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ORIGINAL RESEARCH

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The influence of thyroid status, serum Tg, TSH, and TgAb on FNA-Tg in cervical metastatic lymph nodes of papillary thyroid carcinoma

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Abstract

Objective: Papillary thyroid carcinoma is treated in China mostly with surgery, including total ablation, lobectomy, and lobe and isthmus resection. Therefore, whether thyroid status affects the FNA-Tg cutoff value in the diagnosis of cervical lymph node metastasis deserves our attention. In addition, we investigated the influence of serum Tg, TSH, and TgAb on the accuracy of using FNA-Tg for diagnosis.

Methods: Our retrospective analysis included 189 suspected cervical lymph nodes, and we determined whether the cutoff value of FNA-Tg was affected by thyroid status, sTg, sTSH, and sTgAb.

Results: In thyroid present cases, the optimal cutoff value of FNA-Tg was 2.3 ng/ml (sensitivity 96.2%, specificity 100%), and in the thyroid absent cases, the optimal cutoff value of FNA-Tg was 0.7 ng/ml (sensitivity 97.6%, specificity 96.0%). Although serum Tg, TSH, and TgAb were weakly correlated with FNA-Tg values, they did not affect the diagnostic performance of the optimal cutoff value of FNA-Tg according to thyroid status.

Conclusions: The optimal cutoff value of FNA-Tg should be selected according to the thyroid status (2.3 ng/ml for thyroid present cases and 0.7 ng/ml for thyroid absent cases) to ensure the efficient diagnosis of cervical metastatic lymph nodes of papillary thyroid carcinoma. It was determined that sTg, sTSH, and sTg-Ab cannot influence the diagnostic performance of FNA-Tg. The combination method of FNA-Tg and FNAC is the most optimal choice for the diagnosis of lymph nodes metastasis.

KEYWORDS
cutoff value, fine-needle aspiration, papillary thyroid carcinoma, thyroglobulin, thyroglobulin, thyroglobulin antibody, thyroid-stimulating hormone

1 | INTRODUCTION

In recent years, the incidence of thyroid cancer has rapidly increased all over the world.[1] Papillary thyroid carcinoma (PTC) accounts for 75%–85% of all thyroid cancer cases, and there has been an obvious upward trend for this disease in recent years.[2] Although PTC has a relatively optimistic long-term survival rate, the incidence of cervical and mediastinal lymph node metastases at the initial visit or during follow-up ranged from 20% to 90%.[3,4] Therefore, accurate preoperative and postoperative diagnosis of cervical lymph node metastasis (CLNM) are crucial for PTC patients.

Ultrasonography (US) and fine-needle aspiration cytology (FNAC) are the most commonly used modalities for the diagnosis of CLNM. Although the sensitivity of ultrasonography is high, its specificity is low,[5] and for FNAC, disappointing sensitivity, a high rate of nondiagnostic samples, and a rate as high as 8.6%–24% for false-negative results are the reasons for a lower accuracy.[6] In 1992, fine-needle aspiration thyroglobulin (FNA-Tg) was first proposed by Pacini et al. The procedure consisted of puncturing a suspected cervical lymph node with a needle and measuring the concentration of Tg in the washout fluid. In general, lymph nodes with a high Tg value indicate metastasis, and the procedure produces higher sensitivity and specificity than US or FNAC.[7]

In recent years, there has been increasing use of FNA-Tg, and it has been applied for the diagnosis of CLNM of PTC patients. However, the diagnostic threshold of FNA-Tg and its influencing factors are still controversial. At present, the effects of serum thyroglobulin (sTg), serum thyroid stimulating hormone (sTSH), and serum thyroglobulin antibody (sTgAb) on the FNA-Tg cutoff value have been widely debated around the world, and in the current study, the relationship between them has also been analyzed. In addition, various surgical methods for the thyroid gland are used for PTC in China, including total ablation, lobectomy, lobe, and isthmus resection, etc. And for this reason, we should consider the effect of thyroid status on the cutoff value of FNA-Tg and verify whether the presence of the thyroid gland increases the cutoff value of FNA-Tg. Therefore, this study was conducted to (1) analyze the effects of thyroid status, sTg, sTSH, and sTgAb on FNA-Tg and; (2) compare the diagnostic efficacy of different diagnostic procedures for CLNM.

