

Role of ultrasound-guided fine-needle aspiration cytology combined with thyroid peroxidase and thyroglobulin antibodies in evaluating cervical lymph node metastasis in thyroid cancer

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Abstract. The present study was designed to explore the role of ultrasound-guided fine-needle aspiration (FNA) cytology combined with thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab) in evaluating cervical lymph node metastasis in thyroid cancer. The aim was to propose more effective diagnostic strategies for cervical lymph node metastasis in patients with thyroid cancer. Firstly, the present retrospective case-control study selected 294 patients with thyroid cancer treated at Changzhou Second People's Hospital (Changzhou, China). High-resolution ultrasound equipment was used for ultrasound and FNA examinations. Additionally, a retrospective analysis of the patient's comprehensive thyroid function tests, including TPO-Ab and Tg-Ab levels, was conducted. Subsequently, univariate and multivariate logistic regression models were employed to analyze the association between various factors and cervical lymph node metastasis. The overall diagnostic accuracy of the model was evaluated using the receiver operating characteristic curve and its area under the curve. Finally, the performance of the diagnostic model was quantitatively assessed through calculating sensitivity, specificity, positive predictive and negative predictive values. According to the experimental results, sex, tumor stage and the levels of thyroid autoantibodies were associated with the risk of cervical lymph node metastasis in thyroid cancer. Moreover, ultrasound features, such as cystic lesions,

loss of hilum of the lymph nodes, abundant vascular supply, heterogeneous echo and microcalcification were also closely related to cervical lymph node metastasis. Logistic regression analysis also showed that tumor stage, serum levels of TPO-Ab and Tg-Ab, and cystic lesions were independent predictors of cervical lymph node metastasis. Furthermore, the combined use of ultrasound, FNA, TPO-Ab and Tg-Ab significantly improved diagnostic sensitivity and specificity. Overall, ultrasound-guided FNA combined with TPO-Ab and Tg-Ab may have a significant role in the evaluation of cervical lymph node metastasis in thyroid cancer. This combined diagnostic approach could significantly enhance diagnostic accuracy, providing a more effective strategy for the clinical management of cervical lymph node metastasis in thyroid cancer.

Introduction

Thyroid cancer is one of the most common endocrine malignancies in the world. Owing to a continuously increasing incidence over the past few decades, thyroid cancer has become the third most common type of cancer in China and the eighth most common cancer among women worldwide (1,2). Although patients with thyroid cancer typically have a long survival period, cervical lymph node metastasis significantly affects prognosis. Notably, 20-90% of thyroid cancer cases involve cervical lymph node metastasis, most commonly in the central compartment. Moreover, cervical lymph node metastasis is significantly associated with local recurrence of thyroid cancer (3,4). Therefore, timely and accurate evaluation of lymph node metastasis is crucial to guide the clinical management of patients with thyroid cancer.

Ultrasound-guided fine-needle aspiration (FNA) cytology is a minimally invasive and efficient clinical diagnostic technique widely used for the evaluation of thyroid nodules and cervical lymph nodes. Although FNA demonstrates high sensitivity and specificity in the initial diagnosis of thyroid cancer (5), it presents certain difficulties in evaluating cervical lymph node metastasis. For example, in cases of micrometastasis or microinvasion of lymph nodes, the sensitivity of FNA is suboptimal, potentially leading to false-negative results (6).

In recent years, the application of biomarkers in cancer diagnosis and monitoring has garnered extensive attention (7).

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Abbreviations: FNA, fine-needle aspiration; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody; ROC, receiver operating characteristic; AUC, area under the curve; BMI, body mass index; OR, odds ratio; CI, confidence interval

Key words: ultrasound-guided FNA cytology, TPO-Ab, TG-Ab, thyroid cancer, cervical lymph node metastasis

In particular, biomarkers such as thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab) are considered potential auxiliary diagnostic tools due to their expression characteristics in thyroid cancer cells. Studies have reported that the levels of TPO-Ab and Tg-Ab are associated with the recurrence and metastasis of thyroid cancer (8,9). Therefore, assessing the levels of TPO-Ab and Tg-Ab may enhance the diagnostic accuracy for cervical lymph node metastasis in patients with thyroid cancer.

