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Thyroglobulin measurements in washouts of fine-needle aspiration biopsy in the monitoring of patients with differentiated thyroid carcinoma

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Abstract

Introduction: Approximately 35% patients with papillary thyroid carcinoma (PTC) and 13% with follicular thyroid carcinoma (FTC) present with metastases of cervical lymph nodes (LNs) at the time of diagnosis. In addition, 15–20% of patients treated with total thyroidectomy develop, after an interval of five years, metastases to the neck LNs on ultrasound examination. Fine-needle aspiration biopsy (FNAB) represents the gold standard technique for the detection of cervical LNs metastases. The aim of the study was to evaluate the diagnostic performance of the technique of thyroglobulin (Tg) measurement of washout FNAB (FNAB-Tg) in diagnostics of LNs metastases in different groups of patients with differentiated thyroid carcinoma (DTC).

Material and methods: Two hundred FNAB-Tg samples from 200 patients [158 women; 42 men; mean age 51.37 ± 16.77 (53)] diagnosed with DTC were examined for the assessment of the diagnostic utility of FNAB-Tg from suspicious LNs. FNAB-Tg ranged from 1.96 to 5000 ng/mL in metastatic LNs [mean; 1510 ± 1486 ng/mL (958.5)] and from 0.04 to 635.9 ng/mL in nonmetastatic LNs [mean; 57.86 ± 319.19 ng/mL (1.96)], $p < 0.001$.

Results: The most accurate diagnostic performance was displayed for the concentration of 33.28 ng/mL in FNAB-Tg with AUC of 0.91 and high sensitivity and specificity (0.92 and 0.93). FNAB-Tg in conjunction with the cytopathological examination of suspicious LNs in differentiated thyroid carcinoma (DTC) patients increases the diagnostic accuracy of FNAB (sensitivity 0.99; specificity 0.99; AUC 1.00).

Conclusions: FNAB-Tg may be particularly useful in detecting LN metastases in DTC patients, and in differential diagnosis of various LN metastasizing malignancies. The combination of FNAB and FNAB-Tg measurement has high specificity and sensitivity in the detection of LN metastases of DTC. **(Endokrynol Pol 2021; 72 (6): 601–608)**

Key words: *thyroid carcinoma; thyroglobulin; fine-needle aspiration biopsy; lymph node metastasis; washout of the needle*

Introduction

Differentiated thyroid carcinomas (DTC) usually behave in an indolent fashion and have an excellent prognosis. Nevertheless, DTC is frequently associated with cervical lymph nodes (LNs), and approximately 35% of patients with papillary thyroid carcinoma (PTC) and 13% of patients with follicular thyroid carcinoma (FTC) present LNs metastasis at the time of diagnosis [1]. Moreover 5–20% of patients will develop LN metastases during the first five years after treatment, and recurrences may appear up to 20 years after initial treatment [2].

Ultrasonography (US) is currently the most useful method to detect cervical lymphadenopathy; however, none of the suspicious US findings (round shape, cystic changes, hyperechoic foci or microcalcifications, irregular chaotic vascularization, and absence of the hilus) allows the formulation of a diagnosis of LN metastasis [3, 4]. Consequently, fine-needle aspiration biopsy (FNAB) is usually required to confirm or exclude metastasis because reactive LN enlargement is common in the neck region. However, small LNs are technically difficult to aspirate. On the other hand, evaluation of enlarged neck LNs may be challenging due to the broad spectrum of non-thyroidal lesions presenting as a neck nodule. Moreover, cytological features of enlarged LNs are difficult to evaluate due to the presence of lymphocytes, granulocytes, and multinucleated giant cells, a variable amount of necrosis, and poor epithelial cellularity, particularly in the case of cystic changes [5]. This results in a 6–8%

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false-negative rate and a 5–10% inadequacy rate of FNAB [6].

The thyroglobulin (Tg) measurements in the needle washout (FNAB-Tg) have been reported to increase the sensitivity of FNAB [7]. This technique is particularly useful in detecting cystic and small metastases. FNAB-Tg was found to be helpful in cases of indeterminate cytopathology and in patients suffering from other malignancies beside DTC. The rationale of the technique comes from the fact that Tg is produced only by follicular cells. Consequently, detection of Tg in LNs FNAB suggests the diagnosis of DTC metastasis. This assay was first proposed by Pacini et al., and despite being recommended by many guidelines the technique is not standardized [8]. There are considerable differences between various diagnostic centres regarding the methodology of measurement of Tg levels as well as cut-off values for FNAB-Tg in positive cases (varying from 0.2 ng/mL to 100 ng/mL in different studies) [9].

Nevertheless FNAB-Tg became a gold standard at many centres in the follow-up of DTC patients, with sensitivity and specificity of 92–100% and 89-100%, respectively [10,11]. Current clinical practice guidelines for thyroid carcinoma already incorporate FNAB-Tg as an essential addition to FNAB in suspicious LNs [12].

However, the results of FNAB-Tg may be influenced by many factors, including the presence of a normal thyroid (in patients before surgery or after lobectomy) and the presence of serum Tg antibodies (TgAb) [13–15].