2 | MATERIALS AND METHODS

2.1 | Patients and study design

We retrospectively collected the FNA-Tg results for PTC patients in our hospital from May 2018 to December 2020. We also collected the patients' US reports, pathology, and laboratory results, and a total of 189 lymph nodes were included. Ethical approval was obtained from the Medical Ethics Committee of Hebei General Hospital, Ethical Review No. 2021, Scientific Research Ethics Review No. 12. Before aspiration, all patients signed informed consent forms special treatment. The patients included in the study were selected on the basis of the following criteria: (1) suspicious enlarged lateral cervical lymph nodes were found by US; (2) the results of FNAC, FNA-Tg, sTg, sTSH, and sTgAb were obtained; (3) all malignant lymph nodes and some benign lymph nodes (had suspicious signs on US but then were benign on FNAC or postoperative pathology) were from patients who had undergone surgery at our hospital, and we were in possession of the postoperative pathologic results; (4) US revealed some suspicious signs for benign lymph nodes, and there were negative results for FNAC and FNA-Tg. In addition, there were no suspicious signs during US follow-up longer than 6 months; (5) this study included some patients with intact thyroid glands who initially received treatment in our hospital with thyroid mass and lymph node metastasis and had not undergone thyroid surgery (Figure 1).

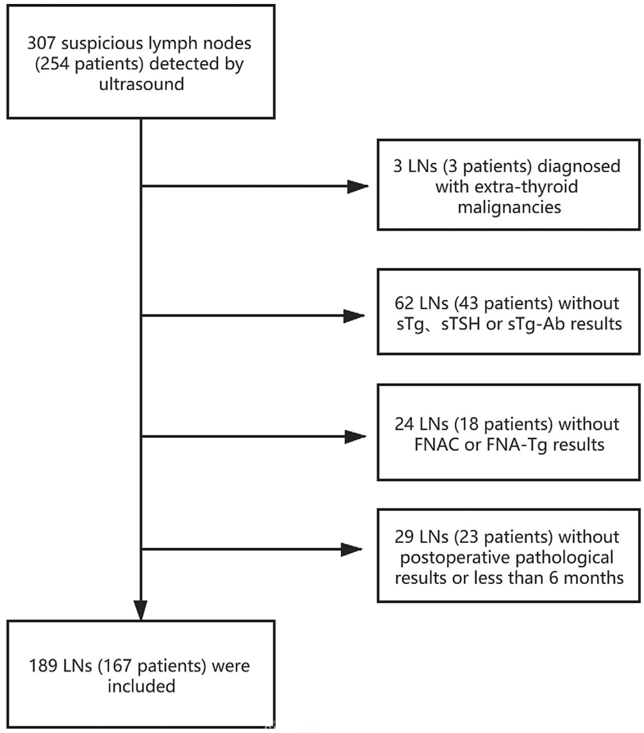

FIGURE 1 Enrollment flow chart of the suspicious lymph nodes and patients

2.2 | FNAC and FNA-Tg measurement

All patients signed informed consent forms before the puncture process. Then, the patients were placed in a supine position with the neck fully exposed. The anterior neck was disinfected, covered with a sterile towel, and local infiltration anesthesia was then administered. For puncturing the target lymph nodes, 22G needles (Ba Guang, Japanese) were used under the real-time guidance of Philips EPIQ 7 ultrasound machine (Royal Philips Electronics, Amsterdam, The Netherlands). We directed the biopsy needle so that the core of the needle quickly penetrated the target lymph nodes several times until the needle absorbed enough cells. Aspiration samples were then dispersed onto slides and fixed. Samples were then stained with either hematoxylin and eosin or Giemsa and evaluated by experienced pathologists. Pathologists categorized the samples according to the Bethesda system. The final sample was rinsed with 1 ml of normal saline, and the washout fluid was submitted for FNA-Tg measurement by an automated electrochemiluminescence immunoassay (Cobas e 601, Roche Diagnostics, Mannheim, Germany). The minimum detectable Tg concentration was 0.04 ng/ml, and the maximum recorded Tg concentration was 500 ng/ml. According to the testing practices in China, 500 ng/ml was much larger than the FNA-Tg cutoff value, and therefore we decided to record the FNA-Tg values above 500 ng/ml as 500+ ng/ml without diluting or examining the sample again.

2.3 | Measurement of sTg, sTSH, and sTgAb

For every patient, 3–5 ml venous blood was extracted, and the blood samples were also measured with an automated electrochemiluminescence immunoassay (Cobas e 601, Roche Diagnostics, Mannheim, Germany). The maximum detectable concentration of sTg was the same as that of FNA-Tg, which was 0.04 ng/ml, and the maximum detectable concentration of sTSH was 0.001 μIU/ml. We defined sTgAb as positive at >115 IU/ml relative to the reference.

