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ORIGINAL RESEARCH

# Value of Ultrasound Combined with Immunohistochemistry Evaluation of Central Lymph Node Metastasis for the Prognosis of Papillary Thyroid Carcinoma

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**Background:** Papillary thyroid carcinoma (PTC) is often accompanied by cervical lymph node metastasis (LNM). The accuracy of the preoperative ultrasound diagnosis of central LNM (CLNM) is limited. LNM is a high-risk factor for local recurrence and may affect the prognosis. Factors not directly related to tumor proliferation are used for risk assessment in the tumor-node-metastasis (TNM) staging system for thyroid cancer. The present study aimed to investigate the value of ultrasound and immunohistochemistry in predicting the presence of CLNM and the prognosis of PTC.

**Patients and Methods:** The ultrasound and immunohistochemistry features of 303 patients with first-ever PTC and who underwent surgery between 01/2014 to 12/2016 were analyzed, as well as the prognosis of the patients. Univariable and multivariable analyses were carried out to determine the risk factors of CLNM and recurrence.

**Results:** Among 303 patients, 125 (41.3%) were pathologically confirmed with CLNM. Multivariable analysis showed that multifocality, taller-than-wide shape, grade III–IV blood flow, capsular invasion, Ki-67 >10%, p53 ≥5%, T2 or T3 stages were independent risk factors for CLNM. The median follow-up was 56 months. Cox regression analysis showed that age ≥55 years, maximum tumor diameter >20 mm, multifocality, capsular invasion, Ki-67 5–10%, Ki-67 >10%, p53 ≥5%, T3 stage and N1a stage were independent risk factors for PTC recurrence. The Kaplan–Meier showed that recurrence-free survival (RFS) was different according to age (P=0.017), tumor size multifocality, capsular invasion, Ki-67, p53, T stage and N stage (all P<0.001).

**Conclusion:** For PTC with rich blood flow, taller-than-wide shape, multifocality, capsular invasion, p53 ≥5%, Ki-67 >10%, T2 or T3 stages prophylactic CLNM dissection might be indicated. Age≥55 years, maximum tumor diameter >20 mm, multifocality, capsular invasion, high Ki-67, p53 ≥5%, T3 and N1a stages affected the clinical outcome.

**Keywords:** papillary thyroid carcinoma, ultrasonic features, Ki-67, p53, central lymph node metastasis, prognosis

## Introduction

Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the thyroid and generally has a good prognosis. Nevertheless, some PTCs are invasive and are associated with lymph node metastasis (LNM), extra-thyroid extension (ETE), local recurrence, distant metastasis, or even death.[1–3] Among patients with PTC, 30–80% exhibit neck LNM at diagnosis, especially in the central region.[4] Even in

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slow-growing PTCs, about 30% have central LNM (CLNM).[5,6] LNM is a high-risk factor for local recurrence and may affect prognosis.[1,7,8] Ultrasound is among the most commonly used imaging methods for the tumor-node-metastasis (TNM) staging of PTC.[9] Ultrasound staging can show tumor size, ETE, LNM, and other features noninvasively, and those characteristics might affect the operation.[9] Notwithstanding, there are limitations in the evaluation of CLNM by ultrasound before PTC surgery. The sensitivity of CLNM diagnosis is <50%,[10,11] and the accuracy is lower than that of LNM in the lateral cervical region and thyroid capsule invasion.[12,13]

The 2015 American Thyroid Association (ATA) guidelines pointed out that the initial treatment goal of PTC is to improve the survival and achieve accurate staging and risk stratification in order to reduce the incidence of treatment-related complications and postoperative recurrence.[1] Nevertheless, currently, there is no reliable method to accurately predict the progression of papillary thyroid microcarcinoma (PTMC). Moreover, no factors directly related to tumor proliferation activity are used for risk assessment in the TNM staging system for thyroid cancer. Tumor proliferative activity is critical for biological evaluation, including prognosis.[14] Ki-67 is a key marker that indicates cell proliferation and a vital index to evaluate the recurrence and metastasis of breast cancer,[15] cervical cancer,[16] colorectal cancer,[17] and lung cancer.[18] A meta-analysis showed that Ki-67 might have an impact on the prognosis of non-Chinese thyroid cancer patients.[19] p53 protein is a tumor suppressor protein that promotes the transformation and excess proliferation of cancer cells.[20] The high expression of p53 is related to PTC ETE and LNM, albeit without statistical significance.[21] Whether Ki-67 and p53 can predict the prognosis of PTC with high differentiation and inactive malignant behavior needs to be investigated further. Positive CK19 staining has been observed in PTC and is recognized as a reliable marker for PTC,[22,23] but its value prognosis of PTC uncertain.[24–28]

Therefore, the present study aimed to explore the risk factors of CLNM and recurrence by the retrospective analysis of the ultrasonic features and immunohistochemical indexes of primary PTC tumors.

# Patients and Methods

## Patients Selection

This retrospective analysis included 303 cases of thyroid PTC, who underwent surgery at the Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, from January 2014 to December 2016, and with complete ultrasound images and histopathological data. This study was approved by the Ethics Committee of Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Science (No. JDKW-2016-W12). This study was conducted in accordance with the Declaration of Helsinki.