In the present study we aimed to evaluate the diagnostic performance of FNAB-Tg and its cut-off value in diagnostic LN metastases in DTC patients.-

Material and methods

Patients

Two hundred LN FNAB-Tg samples from 200 patients [158 women and 42 men; age 51.37 ± 16.77 (53)] with suspicious neck LNs were investigated. All the patients were diagnosed with DTC, treated and followed up at the Department of Oncological Endocrinology and Nuclear Medicine, Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw. The patients were recruited between 2012 and 2015. FNAB and FNAB-Tg measurements were performed at initial diagnosis of thyroid cancer prior to thyroidectomy or during cancer surveillance after thyroidectomy and/or radioactive iodine therapy/or radiotherapy. The inclusion criteria were as follows: confirmed cytopathological (Bethesda VI) or histopathological DTC, suspicious LNs in US. The following exclusion criteria were introduced: 1) metastases to LNs from primary lesions other than DTC, such as anaplastic thyroid carcinoma or medullary thyroid carcinoma; 2) LNs without serum Tg and TgAb at the time of FNAB and FNAB-Tg measurement; and 3) LNs without follow-up at our institution after the first FNAB and FNAB-Tg. This study complied with the ethical standards of the Declaration of Helsinki and was approved by the Ethic Committee of Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw. The requirement for informed consent explaining the study purpose and procedures was waived due to the retrospective design of this study.

US-guided FNAB and FNAB-Tg of suspicious LNs

FNAB was performed by an experienced cytopathologist with a 25-gauge needle attached to a 10 mL disposable plastic syringe under the real-time guidance of a US device (iU22 Philips diagnostic ultrasound system — Philips Ultrasound, with a L12–5 MHz compact linear array transducer). US criteria indicating suspicious LNs included the following: absence of the hilus, prominently hypoechoic, presence of cystic changes, presence of calcifications, a rounded (longitudinal/transverse axis < 2) or polycyclic shape, and irregular and/or peripheral vascularity. The specimen obtained from FNAB was immediately smeared on glass slides after aspiration, fixed with 70% ethanol, and stained with haematoxylin and eosin (H&E) stain.

The remaining aspirate in the syringe and needles were immediately rinsed with 0.5 mL of isotonic 0.9% saline and the washouts were processed to measure Tg using the electrochemiluminescent (ECLIA) assay by an Elecsys cobas e automated analyser (ROCHE). The value below a lower range of the detection limit was < 0.04 ng/mL and above it was > 5000 ng/mL. The threshold values were 3.5–77 ng/mL respondent with 2.5 and 97.5 percentiles. Positive FNAB-Tg was defined as a concentration of Tg in washout above the cut-off value. Normalized washout Tg was defined as the FNAB-Tg divided by the serum Tg level.

Biochemical analysis: Tg and TgAb

Serum Tg, FNAB-Tg, and serum antibodies (TgAb) samples were taken from all the patients. The serum Tg and FNAB-Tg were measured as above, and TgAb was measured using the electrochemiluminescent (ECLIA) assay by an Elecsys cobas e automated analyser (ROCHE).

The value below a lower range of the detection limit was < 10.0 IU/mL and above it was > 4000 IU/mL. The threshold value was 115 IU/mL respondent with the 94th percentile.

Fine-needle aspiration biopsy (FNAB) results and final LN diagnosis

The interpretation of FNAB was performed by experienced pathologists specialized in thyroid cytology. The results of the microscopic examinations were classified into three groups: 1) positive — presence of carcinoma cells; 2) negative — absence of carcinoma cells; and 3) indeterminate or non-diagnostic — poorly cellular smears, presence of macrophages and/or some suspicious epithelial cells without clear (convincing) signs of malignancy, or poor specimen with absence of LN elements and epithelial cells. The patients with indeterminate or non-diagnostic cytopathology results underwent repeated FNAB and FNAB-Tg.

Patients with positive FNAB and/or FNAB-Tg results of the aspirated LNs were qualified for appropriate treatment — surgery, systemic treatment (disseminated diseases), or watchful waiting (patients who did not agree to treatment). Generally, the final LN diagnosis was confirmed by postoperative histopathologic results. Metastatic LNs were defined as those with postoperative metastasis in histopathology in the same compartment that FNAB and FNAB-Tg were performed. If patients did not undergo surgical treatment, metastatic LNs were defined based on metastatic FNAB results combined with a high FNAB-Tg level and results of imaging studies (US, computed tomography — CT, magnetic resonance imaging — MRI). If repeated FNAB showed metastatic carcinoma cells LNs were included in the metastatic group. Benign LNs were confirmed by benign histopathology after post-operative review. If the target LNs were not dissected and follow-up imaging showed decreased size or no interval change in size during a follow-up of at least five years, the final LNs were considered to be benign. If repeat FNAB results revealed benign LNs, the final LNs were also defined as benign. LNs that were negative in FNAB and in FNAB-Tg were subjected to US and clinical follow-up for at least 60 months.