Despite the potential of individual FNA and biomarkers in diagnosing thyroid cancer, their combined application in the evaluation of cervical lymph node metastasis in thyroid cancer has not yet been thoroughly studied. The present study explored the role of ultrasound-guided FNA combined with TPO-Ab and Tg-Ab detection in assessing cervical lymph node metastasis in thyroid cancer, including medullary thyroid cancer. Despite its rarity, medullary thyroid cancer exhibits distinct metastatic behaviors that require thorough evaluation (10). Collectively, the present study may provide more effective diagnostic strategies for cervical lymph node metastasis in patients with thyroid cancer in clinical practice.

Materials and methods

Study design and objective. A retrospective case-control study was performed including 294 patients with thyroid cancer who were diagnosed and treated at Changzhou Second People's Hospital (Changzhou, China) between April 2020 and March 2023. The inclusion criteria were as follows: i) Aged >18 years; ii) confirmed diagnosis of thyroid cancer through surgical pathology; iii) underwent total or near-total thyroidectomy and central lymph node dissection; iv) received preoperative neck lymph node ultrasound examination and ultrasound-guided FNA. The exclusion criteria were as follows: i) A history of neck surgery or radiotherapy; ii) presence of other concurrent malignant tumors; iii) inability to undergo FNA or ultrasound examination; iv) incomplete clinical data. Patients were divided into two groups based on the presence of cervical lymph node metastasis: the cervical lymph node metastasis group (CLNM⁺ group) and the non-cervical lymph node metastasis group (CLNM⁻ group). The present study complied with The Declaration of Helsinki and received ethics approval [approval no. (2024)KY134-01] from the Ethics Committee of Changzhou Second People's Hospital.

Ultrasound and FNA examinations. All ultrasound and FNA procedures were performed by experienced ultrasound physicians using high-resolution ultrasound equipment (PHILIPS EPIQ5 color ultrasound diagnostic system; Philips Healthcare) under sterile conditions. The ultrasonographic characteristics of suspected lymph node metastasis were listed as follows: i) Morphological changes (Poisson's ratio <1); ii) punctate calcifications and cystic changes within the lymph nodes; iii) clustered hyperechoic areas; iv) abnormal vascular distribution within the lymph node; v) disappearance of the lymph node hilum; and vi) heterogeneous echogenicity (11,12). A 25 G disposable syringe was used for FNA, with each suspicious metastatic lymph node being punctured under ultrasound guidance, inserting the needle 10-30 times while applying negative pressure. For cystic lymph nodes,

the original aspiration solution (aspirated fluid) was used for pathological examination. For non-cystic lymph nodes, the sample was aspirated with negative pressure and spread onto a glass slide, immediately fixed with alcohol-free fixative and sent for pathological examination. Additionally, a retrospective analysis of the patient's comprehensive thyroid function tests, including TPO-Ab and Tg-Ab levels, was conducted. All lymph node locations were marked on the skin. In addition, the anatomical regions of these lymph nodes were recorded, and their sizes were measured. Finally, the sizes of these suspicious metastatic lymph nodes were compared with those of intraoperative lymph nodes.

Collection of patient data. The clinical data of the patients were collected, including: i) Basic information, such as age, sex, body mass index (BMI), tumor size, tumor stage, tumor location, bilaterality, tumor foci and histological diagnosis; ii) ultrasonographic characteristics of neck lymph nodes, including Poisson's ratio, cystic lesions, lymph node hilum structure, vascular supply, microcalcification and echogenicity; iii) serum thyroid autoantibodies detected by the Roche Cobas 8000 e 602 fully automated chemiluminescence immunoassay analyzer (Roche Diagnostics).

Statistical analysis. All statistical analyses were performed using SPSS 23.0 software (IBM Corporation). Data that did not follow a normal distribution were expressed as median and interquartile range. Comparisons of non-normally distributed data were performed using the Mann-Whitney U test. Count data are presented as number of cases and percentages, and comparisons were performed using the χ^2 test or Fisher's exact test.