2.4 | Data analysis and statistical analysis

Continuous variables were expressed as mean ± SD or median (range), and discontinuous variables were expressed as positive/negative. We used the *t* test, χ^2 test, and Mann–Whitney *U* test to compare the characteristics of the two groups (malignancy and benign). The χ^2 test and Mann–Whitney *U* test were used to compare the parameters under different thyroid statuses. We performed receiver operating characteristic (ROC) curve analysis to obtain the optimal cutoff value of FNA-Tg for the diagnosis of CLNM under different thyroid statuses and compare the efficiency of various diagnostic procedures. Binary logistic regression was used to analyze the correlation between various parameters and FNA-Tg under different thyroid statuses. The correlation between parameters and FNA-Tg level was analyzed by Spearman correlation. All statistical analyses were performed with SPSS 21.0 software.

We considered the results of postoperative pathology as the "gold standard," and definitive negative results consisted of negative FNAC results and no positive signs after 6 months of follow-up. Then, we compared and analyzed the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of FNAC and FNA-Tg with various cutoff values for the diagnosis of suspicious cervical lymph nodes. Three cutoff values related to FNA-Tg levels, and a fourth one is that a ratio were used: 2.3, 1, 10, and a ratio of FNA-Tg/serum Tg > 1 ng/ml. These are denoted as FNA-Tg[2.3], FNA-Tg[1], FNA-Tg[10], and FNA-Tg [FNA-Tg/sTg > 1].

3 | RESULTS

3.1 | Clinical and cytopathological characteristics

Of the 189 lymph nodes, there were 121 (64%) that were malignant, and 68 (36%) were benign; 122 patients had a thyroid gland, and 67 had no thyroid gland. Overall, the malignant lymph node size was 17.26 ± 4.4 mm, and the benign lymph node size was 13.95 ± 4.8 mm. In the malignancy group, patients age was 51.4 ± 15.1 years, and that in the benign group was 46.6 ± 13.4 years. For FNA-Tg, the mean ± SD in the malignancy group is 447.0 ± 147.6 ng/ml and that in the benign group is 0.18 ± 0.34 ng/ml. There were statistically significant differences between the malignancy group and the benign group for lymph node size, patients' age, and FNA-Tg concentration (all $p < .05$).

For sTg, sTSH, and sTgAb, there was no statistical difference between benign and malignant groups ($p > .05$). Of the 189 lymph nodes, 19 false-negative cases, 2 false-positive, and 4 unsatisfactory sample cases were diagnosed by the use of FNAC (Table 1).

3.2 | Correlation between parameters and thyroid status

In all cases, malignancy cases and benign cases, there were statistically significant differences in sTg and sTSH under different thyroid statuses (all $p < .001$). Thyroid status affected FNA-Tg values in all cases and malignancy cases ($p = .034$ and $p < .001$, respectively). In benign cases, thyroid status had no effect on FNA-Tg level ($p = .668$). Unlike other parameters, sTgAb was not affected by thyroid status in any cases (in all cases $p = .794$, in malignancy cases $p = .985$, and in benign cases $p = .570$, respectively) (Table 2).

3.3 | ROC curve of FNA-Tg according to thyroid status

Because thyroid status has a non-negligible influence on the value of FNA-Tg, we obtained the optimal cutoff value of FNA-Tg in the diagnosis of benign and malignant lymph nodes under different thyroid statuses through the ROC curve. As shown in Figure 2, the optimal cutoff value of FNA-Tg to determine malignant lymph nodes was 2.3 ng/ml in all enrolled cases (sensitivity = 92.6%; specificity = 100%), and the area under the curve (AUC) was 0.993 (95% CI = 0.987–1.0). In 122 cases of lymph nodes with a thyroid gland, the cutoff value for FNA-Tg was 2.3 ng/ml (sensitivity = 96.2%; specificity = 100%; AUC = 0.996; 95% CI = 0.989–1.0), whereas in 67 lymph nodes without a thyroid gland because of previous total thyroidectomy, it was 0.7 ng/ml (sensitivity = 97.6%; specificity = 96.0%; AUC = 0.994; 95% CI = 0.983–1.0).