Statistical analysis

Data analysis was performed using R software (version 3.6.1). The χ^2 test was used to compare the groups of data that did not meet the assumptions of the parametric test (parametric tests were conducted using Student's t-tests). Associations between variables were analysed using Pearson's correlation coefficient (point-biserial correlation when one variable was dichotomous). The receiver operating characteristic (ROC) was used to determine the best cut-off values for diagnostic purposes of the analysed variables. In each case the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were assessed. For each variable, the 95% confidence interval (95% CI) was estimated. In all analyses, the results were considered statistically significant when $p < 0.05$. To measure the predictive capabilities of the models, four-fold cross-validation was performed. The approach was selected to assess how the results of a statistical analysis generalize to an independent dataset (new patients). The main goal of cross-validation is to test the model's ability to predict new data that was not used in the rule selection. This approach is commonly used to overcome overfitting problems or selection bias.

Results

The analysis of the 200 LNs revealed 60 to be metastatic, whereas the remaining 140 lymph nodes had no signs in neoplastic process upon cytopathological and/or histopathological examination. Statistical analysis revealed that patients with the presence of metastatic lymph nodes had significantly higher Tg serum, as well as FNAB-Tg levels; however, the level of TgAb did not differ significantly (Tab. 1). In metastatic cases no significant correlation of Tg serum and FNAB-Tg levels could be disclosed ($r = 0.15$, $p = 0.2467$). However, there was observed correlation in the case of cancer-free patients ($r = 0.2$, $p = 0.0214$). In all analysed lymph nodes the Tg serum also (significantly) correlated with the FNAB-Tg level ($r = 0.26$, $p < 0.001$).

ROC analysis was performed to identify the best markers for discrimination of metastatic LNs (Fig. 1). The analysis revealed that threshold based on FNAB-Tg and FNAB give most accurate results. However, due to the high level of variation of FNAB-Tg (standard deviation = 1082.27), there is high risk of selection bias (another random group of patients can represent slightly different level of FNAB-Tg). In order to overcome this limitation, four-fold cross-validation was performed. The results of cross validation confirm that the addition of FNAB-Tg to cytopathological examination increased the sensitivity and negative predictive value as compared to solitary analysis of FNAB or FNAB-Tg (Tab. 2). Based on the obtained results, the combination with a cut-off value of FNAB-Tg of 33.28 ng/mL was selected. For 100 randomly selected patients, it successfully identified all metastatic LNs; however, two false-positive results were derived. In order to increase the diagnostic potential of FNAB-Tg, a combination of its level and results of cytopathological specimen analyses were combined. Applying the FNAB-Tg threshold (33.28 ng/mL) to the FNAB cytopathology produced more accurate results (Fig. 2).

Patients with follicular thyroid carcinoma (FTC)

Fifteen patients were diagnosed with FTC. In seven patients positive results of the FNAB correlated with elevated FNAB-Tg level. In the follow-up in four of these patients, histopathological examination of the removed LNs disclosed metastases, in two cases with a minor component of the poorly differentiated carcinoma. Three patients with positive correlation of FNAB and elevated FNAB-Tg levels were not operated on because of disseminated neoplastic process or disqualification caused by accompanying diseases. One patient with elevated FNAB-Tg and negative FNAB was operated on with histopathological examination revealing LN metastasis, and in five years of follow-up nodal recurrence was noticed.

Five years of US observation of an additional seven patients with negative FNAB and low FNAB-Tg level indicated a reduction of the enlarged LNs, but in two patients in this group an incomplete biochemical response was observed.

Table 1. *Numeric variable values: mean ± standard deviation (SD) (median)*

Characteristics	Total	Metastatic LNs	Benign LNs	p value	n
Age	51.37 ± 16.77 (53)	55.33 ± 19.11 (57)	49.65 ± 15.41 (52)	0.0448	198
Tg	87.87 ± 531.98 (0.49)	281.17 ± 951.81 (33)	5.82 ± 21.92 (0.16)	0.0302	198
TgAb	57.45 ± 266.23 (10.21)	87.7 ± 367.97 (10.52)	44.52 ± 208.61 (10.05)	0.4007	197
FNAB-Tg	493.54 ± 1082.27 (3.5)	1510.12 ± 1486.36 (958.5)	57.86 ± 319.19 (1.96)	0.0000	200
Tg ratio	310.56 ± 1645.89 (3.98)	479.3 ± 1627.71 (28.89)	238.94 ± 1654.16 (1.5)	0.3463	198
TSH	0.9 ± 2.29 (0.17)	0.94 ± 2.05 (0.19)	0.88 ± 2.42 (0.16)	0.8753	114
Longitudinal/transverse axis	1.77 ± 0.64 (1.63)	1.57 ± 0.68 (1.33)	1.87 ± 0.6 (1.83)	0.0137	144
Longitudinal axis	14.38 ± 7.99 (12)	16.98 ± 10.5 (15)	13.31 ± 6.46 (12)	0.0262	168

LNs — lymph nodes; Tg — thyroglobulin; TgAb —Tg antibodies; FNAB — fine-needle aspiration biopsy; Tg ratio — FNAB-Tg/serum Tg; TSH — thyrotropin

