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Cervical lymph node thyroglobulin measurement in washout of fine-needle aspirates for diagnosis of papillary thyroid cancer metastases

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ABSTRACT

Background: Patients with papillary thyroid cancer (PTC) and enlarged cervical lymph nodes (CLNs) are usually assessed by fine-needle aspiration biopsy cytology (FNAB-C). Thyroglobulin (Tg) is frequently detected in washout of fine-needle aspirates (FNA) of these lymph nodes. The aim of this study was to evaluate the accuracy of the measurement of FNAB-Tg in the washout of FNAB in combination with FNAB-C to detect CLN metastases in PTC.

Methods: We retrospectively evaluated 163 surgically proven CLNs. Ultrasound-guided FNAB-C and FNAB-Tg measurements were performed and the ultrasound features were evaluated.

Results: The sensitivity, specificity and accuracy of FNAB-C, FNAB-Tg and FNAB-C/FNAB-Tg in diagnosis of metastatic CLNs were 85.7, 87.8 and 71.6%, were 80.5, 87 and 82.8% and were 97.1, 96.3 and 95.7%, respectively. The diagnostic sensitivity, specificity and accuracy of FNAB-C/FNAB-Tg for metastatic CLNs was significantly higher than that of FNAB-C or FNAB-Tg alone ($p < 0.01$).

Conclusion: Combined US-guided FNAB-C and FNAB-Tg can improve the accuracy for diagnosis of metastatic CLNs in patients with PTC.

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Introduction

Papillary thyroid carcinomas (PTC) are the most common thyroid cancers and constitute more than 70% of thyroid malignancies.[1] The most common etiologic factor is radiation, but genetic susceptibility and other factors also contribute to the development of this cancer. PTC tends to metastasize preferentially to cervical lymph nodes (CLN).[2] These regional nodal metastases are closely related to the surgery type, extent of surgery and prognosis [3,4] and are considered risk factors for local tumour recurrence and cancer-specific mortality. [5] Therefore, the importance of exact diagnosis of CLN metastases is well recognised in pre-(pro-) operative patients with PTC.

CLN metastases may be detected clinically, but are most often discovered on ultrasonography (US).[6] US criteria distinguishing benign from metastatic or suspicious LNs lack accuracy.[7] When metastatic nodes are suspected by preoperative ultrasound assessment, US fine-needle aspiration biopsy (FNAB) is recommended for the histological diagnosis of metastatic nodes in patients awaiting thyroid surgery.[8] US and US-guided fine-needle aspiration biopsy (US-FNAB) are well-known trustworthy diagnostic tools for cervical metastasis of PTC by preoperative cytological analysis and recurrence after thyroid surgery.[9] Although FNAB is the gold standard technique for the detection of CLN metastasis,[10] in some cases metastatic deposits of PTC in lymph nodes may undergo degeneration and cystic changes. [11] In such instances, the FNAB of LNs even with ultrasound guidance may only show colloid type material, cellular debris and macrophages without any identifiable tumour cells. Therefore, the sensitivity of FNAB is far from optimum, varying from 75 to 85%, and is altered by high rate of nondiagnostic or false-negative samples.[12,13]

Serum thyroglobulin (Tg) measurement only becomes a reliable tumour marker after total thyroidectomy. Preoperative Tg measurement is considered to have limited value, although a number of studies report that an elevated preoperative serum Tg is a risk factor for nodular malignancy.[14,15] In addition, because of the anti-Tg antibody interference, Tg measurement may not accurately reflect the actual content of serum Tg.[16] Recently, it was reported that Tg assessment in the washout of FNAB of suspicious CLN is useful for the early diagnosis of CLN originating from PTC, and it is more sensitive and accurate at detecting metastatic nodes than FNAB, especially in cystic nodal metastases.[17,18]

Numerous studies have demonstrated that measurement of Tg in the washout of the needle used for FNAB

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(FNAB-Tg) increases the sensitivity of FNAB-C in identifying CLN metastasis from PTC.[19–22] It has been shown that lymph node detection of Tg mRNA in fine-needle washout improved the FNAB-C sensitivity for the diagnosis of metastatic CLN from PTC, although this needs to be validated in larger case studies.[23] However, it is unclear whether Tg measurements could be routinely accompanied by US-guided FNAB-C for the diagnosis of metastatic nodes in patients with PTC, and it is unclear whether US features of lymph nodes may help select patients who may benefit from Tg measurements compared with FNAB-C.