3.4 | The influence of sTg, sTSH, and sTgAb on FNA-Tg cutoff value

In Table 3, we performed a binary logistic regression analysis to investigate whether sTg, sTSH, and sTgAb levels independently affected the diagnosis using FNA-Tg. Because thyroid status has a certain influence on FNA-Tg cutoff value, patients were grouped according to thyroid status, and the effects of sTg, sTSH, and sTgAb on FNA-Tg were respectively compared. Nevertheless, regardless of thyroid status, sTg and sTgAb had no influence on the FNA-Tg cutoff value (all $p > .05$). For sTSH, there was no significance between serum TSH and FNA-Tg in all cases and thyroid present cases.

TABLE 1 Clinical characteristics of 189 lymph nodes

	Final diagnosis		
	Malignancy	Benign	*p* value
LNs, n	121	68	–
Size, mm	17.26 ± 4.4	13.95 ± 4.8	<.001[a]
Thyroid gland (present/absent, n)	79/42	43/25	–
Patients			
Sex (males/females, %)	28.1/71.9	35.3/64.7	.303[b]
Age, y	51.4 ± 15.1	46.6 ± 13.4	.039[c]
FNA-Tg (ng/ml)	447.0 ± 147.6	0.18 ± 0.34	<.001[c]
sTg (ng/ml)	0.92 (88.9)	2.38 (163.9)	.762[c]
sTSH (uIU/ml)	7.14 (100)	1.67 (100)	.770[c]
sTg-Ab (±, %)	43.0/57.0	37.2/62.8	.417[c]
FNAC (*n*, %)			
Positive	87 (71.9)	0 (0)	–
Suspicious	13 (10.7)	2 (2.9)	–
Negative	19 (15.7)	64 (94.1)	–
Unsatisfactory	2 (1.6)	2 (2.9)	–

Note: Data are expressed as mean ± SD or median(range).
[a]Derived from a *t* test.
[b]Derived from a χ^2 test.
[c]Derived from a Mann–Whitney *U* test.

TABLE 2 Comparison of parameters according to thyroid status

		Thyroid present	Thyroid absent	*p* value
All cases ($n = 189$)	FNA-Tg, ng/ml	294.3 ± 244.5	189.1 ± 230.7	.034[a]
	sTg, ng/ml	4.88 (163.86)	0.04 (9.85)	<.001[a]
	sTSH, uIU/ml	2.43 (99.32)	0.056 (12.09)	<.001[a]
	sTg-Ab (±, %)	36.9/63.1	38.8/61.2	.794[b]
Malignancy ($n = 121$)	FNA-Tg, ng/ml	454.4 ± 138.1	301.6 ± 225.7	<.001[a]
	sTg, ng/ml	4.34 (88.87)	0.04 (9.85)	<.001[a]
	sTSH, uIU/ml	2.44 (99.24)	0.056 (12.09)	<.001[a]
	sTg-Ab (±, %)	43.0/57.0	42.9/57.1	.985[b]
Benign ($n = 68$)	FNA-Tg, ng/ml	0.20 ± 0.39	0.13 ± 0.23	.668[a]
	sTg, ng/ml	5.21 (163.86)	0.04 (7.05)	<.001[a]
	sTSH, uIU/ml	2.35 (99.32)	0.056 (0.10)	<.001[a]
	sTg-Ab (±, %)	25.6/74.4	32.0/68.0	.570[b]

Note: Data are expressed as mean ± SD or median(range).
[a]Derived from a Mann–Whitney *U* test.
[b]Derived from a χ^2 test.

3.5 | The correlation between sTg, sTSH, sTgAb, and FNA-Tg level

As shown in Table 4, in malignancy cases and benign cases, sTg had a weak positive correlation with FNA-Tg ($\rho = .296$, $p = .001$; $\rho = .396$, $p = .001$). Additionally, there was a weak positive correlation between sTSH and FNA-Tg in all cases and malignancy cases ($\rho = .153$, $p = .035$; $\rho = .325$, $p < .001$). For sTgAb, a weak negative correlation was found in all cases and benign cases ($\rho = -.328$, $p = .006$; $\rho = .521$, $p < .001$).