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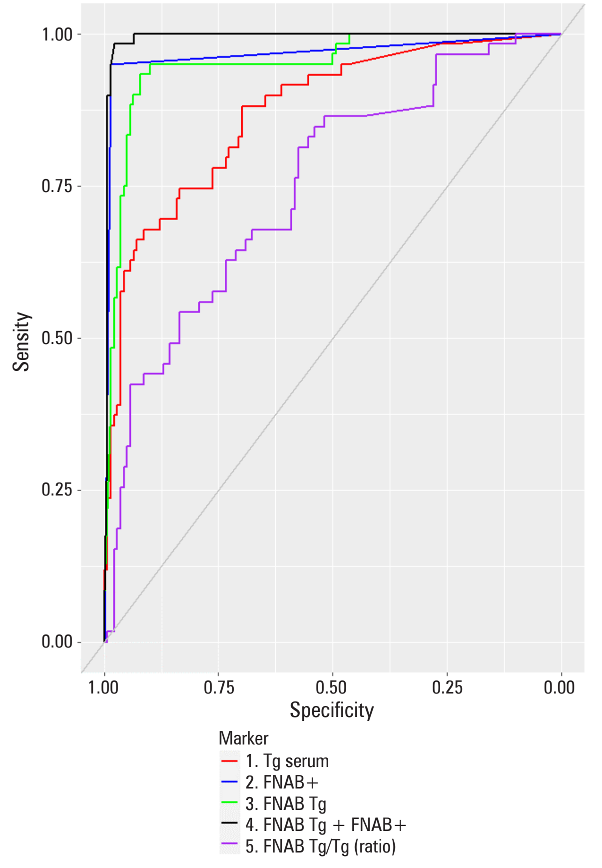

Figure 1. *ROC curve for selected markers (re-substitution method). Tg — thyroglobulin; FNAB — fine-needle aspiration biopsy; Tg ratio — FNAB-Tg/serum Tg*

Patients with Hürthle cell carcinoma

Hürthle cell carcinoma usually has a more aggressive clinical course, often with metastasis to the neck LNs and worse prognosis [16, 17]. Four patients were diagnosed with this classification of thyroid tumours. In two patients, positive results of the FNAB correlated with elevated FNAB-Tg levels. One patient was operated on, and histopathological examination of the removed LNs disclosed metastasis of both Hürthle cell carcinoma and poorly differentiated components; two years after lymphadenectomy the patient died because of disseminated neoplastic disease. The other patient was disqualified from operation because of metastases to the lungs. In an additional two patients FNAB and FNAB-Tg remained negative, and in one patient five-year follow-up US examination indicated benign LNs with incomplete biochemical response; in the second case, recurrence in the thyroid beds and metastases to the lungs were observed.

Patients with papillary thyroid carcinoma (PTC)

In 46 patients with PTC positive results of FNAB correlated with elevated FNAB-Tg levels. In the follow-up in 39 of these patients, histopathological examination of the removed LNs disclosed metastases, in one case with a minor component of the poorly differentiated carcinoma. Thirteen patients displayed discordant results between FNAB and FNAB-Tg. In two of these patients with positive FNAB and low FNAB-Tg levels histopathological examination of the resected LNs revealed either a diffuse sclerosing variant of PTC or widely disseminated PTC. Two of the five patients with high FNAB-Tg level and negative FNAB underwent surgery, and histopathological examination of excised LNs confirmed metastases in one case.

Among 45 patients after lymphadenectomy with histopathologically confirmed LN metastases, 33 patients did not develop biochemical or structural recurrence disease after five years of follow-up, another two patients presented LN metastases after one year of observation, and two cases had nodal recurrence five years after surgical treatment. In six patients in this group incomplete biochemical response was observed after five years of follow-up.

In a group of 13 patients with positive FNAB or/and elevated FNAB-Tg disqualified from lymphadenectomy five patients remained in the five-year follow-up. In two of them not qualified for surgical treatment because of equivocal FNAB or FNAB-Tg recurrence was not observed. Another three patients with inoperable nodal recurrence did not develop progression.

In a group consisting of 123 patients with negative FNAB and FNAB-Tg below cut-off value 104 patients underwent 5-year follow-up. In this group neither

Table 2. *Summary of results for thyroglobulin (Tg), thyroglobulin (Tg) measurements in the needle washout (FNAB-Tg), positive fine-needle aspiration biopsy [FNAB(+)], and FNAB(+) with FNAB-Tg (four-fold cross validation)*

Test	Sensitivity	Specificity	PPV	NPV	AUC	PLR	NLR	p value
Tg	0.84	0.80	0.64	0.93	0.86	33.95	0.19	< 0.000
FNAB-Tg	0.92	0.93	0.84	0.97	0.91	213.31	0.08	0.001
FNAB(+)	0.97	0.99	0.98	0.99	0.98	738.56	0.03	< 0.001
FNAB(+) and FNAB-Tg	0.99	0.99	0.98	1.00	1.00	751.82	0.01	< 0.001

PPV — positive predictive value; NPV — negative predictive value; AUC — area under curve; PLR — positive likelihood ratio; NLR — negative likelihood ratio

