**Interference by circulating thyroglobulin**

Only a few studies have reported on the diagnostic value of FNAB-Tg in the detection of LN metastases of DTC in patients not subjected to thyroidectomy (19, 30, 34, 35, 57), and their results tend to disagree.

Although Frasoldati et al. (19) found 100% specificity in the FNAB-Tg of patients awaiting thyroidectomy and Cunha et al. (27) found 100% PPV in patients before surgery, false-positive results have been reported in patients with high sTg (12, 18, 30). Indeed, Tg as high as 88.8 ng/mL has been found in benign LNs from patients awaiting surgery for thyroid cancer (30, 34) and in patients without any thyroid disease, which could lead to unnecessary surgery for many of those patients (29).

According to Sigstad et al. (35), thyroid manipulation or palpation in patients who conserve all or part of the gland might lead to Tg escaping into the lymph, resulting in false-positive LN FNAB-Tg results. Thyroid manipulation must also be avoided in the examination of the LNs close to the thyroid (e.g., paratracheal, pretracheal, and prelaryngeal LNs) before thyroidectomy, when the risk for the biopsy needle to run through the thyroid is high, resulting in elevated FNAB-Tg independently of the presence of LN malignancy (29). Other possible explanations for the false-positive results include contamination of the LN by circulating sTg and artifacts caused by the method used for Tg detection because of nonspecific binding of the antibodies to various medium components. Such artifacts, described above as matrix effects, depend on the antibodies and the media that are used.

The data obtained by Borel et al. (26) do not support the hypothesis of sTg contamination of the LNs. According to them, even if the sample collected by FNAB was composed of blood alone, then as a function of the dilution, the rate of contamination of FNAB-Tg would be 0.2 times the sTg value, which in that study varied from 0.003 to 0.012% and was thus patently insignificant. To strengthen their argument, they further showed that FNAB-Tg was undetectable in negative controls who were not subjected to previous thyroidectomy and thus had detectable sTg. Finally, they measured FNAB albumin and showed that the contamination of FNAB-

Tg by serum proteins was very low. They concluded that sTg might interfere significantly with the FNAB-Tg values only in patients with high sTg, in whom the measurement of FNAB-Tg is actually not needed because they usually exhibit evident metastases. Other authors, including Bournaud et al. (34) and Kim et al. (57), were also unable to identify any influence of the presence of the thyroid gland on the FNAB-Tg values and found that this test has high sensitivity, specificity, and accuracy in the staging of LNs before thyroidectomy.

In a large-scale study conducted by Kim et al. (30), FNAB-Tg performed before surgery showed less sensitivity and accuracy relative to five assessed cutoff points compared to the measurement after thyroidectomy. According to Sohn et al. (32), it is not yet clear whether the interference of sTg on low or medium FNAB-Tg levels is negligible because the patients assessed by Borel et al. exhibited high FNAB-Tg; thus, the solution to this issue requires more thorough analysis.

Several cutoff points have been suggested for FNAB-Tg before thyroidectomy. According to some authors, who believe in the hypothesis of contamination by circulating Tg, different diagnostic thresholds ought to be established for patients who have undergone thyroidectomy and those awaiting thyroidectomy, whereas others recommend the adoption of a single cutoff point for both categories of patients. According to Borel et al. (26), any detectable level of FNAB-Tg in the neck LNs of patients without elevated sTg is associated with the presence of thyroid cells. That hypothesis is strengthened by the recent results that single FNAB-Tg cutoff points as low as 0.93 ng/mL correctly identified the LNs with DTC metastases.

**Interference by anti-thyroglobulin antibodies (TgAb)**

Doubtlessly, interference from circulating TgAb is the most serious technical complication that hinders the use of sTg as a marker of DTC recurrence (13, 28).

As the methods used to measure FNAB-Tg and sTg are the same, the possible interference of TgAb with FNAB-Tg values was taken into consideration (19, 38), until Boi et al. (28) showed the absence of a significant interaction between the TgAb and the Tg level measured in the neck LNs of patients with DTC, even in those with TgAb detectable inside the LNs, by either active synthesis or blood contamination. Although those authors reported that the presence of TgAb in the samples could result in underestimation of the FNAB-Tg level, such interference actually exerted a

small effect on the diagnostic performance of the test because the FNAB-Tg level in the washout fluid that contained TgAb was much higher than the selected cutoff point in all the evaluated cases (28).

Approximately two years earlier, Baskin et al. (12) reported on the lack of difference in the sensitivity and specificity for the detection of LN metastases between patients with and without serum TgAb. However, the small number of cases assessed in that study precluded any strong conclusion.

The reduced interference of TgAb with FNAB-Tg results compared to the interference exerted on the sTg measurements could be explained by the dilution of samples (most likely being 1:50 or higher) (35); elevated Tg concentrations in the washout fluid, which overcome the interference by saturating the TgAb-binding sites; or the absence or scarcity of TgAb inside the LNs (28).

In 2007, Sigstad et al. (35) studied the TgAb interference and found that those antibodies were absent in the FNAB washout fluid of individuals with positive serum TgAb and no or low FNAB-Tg. The absence of TgAb inside the LNs was also reported by other authors (26, 28, 35, 58).

According to Boi et al. (28), although one cannot rule out the possibility that the presence of TgAb in the FNAB washout fluid will sometimes hinder the detection of Tg in metastatic LNs, this phenomenon was not observed in their large case-series, and thus its frequency might be very low.

In turn, Cappelli et al. (59) recently observed a shift from undetectable to detectable FNAB-Tg in suspected LN metastases on US or WBS following recombinant human (rh)TSH stimulation in two patients with detectable serum TgAb. Those authors believe that in such patients, the presence of TgAb fully prevented the measurement of Tg in the FNAB washout fluid and that the Tg in the LNs increased to levels sufficient to saturate all the TgAb-bindings sites, and thus became detectable, only after stimulation with rhTSH, a possibility that had been suggested previously. Because the presence of TgAb in the FNAB washout fluid of the LNs was not assessed because it is not recommended by most authors and owing to the lack of histological data, that hypothesis could not be confirmed.

Zanella et al. (60) assessed the influence of serum TSH on the FNAB-Tg levels of patients without evidence of LN metastases on cytology who were undergoing levothyroxine-suppressive therapy (TSH = 0.07 mIU/mL)

or were under hypothyroidism to measure stimulated sTg (TSH = 82.2 mIU/mL). According to those authors, there was no significant difference in the median FNAB-Tg value between groups (3.3 ng/mL vs. 3.8 ng/mL, respectively), which suggests a lack of interference of serum TSH on the FNAB-Tg measurements.

**Cutoff points for measuring thyroglobulin in the washout fluid of fine-needle aspiration biopsy**

Although the diagnostic performance of FNAB-Tg is well established, its cutoff points remain controversial. A wide variety of possible diagnostic thresholds ranging from 0.9 to 39.3 ng/mL have been described in the literature, but it is not yet clear whether the same cutoff point ought to be used before and after thyroidectomy and for patients with high sTg. The major hindrances to the definition of one specific cutoff point for FNAB-Tg are the variety of measuring kits available and the different techniques and volumes of fluid used to wash the FNAB syringes. In some studies, the cutoff point was defined as the mean + 2 D) of the FNAB-Tg level of patients with negative cytology (12, 18, 24). Other authors established the cutoff point based on the comparison of the FNAB-Tg and sTg levels, whereby the LNs were considered metastatic when the former was higher than the latter (29, 31, 35, 57), which rules out the possibility of contamination by the circulating Tg. Cunha et al. (27) defined the cutoff point as the functional sensitivity of the Tg assay. In other studies, the cutoff point was established based on the area under the receiver operating characteristic (ROC) curve that best represented the relationship between the sensitivity and specificity of the analysis (30, 32-34). Boi et al. (28) used the highest FNAB-Tg value found in benign LNs that showed ultrasonographic regression in six to 12 months as the cutoff point.

