Influence of Chronic Lymphocytic Thyroiditis and *BRAF V600E* Mutation on Clinicopathological Features in Papillary Thyroid Carcinoma With Different Tumor Size

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AIM: Chronic lymphocytic thyroiditis (CLT) is recognized as the most prevalent inflammatory disorder of the thyroid, while the B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) *V600E* mutation is the most frequently identified genetic alteration in papillary thyroid carcinoma (PTC). This study aims to explore the relationship between CLT and *BRAF V600E* mutation and to assess their combined impact on tumor behavior across different tumor sizes.

METHODS: We conducted a retrospective analysis of clinical and pathological data from 1474 patients who underwent surgical treatment for PTC. Univariate and multivariate logistic regression analyses were applied to identify independent factors influencing tumor characteristics.

RESULTS: CLT was detected in 27.5% (405/1474) of the PTC cases. Multivariate analysis revealed that CLT was significantly associated with female sex; simultaneously, CLT was significantly negatively associated with *BRAF V600E* mutation, extrathyroidal extension (ETE), central lymph node metastasis (CLNM), and advanced disease stage (all $p < 0.05$). The *BRAF V600E* mutation was observed in 80.7% (1189/1474) of patients. Stratified analysis by tumor size showed that *BRAF V600E* mutation independently predicted CLT and advanced tumor-node-metastasis (TNM) stage in papillary thyroid microcarcinoma (PTMC); CLT, ETE, and CLNM in tumors 1–2 cm; CLT, ETE, CLNM, and lateral lymph node metastasis (LLNM) in tumors 2–4 cm; and CLT, ETE, vascular invasion, CLNM, and LLNM in tumors >4 cm (all $p < 0.05$). Notably, PTC patients without CLT but harboring *BRAF V600E* mutation showed a combined association with advanced TNM stage and aggressive features.

CONCLUSIONS: The presence of CLT appears to exert a protective effect in PTC. However, the prognostic significance of *BRAF V600E* mutation varies with tumor size. While CLT-related inflammatory microenvironment may counteract tumor progression in small cancers, it seems insufficient to mitigate the aggressive behavior driven by *BRAF V600E* mutation in larger tumors.

Keywords: papillary thyroid carcinoma; papillary thyroid microcarcinoma; chronic lymphocytic thyroiditis; *BRAF V600E* mutation; aggressiveness

Introduction

Papillary thyroid carcinoma (PTC) represents the most prevalent subtype of thyroid malignancies, comprising roughly 80% of all thyroid cancer cases [1,2] . Over the past few decades, its incidence has shown a steady upward trend [3]. Chronic lymphocytic thyroiditis (CLT), also known as Hashimoto's thyroiditis, is the most frequently observed inflammatory condition affecting the thyroid gland and is a well-established cause of hypothyroidism. Notably, studies have reported CLT coexisting in about 20–35% of PTC cases [4,5]. However, the clinical implications of this association remain debated. While some meta-analyses, such as that by Lee *et al*. [5]. suggest that the presence of CLT may confer a more favorable prognosis in PTC patients, other investigations have found no significant impact of CLT on disease outcomes [6].

The B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) gene, particularly the V600E mutation, is a key driver of tumorigenesis in PTC. This mutation leads to constitutive activation of the MAPK signaling pathway, thereby promoting uncontrolled cellular proliferation and survival [7] . *BRAF V600E* is the most common genetic alteration identified in PTC, with reported prevalence ranging from 76–83% across different populations [8]. Numerous studies have linked this mutation to more aggressive tumor behavior [9,10], including advanced tumor stage, extrathyroidal extension (ETE), lymph node involvement, and higher recurrence rates [11]. Nevertheless, some research has failed to confirm these associations, reporting no significant correlation between *BRAF V600E* mutation and clinical staging, tumor or disease recurrence [12].

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Earlier investigations have indicated that individuals with CLT may have an elevated risk of developing PTC compared to those without thyroiditis [13]. Moreover, *BRAF V600E* mutations have been detected not only in tumor tissues but also in the surrounding thyroid parenchyma affected by CLT, suggesting a potential role of this mutation in the progression from chronic inflammation to malignancy [14]. Given the frequent coexistence of CLT and PTC, understanding their interaction and the resulting clinicopathological features has become a focal point of research. However, the precise relationship between CLT and *BRAF V600E* mutation, and whether their coexistence influences tumor behavior, remains unclear [15]. Additionally, prior study has demonstrated that the predictive significance of *BRAF V600E* mutation for lymph node metastasis may vary depending on tumor size [16]. This suggests that tumor diameter could be a confounding factor in assessing the mutation's impact. Therefore, in this study, we stratified PTC patients based on tumor size to better evaluate the individual and combined effects of CLT and *BRAF V600E* mutation on tumor characteristics.

Methods

Patients

This retrospective cohort study included patients who underwent thyroid surgery for primary PTC at Changzhou First People's Hospital between March 2018 and June 2021. This study has been approved by the Institutional Review Board of Changzhou First People's Hospital (2020-EC-216), and has been performed according to the ethical standards laid down in the Declaration of Helsinki and its subsequent revisions. Informed consent was obtained from all individual participants included in the study. Patients were excluded if they had non-PTC thyroid malignancies (e.g., medullary, follicular, or anaplastic types), variants of PTC other than the classic type (such as mixed subtypes), a history of other cancers prior to thyroid surgery, underwent non-curative or repeat operations, had distant metastasis confirmed by imaging or pathology (e.g., 18F-fluorodeoxyglucose positron-emission tomography–computed tomography (18F-FDG PET/CT)), previous neck radiation exposure, familial thyroid cancer, or incomplete clinical records and follow-up data. Ultimately, a total of 1474 patients met the inclusion criteria and were enrolled in the analysis. Given that obesity has been reported to be associated with thyroid nodules and thyroid cancer risk, body mass index (BMI) was included as a potential influencing factor and patients were categorized into normal weight and overweight groups according to World Health Organization (WHO) criteria [17]. At the time of initial evaluation, BMI was calculated as weight in kilograms divided by the square of height in meters. Based on WHO criteria [17], patients were categorized into normal weight (BMI <25 kg/m^2) and overweight (BMI ≥ 25 kg/m^2) groups [17]. The patient selection process and exclusion criteria are summarized in Fig. 1.

