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Original Article

Diagnostic value of thyroglobulin measurement in fine-needle aspirate washouts for detecting metastatic lymph nodes in patients with differentiated thyroid cancer

Shujian Xu[1,2], Kai Meng[3], Cui Zhao[2], Xilong Wang[2], Zhongming Jia[2], Yong Han[2], Guoqiang Zhang[2], Qifeng Yang[1]

[1]Department of General Surgery, Qilu Hospital, Shandong University, Jinan 250012, Shandong, China; [2]Department of Thyroid and Breast Surgery, Binzhou Medical University Hospital, Binzhou 256603, Shandong, China; [3]Department of Anorectal Surgery, Central Hospital of Taian, Taian 271000, Shandong, China

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Abstract: Objective: Measurement of thyroglobulin (Tg) in the wash-out of the needle used to perform FNAB on cervical lymph nodes (FNAB-Tg) provides evidence for metastatic of differentiated thyroid carcinoma (DTC). However, the cutoff value from preoperative patients is still controversial. The objectives of the study were to (1) determine an optimized cutoff value for FNAB-Tg levels (2) compare fine needle aspiration cytology (FNAC) alone and the combination of FNAC and FNAB-Tg results in a group of 243 patients affected by DTC. Materials and methods: A total of 394 lymph nodes from 243 patients underwent sonographic guided fine-needle aspiration cytology and washout thyroglobulin measurement and the final diagnosis was confirmed by surgical pathology examination. The best FNAB-Tg cutoff level was selected by receiver operating curve (ROC) analysis, and diagnostic performances of FNAC alone and of the combination of FNAC and FNAB-Tg g on the basis of the presence suspicious ultrasonographic findings were compared. Results: 394 lymph nodes (LNs) from 243 patients made up this analysis (120 patients with 1, 95 with 2, and 28 with 3 LNs, respectively; 72 patients without LNs metastases, 171 patients with LNs metastases). Of the 394 lymph nodes, 279 lymph nodes were metastases and 115 lymph nodes were benign. The sensitivity, specificity, PPV, NPV, and accuracy of FNAC were 81.7%, 92.2%, 96.2%, 67.5%, and 84.8%, respectively. The most appropriate cutoff value selected by receiver operating curve analysis for the diagnosis of thyroid cancer metastatic LN was 26.5 ng/mL. The diagnostic sensitivity of FNAB-Tg for metastatic nodes was significantly higher than that of FNAC (P=0.008). Furthermore, combined FNAB-Tg/FNAC significantly increased sensitivity (P < 0.001) and accuracy (P < 0.001) as compared with FNAC alone. Conclusions: Applying the optimized cutoff value of FNAB-Tg of 26.5 ng/mL in patients with suspicious ultrasonographic features facilitated the diagnostic evaluation of neck lymph nodes in preoperative patients with DTC. Combined FNAB-Tg/FNAC is significantly more sensitive and accurate at detecting metastatic nodes than FNAC alone.

Keywords: Differentiated thyroid cancer, lymph node metastasis, fine-needle aspiration, thyroglobulin, cytology

Introduction

During the past several decades, the incidence of thyroid cancer has risen at a higher rate than any other cancer in the United States and many other countries across Europe, Asia, Oceania, and South America [1-3]. Differentiated thyroid cancer (DTC), which includes papillary and follicular cancer, arising from thyroid follicular epithelial cells, comprises the vast majority (> 90%) of all thyroid cancers [4]. Despite its relatively indolent biological behavior, regional lymph nodes (LNs) metastases are common at the time of diagnosis [5-7] and its removal by neck dissection during thyroid surgery is generally recommended [8, 9]. Therefore, an accurate discrimination between metastatic and reactive LNs is essential in the management of thyroid cancer.