Multivariate logistic regression model construction. A logistic regression model was used to analyze the association between various factors and cervical lymph node metastasis. Initially, a univariate analysis was utilized to identify predictor variables significantly associated with cervical lymph node metastasis. These variables included patient demographics (age, sex, BMI), tumor characteristics (tumor size, stage, location, bilaterality, foci, histological diagnosis), ultrasound features (Poisson's ratio, cystic lesions, lymph node hilum structure, vascular supply, microcalcification, heterogeneous echogenicity), and serum thyroid autoantibodies (TPO-Ab, Tg-Ab). Variables with a P-value of <0.05 in the univariate analysis were included in the multivariate logistic regression model. The multivariate logistic regression model was constructed by entering the significant variables from the univariate analysis into the model. Interactions between variables were also considered to identify any potential interactions that could affect the outcome. For example, the interaction between tumor stage and serum Tg-Ab levels was examined to see if their combined effect on metastasis risk differed from their individual effects. The backward elimination method was applied to eliminate non-significant variables and interaction terms stepwise, ensuring that only variables with a significant independent effect remained in the final model. The Hosmer-Lemeshow test was employed to evaluate the model's goodness of fit, while the adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the influence of each variable. Multicollinearity among the predictor variables

was checked using the variance inflation factor (VIF), and any variable with a VIF >10 was removed from the model.

Model performance evaluation. The overall diagnostic accuracy of the model was assessed using the receiver operating characteristic (ROC) curve and the area under the curve (AUC). The AUC value ranges from 0.5 (no diagnostic value) to 1.0 (perfect diagnosis). AUC values closer to 1 indicate a better performance of the diagnostic model. Additionally, the performance of the diagnostic model was quantitatively evaluated with several key indicators, the formulae of which are as follows: Sensitivity (true positive rate)=true positive/(true positive + false negative); Specificity (true negative rate)=true negative/(true negative + false positive); Positive predictive value=true positive/(true positive + false positive); Negative predictive value=true negative/(true negative + false negative). $P < 0.05$ was considered to indicate a statistically significant difference for all statistical analyses.

Results

Clinical characteristics of patients with thyroid cancer. There were no statistically significant differences in age distribution, tumor location, number of tumor foci and histological diagnosis between the two groups of patients (Table I). However, the sex distribution showed that the proportion of female patients in the CLNM⁺ group was much higher than that in the CLNM⁻ group. Furthermore, the proportion of patients with advanced stages (stages III and IV), the proportion of bilateral tumors, and the levels of TPO-Ab and Tg-Ab were markedly higher in the CLNM⁺ group than those in the CLNM⁻ group. These results indicated that sex, tumor stage and thyroid autoantibody levels may be associated with the risk of cervical lymph node metastasis in thyroid cancer.

Ultrasonographic characteristics of cervical lymph nodes in the two groups of patients with thyroid cancer. The ultrasonographic characteristics of cervical lymph nodes showed that there was no statistically significant difference in the Poisson's ratio between the two groups of patients with thyroid cancer (Table II). By contrast, the CLNM⁺ group had significantly higher proportions of cystic lesions, loss of lymph node hilum structure, abundant vascular supply, heterogeneous echogenicity and microcalcification compared with those in the CLNM⁻ group. It could be concluded that these ultrasonographic characteristics were associated with the risk of cervical lymph node metastasis in thyroid cancer. Specifically, the presence of microcalcification and cystic lesions within the lymph nodes suggested possible metastatic involvement, which also indicated a higher likelihood of these features interfering with the pathological diagnosis. Concerning lymph nodes with abnormal vascular distribution, they were marked as suspicious metastatic lymph nodes. Furthermore, the loss of lymph node hilum structure and the heterogeneous echogenicity were indicative of possible malignancy. The ultrasound images of three typical cases from the CLNM⁺ and CLNM⁻ groups are shown in Figs. S1 and S2, respectively.

Analysis of the risk factors related to cervical lymph node metastasis. The logistic regression analysis revealed that

significant independent predictors of cervical lymph node metastasis in thyroid cancer included tumor stage, serum TPO-Ab and Tg-Ab levels, absence of lymph node hilum structure, vascular supply, microcalcification and echogenicity (Table III). Additionally, sex and cystic lesions were identified as significant predictors in the univariate analysis, but lost significance in the multivariate analysis, suggesting that their predictive value might be influenced by other variables. These findings suggested that multiple clinical and ultrasonographic characteristics should be considered when assessing the risk of cervical lymph node metastasis in patients with thyroid cancer.