Surgical Procedures

Preoperative assessment of the primary tumor and cervical lymph nodes was performed using ultrasonography (US), CT, and fine-needle aspiration (FNAC). For patients without clinical evidence of lateral lymph node metastasis (LLNM), the standard surgical approach included total thyroidectomy (TT) or lobectomy combined with central neck dissection (CND). When preoperative evaluation suggested unilateral PTC, patients typically underwent ipsilateral lobectomy; bilateral disease was managed with TT. In cases with suspected or confirmed LLNM, therapeutic lateral neck dissection (LND) was performed in addition to TT and CND. Central compartment dissection included removal of prelaryngeal, pretracheal, and paratracheal lymph nodes [18], while LND covered levels II to V, with preservation of the internal jugular vein, spinal accessory nerve, and sternocleidomastoid muscle [19]. All lymph node specimens were dissected and labeled by the surgeon according to anatomical levels and sent for histopathological examination.

DNA Extraction and BRAF V600E Mutation Analysis

Under ultrasound guidance, fine-needle aspiration was performed by experienced radiologists to obtain cytological samples for molecular analysis. A 22-gauge needle was used, and each lesion was aspirated three to four times. The needle was rinsed with 5 mL of normal saline, and the collected material was centrifuged. The cell pellet was resuspended in TRI reagent (lot no. BCCP2577, Sigma) and stored at –20 °C until DNA extraction, which was carried out following the manufacturer's instructions. *BRAF V600E* mutation status was determined using polymerase chain reaction (PCR) amplification of exon 15, as previously described [20]. The primers used were: forward 5′-*ACCTAAACTCTTCATAATGCTTGCT*-3′ and reverse 5′-*TGATTTTTGTGAATACTGGGAACT*-3′. Bidirectional sequencing was performed by Inhuaiweiji Biological Technology Ltd. (Shanghai, China), and the obtained sequences were compared with the wild-type *BRAF* gene sequence from the GenBank database using Gene Codes sequence assembly software (version 5.2, Gene Codes Corporation, Ann Arbor, MI, USA).

Histopathologic Examination of Surgical Specimens

All surgical specimens were independently reviewed by at least two pathologists. ETE was defined as tumor invasion beyond the thyroid capsule into adjacent soft tissues or strap muscles [21]. Vascular invasion was identified when tumor cells were seen penetrating vessel walls or when intravascular tumor thrombi were present. Multifocality was defined as the presence of two or more distinct tumor foci within the thyroid gland [22,23]. Tumors ≤ 1 cm in greatest dimension were classified as papillary thyroid microcarcinoma (PTMC), while those >1 cm were considered non-PTMC. Tumor staging was performed according to the 8th edition of the American Joint Committee on Cancer (AJCC) tumor-