In the present study, we hypothesise that Tg measurements accompanied by US-guided FNAB-C could enhance the diagnostic accuracy for metastatic nodes in patients with PTC.

Materials and methods

Patients

Between February 2013 and March 2014, 163 consecutive US-guided fine-needle aspirates (FNA) were performed on CLN from 124 patients (23 males, 101 females; age 44.6 ± 14.9 years) at our hospital. All patients had histologically confirmed primary PTC, and attended the Department of Pathology, the affiliated hospital of Qingdao University. The institutional review board approved this study and waived the requirement for informed consent. US-FNAB-C and FNAB-Tg measurements were performed where CLNs where suspected based on the presence of the following US criteria: hyperechogenicity, cystic changes, calcification, abnormal vascularity, heterogeneous echogenicity, a round shape (longitudinal/transverse diameter ratio < 1.5), loss of echogenic hilum and a lymph node with a diameter exceeding 6 mm.[24,25]

Ultrasonography

US examinations were performed by experienced radiologists in all 124 patients before surgery using a 10–12 MHz linear transducer.[26] Ultrasonographic characteristics were described as delineation of multiple lymph nodes, a tendency towards fusion, an internal echo, an irregular margin, the presence of strong echoes and posterior enhancement. The location (levels I-VI) of all cervical LNs were recorded, based on the American Joint Committee on Cancer and the American Academy of Otolaryngology-Head and Neck Surgery nodal classification.[26] When results of the examiners were discordant, agreement was found by conjoint review of clips of the US examinations.

US-guided FNA procedure

US-guided FNA was simultaneously performed by clinicians who were highly experienced in carrying out US-guided FNA using 21-gauge needles; the technique used is described elsewhere.[27] Gradual aspiration was applied by a 20 mL syringe connected to Cameco's device. Some of contents of needles were immediately immersed in 95% alcohol for Papanicolaou staining.[28] The remaining material was rinsed in saline for cell block processing. FNA washout Tg levels were measured by rinsing the same needle and syringe with 1 mL of normal saline.

Washout thyroglobulin

Tg levels in the FNAB washout were measured using the immunoluminometric assay (IRMA) (Cobas 601, Roche Diagnostics, Mannheim, Germany).[29] 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% inter-assay variance, were 0.08 ng/mL and 0.2 ng/mL, respectively. Validated 1.0 ng/mL of Tg is as a cut-off value for diagnosing LN metastasis of PTC.

Analysis of FNAB-C

Following FNAB the needle's material was expelled onto glass slides and smeared with a second slide to spread the material across the surface. The slides were then either air-dried or wet-fixed using the Bio-Fix (Medisham, Shanghai, China). Air-dried slides were stained with a May Grunwald-Giemsa solution, while the wet-fixed slides were stained with the Papanicolaou solution.[30] The results for cytology were classified into three distinct diagnostic categories: ① inadequate or non-diagnostic: presence of blood cells without lymphocytes, plasma cells, histiocytes and epithelial cells ② negative cytology: presence of lymphocytes and occasional plasma cells without malignant epithelial cells ③ positive cytology for PTC metastases: presence of epithelial cells with malignant cytological characteristics.

Statistical analyses

The accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The chi-square ($\chi 2$) test was employed to compare the diagnostic rate of FNAB-C and FNAB-Tg. Statistical analyses were performed using SPSS11.0 software and MedCalc Statistical Software v. 13.2.2 (MedCalc Software, Ostend, Belgium). A two-tailed p-value of < 0.05 was considered statistically significant.

Results

FNAB-C vs. final pathology

The final diagnosis of the 163 LNs was established by final pathology. 108 (66.2%) LNs were diagnosed as

Table 1. Relation between result of FNAB–C, FNAB-Tg, FNAB-C/FNAB-Tg and final pathology.