Now there are many tools proposed for the early detection of clinically silent metastatic lymph nodes before surgery, and the ultrasound (US) and FNAC have been standard diagnostic

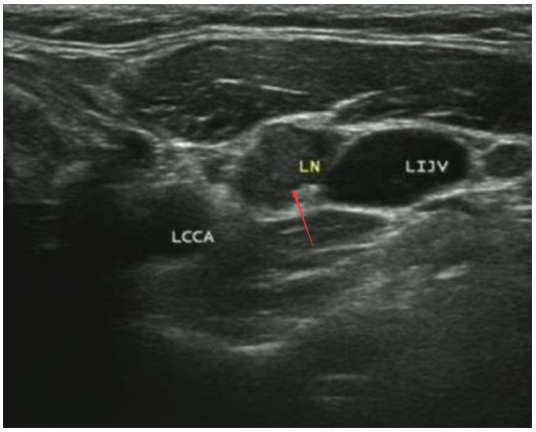

Figure 1. A suspicious solid lymph node (red arrow) with microcalcifications in a patient with papillary thyroid carcinoma. LN, lymph; LCCA, left common carotid artery; LIJV, left internal jugular vein.

modalities to detect and evaluate cervical LNs in patients with thyroid malignancy [9].

Neck US imaging is readily available, non-invasive, cost-effective, and can guide diagnostic and therapeutic procedures with low complication rates. Studies have shown that the sensitivity of US examination ranges from 70% to 100%, but its specificity is only 37% due to its inability to distinguish reactive lymphadenopathy from occult cancer [10, 11]. In addition, its main shortcoming is its operator-dependency [12], proper identification might only be achieved by a skilled and experienced hands.

Ultrasound (US)-guided FNAC has been the most accurate technique for the identification of metastatic lymph nodes originating from thyroid cancer [13]. However, small LNs are technically difficult to aspirate, and cytological features of enlarged nodes are complex to evaluate because of the presence of lymphocytes, granulocytes, multinucleated giant cells, a variable amount of necrosis, and poor epithelial cellularity, particularly in the case of cystic changes [14]. Therefore, this approach is still limited by a false-negative rate of 6% to 8% and a nondiagnostic/unsatisfactory rate of 5% to 10% for FNA cytological samples due to scant cellularity [10, 15-17].

In fact, Tg is produced only by thyroid follicular cells, and its appearance in nonthyroidal tissues is a clue of persistence, recurrence, or metastasis of DTC [18].

During the past two decades, a number of studies indicating the utility of FNAB-Tg for early detection of neck lymph node metastasis in patients with DTC have emerged [13, 19, 20], and the assay is recommended by the 2013 European Thyroid Association Guidelines for Cervical Ultrasound Scan and Ultrasound-Guided Techniques in the Postoperative Management of Patients with Thyroid Cancer [12]. More recently, it has been recommended by the revised American Thyroid Association Guidelines for the follow-up of patients with DTC [9].

However, there have been some concerns that the diagnostic performance could be discounted with the presence of thyroid gland, because that Tg may be detected in FNAB washout fluid from reactive nonmetastatic cervical LNs [19, 21]. As a result, the available information on the diagnostic value of FNAB-Tg in non-thyroidectomized patients is limited [9, 22-24], especially in Chinese population, and the results need to be further investigated. Therefore, in the present work, we evaluated the role of FNAB-Tg for detecting lymph node metastasis in patients with DTC awaiting surgery from the Chinese population.

Patients and methods

Patients

During an 22-month period (July 2013 to April 2015), US-FNAC and FNAB-Tg measurements were performed on 578 suspected metastatic lymph nodes of DTC from 382 consecutive patients (118 males, 264 females; age 44.7± 10.3 years; 220 patients with 1, 128 with 2, and 34 with 3 lesions, respectively) prior to surgery at our institution. We performed US-FNAC and FNAB-Tg evaluation in the LNs with the presence of the following US criteria: Ovoid shape in the longitudinal plane but taller-than wide in the transverse plane, loss of hilum, hypoechogenicity, microcalcifications and cystic components, irregular borders, increased vascularization (**Figure 1**).