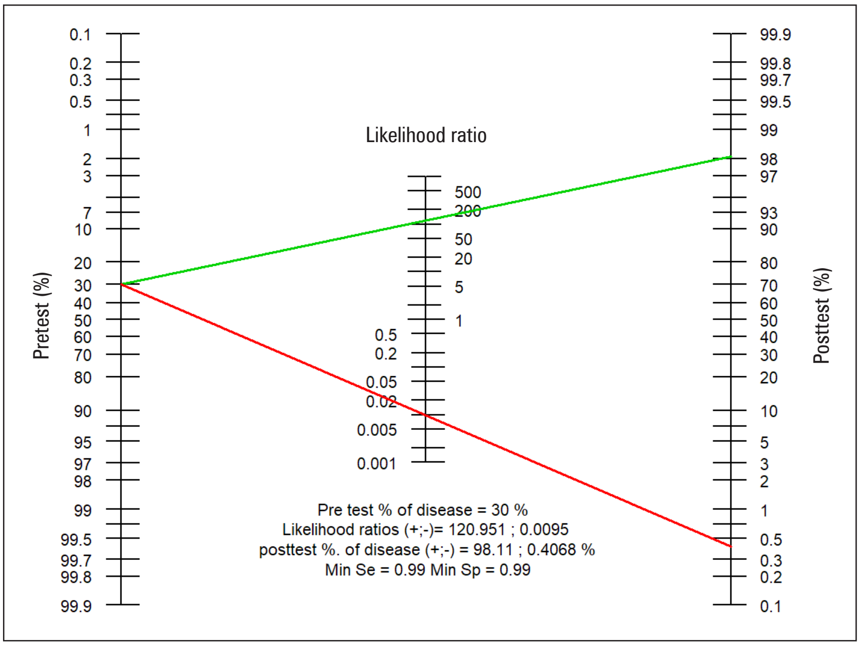

Figure 2. *Nomogram for thyroglobulin (Tg) measurements in the needle washout (FNAB-Tg) level and FNAB(+) cytopathology (four-fold cross-validation). Using combined information about FNAB cytology result and FNAB-Tg level gives a 98% chance that a positively diagnosed patient will be finally diagnosed as metastatic*

biochemical nor structural recurrence was observed. In cases of enlarged LNs in US examination, FNAB or FNAB-Tg did not confirm suspicion of metastases. Another five patients presented only incomplete biochemical response.

Patients after external beam radiotherapy of the neck

Thirty-three patients with locally advanced neoplasms (pT3, pT4, N1, R1) and infiltration of the perinodal adjacent tissue underwent external beam radiotherapy of the neck area. In 20 of these patients radiotherapy was performed before FNAB of suspicious LNs, and in 14 patients positive FNAB correlated with FNAB-Tg above the cut-off value. Seven of them were operated with histopathologically confirmed metastases in resected LNs. The others seven patients were disqualified from surgical treatment because of disseminated neoplastic disease.

Patients with DTC and poorly differentiated carcinoma

In five patients with DTC histopathological examination disclosed an admixture of the poorly differentiated carcinoma. Positive FNAB cytopathology of the suspicious LNs correlated with elevated FNAB-Tg level in four of these patients. In four patients, histopathological examination of the excised LNs confirmed metastases, while one patient did not undergo lymphadenectomy because of disseminated diseases with metastases to the lungs. Only in one operated patient was recurrence of carcinoma not observed in five years of observation; in an additional three cases disseminated neoplastic disease was the cause of death after one to four years of follow-up.

Patients with DTC and additional neoplasm

Eight patients with DTC and a history of additional neoplasm were examined by FNAB and FNAB-Tg of suspicious neck LNs. Positive FNAB of metastatic breast carcinoma, squamous cell carcinoma, and lymphoma correlated with low FNAB-Tg levels in three cases. In one patient with a history of parathyroid carcinoma FNAB of the lump in the neck disclosed follicular-like cells but the FNAB-Tg level was low. Complementary examination disclosed a high level of parathormone (PTH) in the needle washout, indicative of metastasis of the parathyroid carcinoma. Histopathological examination of the excised mass revealed benign parathyroid adenoma. In one patient treated for colonic adenocarcinoma the FNAB-Tg level was clearly elevated (> 2500 ng/mL) in concordance with positive FNAB cytopathology of enlarged LNs. Histopathological examination of the excised LNs disclosed cystic metastasis of the PTC. In one patient with a history of embryonal carcinoma in a testis, positive FNAB cytopathology and elevated FNAB-Tg level were suggestive of PTC metastasis. Subsequent thyroidectomy and neck LN dissection confirmed PTC in the isthmus of a thyroid.

Discussion

Sources of heterogeneity

Although US-guided FNAB is a well establish diagnostic procedure, the accuracy and results of this examination depend on the skill of the radiologists and pathologists performing the aspiration [18].

Almost all authors selected LNs to FNAB on the basis of increased size and suspicious US findings, such as round shape (longitudinal to transverse axis ratio < 2 or transverse axis > 5 mm), the absence of an echogenic hilus, microcalcifications, cystic change, heterogeneous echogenicity, and peripheral or 'chaotic' vascularity [19–21]. The authors confirmed in the study the significant correlation of the existence of some US features in discriminating benign and metastatic LNs (Tab. 3).

Preoperative vs. postoperative assessment

The contamination of the FNAB-Tg washout sample with blood containing Tg may influence the results of the examination and the specificity of this method. Blood in individuals with an intact thyroid or after lobectomy would be expected to have higher circulating serum Tg compared to patients after thyroidectomy [22]. Another study revealed that remnant thyroid tissue has no impact on the diagnostic performance of the FNAB-Tg level [23]. In our group of patients there was no correlation between serum Tg and FNAB-Tg.