The inclusion criteria were: 1) PTC was confirmed by postoperative pathology; 2) all patients underwent central lymph node dissection (CLND); the operation methods were thyroidectomy, isthmectomy, and ipsilateral CLND for unilateral tumor, and total thyroidectomy plus bilateral CLND for bilateral tumor; 3) there was no evidence of cervical lymph node metastasis or distant metastasis on preoperative ultrasound and computed tomography (CT); and 4) the patients were treated with thyrotropin after surgery. At least two experienced pathologists examined the lymph node specimens to determine LNM. The patients were routinely followed at 3 months, 6 months, and 1 year after the first operation, and then every year. Patients were examined by ultrasound, chest X-ray, or computed tomography (CT). LNM occurrence, recurrence of residual thyroid, and distant metastasis, screened by postoperative pathology, biopsy, or imaging methods, were defined as recurrence.

## Evaluation of Ultrasonic Features

GE Logiq E8, Toshiba Aplio 500, and Philips IU22 ultrasound diagnostic systems and high-frequency linear array probes (5–12 MHz) were used to assess the number, size, echogenicity, border, margin, shape, microcalcification, blood flow, and capsular invasion of the tumor. If the patient had multiple lesions, the largest one was selected as the observation object. The tumors were divided into three groups: the largest diameter <10 mm, 10–20 mm, and >20 mm. The blood flow of the tumor was graded by the Adler semiquantitative method,[29] according to the following criteria: Grade I: no obvious blood flow, indicating the absence of obvious blood flow signal inside the tumor; Grade II: small amount of blood flow, showing 1–2 punctate or short rod blood flow signals in the tumor; Grade III: moderate blood flow, showing 3–4 punctate blood flow signals or one long strip of blood flow signal in the tumor; and Grade IV: abundant blood flow, showing >5 punctate blood flow signals, >2 long strip blood flow signals, reticular blood flow signals, or dendritic blood flow signals. At ultrasound, when it was observed that

the tumor was in contact with >25% of the adjacent thyroid capsule, or the thyroid capsule was disrupted by the tumor, or the soft tissue around the thyroid gland was invaded, it was defined as capsular invasion.[6,30,31]

## Evaluation of Immunohistochemistry Indexes

All PTC samples were fixed with 10% neutral formalin, treated routinely, embedded in paraffin, sectioned continuously into 4-μm-thick sections, dewaxed in xylene, hydrated in an ethanol gradient, repaired for antigens at high pressure and temperature for 90 s, incubated with hydrogen peroxide at room temperature for 10 min, thoroughly rinsed and finally used for hematoxylin and eosin staining and immunohistochemistry (all antibodies were from Shanghai Changjia Biotechnology Co., Ltd., Shanghai, China). The primary antibodies were incubated for 60 min, and the secondary antibodies were incubated for 20 min. The protein of interest was revealed using DAB, and the slides were counterstained with hematoxylin. The sections were dehydrated in an ethanol gradient, treated with xylene, and sealed in neutral resin. PBS was used instead of an antibody as negative control. The indexes of CK19, p53 (p53-antibody anti-mutant p53), and Ki-67 were observed under a light microscope. The percentages of positive cells were calculated as: Percentage of positive cells = number of positive cells/the total number of cells × 100%. CK19 expression was divided into four groups (5%, 5%-25%, 25%-75%, and >75%).[32] p53 expression was divided into two groups according to the proportion of nuclear-positive cells <5% and ≥5%.[21] Ki-67 expression was divided into three groups (5%, 5–10%, and >10%).[14,33]

## Statistical Analysis

The data were analyzed using SPSS 24.0 and GraphPad Prism 7.0. The age and maximum diameter of the tumor were expressed as means ± standard deviations ($\bar{x} \pm s$). The age groups, sex, ultrasonic characteristics and immunohistochemical data were expressed as frequency (%). The risk factors of CLNM were determined by conditional forward logistic regression analysis. The multivariable analyses were carried out using the variables with P<0.10 in the univariable analyses. Multivariable Cox regression analysis was used to determine the risk factors for recurrence. The Kaplan–Meier method and Log rank tests were used to compare recurrence-free survival (RFS) between groups. P<0.05 indicated statistical significance.

# Results

## Patient Characteristics

The clinical data of 303 patients with PTC are summarized in Table 1. Their average age was 47.1±13.1 years of age, and the maximum tumor diameter was 13.3±7.3 (4–41) mm. There were 194 patients with a single tumor (64.0%) and 60 patients with unilateral multifocal tumors (19.8%); they all underwent thyroidectomy, isthmectomy, and ipsilateral CLND. On the other hand, 49 patients with bilateral multifocal tumors (16.2%) underwent total thyroidectomy and bilateral CLND. Among the 303 patients, CLNM was confirmed by pathology in 125 (41.3%) patients. No patient received radioiodine. The entire PTC cohort was followed for at least 3 years. The median follow-up was 56 (6–72) months. Thirty-three patients (10.9%) had a recurrence, including eight cases of residual thyroid recurrence, 22 of distant LNM, and three of both local and LNM. No deaths were not recorded. Figure 1 shows the ultrasound and histological characteristics of a typical case.

## Correlation Between CLNM and Ultrasound Features and Immunohistochemical Indexes

Univariable analyses showed that CLNM was associated with the irregular shape of the primary tumor, taller-than-wide shape, grade III–IV blood flow, multifocality, capsular invasion, Ki-67, p53, and T stage (Table 2). Multiple logistic regression analysis was carried out for the indexes with P<0.10 in the univariable analyses. The results showed that taller-than-wide shape, blood flow grade III–IV, multifocality, capsular invasion, Ki-67 >10%, p53 ≥5%, T2 stage, and T3 stage were independently associated with CLNM. The odds ratios (ORs) (95% confidence intervals (CIs)) of the above factors were 2.788 (1.416–5.486), 2.938 (1.506–-5730), 2.908 (1.368–6.184), 4.393 (1.200–16.089), 5.320 (1.634–17.319), 4.160 (1.947–8.888), 2.556 (1.096–5.961), and 8.500 (1.349–53.563), respectively (Table 2).