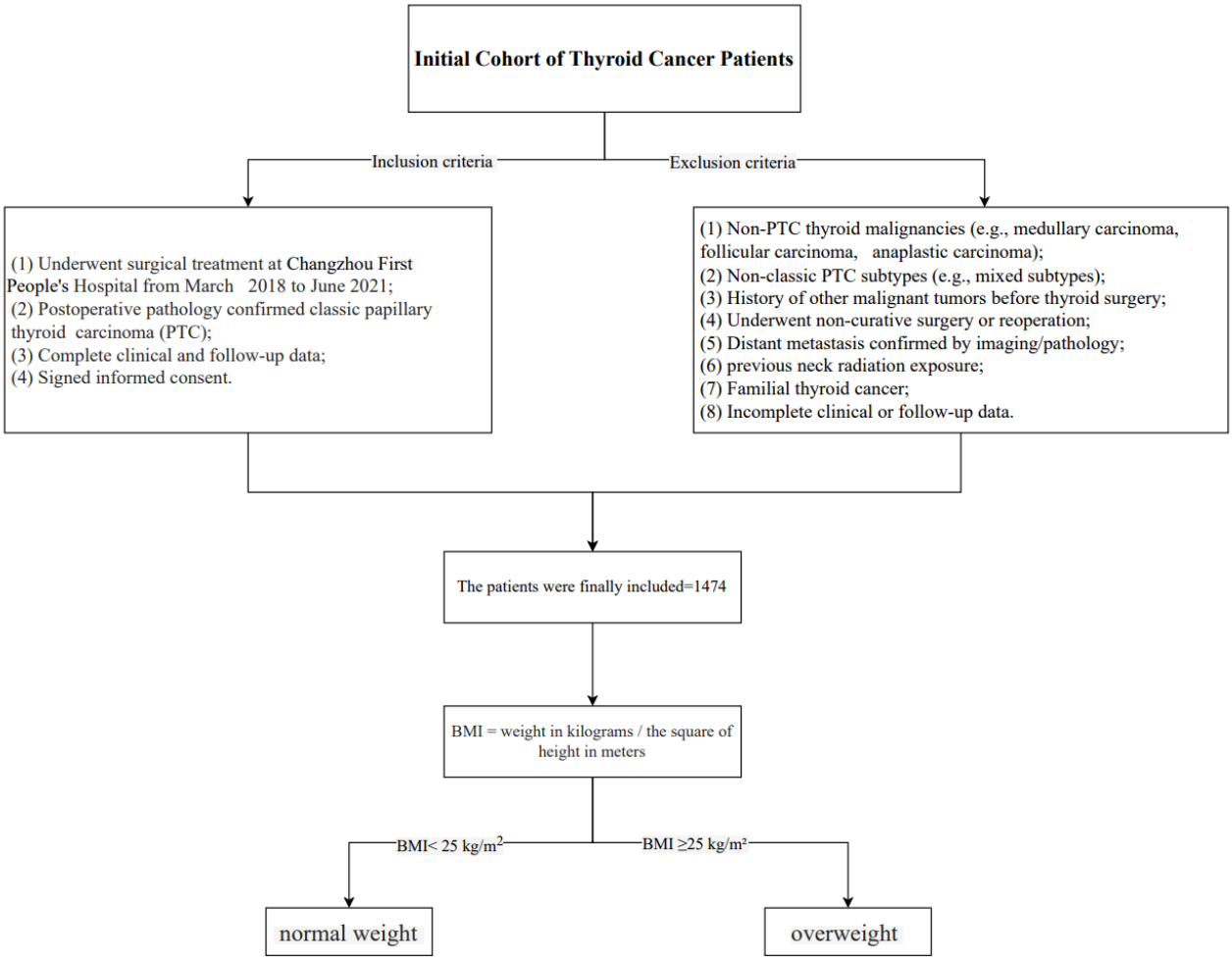

Fig. 1. Flowchart of patient selection based on inclusion and exclusion criteria. BMI, body mass index.

node-metastasis (TNM) classification system [24]. The diagnosis of CLT was based on histological findings, including diffuse lymphocytic infiltration with germinal centers, parenchymal atrophy with oncocytic changes, and variable fibrosis throughout the thyroid tissue [25].

Statistical Analysis

All data analyses were conducted using SPSS version 25.0 (IBM SPSS statistics, Chicago, IL, USA). Normality test for continuous variables: The Shapiro-Wilk test was used. If $p > 0.05$, the variable was considered to follow a normal distribution and expressed as "mean ± standard deviation"; if $p \leq 0.05$, it was considered non-normal and expressed as "median (interquartile range)". All continuous variables in this study passed the Shapiro-Wilk test ($p > 0.05$), so they were uniformly expressed as mean ± SD. For categorical variables: Pearson's chi-square test (sample size ≥40 and all expected frequencies ≥5) or Fisher's exact test (sample size <40 or expected frequency <5) was used; the significance criterion for categorical variables was $p < 0.05$. Variables with a p value < 0.05 in univariate analysis were included in multivariate logistic regression to assess independent associations with CLT or *BRAF V600E* mutation. To evaluate tumor aggressiveness, patients were stratified into four subgroups based on CLT and *BRAF* mutation status: (1) CLT-positive and *BRAF*-negative; (2) CLT-positive and *BRAF*-positive; (3) CLT-negative and *BRAF*-negative; (4) CLT-negative and *BRAF*-positive. Logistic regression analysis was performed to estimate odds ratios (ORs) for aggressive features, adjusting for age (per 10-year increment) and sex.

Results

Clinicopathological Characteristics of 1474 PTC Patients

As shown in Table 1, a total of 1474 patients diagnosed with PTC were enrolled in this study, including 1126 females and 348 males, with a mean age of 46.7 ± 11.9 years. Among them, 497 individuals (33.7%) were aged 55 years or older. The average BMI was 24.21 ± 4.17 kg/m^2, and 547 patients (37.1%) were classified as overweight. The mean tumor diameter was 1.79 ± 0.92 cm, ranging from 0.10 to 6.10 cm. Tumor size distribution was as follows: 843 cases (57.2%) ≤1 cm, 432 cases (29.3%) between 1 and 2 cm, 172 cases (11.7%) between 2 and 4 cm, and 27 cases (1.8%)

Table 1. Clinicopathological characteristics of 1474 PTC patients.

Clinicopathological characteristics	Value
Sex	
Male	348 (23.6%)
Female	1126 (76.4%)
Age (Y), mean ± SD (range)	46.7 ± 11.9 (20–82)
≥55	497 (33.7%)
<55	977 (66.3%)
Maximum tumor size (cm), mean ± SD (range)	1.79 ± 0.92 (0.10–6.10)
≤1	843 (57.2%)
>1 to ≤2	432 (29.3%)
>2 to ≤4	172 (11.7%)
>4	27 (1.8%)
BMI (kg/m^2), mean ± SD (range)	24.21 ± 4.17 (17.22–38.67)
Normal	927 (62.9%)
Overweight	547 (37.1%)
BRAF V600E mutation	
Negative	285 (19.3%)
Positive	1189 (80.7%)
CLT	
Absence	1069 (72.5%)
Presence	405 (27.5%)
Multifocality	
Absence	1080 (73.3%)
Presence	394 (26.7%)
ETE	
Absence	1054 (71.5%)
Presence	420 (28.5%)
Vascular invasion	
Absence	1297 (88.0%)
Presence	177 (12.0%)
No. of removed LNs in CC, mean ± SD (range)	5.7 ± 4.2 (2–27)
No. of removed LNs in LC, mean ± SD (range)	19.4 ± 12.1 (4–56)
No. of metastatic LNs in CC, mean ± SD (range)	2.9 ± 1.8 (0–11)
No. of metastatic LNs in LC, mean ± SD (range)	5.9 ± 4.7 (0–26)
TNM stage (AJCC 8th edition)	
I	1057 (71.7%)
II	225 (15.3%)
III	192 (13.0%)
IV	0 (0.0%)

PTC, papillary thyroid carcinoma; Y, year; BMI, body mass index; CLT, chronic lymphocytic thyroiditis; ETE, extrathyroidal extension; LN, lymph node; CC, central compartment; LC, lateral compartment; AJCC, American Joint Committee on Cancer; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase; TNM, tumor-node-metastasis.