Due to ethical reasons, the patients with benign cytology are not usually subjected to surgery. Thus, in some studies, the cutoff point was based on the FNAB-C results. Nevertheless, cytology could not be used as a gold standard to define the cutoff point of FNAB-Tg because the aim of the studies was to compare the diagnostic performance of both procedures (34).

The use of the sTg value as the cutoff point for FNAB-Tg might be difficult in patients awaiting surgery because most consensuses do not recommend measurement of sTg in such patients. In addition, the blood samples for sTg measurements and FNAB-Tg must be collected simultaneously because the sTg values vary over the course of days to weeks and might thus distort the data. It is also worth keeping in mind that in the presence of TgAb, the sTg level might be higher than the level actually measured by most available assays (12, 28).

Some authors (12, 28, 30) express FNAB-Tg results as nanograms/milliliter (or micrograms/liter), while others (18, 24, 26, 34) use the unit nanograms/punction. According to some authors, the unit nanograms/punction better represents the test, as it reflects the dilution of the Tg left in the needle, whereas nanograms/milliliter might mislead the reader into believing that FNAB-Tg reflects the true concentration of Tg in the LN. Conversely, some authors assert that nanograms/milliliter allows for the comparison of the FNAB-Tg and sTg levels, which is important for decision-making regarding surgical excision of the LNs when the FNAB-Tg cutoff point is defined as the patient's sTg level.

Such differences in the reporting of values, the lack of standardization of the commercially available Tg kits, the heterogeneity of control groups or "negative" patients, and the fact that some authors studied patients subjected to thyroidectomy while others focused on patients awaiting surgery make it difficult to compare published data.

Due to their belief in the possible interference of sTg with FNAB-Tg, some authors suggest establishing different diagnostic thresholds for patients before and after thyroidectomy (19, 28, 33). Frasoldati et al. (19) defined the FNAB-Tg cutoff point as the $97.5^{th}$ percentile of the FNAB-Tg levels of LNs with negative FNAB-C. In the patients awaiting surgery, that value was 39.3 ng/mL, compared with 1.1 ng/mL among those already subjected to thyroidectomy; the sensitivity and specificity, calculated based on patients with histological confirmation, were 84.0% and 95.4%, respectively. Boi et al. (28) defined as the cutoff point the highest FNAB-Tg value found in benign LNs (on histological examination or a combination of reactive FNAB-C and ultrasonographic regression in six to 12 months), which was 36 ng/mL among the patients awaiting surgery and 1.7 ng/mL among the patients subjected to thyroidectomy, with sensitivity and specificity of 100%.

Other studies applied a consistent threshold to FNAB-Tg independently of the status of patients regarding thyroidectomy (27, 29-31, 34, 35, 57). Cunha et al. (27) selected as the cutoff point of FNAB-Tg the functional sensitivity of the method they used to measure Tg (0.9 ng/mL), which resulted in 100% sensitivity in both groups and 100% specificity in the group of patients subjected to surgery. To rule out the possibility of contamination by circulating Tg, other studies (29, 31, 35, 57) used the sTg level as the cutoff point for FNAB-Tg. In the study by Kim et al. (57), the sensitivity and specificity were calculated based on the patients with histological confirmation, and the values found were 95% and 90.9%, respectively, before surgery and 80% and 100% after surgery. Bournaud et al. (34) and Kim et al. (30) suggest using a single and simple threshold. This suggestion agrees with the current guidelines, which do not recommend the measurement of FNAB-Tg in patients with thyroid cancer who are awaiting surgery. In those two studies, the cutoff point was established based on ROC curve analysis.

Independently of the values found, the sensitivity levels reported by the various authors have been similar (84–100% when different cutoff points are established for measurements before and after surgery versus 81–100% when a single cutoff point is used). Nevertheless, one might expect a lower number of false-positive results in the assessment after surgery due to the lack of thyroid tissue and sTg suppression (30).

These issues related to the cutoff point were recently addressed by Kim et al. (30), Bournaud et al. (34), and Sohn et al. (32), who each measured Tg in suspected LNs using the same ultrasensitive immunoradiometric assay based on monoclonal antibodies. Kim et al. (30) studied 168 patients with PTC and neck LNs considered to be potentially malignant on US before and after surgery. The FNAB-Tg results were interpreted based on five different cutoff points (1 ng/mL, 10 ng/mL, 100 ng/mL, the patient's sTg level, and mean + 2 SD of the FNAB-Tg level found in LNs shown not to be metastatic on histological examination, which was equal to 32 ng/mL) and compared to the final diagnosis as established by histological examination, TSH stimulated-Tg measurement, and US follow-up. The authors suggested using the sTg level, 10 ng/mL, or mean + 2 SD of the results of negative patients as the cutoff point for FNAB-Tg, as these criteria exhibited high sensitivity and accuracy in addition to similar diagnostic performance. Using those cutoff points, sensitivity varied from 88.2

to 90.8%, specificity from 84.2 to 89,8%, PPV from 93.9 to 95.6%, NPV from 74.4 to 80.0%, and accuracy from 88.0 to 90.5%.

According to those authors, dissection of the neck LNs must be strongly recommended when FNAB-Tg ≥ 100 ng/mL, as no false-positive results have yet been reported at such levels, and merely suggested when Tg > 10 ng/mL, Tg > sTg, or Tg > mean + 2 SD of the level of negative patients. Nevertheless, when FNAB-Tg < 1 ng/mL with negative FNAB-C, a follow-up study should be considered, and when Tg ranges from 1 to 10 ng/mL, the decision about LN dissection must be made based on the clinical and US findings. All five studied cutoff points were more sensitive and accurate in the assessment before than after thyroidectomy.

In the study by Bournaud et al. (34), 114 patients with thyroid cancer before surgery (n = 13) or throughout follow-up (n = 93) and 16 controls were subjected to FNAB-Tg and FNAB-C. The negative control group comprised 16 individuals with non-thyroid cancers, who exhibited 16 enlarged LNs suspected of metastasis on US performed during follow-up. The sensitivity and specificity of FNAB-Tg and FNAB-C were established for different cutoff points within a range of 0.24-32.60 ng/FNAB by means of ROC curve analysis. The LNs were classified as benign or malignant based on their histological features or, in the patients not subjected to surgery, on the scintigraphy findings and 12-month minimum follow-up. The most adequate cutoff point for diagnosis of LN metastases of thyroid cancer in that study was 0.93 ng/FNAB, which was slightly above the functional sensitivity of the method used to measure Tg. Using that cutoff point, sensitivity was 94.2%, specificity 97.8%, PPV 98.0%, and NPP 93.7%. The use of the method's functional sensitivity as the cutoff point, as suggested by Cunha et al. (27), which in the Bournaud study was 0.69 ng/mL, would have resulted in a similar sensitivity but lower specificity compared to the cutoff point of 0.93 ng/FNAB. Previous studies that used low cutoff points in the assessment of FNAB-Tg also reported excellent performance, with few false-positive or -negative results (12, 25, 27, 28). The adoption of a higher cutoff point in the Bournaud study, as suggested by some authors, could have resulted in absence of false-positive results but would have increased the number of false-negative samples, leading to a significant reduction of the sensitivity (86.5% for the cutoff point of 4.95 ng/FNAB). In that series, the results of the 29 samples of the controls and patients awaiting surgery were similar to each other, as were the results of the 93 samples collected after thyroidectomy.