## Correlation Between Ultrasound Features and Immunohistochemical Indexes and Postoperative Recurrence

Univariable analyses showed that recurrence was associated with age, maximum tumor diameter, taller-than-wide shape, grade III–IV blood flow, multifocality, capsular invasion, Ki-67, p53, T stage, and N stage. Multivariable

**Table 1** Demographics and Clinical Characteristics of the 303 PTC Patients

| Characteristics | n=303 |
|---|---|
| **Age, years** | 47.1±13.1 |
| **Age groups, n (%)** | |
| <55years | 224 (73.9%) |
| ≥55 years | 79 (26.1%) |
| **Sex, n (%)** | |
| Female | 224 (73.9%) |
| Male | 79 (26.1%) |
| **Size on US, mm** | 13.3±7.3 |
| **Size groups, n (%)** | |
| <10 mm | 103 (34.0%) |
| 10–20 mm | 144 (47.5%) |
| >20 mm | 56 (18.5%) |
| **Surgery, n (%)** | |
| Total thyroidectomy+CLND | 49 (16.2%) |
| Thyroidectomy and isthmectomy + CLND | 254 (83.8%) |
| **Multifocality, n (%)** | |
| Single | 194 (64.0%) |
| Ipsilateral | 60 (19.8%) |
| Bilateral | 49 (16.2%) |
| **US features** | |
| **Echogenicity, n (%)** | |
| Isoechoic/Hyperechoic | 13 (4.3%) |
| Hypoechoic/Marked hypoechoic | 290 (95.7%) |
| **Border, n (%)** | |
| Clear | 90 (29.7%) |
| Obscure | 213 (70.3%) |
| **Margin, n (%)** | |
| Regular | 133 (43.9%) |
| Irregular | 170 (56.1%) |
| **Shape, n (%)** | |
| Wide than taller | 159 (52.5%) |
| Taller than wide | 144 (47.5%) |
| **Microcalcification, n (%)** | |
| No | 112 (37.0%) |
| Yes | 191 (63.0%) |
| **Blood flow, n (%)** | |
| I–II | 199 (65.7%) |
| III–IV | 104 (34.3%) |
| **Capsular invasion, n (%)** | |
| No | 245 (80.9%) |
| Yes | 58 (19.1%) |
| **Immunohistochemical index** | |
| **Ki-67, n (%)** | |
| <5% | 203 (70.0%) |
| 5%-10% | 77 (25.4%) |
| >10% | 23 (7.6%) |
| **CK19, n (%)** | |
| <25% | 52 (17.2%) |
| 25–50% | 201 (66.3%) |
| 50–75% | 35 (11.5%) |
| >75% | 15 (5.0%) |
| **p53, n (%)** | |
| <5% | 151 (49.8%) |
| ≥5% | 152 (50.1%) |
| **T stage, n (%)** | |
| T1 | 217 (71.6%) |
| T2 | 43 (14.2%) |
| T3 | 43 (14.2%) |
| T4 | 0 |
| **N stage, n (%)** | |
| N0 | 178 (58.7%) |
| N1a | 125 (41.3%) |
| **M stage, n (%)** | |
| M0 | 303 (100%) |
| M1 | 0 |
| **Recurrence, n (%)** | 33 (10.9%) |

Cox regression analysis showed that age ≥55 years, the largest tumor diameter >20 mm, multifocality, capsular invasion, Ki-67 5%-10%, Ki-67 >10%, p53, T3 stage, and N1a stage were independently associated with recurrence. The hazard ratio (HR) (95% CI) of the above factors was 2.258 (1.069–4.770), 6.339 (1.832–21.929), 2.503 (1.115–5.618), 6.915 (2.974–16.078), 3.631 (1.324–9.961), 5.343 (1.745–16.365), 3.896 (1.252–12.118), 7.220 (2.429–21.467), and 9.368 (1.196–73.393), respectively (Table 3).

## Survival Analysis After PTC

The survival analyses are shown in Figure 2. The Kaplan–Meier analysis showed that age (≥55 and <55 years of age; P=0.017; Figure 2A), tumor size (P<0.001; Figure 2B), multifocality (P<0.001; Figure 2C), capsular invasion (P<0.001; Figure 2D), Ki-67 (P<0.001; Figure 2E), p53 (P<0.001; Figure 2F), T stage (P<0.001; Figure 2G), and N stage (P<0.001; Figure 2H) differed significantly in RFS. The RFS was 95.1%, 89.6%, and 76.8% in patients with tumor