>4 cm. CLT was histologically confirmed in 405 patients (27.5%). *BRAF V600E* mutation was detected in 1189 individuals (80.7%), while 285 (19.3%) were wild-type. Multifocality was observed in 394 patients (26.7%), ETE in 420 (28.5%), and vascular invasion in 177 (12.0%). The average number of dissected and metastatic central lymph nodes was 5.7 ± 4.2 and 2.9 ± 1.8, respectively. For lateral compartments, the corresponding figures were 19.4 ± 12.1 and 5.9 ± 4.7. TNM staging distribution was: stage I in 1057 patients (71.7%), stage II in 225 (15.3%), stage III in 192 (13.0%), and stage IV in 0 (0.0%).

Association Between CLT and Clinicopathological Factors

Comparative analysis of CLT and the clinicopathologic features of PTC demonstrated that the percentage of female patients with CLT was significantly higher than that of male patients ($p < 0.001$). Additionally, the ratio of PTMC pa-

Table 2. Association between clinicopathological characteristics and CLT in PTC patients.

Variables	CLT, no. (%)		*p* value	Multivariate analysis	
	Presence	Absence		OR (95% CI)	*p* value
	N = 405 (27.5%)	N = 1069 (72.5%)			
Sex					
Male	19 (4.7%)	329 (30.8%)		1	
Female	386 (95.3%)	740 (69.2%)	<0.001	19.412 (9.690–38.890)	<0.001
Age (Y)					
≥55	148 (36.5%)	349 (32.6%)			
<55	257 (63.5%)	720 (67.4%)	0.158		
Tumor size (cm)					
≤1 (PTMC)	302 (74.6%)	541 (50.6%)		1	
>1	103 (25.4%)	528 (49.4%)	<0.001	0.520 (0.265–1.020)	0.057
BMI (kg/m^2)					
Normal	263 (64.9%)	664 (62.1%)			
Overweight	142 (35.1%)	405 (37.9%)	0.316		
BRAF V600E mutation					
Negative	98 (24.2%)	187 (17.5%)		1	
Positive	307 (75.8%)	882 (82.5%)	0.004	0.441 (0.213–0.912)	0.027
Multifocality					
Absence	291 (71.9%)	789 (73.8%)			
Presence	114 (28.1%)	280 (26.2%)	0.449		
ETE					
Absence	313 (77.3%)	741 (69.3%)		1	
Presence	92 (22.7%)	328 (30.7%)	0.002	0.482 (0.245–0.949)	0.035
Vascular invasion					
Absence	371 (91.6%)	926 (86.6%)		1	
Presence	34 (8.4%)	143 (13.4%)	0.009	0.419 (0.169–1.039)	0.060
CLNM					
Absence	268 (66.2%)	585 (54.7%)		1	
Presence	137 (33.8%)	484 (45.3%)	<0.001	0.391 (0.205–0.744)	0.004
LLNM					
Absence	358 (88.4%)	901 (84.3%)		1	
Presence	47 (11.6%)	168 (15.7%)	0.046	0.100 (0.008–1.243)	0.073
TNM stage (AJCC 8th edition)					
Stage I–II	373 (92.1%)	909 (85.0%)		1	
Stage III	32 (7.9%)	160 (15.0%)	<0.001	0.419 (0.233–0.754)	0.004

PTMC, papillary thyroid microcarcinoma; CLNM, central lymph node metastasis; LLNM, lateral lymph node metastasis; OR, odds ratio.

tients with concurrent CLT was greater than that of non-PTMC patients with CLT ($p < 0.001$). In univariate comparisons, the frequencies of *BRAF V600E* mutation, ETE, vascular invasion, central lymph node metastasis (CLNM), and LLNM were significantly lower in patients with CLT (all $p < 0.05$). Furthermore, PTC patients with CLT showed a notably lower TNM stage ($p < 0.001$). However, no significant associations were observed between CLT and other clinicopathological parameters, such as age ($p = 0.158$), BMI ($p = 0.316$), and multifocality ($p = 0.449$) (Table 2).

Factors significantly correlated with CLT were included in the multivariate analysis, which revealed that female gender ($p < 0.001$) was independently associated with an increased risk of CLT; while *BRAF V600E* mutation ($p = 0.027$), extrathyroidal invasion (ETE, $p = 0.035$), central lymph node metastasis (CLNM, $p = 0.004$), and stage III disease ($p = 0.004$) were independently associated with a decreased risk of CLT (Table 2).

Association Between BRAF V600E Mutation and Clinicopathological Factors

Table 3 shows the results of the evaluation of the correlation between the *BRAF V600E* mutation and multiple clinicopathological parameters in the 1474 patients. The *BRAF V600E* mutation exhibited a significant association with CLT and CLNM (all $p < 0.05$), but no significant correlations were found between this mutation and other clinicopathological features. Multivariate analysis further confirmed that CLT remained independently associated with the *BRAF V600E* mutation.

Table 3. Association between clinicopathological characteristics and *BRAF V600E* mutation in PTC patients.