Outcomes of diagnosis alone and in combination with various indicators. Differences in sensitivity and specificity for diagnosing cervical lymph node metastasis in thyroid cancer were observed when ultrasound, FNA, TPO-Ab and Tg-Ab were used separately for diagnosis (Tables IV and V). Table IV shows the diagnostic outcomes for cervical lymph node metastasis using different methods, including the number of true positive, false negative, true negative and false positive results for each diagnostic method and their combination. FNA exhibited a certain sensitivity (32.62%) and specificity (79.74%); however, compared with FNA, ultrasound diagnosis had a higher sensitivity and specificity. Furthermore, when thyroid autoantibodies were used for diagnosis, high specificity was observed. In particular, Tg-Ab exhibited the highest specificity among all single diagnostic methods, although its sensitivity was not as high as that of ultrasound. The combination of ultrasound, FNA, TPO-Ab and Tg-Ab markedly improved the sensitivity and specificity of diagnosis, indicating that the combination of these diagnostic tools could more accurately assess cervical lymph node metastasis in thyroid cancer.

AUC, sensitivity and specificity values of each indicator, as determined by the ROC curve analysis. The cut-off values for TPO-Ab and Tg-Ab determined by the ROC curve analysis were 16.265 and 10.350 IU/ml, respectively (Table VI; Fig. S3). Based on these cut-off values, the AUC values for the diagnosis of cervical lymph node metastasis were 0.680 (95% CI: 0.604-0.756) for TPO-Ab and 0.887 (95% CI: 0.832-0.942) for Tg-Ab; the sensitivity values were 0.481 and 0.766, and the specificity values were 0.756 and 0.796, respectively. Furthermore, the AUC value for the combined diagnosis was 0.961 (95% CI: 0.936-0.985), with the sensitivity increasing to 0.922 and the specificity increasing to 0.848, which indicated that the combined use of ultrasound, FNA, TPO-Ab and Tg-Ab provided the highest diagnostic accuracy for cervical lymph node metastasis in thyroid cancer.

Discussion

Thyroid cancer is a malignant tumor with a relatively good prognosis compared with other cancer types; however, the occurrence of cervical lymph node metastasis significantly affects the long-term survival of patients (13). As a non-invasive diagnostic tool, ultrasound is convenient and low-cost; however, ultrasound has limited sensitivity in

Table I. Clinical characteristics of patients with thyroid cancer.

Characteristic	CLNM ⁺ (n=77)	CLNM ⁻ (n=217)	Statistics	P-value
Median age, years (IQR)	42 (37-47)	41 (36-47)	-0.385	0.700
Male/female sex, n	24/53	110/107	8.733	0.003
Median BMI, kg/m ² (IQR)	23.21 (22.22-24.41)	23.44 (22.24-24.62)	-0.739	0.460
Median tumor size, cm (IQR)	1.80 (1.10-2.85)	2.00 (1.20-2.80)	-0.903	0.366
Tumor stage, n (%)			67.203	<0.001
I	9 (11.7%)	117 (53.9%)		
II	14 (18.2%)	52 (24.0%)		
III	28 (36.4%)	34 (15.7%)		
IV	26 (33.8%)	14 (6.5%)		
Tumor location, n (%)			7.158	0.067
Left lobe only	19 (24.7%)	33 (15.2%)		
Isthmus	38 (49.4%)	144 (66.4%)		
Right lobe only	10 (13.0%)	21 (9.7%)		
Bilateral	10 (13.0%)	19 (8.8%)		
Nodes, n (%)			0.690	0.406
Solitary	69 (89.6%)	201 (92.6%)		
Multifocal	8 (10.4%)	16 (7.4%)		
Histological diagnosis, n (%)			0.470	0.948
Papillary cancer	26 (33.8%)	70 (32.3%)		
Follicular cancer	26 (33.8%)	69 (31.8%)		
Medullary cancer	24 (31.2%)	75 (34.6%)		
Undifferentiated cancer	1 (1.3%)	3 (1.4%)		
Median TPO-Ab, IU/ml (IQR)	13.15 (5.00-29.61)	6.96 (3.26-12.31)	-4.697	<0.001
Median Tg-Ab, IU/ml (IQR)	17.81 (11.02-26.82)	5.19 (2.55-7.93)	-10.083	<0.001

Data were presented as median (IQR) or n (%). The values in the Statistics column represent the χ^2 test or Fisher's exact test statistics for the comparison between the CLNM⁺ and CLNM⁻ groups. CLNM⁺, cervical lymph node metastasis; CLNM⁻, non-cervical lymph node metastasis; BMI, body mass index; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody.