To compare the frequency of metastatic and non-metastatic LNs diagnosed by means of FNAB-C and FNAB-Tg in a group of patients in whom the relationship between the FNAB-Tg level and malignancy is not yet well established, Sohn et al. (32) studied 691 consecutive patients with neck LNs bearing traits on US compatible with metastases of PTC. A total of 95 LNs with histological confirmation were included in the study, corresponding to patients with FNAB-Tg values of 0.2-100 ng/mL, because above 100 ng/mL, no false-negative results have been reported. The diagnostic performances of multiple Tg levels (0.7 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 50 ng/ml) were evaluated, and the best diagnostic performance was exhibited by 5.0 ng/mL: the area under the curve (AUC) was 0.76 (95% confidence interval [CI]: 0.6731-0.8476) and the sensitivity, specificity, accuracy, PPV, and NPV were 69.0%, 83.0%, 76.8%, 76.3%, and 77.2%, respectively. However, that cutoff point did not exhibit a significant difference in the sensitivity, specificity, accuracy, PPV, NPV, or AUC compared to 10.0 and 20.0 ng/mL. The cutoff points 0.7, 1.0, and 50 ng/mL were not recommended due to their low sensitivity and AUC. Among the 78 cases with negative cytology, 25 (32%) were confirmed to be metastases on the final diagnosis and exhibited FNAB-Tg varying from 0.22 to 88.34 ng/mL. Although seven cases were classified as negative with all six analyzed FNAB-Tg cutoff points, 13 cases (52%) were identified as positive when a cutoff point of 10 ng/mL was applied, 15 cases (60%) with the cutoff point 5 ng/mL, 18 cases (72%) with the combined cutoff points 0.7 and 1.0 ng/mL, and eight cases (32%) with all six cutoff points.

A large prospective study was recently published, in which 255 neck LNs of 225 patients with neck LN metastases or PTC recurrence (33) were assessed by FNAB-C and FNAB-Tg. The final diagnosis, based on histological examination, was compared to the FNAB-C and FNAB-Tg values before surgery. When the FNAB-Tg cutoff point was established as 1 ng/mL, 200 metastatic LNs were correctly diagnosed, but the method failed to diagnose 55 LNs. The sensitivity, specificity, and diagnostic accuracy of FNAB-Tg (cutoff point = 1 ng/mL) for diagnosis of metastases of thyroid carcinoma before surgery were 100%, 0%, and 78%, respectively. The PPV and NPV were 78% and 0%, respectively. The cutoff point of FNAB-Tg with the best sensitivity and specificity on ROC curve analysis was 28.5 ng/mL, as 253 metastatic LNs were correctly diagnosed and only two were misdiagnosed using that cutoff point. The sensitivity, specificity, and accuracy of FNAB-Tg (cutoff point = 28.5

ng/mL) for diagnosis of metastases of thyroid carcinoma before surgery were 100%, 96%, and 99%, respectively. The PPV and NPV were 99%, and 100%, respectively.

## SUMMARY

FNAB-Tg is simple to perform, is low cost, and improves the diagnostic performance of cytology in the early detection of LN metastases of DTC. This technique allows for diagnosis even in cases of paucicellular samples (metastatic deposits with degeneration and a cystic component) and cases in which cytology fails to provide a diagnosis, and it is not affected by TgAb, is reliable in the case of very small lesions, and exhibits high diagnostic accuracy.

In the case of patients treated with $^{131}I$, the interval between treatment and FNAB-Tg must be sufficient (more than three months) to allow for the definitive destruction of the metastatic LN; otherwise, FNAB-Tg might produce false-positive results. It is also not recommended to measure FNAB-Tg in masses found in the thyroid bed because this test is unable to distinguish thyroid tissue remnants from cancer recurrence.

The diagnostic performance of FNAB-Tg is not affected by the presence of thyroid tissue. Therefore, it might be used for screening LN metastases in patients subjected to thyroidectomy, as well as to perform LN staging in patients with DTC who have not yet undergone initial surgery.

## CONCLUSION

Measuring Tg in the FNAB washout fluid exhibits some advantages over cytology. The combination of FNAB-Tg and FNAB-C is recommended for the staging and follow-up of patients with DTC and suspected neck LNs.

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**Table 1.** Methods of measuring Tg by fine-needle aspiration biopsy (FNAB-Tg)

| | Numbers of patients/lymph nodes | Tg measurement method (kit) | Needle (gauge) | Volume and type of washing fluid | Unit [a] |
|---|---|---|---|---|---|
| Pacine et al. (18) | 35/- | Immunoradiometric (Sorin) | 21 | 0.5 mL kit-supplied diluent | ng/FNAB |
| Frasoldati et al. (19) | 130/216 | Immunoradiometric (Radim) | 22-23 spinal needle | 1 mL kit supplied-diluent or saline solution | ng/mL |
| Snozek et al.(25) | 88/122 | Automatic immunoassay analyzer DXI (Beckman Coulter) or Immulite 2000 automatic immunoassay analyzer (Siemens) | 25 | 0.5-1 mL saline solution | ng/mL |
| Borel et al. (26) | 34/53 | Immunoradiometric (CIS Bio International) | 22 BD spinal ® or 23*BD Microlance 3*® | 1 mL saline solution | ng/mL |
| Cunha et al. (27) | 67/83 | Chemiluminescent immunometric (Immulite 2000, Euro/DPC Ltd) | 22 or 25 | 1 mL saline solution | ng/mL |
| Boi et al. (28) | 73/- | Immunoradiometric (CIS Bio International) or chemiluminescent immunometric (Immulite 2000 – automatic immunoassay analyzer, Siemens) | 22-25 | 0.5 mL kit-supplied diluent | ng/mL |
| Uruno et al. (29) | 111/129 | _ | 22 | 0.5 mL saline solution | ng/mL |
| Kim et al. (30) | 168/168 | Immunoradiometric (CIS Bio International) | 23 | 1 mL saline solution | ng/mL |
| Jeon et al. (31) | 47/76 | _ | 21-23 | 1 mL saline solution | ng/mL |
| Sohn et al. (32) | 92/95 | Immunoradiometric (CIS Bio International) | 23 | 1 mL saline solution | ng/mL |
| Salmashõglu et al. (33) | 225/255 | Immunoradiometric (CIS Bio International) | _ | 3 mL saline solution | ng/mL |
| Bornaud et al. (34) | 89/122 | Immunoradiometric (CIS Bio International) | 27 | 1 mL kit-supplied diluent | ng/FNAB |
| Sigstad et al. (35) | 145/256 | Immunofluorometric | 25 or 27 | 0.5 mL kit-supplied diluent or saline solution | ng/mL |
| Giovanella et al. (56) | 108/156 | Immunoradiometric DYNO test Tg-plus, (BRAHMS Diagnostica GmbH) | _ | 1 mL saline solution | ng/mL |
| Kim et al. (57) | 68/91 | Immunoradiometric (CIS Bio International) | 21-23 | 0.5 mL saline solution | ng/mL |
| Zanella et al. (60) | 43/43 | Electrochemiluminescence (Modular E-170 Roche) | 22-25 | 1 mL saline solution | ng/mL |

FNAB, fine-needle aspiration biopsy.

[a]Unit used to express the results of the measurement of thyroglobulin in the washout fluid of fine-needle aspiration biopsy (FNAB-Tg).