	FNAB-C (*n*)				FNAB-Tg (*n*)			FNAB-C/ FNAB-Tg (*n*)			
Final pathology	Correct	F+	F–	ID	Correct	F+	F–	Correct	F+	F–	ID
+ (*n* = 108)	74	0	18	16	87	0	21	103	0	3	2
– (*n* = 55)	43	6	0	6	48	7	0	53	2	0	0

Note. F–: False-negative; F+: False-positive; ID: inadequate.

Table 2. Diagnostic performance of different tests for diagnosis of CLNs (n-163) from PTC patients.

Test	Sensitivity [95% CI]	Specificity [95% CI]	Accuracy [95% CI]	NPV [95% CI]	PPV [95% CI]
FNAB-C	85.7% [68.3–99.9]	87.8% [71.8–99.8]	71.6% [59.3–93.2]	70.5% [68.1–91.2]	92.5% [71.2–99.6]
Tg	80.5% [65.4–99.9]	87% [63–99.5]	82.8% [67–97.2]*	87.2% [69.6–99.9]*	92.6% [72–100]
FNAB-C/Tg	97.1% [89–100]*	96.3% [81–99.5]*	95.7% [77.0–100]**	94.6% [79–100]**	98.1% [74.3–100]
P value	*$p < 0.01$	$p^* < 0.01$	**$p < 0.001$, *$p < 0.05$	**$p < 0.001$,*$p < 0.05$	$p = 0.089$

Table 3. Summary.

What is known about this subject:
- FNAB for enlarged CLNs is usually used for patients with PTC
- The accuracy of FNAB-C or Tg alone for diagnosis of CLNs is low
- Whether FNAB-C/Tg combination has high diagnostic accuracy for CLNs is unknown

What this study adds
- FNAB-C or Tg alone has higher false-negative rate for diagnosis of CLNs
- FNAB-C or Tg alone has higher false-positive rate for diagnosis of CLNs
- FNAB-C/Tg combination has lower false-negative and false-positive rate for diagnosis of CLNs

metastatic, and the remaining 55 (33.8%) were diagnosed as benign. The FNAB-C diagnosis of the 108 malignant LNs was correctly determined in 74 LNs, with accuracy of 68.5%. FNAB-C was false-nagative in 18 (16.7%) out the 108 metastatic LNs, and inadequate in 16 (14.8%) out the 108 metastatic LNs. For 55 benign LNs, FNAB-C diagnosis was correctly provided in 43 LNs, with accuracy of 78.2%. FNAB-C was inadequate in 6 (10.9%) and 6 (10.9%) was false-positive (Table 1).

Sensitivity was defined as identification of individuals with health problems among individuals actually with health problems (positive/positive + false-negative) × 100, and specificity as identification of healthy individuals within actually healthy individuals (negative/negative + false-positive) × 100. Thus, sensitivity, specificity and accuracy of FNAB-C were 85.7, 87.8 and 71.6%, respectively (Table 2).

Positive predictive value (PPV) was accepted as the percentage of individuals actually with health problems among individuals the test concluded to have health problems(positive/positive + false-positive) × 100, and negative predictive value (NPV) was the percentage of actually healthy individuals among those the test identified as healthy(negative/negative + false-negative) × 100. Thus, the PPV and NPV was 92.5 and 70.5%, respectively (Table 2).

FNAB-Tg vs. final pathology

Tg level in the fine-needle washout was measured in 163 LNs. We adopted a Tg cut-off value of 1 ng/FNAB. [29] The median FNAB-Tg was 463.2 ng/mL in malignant LNs, and 0.21 ng/mL in benign LNs. Based on the validated 1.0 ng/mL of FNAB-Tg as a cut-off value for diagnosing LN metastasis of PTC, the FNAB-Tg value in 87 out of 108 metastatic LNs was more than 1.0 ng/mL, and 21 out of 108 metastatic LNs was less than1.0 ng/mL, the false-negative was 19.4% (21/108) (Table 1). The FNAB-Tg value was less than1.0 ng/mL in 48 out of 55 benign LNs, and 7 out of 55 benign LNs was more than1.0 ng/mL, and the false-positive was 12.7% (7/55). So the sensitivity, specificity, accuracy, PPV and NPV of FNAB-Tg were 80.5, 87 and 82.8, 92.6 and 87.2%, respectively (Table 2).