detecting cervical lymph node metastasis of thyroid cancer, particularly in the detection of metastasis of lymph nodes <5 mm. According to a previous study (14), the sensitivity and specificity of ultrasound in the diagnosis of cervical lymph node metastasis in thyroid cancer are 58 and 86%, respectively. By contrast, FNA has high sensitivity and specificity in the initial diagnosis of thyroid cancer; however, it shows low sensitivity for the evaluation of cervical lymph node metastasis. Especially in cases of tumor micrometastasis or microinvasion of lymph nodes, the sensitivity of FNA can drop to 45% (15). In the present study, the combination of ultrasound, FNA, TPO-Ab and Tg-Ab notably improved the sensitivity and specificity of diagnosis, with the sensitivity increasing to 0.922 and specificity to 0.848. Such improvement reduced the false negative rate, which is particularly important for detecting small or atypical lymph node metastases. Through the ROC analysis, the AUC values for both individual and combined diagnostic methods were calculated to evaluate their overall diagnostic performance. Briefly, the AUC for ultrasound alone was 0.792 and for FNA alone it was 0.580, whereas the AUC for the combination of ultrasound, FNA and biomarkers reached 0.961. The significant AUC values for each diagnostic method

highlight their effectiveness in distinguishing between the CLNM⁺ and CLNM⁻ groups, indicating that the combined diagnostic approach has a higher accuracy than any single tool in diagnosing cervical lymph node metastasis of thyroid cancer. Moreover, improved sensitivity and specificity indicate a more accurate identification of true metastatic cases, thereby avoiding over-treatment or neglecting necessary treatment measures. These findings are crucial for the treatment planning and prognostic assessment of patients with thyroid cancer.

Ultrasound-guided FNA has a high diagnostic rate, offers a satisfactory cell sampling quality and is less affected by lesion sizes compared with CT or MRI (16). Zhang *et al* (17) used FNA of thyroid nodules to predict cervical lymph node metastasis; the results revealed that age, multifocality, lesion size and capsular involvement were independent predictors of cervical lymph node metastasis in thyroid cancer. The present study demonstrated that sex, tumor stage, and the levels of thyroid autoantibodies were associated with the risk of cervical lymph node metastasis in thyroid cancer. Notably, the discrepancies in significance regarding age and sex may arise from differences in baseline data between studies. Furthermore, Ren *et al* (18) employed deep learning models to

Table II. Ultrasonographic characteristics of cervical lymph nodes.

Characteristic	CLNM ⁺ (n=77)	CLNM ⁻ (n=217)	Statistics	P-value
Poisson's ratio			0.025	0.874
≥1	40	115		
<1	37	102		
Cystic lesions			8.706	0.003
No	23	107		
Yes	54	110		
Loss of lymph node hilum structure			18.675	<0.001
No	23	127		
Yes	54	90		
Vascular supply			32.343	<0.001
Not abundant	13	118		
Abundant	64	99		
Microcalcification			81.154	<0.001
No	3	138		
Yes	74	79		
Echogenicity			12.617	<0.001
Homogeneous	21	110		
Heterogeneous	56	107		

Data were presented as the number of cases. The values in the Statistics column represent the χ^2 test statistics for the comparison between the CLNM⁺ and CLNM⁻ groups. CLNM⁺, cervical lymph node metastasis; CLNM⁻, non-cervical lymph node metastasis.

Table III. Logistic regression analysis.

Variable	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (>41.50 years)	1.008 (0.966-1.052)	0.718	-	-
Sex	2.270 (1.309-3.938)	0.004	3.799 (0.809-17.842)	0.091
BMI (>22.85 kg/m ²)	0.925 (0.750-1.140)	0.463	-	-
Tumor size (>3.35 cm)	0.892 (0.672-1.182)	0.425	-	-
Tumor stage (III and IV)	2.924 (2.195-3.897)	<0.001	3.143 (1.643-6.012)	0.001
Tumor location (bilateral)	0.966 (0.703-1.328)	0.831	-	-
Nodes	1.457 (0.597-3.553)	0.408	-	-
Serum TPO-Ab (>16.27 IU/ml)	1.063 (1.040-1.086)	<0.001	1.049 (1.003-1.096)	0.035
Serum Tg-Ab (>10.35 IU/ml)	1.472 (1.324-1.636)	<0.001	1.539 (1.259-1.881)	<0.001
Poisson's ratio	1.043 (0.620-1.755)	0.874	-	-
Cystic lesions	2.284 (1.310-3.982)	0.004	4.321 (0.964-19.374)	0.056
Loss of hilum structure	3.313 (1.897-5.787)	<0.001	6.708 (1.425-31.570)	0.016
Vascular supply	5.868 (3.053-11.278)	<0.001	5.192 (1.051-25.660)	0.043
Microcalcification	43.089 (13.148-141.205)	<0.001	7.596 (3.256-38.25)	0.008
Echogenicity	2.741 (1.554-4.837)	<0.001	5.231 (1.256-21.788)	0.023