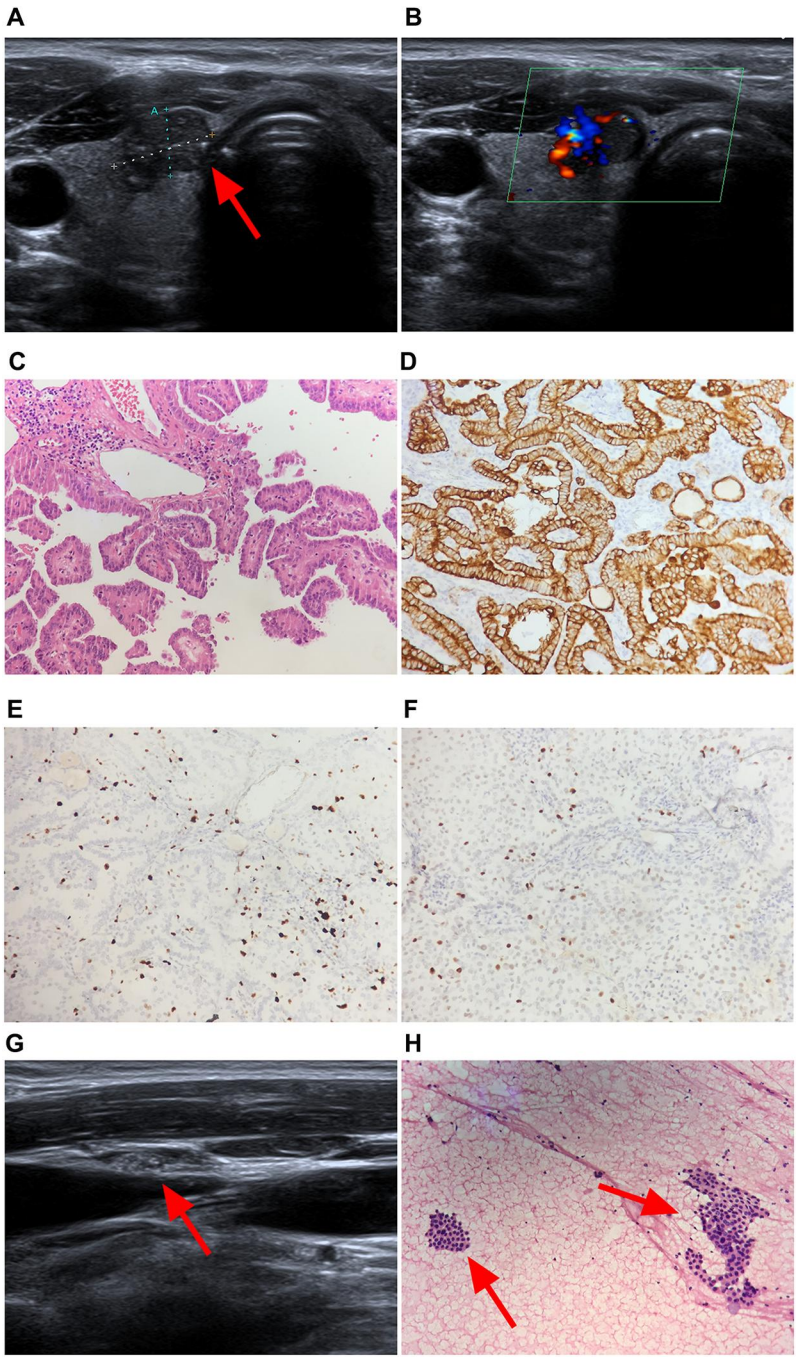


**Figure 1** A 36-years-old woman with PTC. (**A**) Preoperative ultrasound images showing that the size of the tumor was about 7.8×12.0 $mm^2$, the boundary was clear, the morphology was not regular, strong punctate echo (microcalcification) was detected, and the thyroid capsule echo was interrupted (arrow). (**B**) Abundant blood flow signals in the tumor. (**C**) Postoperative pathology was confirmed to be PTC, hematoxylin-eosin (HE) ×200. (**D**) The number of CK19-positive cells was nearly 100%, streptavidin-peroxidase (SP) ×200. (**E**) Ki-67 is about 15%, SP ×200. (**F**) p53-positive cells are about 10% (+), SP ×200. (**G**) After 6 months, the ultrasound showed suspicious LNM in the right lateral jugular vein (V area), about 3×5 $mm^2$ in size (arrow). (**H**) Confirmed by fine-needle aspiration (FNA) as LNM (arrow), H-E ×200.

maximum diameter <10 mm, 10–20 mm, and >20 mm, respectively (<10 vs 10–20 mm, P=0.116; <10 vs >20 mm, P<0.001; 10–20 vs >20 mm, P=0.014). The RFS of single and multifocal tumors was 95.4% and 78.0%, respectively (P<0.001), while it was 96.3% and 58.6% (P<0.001) in patients with and without capsule invasion. Furthermore, the RFS of patients with Ki-67 <5%, 5–10%, and 10% was 96.6%, 77.9%, and 60.9%, respectively (<5% vs 5–10%, P<0.001; <5% vs >10%, P<0.001; 5%-10% vs >10%, P=0.042). The RFS of patients with p53 <5% and p53 ≥5% was 97.4% and 80.9%, respectively (P<0.001). Additionally, the RFS of patients of T1, T2, and T3 stages were 97.7%,