3.6 | Diagnostic performance of different procedures

Table 5 shows that there was high diagnostic accuracy (>90%) for FNA-Tg with different cutoff values. However, the overall diagnostic sensitivity and accuracy of FNAC was lower than that of FNA-Tg with any cutoff value in all cases and in thyroid present cases. The possible reason for this is that FNAC is less effective in the diagnosis of cystic metastatic lymph nodes than FNA-Tg, and cystic lymph nodes account for approximately 30% of all metastatic lymph nodes. As shown in

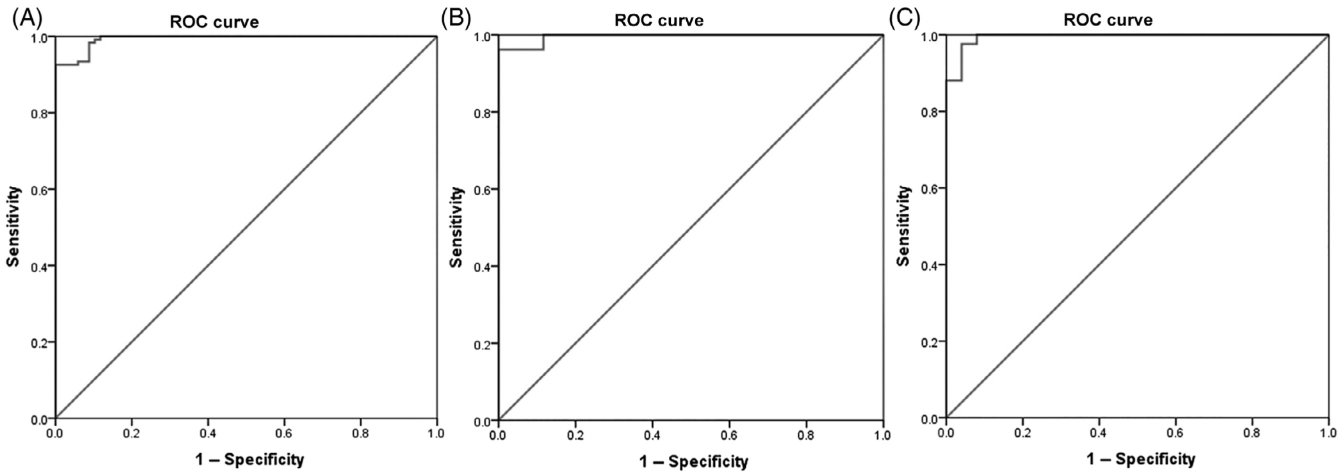

FIGURE 2 ROCs for the cutoff value of FNA-Tg in each case group. (A) ROC curve in all cases, the optimal cutoff value is 2.3 ng/ml (sensitivity 92.6%, specificity 100%). (B) ROC curve in cases thyroid present, the optimal cutoff value is 2.3 ng/ml (sensitivity 96.2%, specificity 100%). (C) ROC curve in cases thyroid absent, the optimal cutoff value is 0.7 ng/ml (sensitivity 97.6%, specificity 96.0%)

TABLE 3 Logistic regression analysis of sTg, sTSH, and sTg-Ab with the diagnosis using different FNA-Tg cutoff value according thyroid status

				95% CI		
			Odds ratio	Lower	Upper	*p* value
FNA-Tg Cutoff Value	All cases FNA-Tg[2.3] (*n* = 189)	sTg	0.995	0.983	1.008	.445
		sTSH	1.004	0.991	1.017	.557
		sTg-Ab	0.759	0.415	1.39	.372
	Thyroid present FNA-Tg[2.3] (*n* = 122)	sTg	0.993	0.98	1.006	.304
		sTSH	1.002	0.989	1.015	.793
		sTg-Ab	0.635	0.292	1.381	.252
	Thyroid absent FNA-Tg[0.743] (*n* = 67)	sTg	1.003	0.73	1.377	.986
		sTSH	–	–	–	–
		sTg-Ab	0.827	0.297	2.301	.716

Note: *p* value derived from a logistic regression.
Abbreviation: 95% CI, 95% confidence interval.

TABLE 4 Spearman correlation analysis the correlation between sTg, sTSH, sTg-Ab, and FNA-Tg concentration

			Correlation coefficient	*p* value
FNA-Tg concentration	All cases(*n* = 189)	sTg	.126	.083
		sTSH	.153	.035
		sTg-Ab	−.328	.006
	Malignancy(*n* = 121)	sTg	.296	.001
		sTSH	.325	<.001
		sTg-Ab	−.079	.282
	Benign(*n* = 68)	sTg	.396	.001
		sTSH	.223	.067
		sTg-Ab	−.521	<.001

Note: *p* value Derived from a spearman correlation analysis.