**Table 2 –** Diagnostic performance of FNAB-Tg and FNAB-C, methods of establishing cutoff points, and their values of FNAB-Tg

| | **Analytic sensitivity/functional sensitivity of the FNAB-Tg measurement kit** | **Method to establish the FNAB-Tg cutoff point** | **FNAB-Tg cutoff point** | **FNAB-C sensitivity/ specificity** | **FNAB-Tg sensitivity/ specificity** | **Gold standard[a]** |
|---|---|---|---|---|---|---|
| Pacine et al. (18) | 3 ng/mL/ - | Mean + 2 SD[b] | 21.7 ng/FNAB | 85.7%/- | 100%/- | Histopathology |
| Frasoldati et al. (19) | 0.25 ng/mL/ - | 97.5th percentile of LNs with benign FNAB-C[c] | 1.1 ng/mL (T) or 39.3 ng/mL (NT) | – | 84.0%/95.4% | Histopathology |
| Snozek et al. (25) | 0.06 or 0.2 ng/mL/0.1 or 0.9 ng/mL | ROC curve | 1 ng/mL | – | 100%/96.2% | Histopathology. Neck US or clinical evidence after more than one-year follow-up |
| Borel et al. (26) | -/1 ng/FNAB | – | High: > 10 ng/mL<br>Intermediate: 1 to 10 ng/mL<br>Undetectable: < 1 ng/mL | – | – | – |
| Cunha et al. (27) | 0.5 or 0.2 ng/mL/0.9 ng/mL | Kit's functional sensitivity | 0.9 ng/mL | 55% (T) and 57% (NT)/100%(T) | 100% (T and NT)/100% (T) | Histopathology |
| Boi et al. (28) | 0.2 (IRMA) or 0.5 ng/mL (ICMA)/- | Greatest FNAB-Tg value in benign LNs[d] | 1.7 ng/mL (T) or 36 ng/mL (NT) | – | 100%/100% | Histopathology or reactive FNAB-C + ultrasonographic regression in 6-12 months |
| Uruno et al. (29) | – | Serum Tg | – | 78%/- | 81%/- | Histopathology |
| Kim et al. (30) | 0.2 ng/mL/0.7 ng/mL | Assessed 5 cutoff points through ROC curve | Serum Tg, mean + 2 SD (32 ng/mL) or 10 ng/mL | 77.3%/98% | 90.8%/89.8% | Histopathology and US follow-up |
| Jeon et al. (31) | – | Based on Uruno et al. | Serum Tg or 36 ng/mL (when serum Tg is unavailable) | 80.4%/100% | 94.6%/90% | Histopathology |
| Sohn et al. (32) | 0.2 ng/mL/0.7 ng/mL | Assessed 6 cutoff points through ROC curve | 5 ng/mL | 40.5%/100% | 69%/83% | Histopathology |
| Salmashõglu et al. (33) | – | ROC curve | 28.5 ng/mL | 95%/40% | 100%/96% | Histopathology |
| Bornaud et al. (34) | 0.2 ng/mL/0.7 ng/mL | Assessed 7 cutoff points through ROC curve | 0.93 ng/FNAB | 71.1%/73% | 94.2%/97.8% | Histopathology or WBS + disappearance/no progression on US |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sigstad et al. (35) | 0.1 ng/mL/- | Serum Tg | Serum Tg | 75%/50% and 100% (NT) | 100%/100% and 83% (NT) | Histopathology |
| Giovanella et al. (56) | – | – | 1 ng/mL | – | 98 or 100%/100% | Histopathology, clinical examination, serum Tg, and neck US |
| Kim et al. (57) | 0.2 ng/mL/0.7 ng/mL | Serum Tg | Serum Tg | 96.4/68.9% (NT) and 100%/90% (T) | 95%/90.9% (NT) and 80%/100% (T) | Histopathology |
| Zanella et al. (60) | 1 ng/mL/- | Based on Kim et al. and Baskin et al. | 10 ng/mL | – | 100%/100% | Histopathology or negative FNAB-C + neck US follow-up; and stimulated Tg for at least one year |

FNAB, fine-needle aspiration biopsy; FNAB-Tg, measurement of Tg in the washout fluid of fine-needle aspiration biopsy; FNAB-C, cytological assessment of samples collected by means of fine-needle aspiration biopsy; Tg, thyroglobulin; US, ultrasound; WBS, whole-body scan; NT, patients awainting for thyroidectomy; T, patients submitted to thyroidectomy; LN, lymph node.

[a]Methods considered gold standards to establish the sensitivity and specificity of FNAB-Tg and FNAB-C.

[b]Mean + two standard deviations of the FNAB-Tg values of benign LNs from patients with history of differentiated thyroid carcinoma (DTC).

[c] $97.5^{th}$ percentile of the FNAB-Tg level of patients with negative FNAB-C.

[d]LNs were classified as benign when they exhibited reactive FNAB-C associated with ultrasonographic regression in 6-12 months or when they had benign histology.

## 5.2 Artigo 2

**TÍTULO:** Thyroglobulin in the lymph node aspirate for the detection of metastases in patients with thyroid papillary carcinoma with non-diagnostic cytology.

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# Thyroglobulin in the lymph node aspirate for the detection of metastases in patients with thyroid papillary carcinoma with non-diagnostic cytology

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| | |

**Title Page**

**Full title:** Thyroglobulin in the lymph node aspirate for the detection of metastases in patients with thyroid papillary carcinoma with non-diagnostic cytology.

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**Abstract**

**Objective:** Metastatic lymph nodes (LNs) are frequent in patients with papillary thyroid carcinoma (PTC). Investigation is made by fine-needle aspiration biopsy cytology (FNAB-C) and supplemented by measuring thyroglobulin in the needle washout fluid (FNAB-Tg). This study evaluates the value of FNAB-Tg for detecting LN metastases from PTC in patients with nondiagnostic FNAB-C.

**Design:** This was a study of 195 LNs from 160 athyrotic PTC patients evaluated from 2009-2010, with follow-up of at least one year.

**Methods:** All patients were evaluated with ultrasonography (US), FNAB-C and FNAB-Tg. LNs were classified as negative or suspicious by US. Ultrasound criteria for suspicion of malignancy included rounded contour, hypoechogenicity, irregular internal echogenicity and/or microcalcifications, and an abnormal color Doppler pattern. Negative LNs with suspicious FNAB-C and high FNAB-Tg were excised, as suspicious LNs with suspicious FNAB-C and/or high FNAB-Tg.

**Results:** Of 195 LNs, 55 were suspicious. Median of the FNAB-Tg values in metastatic LNs was 1841.5 ng/dl. FNAB-C was nondiagnostic in 10 negative LNs with low FNAB-Tg, and in 5 suspicious LN with high FNAB-Tg and malignant in histopathology. One negative LN with benign FNAB-C and high FNAB-Tg receded in the follow-up. One suspicious LN with malignant FNAB-C and low FNAB-Tg was malignant in histopathology.

**Conclusions:** FNAB-Tg substitutes FNAB-C in unsatisfactory samples, but should be interpreted with caution if FNAB-C is benign. Further studies are required to confirm this.

## Introduction

Papillary thyroid carcinoma (PTC) is the most frequent malignant tumor in the thyroid gland and although the chances of long term survival are favorable, many of these patients have

metastatic cervical lymph nodes (LN) which are found before or many years after the initial surgery in approximately 5% to 20% of the cases, so that long term follow-up is required (1,2). However, the clinical investigation of cervical LN enlarged at the beginning or during the following of patients with PTC is no easy task, since inflammatory lymphadenopathy is extremely frequent and metastases of non-thyroid cancers are also relatively common (3).

The different tools available for this follow-up include basal and thyrotropin-stimulated (TSH) serum thyroglobulin (sTg), whole body scan with $I^{131}$ (WBS), neck ultrasound (US), and fine needle aspiration with cytology analyses of the LNs guided by US (FNAB-C), yet none of these tools is perfect. Serum Tg has a very high specificity for detection of recurrences (1, 4), but it is not reliable in patients with circulating anti-thyroglobulin antibodies (TgAb) and does not locate the neoplastic foci. The WBS may be affected by a number of variables, including the ability to capture the $I^{131}$ and there is evidence that 20-40% of cervical metastases do not capture $I^{131}$ even after the administration of high doses of this radiopharmaceutical (5). The specificity of cervical US is not ideal, although it has recently been improved by identifying several characteristics whose association is highly specific for malignancy (3).