**Table 2** Univariable and Multivariable Analyses Results of Ultrasound Features, Immunohistochemical Indexes and CLNM

| Characteristics | CLNM+ (n=125) | CLNM- (n=178) | Univariable Analysis | | Multivariable Analysis | |
|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P-value | OR (95% CI) | P-value |
| **Age** | | | | | N/A | |
| <55 years | 94 (75.2%) | 130 (73.0%) | 1 | | | |
| ≥55 years | 31 (24.8%) | 48 (27.0%) | 0.893 (0.529–1.508) | 0.672 | | |
| **Sex** | | | | | N/A | |
| Female | 90 (72.0%) | 134 (75.3%) | 1 | | | |
| Male | 35 (28.0%) | 44 (24.7%) | 1.184 (0.705–1.988) | 0.522 | | |
| **Size groups** | | | | | N/A | |
| <10 mm | 32 (25.6%) | 71 (39.9%) | 1 | | | |
| 10–20 mm | 62 (49.6%) | 82 (46.1%) | 1.678 (0.986–2.856) | **0.057** | | |
| >20 mm | 31 (24.8%) | 25 (14.0%) | 2.751 (1.405–5.388) | **0.003** | | |
| **Echogenicity** | | | | | N/A | |
| Isoechoic/Hyperechoic | 5 (4.0%) | 8 (4.5%) | 1 | | | |
| Hypoechoic/Marked hypoechoic | 120 (96.0%) | 170 (95.5%) | 2.743 (0.864–8.711) | 0.834 | | |
| **Border** | | | | | N/A | |
| Clear | 30 (24.0%) | 60 (33.7%) | 1 | | | |
| Obscure | 95 (76.0%) | 118 (66.3%) | 1.610 (0.962–2.694) | 0.070 | | |
| **Margin** | | | | | N/A | |
| Regular | 46 (36.8%) | 87 (48.9%) | 1 | | | |
| Irregular | 79 (63.2%) | 91 (51.1%) | 1.642 (1.029–2.620) | **0.038** | | |
| **Shape** | | | | | | |
| Wide than taller | 48 (38.4%) | 111 (62.4%) | 1 | | 1 | |
| Taller than wide | 77 (61.6%) | 67 (37.6%) | 2.658 (1.659–4.257) | **<0.001** | 2.788 (1.416–5.486) | **0.003** |
| **Microcalcification** | | | | | N/A | |
| No | 39 (31.2%) | 73 (41.0%) | 1 | | | |
| Yes | 86 (68.8%) | 105 (59.0%) | 1.533 (0.947–2.483) | 0.082 | | |
| **Blood flow** | | | | | | |
| I–II | 63 (50.4%) | 136 (76.4%) | 1 | | 1 | |
| III–IV | 62 (49.6%) | 42 (23.6%) | 3.187 (1.947–5.215) | **<0.001** | 2.938 (1.506–5730) | **0.002** |
| **Capsular invasion** | | | | | | |
| No | 76 (60.8%) | 169 (94.9%) | 1 | | 1 | |
| Yes | 49 (39.2%) | 9 (5.1%) | 12.107 (5.659–25.903) | **<0.001** | 4.393 (1.200–16.089) | **0.025** |
| **Multifocality** | | | | | | |
| No | 56 (44.8%) | 138 (77.5%) | 1 | | 1 | |
| Yes | 69 (55.2%) | 40 (22.5%) | 4.251 (2.583–6.996) | **<0.001** | 2.908 (1.368–6.184) | **0.006** |
| **Ki-67** | | | | | | |
| <5% | 63 (50.4%) | 140 (78.7%) | 1 | | 1 | |
| 5%-10% | 45 (36.0%) | 32 (18.0%) | 3.125 (1.817–5.374) | **<0.001** | 1.367 (0.663–2.821) | 0.397 |
| >10% | 17 (13.6%) | 6 (0.3%) | 6.296 (2.370–16.727) | **<0.001** | 5.320 (1.634–17.319) | **0.006** |
| **CK19** | | | | | N/A | |
| <25% | 18 (14.4%) | 34 (19.1%) | 1 | | | |
| 25–50% | 85 (68.0%) | 116 (65.2%) | 1.384 (0.733–2.615) | 0.317 | | |
| 50–75% | 15 (12.0%) | 20 (11.2%) | 1.417 (0.588–3.416) | 0.438 | | |

(*Continued*)

**Table 2** (Continued).

| Characteristics | CLNM+ (n=125) | CLNM- (n=178) | Univariable Analysis | | Multivariable Analysis | |
|---|---|---|---|---|---|---|
| | | | OR (95% CI) | P-value | OR (95% CI) | P-value |
| >75% | 7 (5.6%) | 8 (4.5%) | 1.653 (0.516–5.294) | 0.398 | | |
| **p53** | | | | | | |
| <5% | 31 (24.8%) | 120 (67.4%) | 1 | | 1 | |
| ≥5% | 94 (75.2%) | 58 (32.6%) | 6.274 (3.757–10.477) | **<0.001** | 4.160 (1.947–8.888) | **<0.001** |
| **T stage** | | | | | | |
| T1 | 65 (19.8%) | 152 (80.2%) | 1 | | 1 | |
| T2 | 20 (48.8%) | 23 (51.2%) | 3.617 (1.653–7.915) | **0.001** | 2.556 (1.096–5.961) | **0.030** |
| T3 | 40 (93.2%) | 3 (6.8%) | 51.789 (14.448–185.636) | **<0.001** | 8.500 (1.349–53.563) | **0.023** |

**Note:** Bold P-values are statistically significant (P<0.05).
**Abbreviations:** OR, odds ratio; CI, confidence interval; N/A, not available.

86.1%, and 38.8% (P<0.001); those of N0 and N1a were 99.4% and 74.4% (P<0.001), respectively (Figure 2).