Table 3. Variable ***correlation with benign/metastatic lymph nodes (LN) results***

Variable	Pearson's χ^2	p value	n
Presence of hilus	12.30	0.0005	149
Heterogenous echogenicity	7.73	0.0054	137
Spherical shape	0.20	0.6543	136
Oval shape	0.27	0.6065	134
Spindle shape	0.01	0.9098	137
Polycyclic	1.12	0.2906	124
Microcalcifications	7.22	0.0072	130
Hypoechogenic	0.24	0.6262	135
Hyperechogenic	0.00	1.0000	127
Cystic change	2.45	0.1175	128
Hilar vascularity	1.98	0.1598	80
Mixed central-peripheral vascularity	0.00	0.9845	80
Chaotic vascularity	8.01	0.0047	80
Peripheral vascularity	0.00	1.0000	80
Transverse axis > 5 mm	4.01	0.0453	144
Longitudinal/transverse axis > 2	6.68	0.0097	144
Sex	2.99	0.0840	200

FNAB-Tg above the cut-off level was observed in patients with low Tg serum level, which could not be explained by blood contamination. However, in patients before thyroidectomy the authors revealed statistically higher FNAB-Tg levels. Furthermore, it was hypothesized that the serum TSH level could also affect FNAB-Tg, independently of its effect on the serum Tg level. In other terms, in patients after thyroidectomy, serum TSH suppression may play a role in the lowering of both serum Tg and FNAB-Tg, together with the absence of thyroid tissue [22]. Most patients in this study underwent FNAB after thyroidectomy and under TSH suppression.

Interference of antibodies

The interference of TgAb when measuring serum Tg is well known, and FNAB-Tg might also be affected by positive TgAb, which occurs in approximately 20–25% of patients with DTC and in approximately 10% of the whole population [24, 25]. The presence of TgAb may be a result of contamination of the wash-up sample with blood, but also by antibodies produced in the lymph nodes [26]. In previous publications some authors excluded patients with high levels of serum TgAb from the FNAB-Tg examination. However, Baskin et al. concluded in their study that FNAB-Tg was not affected by positive TgAb in the serum [26]. Boi et al. found that the presence of TgAb washouts of FNAB may cause an underestimation of the FNAB-Tg level, but this interference has little influence on the test and no false-negative case was recorded [25]. The results of our study confirm a limited effect of TgAb on the diagnostic significance of FNAB-Tg, and there was no significant discordant results between elevated TgAb level and FNAB-Tg (r = –0.17, p = 0.4434).

Cut-off levels

The last meta-analysis based on the largest published studies found that a FNAB-Tg cut-off value of 1 ng/mL had highest sensitivity and 40 ng/mL had highest specificity [9]. An FNAB-Tg cut-off value has not been unanimously established. Cut-off values ranging from 0.2 to 100 ng/mL have been suggested for FNAB-Tg. Some authors set the threshold to the mean value of 2 SD for the negative patients, while others used the highest Tg concentration measured in patients with reactive LNs, the upper value of reference range of serum Tg, the functional sensitivity of the assay, or the best cut-off derived by a receiver operating characteristic (ROC) curve analysis. Some studies adopted an FNAB-Tg cut-off equal to the serum Tg concentration [22]. Our subgroup analysis supports the choice of a 33.28 ng/mL cut-off value for FNAB-Tg, but the results are affected by a significant heterogeneity between

Table 4. *Confusion matrix for all patients and thyroglobulin (Tg) measurements in the needle washout (FNAB-Tg) cut-off value 33.28 ng/mL*

	Metastatic LNs	Benign LNs
FNAB-Tg level+	53	9
FNAB-Tg level–	7	131

LNs — lymph nodes

studies (Tab. 4). An FNAB-Tg level below the cut-off value was observed in seven metastatic patients. The lowest values of FNAB-Tg in the FNAB cytopathologically positive group were observed in patients with PTC 'tall cell' variant (1.56 ng/mL) and in patients with Hürthle cell carcinoma with a minor component of the poorly differentiated carcinoma (1.96 ng/mL). It should be taken into consideration that in the case of positive FNAB cytopathology, the low FNAB-Tg value should not influence the therapeutic decision on lymphadenopathy because of decreased Tg production by some aggressive variants of DTC and poorly differentiated carcinoma [27, 28]. Further studies are needed to clarify the FNAB-Tg cut-off value and to elucidate whether this should be different in patients before and after thyroidectomy.