OR, odds ratio; CI, confidence interval; BMI, body mass index; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody.

improve the predictive performance of FNA for central lymph node metastasis; however, such radiomics algorithms require large data support and depend on certain computing resources. Overall, further research is still required to achieve more

efficient and convenient diagnosis of lymph node metastasis in thyroid cancer.

In the present study, the diagnostic sensitivity and specificity values of ultrasound-guided FNA for suspicious lymph

Table IV. Diagnostic outcomes of cervical ultrasound, FNA, TPO-Ab, Tg-Ab and ultrasound + FNA + TPO-Ab + Tg-Ab.

A, Ultrasound			
Pathological results	Positive	Negative	Total
Positive	56	21	77
Negative	31	186	217
Total	87	207	
B, FNA			
Pathological results	Positive	Negative	Total
Positive	46	31	77
Negative	95	122	217
Total	141	153	
C, TPO-Ab			
Pathological results	Positive	Negative	Total
Positive	52	25	77
Negative	39	178	217
Total	91	203	
D, Tg-Ab			
Pathological results	Positive	Negative	Total
Positive	48	29	77
Negative	11	206	217
Total	59	235	
E, Combination ^a			
Pathological results	Positive	Negative	Total
Positive	63	14	77
Negative	6	211	217
Total	69	225	

Data are presented as the number of cases. ^aCombination: ultrasound + FNA + TPO-Ab + Tg-Ab. FNA, fine-needle aspiration; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody.

nodes were suboptimal. This was mainly due to the presence of atypical histiocytic cells caused by cystic degeneration of the lymph nodes, which severely interfered with pathological diagnosis. The results showed that 55.8% (164/294) of patients had lymph node cystic degeneration, consistent with a previous report (19). Cystic thyroid cancer is characterized by varying cytological features, consisting of various components of hemosiderin-rich macrophages, cellular debris,

watery colloid and tumor cells. Unlike the classic nuclear characteristics of thyroid cancer (such as powdery chromatin and pseudoinclusion), cystic thyroid cancer cells exhibit enlarged round nuclei and occasional nucleoli (small, dense structures within the nucleus that are irregularly present in tumor cells), accompanied by abundant vacuolated, dense or granular nucleoplasm and hard nuclear membranes (rigid nuclear envelopes), making diagnosis difficult. These characteristics overlap with features of other types of thyroid cancer and benign conditions, complicating the differentiation and accurate diagnosis. Hence, the importance of biomarker detection is highlighted in improving the accuracy of FNA diagnosis.

Generally, thyroid autoantibodies are considered specific markers for autoimmune thyroid disease; however, previous research has identified their potential in monitoring thyroid cancer (20,21). Shield *et al* (22) utilized TPO-Ab for immunohistochemical staining of metastatic tumor cells. This previous study revealed strongly positive TPO-Ab staining in metastatic thyroid cancer, suggesting the potential of TPO-Ab as a diagnostic marker for thyroid cancer metastasis. Subsequently, Li and Cheng (8) demonstrated through bioinformatics analysis that decreased expression of TPO-Ab was significantly associated with lymph node metastasis and recurrence in patients with thyroid cancer. In addition to tissue sample testing, de Meer *et al* (9) further monitored serum levels of Tg-Ab in patients with thyroid cancer, and indicated that elevated serum Tg-Ab levels may be associated with recurrence in these patients. Furthermore, Wu *et al* (23) explored the predictive value of serum Tg-Ab for cervical lymph node metastasis in thyroid cancer and found that elevated levels of Tg-Ab were significantly associated with an increased risk of cervical lymph node metastasis.