## Discussion

Although preoperative ultrasound is routinely used for thyroid cancer, and acute lymph node staging, the accuracy of CLNM ultrasound is not high. In this study, the precise diagnosis of PTC combined with factors directly related to tumor proliferation activity was carried out for risk assessment, and the factors independently associated with CLNM and prognosis were identified, which could be helpful to subdivide high-risk or low-risk PTC. The 2015 ATA guidelines recommend CLND for patients with PTC and suspected LNM or advanced stage metastasis.[1] Nevertheless, preventive CLND for patients without evidence of lymph node metastasis is still controversial because of the risks of complication and morbidity. Because CLND has uncertain efficacy and high risk of complications,[34,35] reoperation of PTC recurrence may significantly increase the surgical complications and affect the quality of life of the patients.[36] Therefore, identifying the specific risk factors of CLNM is crucial. Some recent studies tried to predict CLNM in patients with thyroid cancer based on ultrasound features, but the specificity is low.[6,37] Hence, in the present study, we included indicators such as ultrasound features and immunohistochemistry, for an in-depth analysis of the factors associated with CLNM. The results showed that capsule invasion was the strongest factor among ultrasonic features (OR=4.493), which was consistent with the previous results.[5,6] Taller-than-wide shape and blood flow were reported by Xu et al[6] but the present study reached different conclusions, possibly because of case selection since their research was limited to PTMC. The cases with taller-than-wide shape accounted for 60.7%,[6] which was significantly higher than in the present study (47.5%). In addition, PTMC is characterized by the lack of blood supply due to the lack of internal neovascularization.[38] High contrast-enhanced or iso-enhanced contrast-enhanced ultrasound is an index to predict CLNM,[39] supporting the present study. Multifocality has been recognized as a risk factor for LNM,[5,40] and this was observed here. Since the thyroid is a rich network of lymphatic channels, multifocal tumors increase the chance of intraglandular metastasis and LNM.[5] T2 and T3 tumors were associated with CLNM, but 31.1% of the patients in the <10-mm group still had CLNM, indicating that smaller tumors are still invasive. This is supported by the fact that PTC size is indeed associated with metastases.[41] In addition, we confirmed that p53 ≥5% and Ki-67 >10% were independent risk factors of LNM, but CK19 was not related to CLNM. The usefulness of CK19 as a postsurgical prognostic factor of PTC in various populations has so far yielded conflicting results.[24–28] Some studies showed that the expression of Ki-67 and p53 was not related to tumor size.[42,43] Therefore, the evaluation of the proliferation activity of the tumor cell is more reliable for determining the biological behavior rather than relying on tumor size alone. Indeed, a tumor of 10 mm, for example, can be the results of an aggressive tumor that grew over a few months or of an indolent tumor that grew over a few years. The biological markers might indicate the aggressiveness of a tumor.

PTC is characterized by low invasiveness, with a 10-year survival rate of >90%, but as high as 22–30% of patients show relapse, and most of them occur within 3 years.[44,45] In order to determine the factors associated with recurrence, we followed the patients for at least 3 years. The overall prognosis was

**Table 3** Results of Univariable and Multivariable Analyses of Ultrasound Features, Immunohistochemical Indexes and PTC Recurrence

| Characteristics | Recurrence (n=33) | Non-Recurrence (n=270) | Univariable Analysis | | Multivariable Analysis | |
|---|---|---|---|---|---|---|
| | | | HR (95% CI) | P-value | HR (95% CI) | P-value |
| **Age (years)** | | | | | | |
| <55 years | 17(51.5%) | 207(76.7%) | 1 | | 1 | |
| ≥55 years | 16(48.5%) | 63(23.3%) | 2.836 (1.433–5.614) | 0.003 | 2.258 (1.069–4.770) | 0.033 |
| **Sex** | | | | | N/A | |
| Female | 24 (72.7%) | 200 (74.1%) | 1 | | | |
| Male | 9 (27.3%) | 70 (25.9%) | 1.077 (0.501–2.317) | 0.849 | | |
| **Size groups** | | | | | | |
| <10 mm | 6 (18.2%) | 97 (35.9%) | 1 | | **1** | |
| 10–20 mm | 15 (45.4%) | 129 (47.8%) | 2.198 (0.779–6.048) | 0.127 | 2.556 (0.869–7.517) | 0.088 |
| >20 mm | 12 (36.4%) | 44 (16.3%) | 5.427 (1.934–15.228) | **0.001** | 6.339 (1.832–21.929) | **0.004** |
| **Echogenicity** | | | | | N/A | |
| Isoechoic/Hyperechoic | 3 (9.1%) | 10 (3.7%) | | | | |
| Hypoechoic/Marked hypoechoic | 30 (90.9%) | 260 (96.3%) | 0.377 (0.115–1.237) | 0.108 | | |
| **Border** | | | | | N/A | |
| Clear | 6 (18.2%) | 84 (31.1%) | 1 | | | |
| Obscure | 27 (81.8%) | 186 (68.9%) | 2.444 (0.944–6.331) | 0.066 | | |
| **Margin** | | | | | N/A | |
| Regular | 9 (27.3%) | 124 (45.9%) | 1 | | | |
| Irregular | 24 (72.7%) | 146 (54.1%) | 2.135 (0.992–4.593) | 0.052 | | |
| **Shape** | | | | | N/A | |
| Wide than taller | 11 (33.3%) | 148 (54.8%) | 1 | | | |
| Taller than wide | 22 (66.7%) | 122 (45.2%) | 2.295 (1.113–4.733) | **0.024** | | |
| **Microcalcification** | | | | | N/A | |
| No | 12 (36.4%) | 100 (37.0%) | 1 | | | |
| Yes | 21 (63.6%) | 170 (63.0%) | 1.035 (0.509–2.103) | 0.925 | | |
| **Blood flow** | | | | | N/A | |
| I–II | 16 (48.5%) | 183 (67.8%) | 1 | | | |
| III–IV | 17 (51.5%) | 87 (32.2%) | 2.196 (1.110–4.374) | **0.024** | | |
| **Capsular invasion** | | | | | | |
| No | 9 (27.3%) | 236 (87.4%) | 1 | | **1** | |
| Yes | 24 (72.7%) | 34 (12.6%) | 13.976 (6.488–30.107) | **<0.001** | 6.915 (2.974–16.078) | **<0.001** |
| **Multifocality** | | | | | | |
| No | 9 (27.3%) | 185 (68.5%) | 1 | | **1** | |
| Yes | 24 (72.7%) | 85 (31.5%) | 5.281 (2.454–11.366) | **<0.001** | 2.503 (1.115–5.618) | **0.026** |
| **Ki-67** | | | | | | |
| <5% | 7 (21.2%) | 196 (72.6%) | 1 | | **1** | |
| 5%-10% | 17 (51.5%) | 60 (22.2%) | 7.071 (2.931–17.058) | **<0.001** | 3.631 (1.324–9.961) | **0.012** |
| >10% | 9 (27.3%) | 14 (5.2%) | 16.070 (5.973–42.237) | **<0.001** | 5.343 (1.745–16.365) | **0.003** |
| **CK19** | | | | | N/A | |
| <25% | 2 (6.1%) | 50 (18.5%) | 1 | | | |
| 25–50% | 24 (72.7%) | 177 (65.6%) | 3.219 (0.761–13.621) | 0.112 | | |
| 50–75% | 4 (12.1%) | 31 (11.5%) | 3.033 (0.556–16.561) | 0.200 | | |
| >75% | 3 (9.1%) | 12 (4.4%) | 5.774 (0.964–34.574) | 0.055 | | |
| **p53** | | | | | | |
| <5% | 4 (12.1%) | 147 (54.4%) | 1 | | **1** | |