Figure 3, the diagnostic result of FNAC for cystic lymph nodes was no cancer cells, although the FNA-Tg value was >500 ng/ml. The final postoperative pathological result was PTC metastatic lymph node. We can draw a conclusion from Table 5 that there was greater effectiveness of diagnosis from the optimal cutoff values of FNA-Tg according to thyroid status (2.3 or 0.7 ng/ml) than that of other cutoff values.

TABLE 5 Evaluation of metastatic lymph node according to the diagnostic modality

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC
All cases	FNA-Tg[2.3]	92.6	100	100	88.3	95.2	0.962
	FNA-Tg[1]	92.6	97.1	98.2	88	94.2	0.947
	FNA-Tg[10]	89.3	100	100	84	93.1	0.945
	FNA-Tg[FNA-Tg/sTg > 1]	93.4	97.1	98.3	89.2	94.7	0.951
	FNAC	84	97	98	77.1	88.6	0.905
	FNAC+FNA-Tg[2.3]	99.1	97	98.3	98.5	98.4	0.981
Thyroid present	FNA-Tg[2.3]	96.2	100	100	93.5	96.2	0.981
	FNA-Tg [1]	96.2	95.3	97.4	93.2	95.9	0.957
	FNA-Tg [10]	94.9	100	100	91.5	96.7	0.974
	FNA-Tg[FNA-Tg/sTg > 1]	94.9	97.7	98.7	91.3	95.9	0.962
	FNAC	81.8	95.1	96.9	73.6	86.4	0.885
	FNAC+FNA-Tg[2.3]	98.7	95.1	97.4	97.5	97.4	0.97
Thyroid absent	FNA-Tg[0.743]	97.6	96	97.6	96	97	0.967
	FNA-Tg [1]	85.7	100	100	80.6	91	0.929
	FNA-Tg [10]	78.6	100	100	73.5	86.6	0.893
	FNA-Tg[FNA-Tg/sTg > 1]	90.5	96	97.4	85.7	92.5	0.932
	FNAC	88.1	100	100	82.8	92.4	0.940
	FNAC+FNA-Tg[0.743]	100	95.8	97.7	100	98.5	0.979

Abbreviation: AUC: area under curve; FNA-Tg[2.3, 1, 10, FNA-Tg/sTg > 1, 0.743], the cutoff value of interpretation for test positivity was 2.3, 1, 10 ng/ml, FNA-Tg/serum Tg > 1, 0.743 ng/ml, respectively; FNAC+FNA-Tg[2.3], combination of FNA-Tg[2.3] and FNAC; NPV, negative predictive value; PPV, positive predictive value.

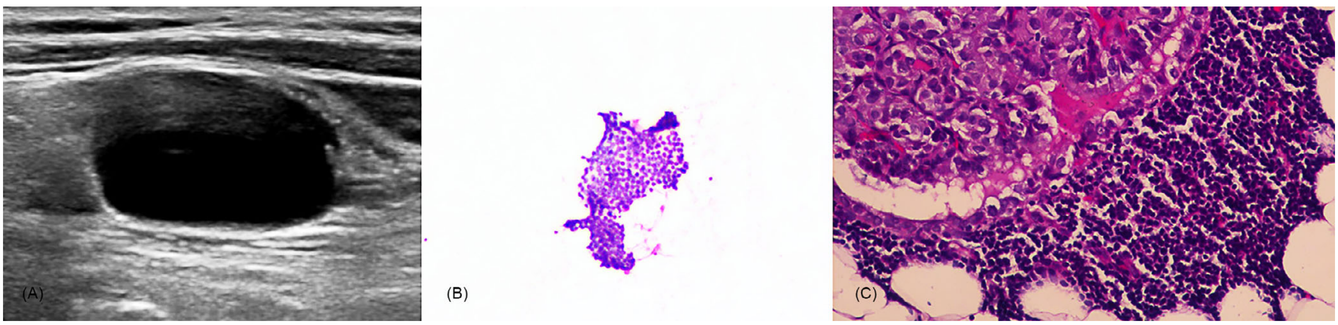

FIGURE 3 Image of a 53-year-old female with an abnormal lymph node in area IV of the lateral neck. (A) The ultrasound image showed the lymph node is completely cystic, full in shape, and enlarged in size. (B) The cytological pathology found no cancer cells (HE ×400). (C) Histopathology showed lymph node metastasis (HE ×400)

Additionally, the combination method of FNA-Tg with the optimal cutoff value and FNAC is the most optimal choice for the diagnosis of CLNM of PTC.