The heterogeneity of the treatment groups vs. FNAB-Tg assessment

The majority of patients in our study were diagnosed with PTC (n = 181), similarly to its relative worldwide incidence. Statistical analysis revealed that this group was younger ($p = 0.02$) and characterised with higher, but not statistically significant, FNAB-Tg value ($p = 0.75$) and lower Tg serum value ($p = 0.08$), which might be the effect of the strength of this group and the fact that metastases in LNs were confirmed in 60 patients in the whole group. The majority of patients with positive FNAB and FNAB-Tg had histopathologically confirmed LN metastases. Patients with benign LNs did not develop recurrence in five years of follow-up. The group of 15 patients diagnosed with FTC were older compared with the whole group of patients ($p = 0.19$) and were characterised by higher FNBA-Tg ($p = 0.96$) and Tg serum values ($p = 0.08$). Patients with FTC developed a more aggressive course of neoplastic disease precluding surgical treatment in some cases. Four out of 7 patients with positive FNAB and FNAB-Tg, who underwent lymphadenectomy, were diagnosed histopathologically with LNs metastases. Four patients from all the cases in the study were diagnosed with Hürthle cell carcinoma. Those patients were statistically significantly older ($p = 0.02$) with lower both FNAB-Tg ($p < 0.05$) and Tg serum ($p = 0.25$). Two patients in this group had positive FNAB and FNAB-Tg; one of them was operated with histopathological confirmation of LN metastases. In one case from this group, with negative FNAB and FNAB-Tg, we observed recurrence in the thyroid bed, which confirmed a more aggressive course of disease in Hürthle cell carcinoma. According to our statistical analysis, we confirmed the usefulness of FNAB-Tg in the diagnosis of LN metastases in patients who underwent external beam radiotherapy of the neck before FNAB. This group of patients were characterised by older age, more aggressive course of neoplastic disease, and what is worth emphasizing, statistically significantly higher FNAB-Tg value ($p = 0.01$). We confirmed in our study a high sensitivity and specificity of the combination of FNAB and FNAB-Tg in the whole group of patients, and the heterogeneity of their strength precludes the establishment of different FNAB-Tg cut-off values in each group. For this purpose, further research should be done.

Conclusions

A combination of FNAB and FNAB-Tg is an excellent diagnostic procedure for LN metastasis of DTC. Compared to FNAB and FNAB-Tg alone, the combination could statistically improve the diagnostic performance. This procedure should be recommended for patients with suspicious LN metastases of DTC after initial therapy.

References

1. Mazzaferri EL, Kloos RT. Clinical review 128: Current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab. 2001; 86(4): 1447–1463, doi: 10.1210/jcem.86.4.7407, indexed in Pubmed: 11297567.
2. Schlumberger MJ. Papillary and follicular thyroid carcinoma. N Engl J Med. 1998; 338(5): 297–306, doi: 10.1056/NEJM199801293380506, indexed in Pubmed: 9445411.
3. Choi Y, Park KJ, Ryu S, et al. Papillary thyroid carcinoma involving cervical neck lymph nodes: correlations with lymphangiogenesis and ultrasound features. Endocr J. 2012; 59(10): 941–948, doi: 10.1507/endocrj.ej12-0178, indexed in Pubmed: 22785182.
4. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016; 26(1): 1–133, doi: 10.1089/thy.2015.0020, indexed in Pubmed: 26462967.
5. Hall TL, Layfield LJ, Philippe A, et al. Sources of diagnostic error in fine needle aspiration of the thyroid. Cancer. 1989; 63(4): 718–725, doi: 10.1002/1097-0142(19890215)63:4<718::aid-cncr2820630420>3.0.co;2-n, indexed in Pubmed: 2914278.
6. Frasoldati A, Valcavi R. Challenges in neck ultrasonography: lymphadenopathy and parathyroid glands. Endocr Pract. 2004; 10(3): 261–268, doi: 10.4158/EP.10.3.261, indexed in Pubmed: 15310545.
7. Torres MR, Nóbrega Neto SH, Rosas RJ, et al. Thyroglobulin in the washout fluid of lymph-node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma? Thyroid. 2014; 24(1): 7–18, doi: 10.1089/thy.2013.0244, indexed in Pubmed: 24044517.
8. Pacini F, Fugazzola L, Lippi F, 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(6): 1401–1404, doi: 10.1210/jcem.74.6.1592886, indexed in Pubmed: 1592886.