(*Continued*)

**Table 3** (Continued).

| Characteristics | Recurrence (n=33) | Non-Recurrence (n=270) | Univariable Analysis | | Multivariable Analysis | |
|---|---|---|---|---|---|---|
| | | | HR (95% CI) | P-value | HR (95% CI) | P-value |
| ≥5% | 29 (87.9%) | 123 (45.6%) | 7.889 (2.773–22.466) | **<0.001** | 3.896 (1.252–12.118) | **0.019** |
| **T stage** | | | | | | |
| T1 | 5 (15.1%) | 212 (78.5%) | 1 | | 1 | |
| T2 | 6 (18.2%) | 37 (13.7%) | 6.455 (1.970–21.153) | **0.002** | 2.687 (0.584–12.352) | **0.204** |
| T3 | 22 (66.7%) | 21 (7.8%) | 30.480 (11.518–80.660) | **<0.001** | 7.220 (2.429–21.467) | **<0.001** |
| **N stage** | | | | | | |
| N0 | 1 (3.0%) | 177 (65.6%) | 1 | | 1 | |
| N1a | 32 (97.0%) | 93 (34.4%) | 52.472 (7.168–384.091) | **<0.001** | 9.368 (1.196–73.393) | **0.033** |

**Note:** Bold P-values are statistically significant (P<0.05).
**Abbreviations:** HR, hazard ratios; CI, confidence interval; N/A, not available.

favorable, and no deaths were recorded. Therefore, we only analyzed the difference in RFS. The Kaplan–Meier analysis showed that Ki-67 5%-10% or >10% and p53 ≥5% were associated with relapse, with recurrence rates of 22.1%, 39.1%, and 19.1%, respectively. Moreover, multiple factors were also confirmed; the most important factor for CLNM was Ki-67 >10%, consistent with previous findings.[14,33] The above conclusions further clarified that proliferative activity is a critical factor in the assessment of PTC recurrence. A positive correlation was observed between tumor size and recurrence or tumor-specific mortality,[1] but no significant difference was detected between the 10–20 and <10 mm groups. Thus, the present study supports the >20-mm threshold used by the AJCC staging. In the present study, T3 tumors and N1a involvement were associated with recurrence. T staging and N staging are recognized prognostic factors for PTC[46,47] and were confirmed in the present study, suggesting the reliability of the research results. Imaging findings (which are macroscopic observations) showed that capsule invasion was consistent with histopathological (microscopic) ETE, and multifocality could potentially cause invasion of the capsule and effectuate histological characteristics of ETE; interestingly, the presence of ETE was related to local recurrence and distant metastasis.[48,49] We also confirmed that capsular invasion and multifocal tumors were independently associated with recurrence. Therefore, ultrasound examination of PTC patients should be focused on the evaluation of the above features. In order to prevent the over-staging of low-risk patients and reduce the excess treatment, the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) staging system adjusted the age threshold from 45 to 55 years.[50] Surprisingly, the results showed that age was not related to CLNM, but it was associated with prognosis. Nevertheless, the predictive value of age for prognosis is yet controversial, as mentioned previously.[51,52]

The present study has some limitations. First, 34.0% and 47.5% of the cases were maximum tumor diameter >10 and 10–20 mm, respectively, while only 18.5% were >20 mm, which might be attributed to a bias in case selection. Second, the World Health Organization (WHO) classifies PTC into classic, follicular, diffuse sclerotic, high cell, and columnar cell subtypes. The degree of invasion of different subtypes of PTC varies,[1] but this could not be analyzed because of the two small numbers of patients in some subtypes. Furthermore, the median follow-up was <5 years, and there is a possibility of occult micrometastases, and may not be sufficient to evaluate the variation in RFS. Third, the detection of the tumor proliferation indexes id valuable in increasing the accuracy of the diagnosis of auxiliary fine-needle aspiration cytology,[53,54] which provides the feasibility for the preoperative acquisition. In this retrospective study, we analyzed the immunohistochemical indicators detected after surgery, and the ultrasound features and immunohistochemical indicators were subjective evaluations.