4 | DISCUSSION

In 1992, Pacini et al. proposed that FNA-Tg could be used to detect CLNM of PTC.[7] In recent years, a number of studies has shown that FNA-Tg exhibits outstanding performance in the diagnosis of CLNM. The 2013 and 2015 guidelines of the American Thyroid Association and the European Thyroid Association for thyroid carcinoma treatment strongly recommend FNA-Tg for the diagnosis of metastatic lymph nodes.[8,9] However, due to the differences in research methods, operational approaches, sensitivity, and assay kits of past studies, there are still disputes on the cutoff value of FNA-Tg. Researchers currently use a variety of FNA-Tg cutoff values, including the optimal cutoff value obtained by drawing the ROC curve of data,[10] the fixed cutoff value of 1 or 10 ng/ml,[11] and the ratio cutoff values of FNA-Tg/serum Tg > 1. Although each cutoff value has its advantages and disadvantages, its overall diagnostic efficiency cannot satisfy all researchers.

In western countries, the most common management regimen for PTC is surgery and radioiodine ablation of cancerous and non-cancerous thyroid tissue. Therefore, the influence of thyroid status can be ignored. However, in the Chinese population, physicians will perform different surgical procedures depending on the location, number and size of the tumor, including total ablation, lobectomy, lobe and isthmus resection, and affected lobe and isthmus resection plus opposite-side subtotal lobectomy.[12] In our study, 67 lymph nodes were from patients who had undergone total thyroidectomy, and 122 lymph nodes were from patients who had not undergone thyroid surgery or who underwent thyroid partial resection. Therefore, the effect of residual thyroid on the FNA-Tg cutoff value should be noted. The research findings of Moon and Zhao Huan show that the cutoff value of FNA-Tg in cases with thyroid tissue is higher as compared to that in patients who underwent thyroidectomy,[12,13] which is consistent with the results of this study (for thyroid present cases, the optimal cutoff value was 2.3 ng/ml; for thyroid absent cases, the optimal cutoff value was 0.7 ng/ml).

The influence of sTg, sTSH, and sTgAb on FNA-Tg is also controversial in multiple studies. Moon showed that sTg and sTSH were positively correlated with FNA-Tg.[12] In contrast, the study results of Lee and Duval indicated that there was no statistical correlation between sTg, sTgAb, sTSH, and FNA-Tg in all cases,[14,15] and Boi, Sigstad, and Pak found that the diagnostic ability of FNA-Tg was not affected by sTgAb.[16–18] Our study found that sTg concentration was weakly and positively correlated with FNA-Tg value in malignancy cases and benign cases. In all cases and malignancy cases, sTSH level was weakly and positively correlated with FNA-Tg value. There also was a weak negative correlation between sTgAb level and FNA-Tg concentration in all cases and benign cases. Also, it is likely that blood contamination during the process of puncture may be an important reason for the influence of sTg, sTSH, and sTgAb on the FNA-Tg level. Although experienced physicians try to prevent blood from entering the needle, we can still detect a weak influence of sTg, sTSH, and sTgAb on the FNA-Tg concentration because the current analytical instrument is equipped with high sensitivity. However, it is worth noting that regardless of thyroid status, sTg, sTSH, and sTgAb cannot affect the performance of the optimum cutoff value of FNA-Tg (2.3 or 0.7 ng/ml, all $p > .05$).

For the diagnostic cutoff value of FNA -Tg for CLNM, 500 ng/ml far exceeds it, and therefore many hospitals in China, set the maximum detectable Tg concentration at 500 ng/ml, and any value greater than 500 ng/ml is marked as 500+ ng/ml. This is performed because obtaining a higher value requires another dilution and examination of the sample. This increases the burden of human and material resources and is unnecessary in clinical diagnosis. The current study is based on clinical practice, and the optimum cutoff results are more practical and reliable, which can be better applied in clinical operation.

TSH promotes the function of the thyroid gland. It promotes the release of thyroid hormone, and the synthesis of T4 and T3, including strengthening the activity of the iodine pump, enhancing the activity of peroxidase, and promoting the synthesis of thyroglobulin (Tg). Because of the presence of Tg-secreting cells in metastatic lymph nodes, high TSH levels can also lead to an increase in FNA-Tg value, although the effect is very weak. There was a statistically significant difference in serum TSH between the thyroid present cases and the thyroid absent cases (all $p < .001$). The reason for this is because most thyroidectomy patients received TSH inhibition therapy, which resulted in very low sTSH level (most of the values were less than 0.1 μIU/ml). And that is the reason why the 95% confidence interval (95% CI) of sTSH in logistic regression is too wide (0.000–1,088,383.8), and we abandoned it. In addition, there was no statistical difference in sTSH between the metastatic lymph node cases and the non-metastatic lymph node cases, and sTSH could not reduce the diagnostic ability of FNA-Tg with the optimum cutoff value.