Of all 382 patients with 578 lymph nodes, 184 lymph nodes in 139 patients were excluded because: 1) the patients were not diagnosed with DTC (82 cases); 2) the patients did not accept surgery at our institution after FNA evaluation (27 cases); 3) there was no subsequent

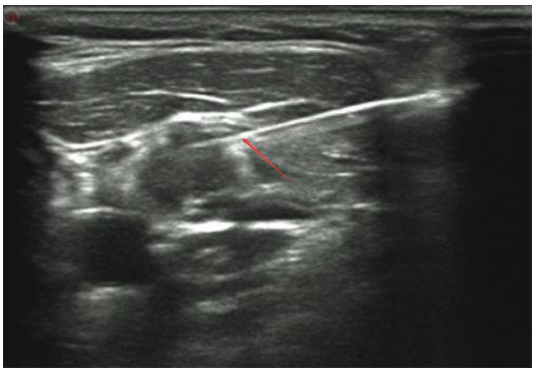

Figure 2. Thyroglobulin washout procedure. A 22-G needle (red arrow) is inserted inside an ovoid, hypoechoic lymph node and it is being aspirated to obtain a small sample.

surgical histology confirmation) (19 cases); 4) the patients had accepted thyroid surgery or biopsy before (11 cases). Finally, the remaining 394 LNs from 243 patients made up this analysis (papillary, n=209; follicular, n=34; Hŭrthle cell carcinomas, n=5; 74 males, 169 females; 120 patients with 1, 95 with 2, and 28 with 3 LNs, respectively). The interval between FNAB and surgery was 4.1±1.8 days.

The FNAB procedure was well tolerated by all patients and no complication was noted, apart from mild subcutaneous swelling in 37 patients. In all 243 patients, preoperative skin marking was performed for lymph nodes where FNAB was performed. Surgeons were aware of the US, FNAC, and FNAB-Tg findings and removed all skin-marked lymph nodes.

The institutional review board approved this retrospective study and written informed consent was obtained from all patients before FNAB.

FNAC and FNAB-Tg measurement

US examinations were performed by the same skilled operator in all 243 patients prior to surgery using a 10-15-MHz linear array transducer (PREIRUSC; HITACHI Medical Systems, Japan), when the patients placed on supine position with the neck hyperexnded. US examination was performed on both thyroid lobes and the neck, including all neck levels (level I to level VI) and the supraclavicular fossa. US-FNAB was performed on metastatic nodes determined by US criteria using 22 gauge needle attached to a 10 ml syringe under continuous real-time US guidance with local anesthesia. Once the needle was introduced into the lymph node, 3-5 ml of negative syringe pressure was applied (**Figure 2**). Each lymph node was aspirated at least twice. Based on available clinical information, the total number of passes for each case varied (mean, 3.3 passes; range, 2-6 passes). Immediately after the aspiration, samples obtained from FNAB were smeared on glass slides and fixed by air-drying. All smears were placed in 95% alcohol for hematoxylin-eosin staining and evaluated by an experienced cytopathologist of our hospital. The needle used was washed with 1 mL of normal saline solution, and the wash-out was submitted for Tg measurement (FNAB-Tg) immediately. When the aspirates were serous fluid, Tg was measured without dilution. The measured values were evaluated without considering dilution. FNAB-Tg concentrations were assayed with an immunoradiometric assay (IRMA) based on coated tubes with monoclonal antibodies directed against distinct epitopes of the molecule of Tg (DYNO test Tg-plus, BRAHMS Diagnostic GmbH, Berlin, Germany). With this measurement, analytic sensitivity, defined as the detectable minimum concentration different from zero (mean value+2 standard deviation), and functional sensitivity, defined as the lowest value that was measured with the precision of a maximum 20% interassay variance, were 0.08 ng/mL and 0.2 ng/mL, respectively. We did not measure Tg antibodies (TgAb) because previous studies have reported that the clinical performance of FNAB-Tg is unaffected by serum TgAb [25]. Positive cytology was categorized as positive for metastatic DTC, negative cytology included benign cytology such as reactive lymph nodes or other benign lymphadenitis, and non-diagnostic due to the inadequate cytology. We treated non-diagnostic cytology as negative for malignancy because non-diagnostic cytology alone does not indicate surgical excision. Final lymph node status was determined from histology reports of surgical lymph node specimens. The reference standards were set by the pathology results of the lymph node dissection.