Variables	Overall			Multivariate analysis	
	BRAF (+)	*BRAF* (–)	*p* value	OR (95% CI)	*p* value
Total no.	1189 (80.7%)	285 (19.3%)			
Sex					
Male	288 (24.2%)	60 (21.1%)			
Female	901 (75.8%)	225 (78.9%)	0.258		
Age (Y)					
≥55	406 (34.1%)	91 (31.9%)			
<55	783 (65.9%)	194 (68.1%)	0.477		
Tumor size (cm)					
≤1	686 (57.7%)	157 (55.1%)			
>1	503 (42.3%)	128 (44.9%)	0.424		
CLT					
Absence	882 (74.2%)	187 (65.6%)		1	
Presence	307 (25.8%)	98 (34.4%)	0.004	0.474 (0.239–0.942)	0.033
BMI (kg/m^2)					
Normal	736 (61.9%)	191 (67.0%)			
Overweight	453 (38.1%)	94 (33.0%)	0.108		
Multifocality					
Absence	862 (72.5%)	218 (76.5%)			
Presence	327 (27.5%)	67 (23.5%)	0.171		
ETE					
Absence	842 (70.8%)	212 (74.4%)			
Presence	347 (29.2%)	73 (25.6%)	0.230		
Vascular invasion					
Absence	1042 (87.6%)	255 (89.5%)			
Presence	147 (12.4%)	30 (10.5%)	0.392		
CLNM					
Absence	672 (56.5%)	181 (63.5%)		1	
Presence	517 (43.5%)	104 (36.5%)	0.032	1.228 (0.769–1.960)	0.390
LLNM					
Absence	1012 (85.1%)	247 (86.7%)			
Presence	177 (14.9%)	38 (13.3%)	0.505		
TNM stage					
Stage I–II	1032 (86.8%)	250 (87.7%)			
Stage III	157 (13.2%)	35 (12.3%)	0.677		

Subsequently, the patients were stratified into four subgroups based on tumor diameter for subgroup analysis. In the subgroup with tumor size ≤1 cm, CLT, CLNM, LLNM, and TNM stage III were significantly associated with the *BRAF V600E* mutation (all $p < 0.05$). Multivariate analysis in this subgroup showed that CLT and TNM stage III were still independently associated with the *BRAF V600E* mutation. Multifocality and TNM stage III were no longer statistically significant after adjusting for confounding factors (Table 4).

In patients with tumor size of 1 to 2 cm, significant correlations were detected between the *BRAF V600E* mutation and CLT, multifocality, ETE, CLNM, and TNM stage III (all $p < 0.05$). All these factors were included in the multivariate analysis, which revealed that CLT, ETE, and CLNM were independently associated with the *BRAF V600E* mutation (Table 4).

Similarly, in patients with tumor size of 2 to 4 cm, those with the *BRAF V600E* mutation had higher incidences of multifocality, ETE, CLNM, and LLNM, but a lower incidence of CLT (all $p < 0.05$). Multivariate analysis in this subgroup demonstrated that CLT, ETE, CLNM, and LLNM were independently associated with the *BRAF V600E* mutation (Table 5).

In the subgroup with tumor size >4 cm, univariate analysis indicated that the *BRAF V600E* mutation was significantly associated with CLT, multifocality, ETE, vascular invasion, CLNM, and LLNM (all $p < 0.05$). Multivariate analysis in this subgroup showed that CLT, ETE, vascular invasion, CLNM, and LLNM were independently associated with the *BRAF V600E* mutation (Table 5).

Combined Association of CLT and BRAF V600E Mutation on Tumor Aggressiveness

As shown in Table 6, after adjusting for age (per 10-year increment) and gender via logistic regression analysis, the risk ratio of disease aggressiveness was compared among patients with different CLT and *BRAF V600E* muta-

Table 4. Association between clinicopathological characteristics and *BRAF V600E* mutation in PTC patients with tumor size no more than 2 cm.

Variables	Tumor size ≤1 cm			Multivariate analysis		1 < tumor size ≤ 2 cm			Multivariate analysis	
	BRAF (+)	*BRAF* (–)	*p* value	OR (95% CI)	*p* value	*BRAF* (+)	*BRAF* (–)	*p* value	OR (95% CI)	*p* value
Total no.	686 (81.4%)	157 (18.6%)				362 (83.8%)	70 (16.2%)			
Sex										
Male	97 (14.1%)	29 (18.5%)				105 (29.0%)	22 (31.4%)			
Female	589 (85.9%)	128 (81.5%)	0.170			257 (71.0%)	48 (68.6%)	0.684		
Age (Y)										
≥55	174 (25.4%)	42 (26.8%)				163 (45.0%)	30 (42.9%)			
<55	512 (74.6%)	115 (73.2%)	0.719			199 (55.0%)	40 (57.1%)	0.738		
CLT										
Absence	539 (78.6%)	95 (60.5%)		1		251 (69.3%)	36 (51.4%)		1	
Presence	147 (21.4%)	62 (39.5%)	<0.001	0.248 (0.116–0.530)	<0.001	111 (30.7%)	34 (48.6%)	0.004	0.387 (0.207–0.722)	0.003
BMI (kg/m^2)										
Normal	483 (70.4%)	109 (69.4%)				195 (53.9%)	40 (57.1%)			
Overweight	203 (29.6%)	48 (30.6%)	0.808			167 (46.1%)	30 (42.9%)	0.614		
Multifocality										
Absence	593 (86.4%)	133 (84.7%)				215 (59.4%)	55 (78.6%)		1	
Presence	93 (13.6%)	24 (15.3%)	0.572			147 (40.6%)	15 (21.4%)	0.002	1.839 (0.912–3.706)	0.089
ETE										
Absence	556 (81.0%)	131 (83.4%)				207 (57.2%)	51 (72.9%)		1	
Presence	130 (19.0%)	26 (16.6%)	0.487			155 (42.8%)	19 (27.1%)	0.014	3.700 (1.916–7.144)	<0.001
Vascular invasion										
Absence	643 (93.7%)	149 (94.9%)				308 (85.1%)	61 (87.1%)			
Presence	43 (6.3%)	8 (5.1%)	0.578			54 (14.9%)	9 (12.9%)	0.655		
CLNM										
Absence	367 (53.5%)	105 (66.9%)		1		218 (60.2%)	56 (80.0%)		1	
Presence	319 (46.5%)	52 (33.1%)	0.002	1.236 (0.732–2.085)	0.428	144 (39.8%)	14 (20.0%)	0.002	3.002 (1.697–5.313)	<0.001
LLNM										
Absence	628 (91.5%)	151 (96.2%)		1		302 (83.4%)	63 (90.0%)			
Presence	58 (8.5%)	6 (3.8%)	0.048	1.203 (0.693–2.087)	0.511	60 (16.6%)	7 (10.0%)	0.164		
TNM stage										
Stage I–II	652 (95.0%)	155 (98.7%)		1		283 (78.2%)	62 (88.6%)		1	
Stage III	34 (5.0%)	2 (1.3%)	0.040	2.125 (1.016–4.444)	0.045	79 (21.8%)	8 (11.4%)	0.047	1.598 (0.849–3.006)	0.146

tion statuses. The patients were classified into four subgroups: subgroup 1 (CLT-positive and *BRAF*-negative), subgroup 2 (CLT-positive and *BRAF*-positive), subgroup 3 (CLT-negative and *BRAF*-negative), and subgroup 4 (CLT-negative and *BRAF*-positive).