ORIGINAL PAPER

9. Zhu XH, Zhou JN, Qian YY, et al. Diagnostic values of thyroglobulin in lymph node fine-needle aspiration washout: a systematic review and meta-analysis diagnostic values of FNA-Tg. Endocr J. 2020; 67(2): 113–123, doi: 10.1507/endocrj.EJ18-0558, indexed in Pubmed: 31723088.
10. Kim MJ, Kim EK, Kim BM, et al. Thyroglobulin measurement in fine-needle aspirate washouts: the criteria for neck node dissection for patients with thyroid cancer. Clin Endocrinol (Oxf). 2009; 70(1): 145–151, doi: 10.1111/j.1365-2265.2008.03297.x, indexed in Pubmed: 18466347.
11. Sohn YM, Kim MJ, Kim EK, et al. Diagnostic performance of thyroglobulin value in indeterminate range in fine needle aspiration washout fluid from lymph nodes of thyroid cancer. Yonsei Med J. 2012; 53(1): 126–131, doi: 10.3349/ymj.2012.53.1.126, indexed in Pubmed: 22187242.
12. Filetti S, Durante C, Hartl D, et al. ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2019; 30(12): 1856–1883, doi: 10.1093/annonc/mdz400, indexed in Pubmed: 31549998.
13. Bournaud C, Charrié A, Nozières C, et al. Thyroglobulin measurement in fine-needle aspirates of lymph nodes in patients with differentiated thyroid cancer: a simple definition of the threshold value, with emphasis on potential pitfalls of the method. Clin Chem Lab Med. 2010; 48(8): 1171–1177, doi: 10.1515/CCLM.2010.220, indexed in Pubmed: 20441483.
14. Borel AL, Boizel R, Faure P, et al. Significance of low levels of thyroglobulin in fine needle aspirates from cervical lymph nodes of patients with a history of differentiated thyroid cancer. Eur J Endocrinol. 2008; 158(5): 691–698, doi: 10.1530/EJE-07-0749, indexed in Pubmed: 18426828.
15. Jeon MJi, Park JW, Han JiM, et al. Serum antithyroglobulin antibodies interfere with thyroglobulin detection in fine-needle aspirates of metastatic neck nodes in papillary thyroid carcinoma. J Clin Endocrinol Metab. 2013; 98(1): 153–160, doi: 10.1210/jc.2012-2369, indexed in Pubmed: 23144473.
16. Grani G, Lamartina L, Durante C, et al. Follicular thyroid cancer and Hürthle cell carcinoma: challenges in diagnosis, treatment, and clinical management. Lancet Diabetes Endocrinol. 2018; 6(6): 500–514, doi: 10.1016/S2213-8587(17)30325-X, indexed in Pubmed: 29102432.
17. Kure S, Ohashi R. Thyroid Hürthle Cell Carcinoma: Clinical, Pathological, and Molecular Features. Cancers (Basel). 2020; 13(1), doi: 10.3390/cancers13010026, indexed in Pubmed: 33374707.
18. Florentine BD, Staymates B, Rabadi M, et al. Cancer Committee of the Henry Mayo Newhall Memorial Hospital. The reliability of fine-needle aspiration biopsy as the initial diagnostic procedure for palpable masses: a 4-year experience of 730 patients from a community hospital-based outpatient aspiration biopsy clinic. Cancer. 2006; 107(2): 406–416, doi: 10.1002/cncr.21976, indexed in Pubmed: 16773630.
19. Machens A, Hinze R, Thomusch O, et al. Pattern of nodal metastasis for primary and reoperative thyroid cancer. World J Surg. 2002; 26(1): 22–28, doi: 10.1007/s00268-001-0176-3, indexed in Pubmed: 11898029.
20. Yeh MW, Bauer AJ, Bernet VA, et al. American Thyroid Association Surgical Affairs Committee Writing Task Force. American Thyroid Association statement on preoperative imaging for thyroid cancer surgery. Thyroid. 2015; 25(1): 3–14, doi: 10.1089/thy.2014.0096, indexed in Pubmed: 25188202.
21. Roy R, Kouniavsky G, Venkat R, et al. The role of preoperative neck ultrasounds to assess lymph nodes in patients with suspicious or indeterminate thyroid nodules. J Surg Oncol. 2012; 105(6): 601–605, doi: 10.1002/jso.22115, indexed in Pubmed: 22006435.
22. Moon JH, Kim YIl, Lim JAh, et al. Thyroglobulin in washout fluid from lymph node fine-needle aspiration biopsy in papillary thyroid cancer: large-scale validation of the cutoff value to determine malignancy and evaluation of discrepant results. J Clin Endocrinol Metab. 2013; 98(3): 1061–1068, doi: 10.1210/jc.2012-3291, indexed in Pubmed: 23393171.
23. Torres MR, Nóbrega Neto SH, Rosas RJ, et al. Thyroglobulin in the washout fluid of lymph-node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma? Thyroid. 2014; 24(1): 7–18, doi: 10.1089/thy.2013.0244, indexed in Pubmed: 24044517.
24. Ringel MD, Nabhan F. Approach to follow-up of the patient with differentiated thyroid cancer and positive anti-thyroglobulin antibodies. J Clin Endocrinol Metab. 2013; 98(8): 3104–3110, doi: 10.1210/jc.2013-1412, indexed in Pubmed: 23922347.
25. Boi F, Baghino G, Atzeni F, et al. The diagnostic value for differentiated thyroid carcinoma metastases of thyroglobulin (Tg) measurement in washout fluid from fine-needle aspiration biopsy of neck lymph nodes is maintained in the presence of circulating anti-Tg antibodies. J Clin Endocrinol Metab. 2006; 91(4): 1364–1369, doi: 10.1210/jc.2005-1705, indexed in Pubmed: 16434461.
26. Baskin HJ. Detection of recurrent papillary thyroid carcinoma by thyroglobulin assessment in the needle washout after fine-needle aspiration of suspicious lymph nodes. Thyroid. 2004; 14(11): 959–963, doi: 10.1089/thy.2004.14.959, indexed in Pubmed: 15671775.
27. Purkait S, Agarwal S, Mathur SR, et al. Fine needle aspiration cytology features of poorly differentiated thyroid carcinoma. Cytopathology. 2016; 27(3): 176–184, doi: 10.1111/cyt.12270, indexed in Pubmed: 26662642.
28. Cameselle-Teijeiro JM, Eloy C, Sobrinho-Simões M. Pitfalls in Challenging Thyroid Tumors: Emphasis on Differential Diagnosis and Ancillary Biomarkers. Endocr Pathol. 2020; 31(3): 197–217, doi: 10.1007/s12022-020-09638-x, indexed in Pubmed: 32632840.