FNAB-Tg in combination with FNAB-C vs. final pathology

Combined FNAB-C/FNAB-Tg examination correctly identified 103 metastatic LNs out of 108 metastatic LNs, 3 LNs was false-negative out of 108 metastatic LNs, and 2 LNs was inadequate in 108 metastatic LNs (Table 1). For 55 benign LNs, combined FNAB-C/ FNAB-Tg was negative in 53 LNs, and false-positive in 2 LNs (Table 1). So the sensitivity, specificity, accuracy, PPV and NPV of combined FNAB-C/FNAB-Tg were 97.1, 96.3, 95.7, 98.1 and 94.6% (Table 2).

Discussion

Accurate discrimination between metastatic and reactive LNs is essential in the management of thyroid cancer. US is the primary imaging modality for preoperative assessment of thyroid malignancy, and computed tomography (CT) has also been commonly used for evaluation of metastatic CLNs.[17,18] However, several studies have

shown that US or CT sometimes was not easy to differentiate benign from malignant lymph nodes.[19–21] In addition, CT is not widely used because of its high cost and unavailability (Table 3).

Cytological examination of FNAB-C samples disclosed by US has been the most accurate method to diagnose a cervical LN. US-guided FNAB-C has high sensitivity, specificity and accuracy for the detection of metastatic CLNs in general head and neck malignancies.[22,23] Moreover, FNAB-C has a high sensitivity, specificity and an accuracy for the diagnosis of metastatic CLNs from thyroid carcinoma. However, thyroid FNA cytology has some limitations in cases of suspicious, inadequate and indeterminate cytology. In addition, there are false-positive and false-negative results. The technique accuracy, highly dependent on the experience and ability of the cytopathologist, has been reported to vary from 73 to 94%.[24,25] In the present study 13.5% (22/163) of samples were non-diagnostic, 6 were false-positive and 18 false-negative by FNAB-C measurement. In addition, we found the accuracy and sensitivity of FNAB-C was low. Therefore, other methods are needed to compensate for these limits, and FNAB-Tg measurement provides useful information because of its sensitivity for cystic components in lymph nodes.

Over the last 10 years, following clinical evidence showing that FNAB-Tg in fine-needle washout improves the accuracy of FNAB-C in the evaluation of LN metastases of PTC, the routine association of FNAB-Tg with FNAB-C in the preoperative diagnosis of suspicious LNs has been recommended.[31–33] In thyroid carcinoma, Tg has been found to be more sensitive than FNAB-C alone.[29,34,35] In our present study, we chose a cut-off value for Tg (>1.0 ng/mL). On 108 LNs harbouring metastases from PTCs, FNAB-Tg had 82.8% accuracy, that is higher than FNAB-C alone ($p < 0.05$). However, there were 7 false-positive cases and 21 false-negative cases by FNAB-Tg measurement. The reason for these cases might be complicated in that FNAB-Tg in the blood was influenced by TSH and TgAb levels.[16] This resulted in lower levels of the detected FNAB-Tg.

Previous studies have found that in cases of the presence of false positive and false negative results in FNAB-Tg, some cases have positive FNAB-C results, and vice versa.[36] In our study, we found that the sensitivity, specificity, accuracy and NPV of FNAB-C/ FNAB-Tg is higher than that of FNAB-C or FNAB-Tg alone, suggesting that the combined application of US-guided FNAB-C and FNAB-Tg has better effect.

In conclusion, combined US-guided FNAB-C and FNAB-Tg can improve the accuracy for diagnosis of metastatic CLNs in patients with thyroid cancer. This work represents an advance in biomedical science because it shows that combined US-guided FNAB-C and FNAB-Tg methods compensated for the deficiencies of each technique.

Disclosure statement

No potential conflict of interest was reported by the authors.

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