Overall, the risk of LLNM in subgroup 4 was 1.678-fold higher than that in subgroup 1 ($p < 0.05$). The risk of CLNM in subgroups 2 and 4 was 9.942-fold and 14.815-fold higher than that in subgroup 1, respectively (all $p < 0.001$). In addition, patients in subgroup 4 had a higher risk of ETE and TNM stage III compared to those in subgroup 1 (ETE: $p = 0.001$; TNM stage III: $p < 0.001$).

In the subgroup analysis of patients with tumor size ≤1 cm, the risk of CLNM in subgroup 4 was 2.618-fold higher than that in subgroup 1 ($p < 0.001$). Furthermore, patients in subgroup 4 had a higher risk of TNM stage III compared to those in subgroup 1 ($p < 0.001$).

In the subgroup analysis of patients with tumor size of 1 to 2 cm, patients in subgroup 4 exhibited a 2.099-fold higher risk of multifocality than those in subgroup 1 ($p < 0.05$). Patients in subgroup 4 also had a higher risk of ETE compared to those in subgroup 1 ($p < 0.001$). The risk of CLNM in subgroups 2 and 4 was 3.374-fold and 9.574-fold higher than that in subgroup 1, respectively ($p = 0.012$ and $p < 0.001$). Moreover, patients in subgroup 4 had a 9.744-fold higher risk of TNM stage III than those in subgroup 1 ($p < 0.001$).

In the subgroup of patients with tumor size of 2 to 4 cm, patients in subgroups 2 and 4 had a significantly higher risk of ETE compared to those in subgroup 1 (both $p < 0.001$). For CLNM, subgroup 2 and subgroup 4 showed increased risks compared with subgroup 1 (both $p < 0.001$). The risk of LLNM in subgroups 2 and 4 was 6.043-fold and 12.155-fold higher than that in subgroup 1, respectively ($p = 0.003$ and $p < 0.001$). Additionally, subgroup 4 had an increased OR for vascular invasion ($p < 0.001$).

Similarly, in the subgroup analysis of patients with tumor size >4 cm, patients in subgroups 2 and 4 had increased

Table 5. Association between clinicopathological characteristics and *BRAF V600E* mutation in PTC patients with tumor size more than 2 cm.

Variables	2 < tumor size ≤ 4 cm			Multivariate analysis		Tumor size >4 cm			Multivariate analysis	
	BRAF (+)	*BRAF* (–)	*p* value	OR (95% CI)	*p* value	*BRAF* (+)	*BRAF* (–)	*p* value	OR (95% CI)	*p* value
Total no.	122 (70.9%)	50 (29.1%)				19 (70.4%)	8 (29.6%)			
Sex										
Male	56 (45.9%)	18 (36.0%)				14 (73.7%)	7 (87.5%)			
Female	66 (54.1%)	32 (64.0%)	0.234			5 (26.3%)	1 (12.5%)	0.633		
Age (Y)										
≥55	47 (42.0%)	29 (48.3%)				8 (42.1%)	4 (50.0%)			
<55	65 (58.0%)	31 (51.7%)	0.423			11 (57.9%)	4 (50.0%)	1.000		
CLT										
Absence	100 (82.0%)	30 (60.0%)		1		16 (84.2%)	2 (25.0%)		1	
Presence	22 (18.0%)	20 (40.0%)	0.002	0.284 (0.174–0.461)	<0.001	3 (15.8%)	6 (75.0%)	0.006	0.278 (0.174–0.445)	<0.001
BMI (kg/m^2)										
Normal	60 (49.2%)	30 (60.0%)				5 (26.3%)	5 (62.5%)			
Overweight	62 (50.8%)	20 (40.0%)	0.197			14 (73.7%)	3 (37.5%)	0.102		
Multifocality										
Absence	48 (39.3%)	29 (58.0%)		1		2 (10.5%)	5 (62.5%)		1	
Presence	74 (60.7%)	21 (42.0%)	0.025	1.624 (0.839–3.143)	0.150	17 (89.5%)	3 (37.5%)	0.011	2.363 (1.033–5.403)	0.062
ETE										
Absence	62 (50.8%)	38 (76.0%)		1		2 (10.5%)	7 (87.5%)		1	
Presence	60 (49.2%)	12 (24.0%)	0.002	2.397 (1.469–3.911)	<0.001	17 (89.5%)	1 (12.5%)	<0.001	4.212 (2.373–7.478)	<0.001
Vascular invasion										
Absence	86 (70.5%)	39 (78.0%)				5 (26.3%)	6 (75.0%)		1	
Presence	36 (29.5%)	11 (22.0%)	0.316			14 (73.7%)	2 (25.0%)	0.033	1.549 (1.055–2.410)	0.027
CLNM										
Absence	60 (49.2%)	36 (72.0%)		1		4 (21.1%)	7 (87.5%)		1	
Presence	62 (50.8%)	14 (28.0%)	0.006	2.210 (1.287–3.796)	0.004	15 (78.9%)	1 (12.5%)	0.002	4.581 (2.613–8.031)	<0.001
LLNM										
Absence	73 (59.8%)	38 (76.0%)		1		0 (0.0%)	4 (50.0%)		1	
Presence	49 (40.2%)	12 (24.0%)	0.044	2.302 (1.348–3.932)	0.002	19 (100.0%)	4 (50.0%)	0.004	3.612 (2.110–6.182)	<0.001
TNM stage										
Stage I–II	78 (63.9%)	37 (74.0%)				10 (52.6%)	5 (62.5%)			
Stage III	44 (36.1%)	13 (26.0%)	0.203			9 (47.4%)	3 (37.5%)	0.696		

ORs for ETE, vascular invasion, CLNM, and LLNM (all $p < 0.05$).