Surgical protocol and histopathological analyses

During the operation of patients with DTC, a unilateral modified neck dissection was performed on a level-by-level basis based on pre-

Table 1. Patient characteristics according to final diagnosis

	Metastasis	Benign	*P* value
Lymph nodes, n	279	115	
Patients			
Sex (male/female), %	31.6/68.4	28.8/71.2	< 0.01a
Age, years	44.26±9.68	42.51±10.47	< 0.01b
Primary tumor			
Size, mm	14.72±11.03	12.14±8.62	< 0.01b
Multiplicity (yes/no), %	69.2/30.8	73.6/26.4	< 0.01a
Lymphatic invasion (yes/no), %	56.9/43.1	38.7/61.3	< 0.01a
Vascular invasion (yes/no), %	24.7/65.3	16.5/83.5	< 0.05a
Extrathyroid extension (yes/no), %	76.5/23.5	56.9/43.1	< 0.01a

Data are expressed as mean ± SD or median (interquarter range). a, Derived from χ^2 test; b, Derived from Student's test.

operative or intraoperative clinical palpation, US features, FNAC, and FNAB-Tg results. However, a selective frozen section was performed as the initial thyroid surgery in patients with lymph nodes with suspicious US features but negative cytology. In addition, routine central lymph node dissections were performed at the time of thyroidectomy in all cases. We evaluated the final results of the aspirated lymph nodes in a level-by-level analysis, and compared them to the pathology reports. On pathologic results, positive lymph nodes were defined as metastatic and negative lymph nodes were defined as nonmetastatic.

Data analysis

Diagnostic performance (sensitivities, specificities, accuracies, positive predictive values [PPV], and negative predictive values [NPV]) of FNAC, FNAB-Tg at the best cutoff value, and the combination of FNAC and FNAB-Tg was assessed by comparing the result of each diagnostic strategy to the final diagnosis of the patients using node-by-node analysis. Values with normal distribution were expressed as mean ± SD, and values with non-normal distribution were expressed as median (interquartile range). Student's t test and χ^2 test were used for the comparison of patient characteristics between 2 groups. The statistical difference in Tg measurements of metastatic and nonmetastatic lymph nodes in the final diagnosis was assessed by Wilcoxon rank-sum test because these measurements had no normal distribution. For dot plots, undetectable Tg values of < 0.2 ng/mL were represented using values of 0.19 ng/mL. The FNAB-Tg receiver operating characteristic curve was developed to determine the cutoff value of FNAB-Tg for the diagnosis of malignant LNs. McNemar's test was used to perform statistical comparison of Diagnostic performance between the 3 diagnostic strategies. *P*-values < 0.05 were considered statistically significant differences. All statistical analyses were conducted with SPSS software (version 17.0).

Results

Patient characteristics and FNAB-Tg according to the final diagnosis of LNs

In the current study, 394 Tg measurements of FNA needle rinses were performed under US guidance in 243 patients with DTC awaiting surgery. Based on the final diagnosis, a total of 279 LNs were diagnosed as metastatic LN, and 115 LNs were diagnosed as benign. **Table 1** shows the patient characteristics according to the final diagnosis of LNs. There was no difference with regard to the male-to-female ratio (1:1.88 in patients with benign vs. 1:1.90 in patients with metastatic DTC) or age (mean, 44.9 years in patients with benign vs. 45.1 years in patients with metastatic DTC) between nonmetastatic and metastatic cases. The patient characteristics according to the final diagnosis of LNs are summarized in **Table 1**.

Based on preoperative evaluation with FNAC, 234 (59.4%) were positive, 104 (26.4%) negative, and 56 (14.2%) nondiagnostic. In positive cytologic results, the frequency of metastatic lymph nodes was 97.4% (n=228) and that of nonmetastatic lymph nodes was 2.6% (n=6); in negative cytologic results, they were 21.2% (n=22) and 78.8% (n=82), respectively.