The FNAB-C is the most reliable method for diagnosing cervical LN metastases in patients with recently diagnosed malignant neoplasm of the thyroid or previously treated thyroid cancer, but its sensitivity is far from great and is limited to presenting up to 20% of unrepresentative samples, with poor cellularity (such as cystic lesions), and 6% to 8% of false negative results, depending on the experience and skill of the cytopathologist (4, 6, 7, 8, 9, 10). Thyroglobulin measurement in washout fluid from fine needle aspirations of LNs (FNAB-Tg), initially suggested by Pacini et al in 1992 and confirmed in several studies, solves most of these issues (2, 3), with sensitivity and specificity of up to 100% in the detection of lymph node metastases whereas cytology alone had a sensitivity of only 85% (11, 12). However, studies to validate the benefit of FNAB-Tg over isolated FNAB-C are still scarce.

Thus, the objective of this study was to evaluate the diagnostic value of FNAB-Tg for detecting cervical lymph node metastases from PTC in patients with nondiagnostic FNAB-C.

## Material and methods

One hundred ninety-five lymph nodes from 160 patients with PTC that underwent a total or near-total thyroidectomy and subsequent ablation with Iodine$^{131}$, evaluated at the Department of Endocrinology and Diabetes of University Hospital Alcides Carneiro between June 2009 and June 2010, were referred to the diagnostic imaging service of the Dr. Wanderley Radiology Clinic for the investigation of central or lateral lymph node cervical metastases. All patients were submitted to high resolution neck US for the performance of guided FNAB-C and FNAB-Tg of cervical lymph nodes.

The patients were divided into two subgroups according to the ultrasonography aspect of the lymph nodes. Subgroup 1 consisting of patients with lymph nodes of reactive or negative aspect, and subgroup 2 with lymph nodes suspected of cervical PTC metastasis. Lymph nodes were considered suspicious for cervical PTC metastasis if they had an oval shape (ratio between minor and major axis > 0.7), hypoechogenicity, heterogeneous pattern (including cystic areas) and/or microcalcifications, in addition to anomalous vascularity (diffused hypervascularization to the color Doppler). A lymph node was considered negative when it had an elongated or fusiform shape, homogeneous pattern, presence of central echogenic hilum, and normal vasculature to the Doppler.

The ultrasound scans were performed by a single sonographer using a Toshiba Neumio 17® device, equipped with color Doppler and with a 12-14 mHz linear transducer. The examination was performed with the patient in a supine position and the neck in hyperextension. FNAB guided by US was performed by a single operator, without the use of anesthesia, by using 25 X 7 needles or a 22-gauge needle connected to a 10 mL syringe in the aspiration technique (14, 15), and using only the needle in the capillarity technique (cells move into the needle via capillary action) (15, 16, 17). All lymph nodes were aspirated by the two techniques.

Contents of the biopsy needles were expelled onto glass slides and smeared with a second slide to spread the fluid across the surface (6-12 smears per lymph node), immediately fixed in alcohol 96º, stained with haematoxylin and eosin and sent for cytological examination.

The FNAC results were interpreted by one cytopathologist, who specialized in thyroid cytology. According to the cytological criteria, the smears were classified as inadequate for diagnosis, negative, or suspicious of metastatic PTC. After collection of the cytology samples, each FNAB needle was then washed with 1.0 ml of normal saline and sent to the laboratory for measurement of thyroglobulin. FNAB needle wash Tg was assayed with a solid phase immunometric assay, chemiluminescent, (Thyroglobulin IMMULITE) with analytical sensitivity of 0.2 ng/ml. The cutoff point was 10 ng/ml (16, 20, 21, 29, 30).

The samples of FNAB-Tg were not tested for the presence of TgAb because it has been proved that FNAB-Tg is not affected by TgAb (25, 31, 32).

One hundred thirty-seven of 160 patients with negative and suspicious lymph nodes by the US, that had benign or unsatisfactory cytology and negative FNAB-Tg were followed up clinically and with cervical US for at least one year, and recurrence of PTC was not observed in any of them. The 2 patients with negative lymph nodes to the US, with suspicious cytology for metastasis and high FNAB-Tg were referred to surgery, and malignancy was confirmed in both. One patient with a negative lymph node to the US, benign cytology and FNAB-Tg of 51.4 ng/dl was followed up clinically and with US for one year, and the involution of the lymph node was observed.

The 50 lymph nodes considered suspicious to the US, with a cytology suspicious for malignancy and/or high FNAB-Tg, were referred to surgery.

In the statistical analysis, descriptive methods (frequency, percentages, averages, medians, standard deviation and data variance) were used for each continuous variable. The performance of each diagnostic method (US, FNAB-C, and FNAB-Tg) was evaluated for sensitivity, specificity, positive predictive value, negative predictive value and accuracy, separately, and then compared among them. Data were analyzed using SPSS 17.0 for Windows. Comparisons of the data were carried out using Chi-square tests. The results were considered statistically significant when the two-tailed p value was less than 0.05 with a confidence interval of 95%. The analysis of concordance between the results of FNAB-C and FNAB-Tg was performed by using the Kappa index.

The study plan was reviewed and approved by our institutional ethics committee, and informed consent was obtained from all patients.

**Results**

The average age of the patients was 49.4 ± 13.2 years, ranging between 20 and 82 years of age and the female/male ratio was 3.8/1 (n = 127/33).

Of the 195 lymph nodes evaluated, considering the ultrasound aspects, 140 (71.8%) were considered negative or reactive, and 55 (28.2%) were defined as suspected metastases.

The average diameter of lymph nodes was 1.22 ± 0.5 cm, ranging from 0.5 to 3.8 cm. The lymph nodes suspected by US showed an average diameter of 1.67 ± 0.5 cm and the negative lymph nodes measured 1.05 ± 0.3 cm. The average of the FNAB-Tg in the 52 (26,6%) metastatic lymph nodes was 3241.8 ± 5168.6 ng/dl, with a median of 1841.5 ng/dl, and in the 143 (73,3%) benign lymph nodes the average of the FNAB-Tg was 0.7 ± 4.3 ng/dl, with a median of 0.2 ng/dl.

**Results of aspirate cytology (FNAB-C) and thyroglobulin from the fine-needle aspiration washout fluid (FNAB-Tg) compared to high resolution ultrasonography (US) and histopathology.**

Considering the FNAB-C of the 140 lymph nodes reactive or negative to the US, 128 (91.4%) had benign FNAB-C, and in these, 127 (90.7%) had needle-wash Tg < 10 ng/dl and one had needle-wash Tg of 51.4 ng/dl. In the follow-up with US, there was involution of that lymph node, confirming that it was reactive lymphadenitis. The cytology was nondiagnostic in 10 (7.14%) lymph nodes, all with FNAB-Tg < 10 ng/dl. In two LNs (1.42%) the cytology was suspicious for malignancy, with elevated thyroglobulin at FNAB-Tg and malignancy was confirmed in histopathology. Of the 140 lymph nodes that were reactive to the US, 2 were malignant, which means 1.42% of false negative results for US.

As to the 55 lymph nodes considered suspicious to the US, 45 (81.8%) had cytology compatible with PTC metastases; 7 (12.7%) had nondiagnostic cytology; and 3 (5.45%) had

benign cytology and FNAB-Tg < 10 ng/dl. Of the 45 lymph nodes with cytology compatible with metastases, 44 had high FNAB-Tg and one had FNAB-Tg of 2.98 ng/dl. PTC metastasis was confirmed by histopathology in all of them.