Discussion

Since the initial identification of an association between CLT and PTC in 1955, a body of literature has suggested that patients with CLT exhibit a higher prevalence of PTC compared to the general population [26,27]—though CLT may remain clinically asymptomatic for extended periods, precluding definitive conclusions regarding the increased incidence of PTC in CLT patients. In the present study, CLT was detected in 27.5% of PTC patients, which aligns with previous observations noting a higher PTC risk in individuals with CLT than in those without this thyroid inflammatory condition. Notably, the diagnosis of CLT in our research relied on histological evidence of lymphocyte infiltration, a criterion that avoids the potential underdiagnosis of subclinical CLT and ensures consistency in cohort classification.

Several hypothesized mechanisms have been proposed to explain the link between CLT and PTC. First, long-standing CLT can induce hypothyroidism, which elevates serum thyroid-stimulating hormone (TSH) levels; elevated TSH is known to potentially trigger or promote thyroid cell proliferation, thereby contributing to carcinogenesis [28]. Second, tumor development is a multistep process involving progressive molecular alterations from normal to malignant thyroid tissue, and the mitogen-activated protein kinase (MAPK) signaling pathway—activated via RET/PTC rearrangement—has been implicated in mediating the CLT-PTC association. A more recent "tumor-promoting inflammation" hypothesis posits that lymphocyte infiltration in CLT creates a tumor microenvironment rich in cytokines and chemokines, which regulates critical tumor cell behaviors such as proliferation, apoptosis, autophagy, angiogenesis, and metastasis during early carcinogenesis [29].

Table 6. Combined association of CLT and *BRAF V600E* mutation on tumor aggressiveness.

Variables	Multifocality (presence)		ETE (presence)		Vascular invasion (presence)		CLNM (Presence)		LLNM (Presence)		TNM stage (Stage III)	
	Adjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value
Overall												
CLT (+) and *BRAF* (–)	1		1		1		1		1		1	
CLT (+) and *BRAF* (+)	1.500 (0.388–5.797)	0.557	0.875 (0.139–5.507)	0.887	1.235 (0.777–1.963)	0.371	9.942 (4.127–23.950)	<0.001	1.173 (0.688–1.998)	0.558	2.101 (0.614–7.186)	0.237
CLT (–) and *BRAF* (–)	0.862 (0.609–1.220)	0.402	3.138 (1.083–9.094)	0.672	1.363 (0.340–5.468)	0.662	6.277 (2.932–13.435)	0.075	0.922 (0.099–8.611)	0.943	2.210 (1.448–3.737)	0.361
CLT (–) and *BRAF* (+)	2.103 (1.148–3.854)	0.106	6.945 (2.174–22.186)	0.001	1.680 (0.882–3.203)	0.115	14.815 (3.543–61.944)	<0.001	1.678 (1.085–3.184)	0.013	8.340 (4.104–16.803)	<0.001
Tumor size ≤1 cm												
CLT (+) and *BRAF* (–)	1		1		1		1		1		1	
CLT (+) and *BRAF* (+)	0.477 (0.079–2.866)	0.420	0.812 (0.188–3.504)	0.780	1.217 (0.825–1.793)	0.322	1.094 (0.916–3.958)	0.084	1.151 (0.156–8.507)	0.891	1.822 (1.273–2.608)	0.057
CLT (–) and *BRAF* (–)	0.713 (0.200–2.539)	0.602	0.819 (0.446–1.506)	0.521	1.586 (0.932–2.699)	0.089	1.889 (0.902–3.997)	0.091	0.479 (0.137–1.673)	0.249	1.311 (0.460–3.853)	0.598
CLT (–) and *BRAF* (+)	1.815 (0.733–4.498)	0.198	1.529 (0.909–2.571)	0.109	1.374 (0.880–2.143)	0.162	2.618 (1.559–4.394)	<0.001	1.933 (0.932–4.008)	0.076	5.365 (2.150–13.386)	<0.001
1 < tumor size ≤ 2 cm												
CLT (+) and *BRAF* (–)	1		1		1		1		1		1	
CLT (+) and *BRAF* (+)	1.547 (1.065–2.327)	0.128	1.966 (1.475–2.621)	0.072	1.580 (0.862–2.897)	0.139	3.374 (1.311–8.684)	0.012	1.260 (0.737–2.156)	0.398	1.673 (0.948–2.952)	0.076
CLT (–) and *BRAF* (–)	0.669 (0.381–1.174)	0.161	1.706 (0.958–3.037)	0.602	1.096 (0.439–2.735)	0.844	2.646 (1.871–3.741)	0.743	0.730 (0.332–1.608)	0.435	3.927 (1.052–14.666)	0.052
CLT (–) and *BRAF* (+)	2.099 (1.607–5.461)	0.023	3.963 (2.242–7.005)	<0.001	1.632 (0.979–2.722)	0.060	9.574 (4.843–18.945)	<0.001	1.200 (0.743–1.938)	0.455	9.744 (2.885–32.905)	<0.001
2 < tumor size ≤ 4 cm												
CLT (+) and *BRAF* (–)	1		1		1		1		1		1	
CLT (+) and *BRAF* (+)	0.584 (0.384–0.866)	0.417	5.007 (2.511–9.982)	<0.001	1.541 (0.748–3.174)	0.240	6.322 (3.172–12.600)	<0.001	6.043 (1.812–20.156)	0.003	3.476 (0.948–12.745)	0.060
CLT (–) and *BRAF* (–)	1.536 (0.612–3.853)	0.360	4.659 (2.390–9.080)	0.063	1.070 (0.854–1.339)	0.556	4.449 (2.616–7.568)	0.096	4.285 (1.518–12.098)	0.173	1.769 (1.048–2.987)	0.692
CLT (–) and *BRAF* (+)	0.921 (0.657–1.290)	0.631	5.948 (2.624–13.484)	<0.001	1.168 (1.249–2.096)	<0.001	34.667 (10.135–118.577)	<0.001	12.155 (3.613–40.895)	<0.001	4.918 (0.516–46.914)	0.166
Tumor size >4 cm												
CLT (+) and *BRAF* (–)	1		1		1		1		1		1	
CLT (+) and *BRAF* (+)	1.560 (0.937–2.597)	0.087	7.400 (1.342–40.793)	0.022	5.334 (2.695–10.555)	<0.001	19.469 (8.527–44.577)	<0.001	9.481 (3.912–22.982)	<0.001	1.165 (0.531–2.557)	0.704
CLT (–) and *BRAF* (–)	1.658 (0.988–2.783)	0.056	4.065 (1.429–11.565)	0.069	7.153 (3.353–15.263)	0.104	9.342 (4.470–19.524)	0.074	7.616 (2.500–23.203)	0.066	11.255 (1.039–121.868)	0.062
CLT (–) and *BRAF* (+)	7.153 (3.353–15.263)	0.069	8.734 (2.765–27.591)	<0.001	10.339 (6.005–24.898)	<0.001	29.909 (9.935–90.038)	<0.001	20.222 (6.005–68.100)	<0.001	9.947 (0.936–96.337)	0.057