Of the 56 (14.2%) cases categorized as "nondiagnostic", 29 cases (52.7%) were confirmed as metastases by surgery, 10 cases had a histological diagnosis of normal thyroid tissue, and 17 cases were labeled as "neck LN". Finally, FNAC correctly diagnosed 228 metastatic lymph nodes but failed to diagnose 51 of them. The sensitivity, specificity and diagnostic accuracy values of FNAC for the preoperative identification of the LNs involved were 81.7%, 94.8%

Table 2. The result of FNAC according to the final diagnosis of lymph nodes

	Metastatic	Non-metastatic
Positive	228	6
Negative	22	82
Nondiagnostic	29	27

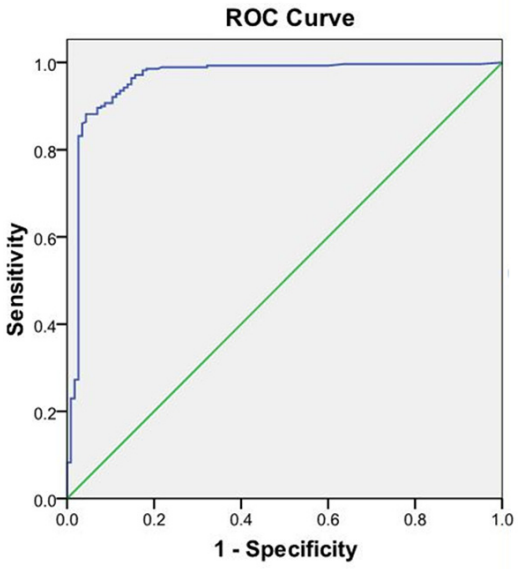

Figure 3. Receiver operating characteristic analysis for FNAB-Tg of lymph nodes according to the final diagnosis is shown. The area under the curve is 0.961 (95% confidence interval 0.936-0.987).

and 85.5%, respectively. The PPV and the NPV were found to be 97.4% and 68.1%, respectively. The results of FNAC according to the final diagnosis of LNs are summarized in **Table 2**.

The cutoff value derived from the ROC curves

The FNAB-Tg level ranged from < 0.2 ng/mL to 80.9 ng/mL in nonmetastatic lymph nodes, except in 3 cases in which the FNAB-Tg levels were 3271.8 ng/mL, 2575 ng/mL and 4863.5 ng/mL, respectively, and < 0.2 ng/mL to 584135 ng/mL in metastatic lymph nodes. Taken together, the levels of FNAB-Tg in the cases with metastatic DTC were significantly different when compared with the FNAB-Tg levels of cytological benign cases ($P < 0.01$).

We performed a ROC analysis comparing the metastatic and nonmetastatic groups (**Figure 3**) that revealed an area under the curve of 0.961 (95% confidence interval 0.936-0.987). The best cutoff value derived from the ROC curves was 26.5 ng/ml for the FNAB-Tg level, at which the accuracy and the sum of sensitivity and specificity were highest [26]. If the given Tg value was larger than that of the respective threshold Tg value, it was deemed a positive Tg value. FNAB-Tg correctly distinguished 250 metastatic lymph nodes but failed to diagnose 29 of them. The sensitivity, specificity and diagnostic accuracy values of FNAB-Tg (cutoff= 26.5 ng/ml) for the identification of metastatic lymph nodes originating from thyroid cancer were 89.2%, 92.3% and 91.1%, respectively. The PPV and the NPV were found to be 96.5% and 78.5%, respectively (**Table 3**).

Comparison of diagnostic performance of FNAC, FNAB-Tg, and the combination of FNAC and FNAB-Tg

Higher sensitivity and lower specificity were obtained from a diagnostic strategy using FNAB-Tg when compared to FNAC alone (**Table 4**). We combined the 2 criteria of FNAC and FNAB-Tg, and malignancy was determined if the malignancy criteria were met in either criteria. The combined criteria resulted in more optimal diagnostic power when compared with diagnostic strategy using either FNAC or FNAB-Tg alone (**Table 4**).