Of the 7 lymph nodes (12.7%) suspicious to the US that had nondiagnostic cytology, two had FNAB-Tg < 10 ng/dl and five of them had high FNAB-Tg (1119, 1980, 2414, 4534 e 6300 ng/dl, average 3806.0 ± 2002.76 ng/dl), with metastasis confirmed by histopathology in all five. Therefore, FNAB-C failed to diagnose 5 lymph nodes considered suspicious for metastases by the US and in these patients the FNAB-Tg was fundamental for the diagnosis of metastasis of papillary thyroid carcinoma. Thus, the histopathological exam confirmed metastasis in 50 (90.9%) of the 55 lymph nodes that were suspicious to the US. For the cutoff point suggested in this study (10ng/dl), the FNAB-Tg diagnosed 51(98%) metastatic lymph nodes correctly from a total of 52, and had 0.69% cases of false positive among the benign lymph nodes (Fig. 1).

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy for the detection of lymph node metastasis of PTC from the US results were 96.1%, 96.5%, 90.9%, 98.6%, and 96.4%, respectively.

The sensitivity, specificity, PPV, NPV and diagnostic accuracy for the detection of lymph node metastasis of papillary thyroid carcinoma from the FNAB-C results were 90.4%, 100%, 100%, 96.6%, and 97.4%, respectively.

FNAB-Tg achieved sensitivity of 98.1%, specificity of 99.3%, PPV of 98.1% and NPV of 99.3%, and an accuracy of 98.9%.

When we evaluated the serum stimulated Tg, its sensitivity, specificity, PPV, NPV, and accuracy were respectively 60%, 78.9%, 52.9%, 83.3% and 73.5%.

Associating high resolution US with FNAB-C and FNAB-Tg increased the specificity and PPV to 100% (Table 1).

The concordance analysis of the results between FNAB-Tg and FNAB-C showed that in 189 lymph nodes (96.4%) the results of the two methods were in accordance, and discordant in only 6 lymph nodes (3.1%), which means a high concordance with a *k* of 0.9 (Table 2).

192 **Discussion**

193 Since Pacini et al. reported that high Tg concentrations are detectable in metastatic
194 thyroid carcinoma LNs, about 20 years ago, a number of studies have shown that the
195 determination of FNAB-Tg is more sensitive than FNAB-C for the diagnosis of lymph node
196 metastases of differentiated thyroid carcinoma (DTC) (7, 13, 14, 15).

197 In 2003, Cignarelli et al. (8) demonstrated that FNAB-Tg can also contribute to the
198 diagnosis of the paucicellular material obtained from cystic metastases, while reporting that two
199 out of six cases of cervical lymph nodes with cystic metastasis from papillary thyroid carcinoma
200 who participated in their study were diagnosed by FNAB-Tg, but not by cytology.

201 Bournaud et al. (16) evaluated 114 consecutive patients with thyroid cancer and 16
202 control individuals. The FNAB-Tg showed sensitivity of 94.2% and specificity of 97.8%.The
203 FNAB-C, in turn, despite having 100% specificity, showed sensitivity of 71%, mainly due to
204 the existence of inadequate samples.

205 Salmaslhoğlu et al. (1) compared the final diagnoses of 255 lymph nodes confirmed by
206 histologic exam, with the results of pre-surgery FNAB-C and FNAB-Tg and found that while
207 17% of the metastatic lymph nodes were not diagnosed by the FNAB-C, only 1% were not
208 detected by the FNAB-Tg. According to the authors, specificity, accuracy, positive predictive
209 value and negative predictive value of FNAB-Tg were significantly higher than the
210 corresponding values of FNAB-C.

211 In this study, we demonstrated that of 52 metastatic LNs, FNAB-C failed to diagnose
212 five (9.6%) that were suspicious in the US, and that were adequately diagnosed by FNAB-Tg,
213 which failed in the diagnostic of only one LN (1.9%), with sensitivity of 98.1%, accuracy of
214 98.9% and NPV of 99,3%, significantly higher values than those corresponding to FNAB-C.
215 The FNAB-C, in spite of having achieved a specificity of 100%, showed a sensitivity of 90.4%
216 and NPV of 96.6%.

217 However, although the performance of FNAB-Tg is well established, the value of the
218 cutoff remains controversial. For ethical reasons, the patients with benign cytology, in general,

are not submitted to surgery. As a result, some studies have established their cutoff points from the results of FNAB-C, such as Boi et al. (18), who determined the cutoff to be the highest value of FNAB-Tg found in lymph nodes with benign cytology that demonstrated ultrasound regression in 6 to 12 months.

Kim et al. (19) studied 168 patients with PTC and cervical lymph nodes identified as potentially malignant by pre or post-operative. The results of FNAB-Tg were interpreted according to five different cutoff points (1 ng/ml, 10 ng/ml, 100 ng/ml, the patient's own serum Tg level and average + 2 standard deviations of the FNAB-Tg levels found in lymph nodes that proved not to be metastatic in histological exam, corresponding to 32 ng/ml) and concluded that the best value for the diagnosis of persistent/recurrent DTC was < 10 ng/mL (20).

In our study, despite the outstanding performance of the cutoff of 10 ng/dl, one patient with lymph node suspicious to the US and with malignant cytology, confirmed as metastasis in the final pathology, had a level of FNAB-Tg inferior to the cutoff point suggested (2.98 ng/dl). The likely explanation for this low level of FNAB-Tg may be that little differentiation may occur in some cases of PTC, which are incapable of synthesizing/secreting quantifiable levels of Tg (21). Corroborating that possibility in this case, is the fact that this patient had tumors in the dorsum compatible with PTC metastasis, since this was an unusual site of metastatic implants of PTC.

Similarly to that found in other studies (21, 7, 13, 16), we had in this study a patient without metastasis, with benign FNAB-C and LN of reactive aspect in the US, who had a level of FNAB-Tg of 51,4ng/dl. Although the reason for this is not clear, some authors suggest that the reason is the contamination of the tissue by serum Tg or the presence of artifacts (matrix effect) which increased by up to 25% the value of the FNAB-Tg when the needle is washed with saline solution, which was used in this study (9, 10, 15, 26, 35). The first possibility is unlikely in this case, since the patient had undetectable serum Tg.

This finding, despite having been observed in only one patient, draws attention to the fact that it is probable, according to our data and to that of other studies, that FNAB-Tg solves the dilemma of the nondiagnostic samples by FNAB-C, but should be interpreted with caution

in patients with benign FNAB-C, image of reactive lymph node in the US, and slightly elevated levels of FNAB-Tg, seeming that in these cases the result of the FNAB-C should prevail.

Snozek et al. (4) evaluated 122 samples of FNAB in 88 patients submitted to thyroidectomy with a history of differentiated thyroid carcinoma, showed that all the cases with confirmed histological diagnosis of lymph node metastasis had highly suspicious ultrasonography findings, supporting the role of the US as a guide for diagnosis.

In the present study, 52 (26.6%) of 195 LNs were malignant. Among these, the US was able to identify 50 (96.1%), that is, it had a sensitivity of 96.1%, superior to what was reported by most studies (70 to 100%) (27, 28). However, the accuracy of the method has proved to be low, and confirmation of malignancy of the suspicious LN found in an US is generally recommended by FNAB-C and FNAB-Tg (28).

Still diverging from findings in literature (27, 28), our false positive rate from US was of only 9.1% (5 LNs), with a specificity higher than that reported by most studies (96.5%) and an accuracy of 96.4%. Of 140 reactive lymph nodes in the US, two were suspected to be PTC metastasis in the FNAB-C, both with high FNAB-Tg, and were confirmed malignant in the final pathology, which means only 1.42% of false negative result from US.