## Conclusion

For patients with PTC and rich blood flow, taller-than-wide shape, multifocality, capsular invasion, p53 ≥5%, Ki-67 >10%, T2 or T3 stages prophylactic CLND might be recommended. Moreover, the age≥55 years, maximum tumor diameter >20 mm, multifocality, capsular invasion, high Ki-67, p53 ≥5%, T3 stage and N1a stage affected the

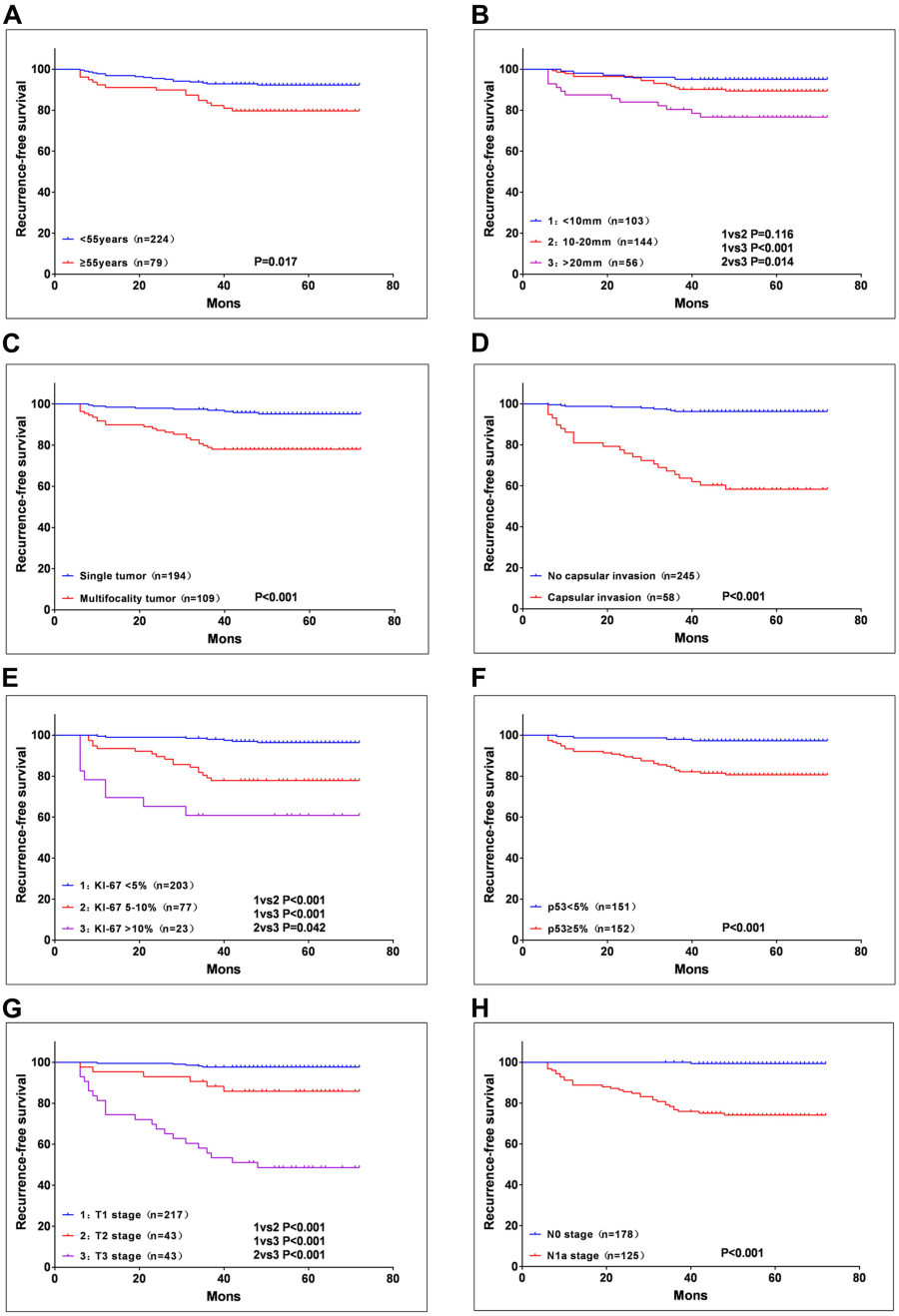


**Figure 2** Kaplan–Meier curve of RFS in PTC patients. (**A**) Comparison between <55-years-old and ≥55-years-old patients. (**B**) Comparison of tumor maximum diameter <10, 10–20 mm, and >20 mm. (**C**) Comparison of multifocal tumors with single tumors. (**D**) Comparison of tumor invasion and non-invasion. (**E**) Comparison of Ki-67 <5%, Ki-67 5%-10%, and Ki-67 >10%. (**F**) Comparison of p53 <5% and p53 ≥5%. (**G**) Comparison of T1, T2, and T3. (**H**) Comparison of N0 and N1a.

clinical outcome. Such patients would benefit from expanding the scope of surgery that would help avoid the high incidence of complications caused by a second surgery.

## Abbreviations

PTC, Papillary thyroid carcinoma; LNM, lymph node metastasis; ETE, extra-thyroid extension; CLNM, central LNM; ATA, American Thyroid Association; CLND, central lymph node dissection.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Science (No. JDKW-2016-W12). All participants provided written informed consent.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

All authors declare that they have no conflicts of interest for this work.

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