Discussion

In fact, Tg is a glycoprotein synthesized by the thyroid follicular cell, in addition, its synthesis and secretion is also the characteristic of a differentiated follicular cell [20]. Tg specifically represents thyroid tissue as there is no evidence of transcription of Tg gene by non-thyroidal tissues. Hence Tg expression is a reliable specific indicator of the presence of thyroidal epithelial cells, either benign or malignant [27, 28]. Detectable levels of Tg in the neck lymph nodes, especially in the high levels, raise concern about the presence of thyroid tissue and/or a recurrent/metastatic cancer.

In 1992, Pacini first reported the benefit of measuring Tg in needle aspirates of neck lymph nodes (FNAB-Tg) for the detection of metastatic differentiated thyroid cancer. Their study demonstrated that elevated FNAB-Tg levels in cervical LNs strongly suggested the diagnosis of metastatic DTC in cases both with and without a history of thyroidectomy [19]. Besides, several studies have reported that FNAB-Tg

Table 3. Diagnostic accuracies of FNAC, FNAB-Tg, and combined FNAB-Tg/Cytology

Tools	Diagnostic value				
	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
FNAC	81.7	94.8	97.4	68.1	85.5
FNAB-Tg	89.2	92.3	96.5	78.5	91.1
FNAC+FNAB-Tg	97.1	90.4	96.1	92.9	95.2

Abbreviation: FNAC+FNAB-Tg, combination of FNAB-Tg and FNAC.

Table 4. Statistical analysis for assessment of cervical node metastasis by FNAB-Tg, FNAC and combined FNAB-Tg/cytology

Tool	Diagnostic value				
	Sensitivity	Specificity	PPV	NPV	Accuracy
FNAB-Tg vs. FNAC	0.008	0.423	0.057	0.045	0.038
FNAC+FNAB-Tg vs. FNAC	< 0.001	0.208	0.397	< 0.001	< 0.001
FNAC+FNAB-Tg vs. FNAB-Tg	< 0.001	0.640	0.793	0.002	0.009

P-values were Derived from McNemar's test and < 0.05 were considered statistically significant differences.

identifies DTC metastases of the neck with higher sensitivity and specificity than FNAC [13, 19, 22, 24]. Detection of FNAB-Tg in neck lymph nodes should therefore be regarded as a specific marker of DTC. In addition, measuring Tg in the FNAB washout fluid exhibits some advantages over cytology. FNAB-Tg is simple to perform, low cost, and improves the diagnostic performance of cytology in the early detection of LNs metastases of DTC. This technique allows 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 [29].

In the current study, the doubtful neck lymph nodes in patients with DTC were assessed by FNAC and FNAB-Tg. 18.3% (n=51) of metastatic lymph nodes were not diagnosed by FNAC, whereas only 10.4% (n=29) of metastatic lymph nodes were not diagnosed by FNAB-Tg. The role of FNAC is very valuable when cytology is inadequate. However, FNAB-Tg could contribute especially to the diagnosis of poor cellular material obtained from cystic metastasis [30]. Therefore, FNAC with FNAB-Tg performed on the lymph nodes that have cystic change by US findings can accurately evaluate the nodes and make up for the inadequate cytology. Accordingly, the combination of FNAB-Tg measurement and cytology increased FNAC diagnostic sensitivity by 15.4%, allowing the detection of lymphatic metastasis in 43 of 51 patients awaiting surgery. It could be concluded that FNAB-Tg is more sensitive and has a higher negative predictive value than FNAC, and FNAB-Tg/cytology increased accuracy, sensitivity, and specificity. Our observations are in accordance with previous studies. In these patients, Baldini [31] showed a 92.3% FNAB-Tg sensitivity in detecting lymph node metastasis versus a 83.3% sensitivity of cytology alone. Lee [32] obtained false-negative results with either method; however, the combination of FNAB-Tg plus cytology attained a 96.4% sensitivity and 90% specificity. This study demonstrates that FNAB-Tg/cytology significantly increased sensitivity and accuracy for the detection of metastatic nodes from DTC as compared with FNAB-cytology alone.