Salmaslhoğlu et al. (1), when studying 225 patients with metastases of cervical lymph nodes or recurrence of PTC, demonstrated that, as in other studies, serum Tg is undetectable in 20% of patients with isolated lymph node metastases during treatment with levothyroxine and in 5% after hormone withdrawal and does not find the metastatic foci. In our study, serum Tg also showed unsatisfactory performance, reaching a low diagnostic performance.

In the study of Snozek et al. (4) eighteen of the 19 cases (94.7%) with cytological evaluations not performed or nondiagnostic were correctly classified by FNAB-Tg, a result which made the authors suggest replacing the FNAB-C with the dosing of Tg in the washout of the needle of FNAB to evaluate metastases in cervical lymph nodes. Similarly, in this study when the degree of correlation between FNAB-C and FNAB-Tg was assessed, there was a high concordance (96.9%, $k = 0.9$), meaning that FNAB-Tg can substitute FNAB-C in the diagnosis of lymph node metastases in patients with PTC.

In the present study, 8.7% of cytological samples were nondiagnostic, a value much lower than that shown in most studies. The reason for this is that the procedures are performed by a team consisting of an endocrinologist, responsible for the FNAB, a sonographer who guides the procedure, and a cytologist specialized in thyroid, all of them members of a healthcare service specialized thyroid diseases.

A possible limitation to our study was the non-surgical removal of benign lymph nodes, an approach also adopted in other studies (16, 21), since it is ethically unacceptable to remove lymph nodes surgically without cytological or ultrasound evidence of malignancy. To overcome this limitation, we followed up on these patients clinically and with high resolution US for at least one year, and no alterations suggestive of metastasis were found in any of them.

Our results show that FNAB-Tg is the best technique for the early diagnosis of PTC metastases and is fundamental in lesions where the FNAB-C does not provide the diagnosis; in these cases, FNAB-Tg can replace FNAB-C.



**Declaration of interest**

No competing financial interests exist.

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405 **Figure legends**

406 Fig. 1 Study design and summary of results.

407 [a]High FNA-Tg levels are ones above the cutoff point (10 ng/dl).

408 [b]Low FNA-Tg levels are ones below 10 ng/dl.

**Table 1.** Performance of diagnostics modalities to evaluation of metastatic lymph nodes

| | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|
| Cervical US | 96,1 | 96,5 | 90,9 | 98,6 | 96,4 |
| FNAB-C | 90,4 | 100,0 | 100,0 | 96,6 | 97,4 |
| FNAB-Tg | 98,1 | 99,3 | 98,1 | 99,3 | 98,7 |
| Cervical US + FNAB-C + FNAB-Tg | 84,6 | 100,0 | 100,0 | 94,7 | 95,8 |
| Stimulated sTg | 60,0 | 78,9 | 52,9 | 83,3 | 73,5 |

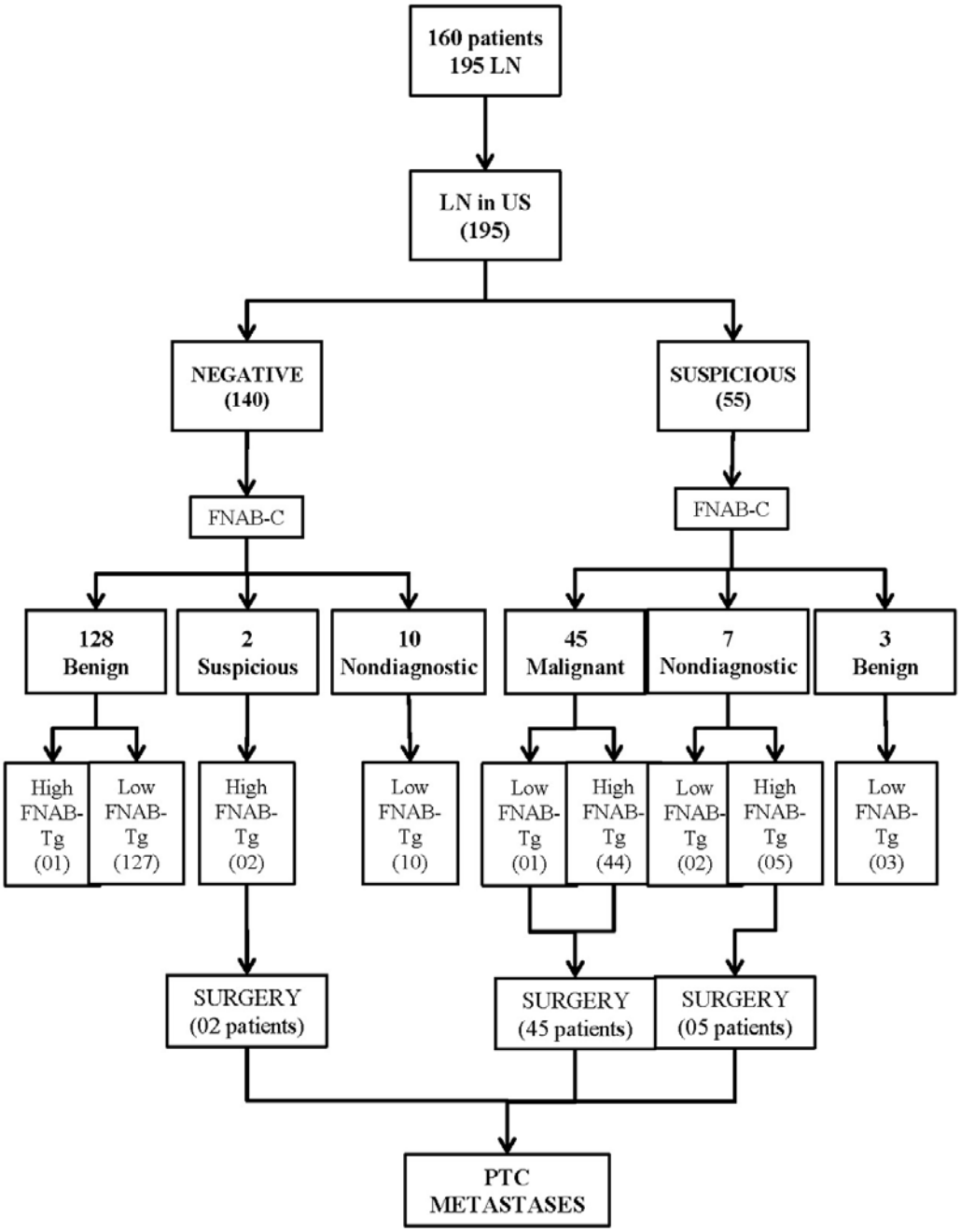
US, ultrasonography; FNAB-C, fine needle aspiration with cytology analyses; FNAB-Tg, tyroglobulin measurement in washout fluid from fine needle aspiration biopsy; sTg, serum tyroglobulin.

**Table 2.** Evaluation of the concordance between FNAB-C and FNAB-Tg

| | | FNAB-Tg | |
|---|---|---|---|
| | | Positive n (%) | Negative n (%) |
| **FNAB-C** | Positive n (%) | 46 (23,6) | 2 (1,0) |
| | Negative n (%) | 5 (2,6) | 142 (72,8) |

FNAB-C, fine needle aspiration with cytology analyses; FNAB-Tg, tyroglobulin measurement in washout fluid from fine needle aspiration biopsy.