In a study by Cabibi *et al* (24), serum TPO-Ab was associated with the nuclear features of thyroid cancer precursors, such as nuclear enlargement, overlapping, crowding, elongation and irregular nuclear membranes. These nuclear features highly overlapped with those of cystic lesions in thyroid cancer cells, indicating a possible association of the molecular mechanisms underlying lymph node metastasis with thyroid autoantibodies. The findings of the present study demonstrated that, although ultrasound, FNA and serum autoantibodies all had some value in evaluating cervical lymph nodes in thyroid cancer, there were limitations in the precise diagnosis. By contrast, the combination of ultrasound, FNA and biomarkers effectively enhanced the sensitivity and specificity of diagnosis of cervical lymph node metastasis, providing a more accurate clinical diagnostic strategy.

One notable aspect of the present study is the atypical distribution of thyroid cancer types within the patient cohort. This may be attributed to the specific nature of the tertiary referral hospital, which frequently manages more complex and advanced cases of thyroid cancer. Consequently, the patient population in the present study may not reflect the general population due to referral patterns and inclusion criteria. This higher proportion of advanced cases could explain the observed distribution discrepancies compared with broader epidemiological studies (25,26).

Additionally, the present study had certain limitations. Firstly, as a retrospective study, inherent biases are

Table V. Diagnostic values of cervical ultrasound, FNA, TPO-Ab, Tg-Ab and ultrasound + FNA + TPO-Ab + Tg-Ab.

Diagnostic method	Sensitivity (%)	Specificity (%)	Positive predictive rate (%)	Negative predictive rate (%)
Ultrasound	64.37	89.86	72.73	85.71
FNA	32.62	79.74	59.74	56.22
TPO-Ab	81.36	87.66	62.34	94.93
Tg-Ab	57.14	87.68	67.53	82.03
Combination ^a	91.30	93.78	81.82	97.24

Data are presented as percentages. ^aCombination: ultrasound + FNA + TPO-Ab + Tg-Ab. FNA, fine-needle aspiration; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody.

Table VI. AUC, sensitivity and specificity values of the receiver operating characteristic curve analysis.

Diagnostic method	AUC (95% CI)	P-value	Cut-off	Sensitivity (%)	Specificity (%)
Ultrasound	0.792 (0.728-0.856)	<0.001	-	72.7	80.1
FNA	0.580 (0.506-0.654)	0.037	-	59.7	61.5
TPO-Ab	0.680 (0.604-0.756)	<0.001	16.265	48.1	75.6
Tg-Ab	0.887 (0.832-0.942)	<0.001	10.350	76.6	79.6
Combination ^a	0.961 (0.936-0.985)	<0.001	-	92.2	84.8

Data are presented as percentages. ^aCombination: ultrasound + FNA + TPO-Ab + Tg-Ab. AUC, area under the curve; CI, confidence interval; FNA, fine-needle aspiration; TPO-Ab, thyroid peroxidase antibody; Tg-Ab, thyroglobulin antibody.

unavoidable. Secondly, the small sample size may impact the generalizability and reliability of the results, potentially introducing selection bias and making the findings less representative of the broader population. Therefore, future research should aim to validate these findings through prospective trials in larger and more diverse patient populations. Thirdly, the presence of thyroid autoimmune diseases, which often coexist with thyroid cancer, may interfere with the levels of thyroid autoantibodies. Hence, future studies need to take the impact of these confounding factors into account to better understand the relationship between thyroid autoantibodies and cervical lymph node metastasis in thyroid cancer (27). Finally, the lack of long-term follow-up data study limits the ability to assess the impact of the combined diagnostic method on patient prognosis. As a result, future studies should include long-term follow-up data to evaluate the prognostic significance and long-term outcomes associated with the combined diagnostic approach.

In conclusion, the present study demonstrated that ultrasound-guided FNA combined with TPO-Ab and Tg-Ab provided high sensitivity and specificity in diagnosing cervical lymph node metastasis in thyroid cancer, indicating the importance of integrating multiple diagnostic tools. Furthermore, the study offered a more precise strategy compared with using ultrasound-guided FNA alone for better assessing the severity of the disease and developing appropriate treatment plans. Future research is still required to validate the present findings and to explore how these diagnostic techniques can be applied routinely in clinical practice.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LW conceived and designed the study; analyzed and interpreted the data; drafted the manuscript; performed critical revision of the manuscript for important intellectual content; conducted statistical analysis. LW confirms the authenticity of all the raw data. The author has read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study complied with The Declaration of Helsinki and received ethics approval from the Ethics Committee of Changzhou Second People's Hospital [approval no. (2024) KY134-01]. All patients provided written informed consent before participating in the study.

Patient consent for publication

Not applicable.

Competing interests

The author declares that they have no competing interests.

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