Although FNAB-Tg is obviously more sensitive than FNAB-cytology alone, a number of false-positive cases (9 of 115) are encountered, especially in level-VI, the most commonly involved lymph nodes in thyroid carcinoma [33]. The reason for false-positive cases is uncertain. However, the most likely explanation is needle contamination by peripheral blood with high Tg levels or the presence of thyroid tissue during FNAB. If normal thyroid tissue or blood with high Tg levels is aspirated, FNAB -Tg will obviously result in positive, leading to a misdiagnosis of LN metastasis [14]. Therefore, the needle track should avoid intersecting thyroid tissue or the blood vessel during aspiration in patients prior to thyroidectomy. Besides, small clusters of microscopically normal thyroid follicles within cervical lymph nodes has been very occasionally encountered during histological examination [34-36], and these histologically benign thyroid follicles in cervical lymph nodes may be another cause of false positive results. Thirdly, we employed 1 mL saline solution to wash out the needles. Studies by Snozek [15]

found that the Tg values increased by approximately 25% when the FNAB-Tg needle was washed with saline solution, due to the effect of matrix effect, which might result in the high Tg levels in benign LNs. Therefore, FNAB-Tg positive result, especially in Level-VI, should be carefully interpreted because a false-positive result may indicate an unnecessary neck dissection with a higher risk of hypoparathyroidism and recurrent nerve injury, without notable reduction of the local recurrence in thyroid cancer [37].

In the current study, we also found false-negative cases (29 of 279) with the FNAB-Tg level of < 26.5 ng/mL, but after reevaluation of US and cytological findings, these cases were confirmed to be metastatic DTC. Dedifferentiation of the metastatic tissue with lower Tg local production or focal distribution of malignant tissue in LNs could result in the false-negative FNAB-Tg results [34, 36]. Besides, variations in cell density among different specimens may contribute to these false-negative results, and other possible explanations should be considered, such a technique error. Therefore, the use of FNAB-Tg should not replace FNAC because of the possibility of both false-positive and false-negative results. Rather, the 2 tests should be used in concert with each other to aid the diagnosis of metastatic DTC.

There were several potential limitations to this study. First, the presence of serum anti-Tg antibody was not considered in our study. Despite the fact that TgAb affect the detection of serum Tg by immunometric assays, FNAB-Tg values in patients who were positive for serum TgAb did not significantly differ from those in patients who were negative for serum TgAb, as reported by Baskin [21]. However, a cautious approach suggests evaluating TgAb in the FNA washout solution of TgAb-positive patients [25]. Second, even though we used 1 mL rinsed washout with normal saline, there may be a difference in the dilution rate of the washout, because the volume of residual sample in the washout of the needle varies. A more accurate quantitative analytic method should be developed to compensate for this limitation. Third, the device for measuring FNAB-Tg varies from one institution to another. Therefore, further investigations are needed to determine the optimal cutoff value in patients in other institution. Fourth, selection bias among neck lymph node investigated was observed, as is common in other retrospective studies. FNAC and FNAB-Tg might be performed in more suspicious LNs, based on US.

In conclusion, the FNAB-Tg is an effective ancillary diagnostic tool for the early detection of LNs metastases of DTC before surgery when it is used in LNs with suspicious sonographic features, even in cases of paucicellular samples and very small lesions. In addition, the combination of FNAC and FNAB-Tg yield better diagnostic performance in the detection of metastatic lymph nodes than either one used alone. Accordingly, FNAC, associated with FNAB-Tg measurement, should be performed actively for the identification of metastatic cervical LNs from DTC.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qifeng Yang, Department of Breast Surgery, Qilu Hospital of Shandong University, 107 West Wenhua Road, Jinan 250012, Shandong, China. Fax: +86-531-8295-9051; E-mail: yqifeng123@sina.com

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