*$k$ = 0,9


160 patients
195 LN
LN in US
(195)
NEGATIVE
(140)
SUSPICIOUS
(55)
FNAB-C
FNAB-C
128
Benign
2
Suspicious
10
Nondiagnostic
45
Malignant
7
Nondiagnostic
3
Benign
High FNAB-Tg (01)
Low FNAB-Tg (127)
High FNAB-Tg (02)
Low FNAB-Tg (10)
Low FNAB-Tg (01)
High FNAB-Tg (44)
Low FNAB-Tg (02)
High FNAB-Tg (05)
Low FNAB-Tg (03)
SURGERY
(02 patients)
SURGERY
(45 patients)
SURGERY
(05 patients)
PTC
METASTASES

## 6 CONCLUSÕES

- A Tg-PAAF é o melhor método para o diagnóstico precoce de metástase de carcinoma papilífero de tireóide.
- A Tg-PAAF deve ser associada à PAAF-C no seguimento de pacientes com carcinoma papilífero de tireóide porque aumenta significativamente a sua sensibilidade e pode substituí-la no caso das citologias não diagnósticas.
- A Tg-PAAF deve ser interpretada com cautela quando elevada em pacientes com citologia benigna, em LNs com aspecto reacional à ultrassonografia pelo risco de falso positivo.

## 7 CONSIDERAÇÕES FINAIS

O presente estudo alcançou os objetivos propostos e trouxe uma questão ainda pouco discutida na literatura a ser respondida em futuras pesquisas, qual seja, a necessidade de se explicar os casos de Tg-PAAF falsamente elevada, assim como propor formas de evitá-los.

Todavia apresenta duas consideráveis limitações: o fato de ter sido puncionado apenas um LN de cada paciente quando havia mais de um LN com características semelhantes, embora tenhamos escolhido o maior deles nesses casos, e a não remoção cirúrgica dos LNs negativos, embora saibamos ser eticamente inadmissível a retirada cirúrgica de LNs sem evidência de malignidade.

## 8 PERSPECTIVAS DE ESTUDOS

Avaliar se é realmente o efeito matriz o responsável pelos casos de Tg-PAAF falsamente elevada, através de um estudo utilizando duas soluções para medida da Tg-PAAF, a solução salina e o líquido fornecido pelo Kit de dosagem de tireoglobulina (tampão do ensaio ou solução livre de tireoglobulina), em um mesmo grupo de pacientes, ou seja, cada paciente seria o controle se si mesmo, todos com carcinoma papilífero e tireoglobulina sérica indetectável, para afastar a possibilidade de contaminação.

# 9 ANEXOS

## 9.1 Anexo A – Parecer do Comitê de Ética


**Comitê de Ética em Pesquisa em Seres Humanos**


**DECLARAÇÃO**

*Declaro para os devidos fins que em reunião de 22/ 12/ 2009 foi aprovado por unanimidade o Projeto de Pesquisa: Tireoglobulina Obtida do Lavado de Instrumento de Gânglio Cervical para Diagnóstico de Câncer Metastático de Tireóide. Projeto a ser realizado no período de: Fevereiro de 2010 a Dezembro de 2010.*

*Após conclusão da pesquisa deve ser encaminhado ao CEP/ HUAC, em 30 dias (trinta dias), relatório final de conclusão, antes de envio do trabalho para publicação. Haverá apresentação pública do trabalho no Centro de Estudos HUAC em data a ser acordada entre pesquisador e CEP/ HUAC.*

*Relator (a):*
*Maria das Graças Vieira de Souza e Cavalcanti de Castro*

*Maria das Graças Vieira de Souza e Cavalcanti de Castro*
*Coordenadora CEP/ HUAC/ UFCG.*

*Campina Grande - PB, 07 de Janeiro de 2010.*

Rua.: Dr. Carlos Chagas s/ n – São José – Campina Grande – Paraíba
Telefone.: 0xx 83.2101.5500 – Ramal - 5545
E-mail.: cep@huac.ufcg.edu.br - cep.huac@ig.com.br

Aprovado pelo parecer
22/01/10

## 9.2 Anexo B – Termo de Consentimento Livre e Esclarecido

**Universidade Federal de Campina Grande – UFCG**

**Universidade Federal da Bahia - UFBA**

**Doutorado Interinstitucional - DINTER**

**Título da Pesquisa:** Tireoglobulina obtida do lavado de instrumento de gânglio cervical para diagnóstico de câncer metastático de tireóide

**Durante a leitura do documento abaixo fui informado (a) que posso interromper para fazer qualquer pergunta, para tirar dúvidas e o meu melhor entendimento.**

Eu,_____
brasileiro(a), RG:_____, estado civil:_____,
residente em _____
fui procurado(a) pela médica Dra. Maria Roseneide dos Santos Torres do Hospital Universitário, da Universidade Federal de Campina Grande. PB, médica com registro 3996 no Conselho Regional de Medicina do estado da Paraíba, para participar do projeto de pesquisa com o título acima citado, coordenado pela mesma. Neste estudo, eu_____, fui selecionado(a) (sob minha inteira responsabilidade) por encaminhamento do meu médico assistente para realização de punção de LNs cervicais.

A doutora Maria Roseneide dos Santos Torres explicou-me que as pessoas que tiveram câncer de tireóide devem fazer um acompanhamento para descobrir o reaparecimento da doença (metástase). Também fui informado que esse exame descobre o tipo mais freqüente de metástase que ocorre nesse câncer, permitindo assim o tratamento e o controle da doença.

Fui informado (a) que o exame é feito com uma agulha e uma seringa que irá retirar um líquido do caroço para ser examinado e que cada pessoa recebe duas ou mais furadas, dependendo do número e das características dos caroços que ela tenha no pescoço. Ela informou ainda que esse exame não é perigoso e que eu não corro risco de vida durante ou após a sua realização e que a possibilidade de que eu tenha alguma complicação é muito pequena, embora exista. Porém, ela também esclareceu que essas complicações, quando surgem, são facilmente resolvidas e que se eu apresentá-las serei rapidamente atendido(a).

Estou ciente de que caso tenha alguma reclamação posso procurá-la a qualquer momento, assim como também posso me recusar a realizar o exame. Declaro ainda não ter recebido nenhum benefício, qualquer que seja ele.

Também estou ciente que os resultados da pesquisa serão publicados mas meu nome ou endereço não aparecerão na publicação. Fui informado(a) que posso conhecer os resultados da pesquisa e, caso me sinta prejudicado (a), poderei recorrer ao Conselho Regional de Medicina ou ao Comitê de Ética em Pesquisa.

Assim, após ouvir e entender o que me foi dito, vagarosamente, considero-me satisfeito (a) com as explicações desse documento e concordo em participar dessa pesquisa.

COMO TENHO DIFICULDADE PARA LER ([ ] sim ou [ ] não), O ESCRITO ACIMA, ATESTO TAMBÉM QUE A DRA. MARIA ROSENEIDE DOS SANTOS TORRES QUANDO DA LEITURA PAUSADA DESSE DOCUMENTO ESCLARECEU ÀS MINHAS DÚVIDAS E COMO TEM A MINHA CONCORDÂNCIA PARA PARTICIPAR DO ESTUDO, CONCORDEI COLOCAR ABAIXO A MINHA IMPRESSÃO DO DEDO POLEGAR.

Campina Grande (PB), _____, de _____ de _____.

Nome: _____

Assinatura:_____

Ou

| |
|---|
| |

(impressão digital ou datiloscópica)

**Testemunhas:**

1. Nome:_____

_____
Assinatura

2. Nome:_____

_____
Assinatura

_____
Maria Roseneide dos Santos Torres (CRM-PB)

**DOCUMENTO DISPONÍVEL EM DUAS (2) VIAS, UMA PARA SER ENTREGUE A PESSOA OU RESPONSÁVEL QUE VAI PARTICIPAR DA PESQUISA.**