It is quite possible that the FNA-Tg concentration was underestimated by the presence of sTgAb,[19] because a small amount of blood may contaminate the washout fluid during the puncture process, or some active lymph nodes can synthesize TgAb. The study by Boi showed that FNA-Tg concentrations were clearly lower in two cases with TgAb detectable in the washout fluid.[16] However, Wang noted that sTgAb did not affect the FNA-Tg measurements when diagnosing CLNM.[20] In this study, we found that sTgAb can slightly influence the FNA-Tg level, but cannot alter the result of the diagnosis of FNA-Tg. The most probable explanation is that the exceedingly elevated Tg concentration in positive FNA-Tg is able to saturate TgAb binding sites.

Our results supported the hypothesis that diagnosability of the optimum cutoff value, which we obtained by ROC curve (2.3 and 0.7 ng/ml), was preceded by other common cutoff values. Regardless of thyroid status, the combination of FNA-Tg with the optimum cutoff value and FNAC provided a performance that was stronger than any other single diagnostic method, and these results were the same as those of other published studies.[21] Moreover, some studies have noted that there is a stronger diagnostic performance of FNA-Tg as compared to that of FNAC. Especially in the cases of cystic metastatic lymph nodes,[22] our results showed that the diagnostic capability of FNA-Tg (with every cutoff value) was higher than that of FNAC in all cases and thyroid present cases. However, because the sensitivity and negative predictive value of FNA-Tg when the cutoff value was 1 or 10 ng/ml is lower than that of FNAC, the accuracy of FNA-Tg displayed a descending trend in thyroid absent cases.

This study has several potential limitations. First, the gold standard of some benign cases is not based on postoperative pathology, but on cytological diagnosis and follow-up, and therefore, it cannot guarantee that all of these cases are benign, which may cause deviation of the results. Second, although 500+ ng/ml can be grouped as clearly "positive," the maximum FNA-Tg value was 500 ng/ml, may underestimate the influence of sTg on Tg-Ab concentration. Third, the number of cases is small, which may cause insufficient accuracy of the cutoff value of FNA-Tg. Fourth, in the thyroid absent group, the inhibition of sTSH resulted in a small variation in sTSH (most sTSH values<0.1 μIU/ml), which resulted in a too wide 95% CI for the logistic regression analysis (0.000–1,088,383.8), that indicated the binary logistic regression cannot well analysis the correlation between the sTSH with too low values and FNA-Tg cutoff value. Finally, we removed it.

5 | CONCLUSION

We determined that FNA-Tg combined with FNAC is an ancillary test that exhibited satisfactory performance to identify CLNM of PTC. The cutoff value of FNA-Tg is affected by thyroid status and a higher cut-off value is recommended for patients with thyroid tissue than for patients that underwent thyroidectomy. Although there was a certain influence by sTg, sTSH and sTg-Ab on the FNA-Tg concentration, the influence was weak and did not seem to affect the diagnostic performance of the optimal cutoff value of FNA-Tg.

6 | SUMMARY

- FNA-Tg is an important supplement to FNA.
- FNA-Tg exhibited a satisfactory diagnostic performance for PTC cervical lymph node metastasis, especially for cystic lymph nodes.
- Thyroid status had a non-negligible influence on the cut-off value of FNA-Tg, and the cut-off value in the thyroid present cases is higher than that in the thyroid absence cases.
- sTg, sTSH, and sTg-Ab exerted a weak influence on FNA-Tg concentration, but they do not seem to affect the diagnostic performance of the FNA-Tg cutoff value.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Conception and design: Li Li, Jingjing Sun. *Performed the process of puncturing lymph nodes*: All authors. *Collection and assembly of data*: Peipei Li, Xiao Chen, Qiujie Yu. *Data analysis and interpretation*: Jingjing Sun. *Manuscript writing*: All authors. *Final approval of manuscript*: All authors.

DATA AVAILABILITY STATEMENT

Our data can be shared publicly through the mailbox of the corresponding author. However, to prevent the disclosure of the original confidential data, our organization allows us to upload the original data only after the article is accepted.

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