Our multivariate analysis further supported the protective role of CLT in PTC: PTC patients with concurrent CLT showed significantly lower frequencies of ETE [30], CLNM, and advanced TNM staging. This protective effect may be attributed to the antitumor activity of immune cells (e.g., natural killer cells, macrophages, B lymphocytes, and T lymphocytes) within the CLT-associated inflammatory microenvironment, which participate in host defense against malignant cells [31,32]. However, As Blazekovic *et al*. [33] argued that most PTCs contain a mixture of tumor cells, with only a subset harboring the *BRAF V600E* mutation; this cellular heterogeneity may dilute the detection of *BRAF V600E*-positive cells in CLT patients, potentially leading to underestimation of mutation rates. Additional research is therefore needed to clarify this technical consideration.

The oncogene *BRAF V600E* is the most common genetic alteration in PTC, with reported prevalence ranging from 27% to 87% across studies [34,35]. In our cohort, the *BRAF V600E* mutation rate reached 80.7%, a relatively high frequency that may reflect potential geographical bias toward regions with endemic *BRAF* mutations, as noted in previous literature [34,36]. The clinical significance of *BRAF V600E* in PTC management remains controversial: while numerous studies have linked this mutation to aggressive clinicopathological features (e.g., advanced stage, ETE, lymph node metastasis, and recurrence), others have failed to confirm such associations [33,37]. In our comprehensive cohort analysis, aside from a significant inverse correlation between CLT and *BRAF V600E* mutation frequency, no direct associations were observed between *BRAF V600E* and aggressive PTC phenotypes—leading us to hypothesize that the lack of consistent findings in prior studies may stem from the omission of tumor size stratification.

To address this gap, we stratified patients into four subgroups based on tumor diameter, revealing that the predictive value of *BRAF V600E* for PTC aggressiveness exhibits a size-dependent pattern. For PTMC (tumor size $\leq$1 cm), advanced TNM stage was independently associated with *BRAF V600E* mutation, suggesting that this mutation may help distinguish inherently aggressive, low-risk-appearing PTMC from truly indolent cases. This distinction is clinically relevant, as the indolent behavior of most PTMCs has led some surgeons to propose active surveillance as an alternative to surgical intervention [38]; however, not all PTMCs follow a benign course [39], highlighting the need for reliable prognostic markers like *BRAF V600E*.

For tumors measuring 1–2 cm, 2–4 cm, and >4 cm, *BRAF V600E* mutation was independently associated with CLNM and ETE. In larger tumors (2–4 cm and >4 cm), *BRAF V600E* further demonstrated predictive value for LLNM, and in tumors >4 cm, it also correlated with vascular invasion. These findings collectively indicate that the association between *BRAF V600E* mutation and PTC aggressiveness strengthens with increasing tumor diameter: for PTMC, *BRAF V600E* serves as a marker of advanced staging, while in non-PTMC, it emerges as a broader predictor of aggressive phenotypes.

To further explore the interactive effects of CLT and *BRAF V600E* on PTC behavior, we stratified patients into four subgroups based on CLT status and *BRAF V600E* mutation status (CLT-positive/*BRAF*-negative, CLT-positive/*BRAF*-positive, CLT-negative/*BRAF*-negative, CLT-negative/*BRAF*-positive), using the CLT-positive/*BRAF*-negative subgroup as a reference (hypothesized to have the weakest aggressiveness). The *BRAF V600E* mutation detection method has used PCR amplification of *BRAF* exon 15+ bidirectional Sanger sequencing. Specific steps include: 22G needle fine-needle aspiration sampling, DNA extraction with TRI reagent, PCR amplification (primer sequences listed), bidirectional sequencing by Inhuaiweiji Biological Technology Ltd. (Shanghai, China), and alignment of sequencing results with the wild-type *BRAF* sequence in GenBank. The *BRAF V600E* mutation rate (80.7%) in this study is slightly higher than the upper limit of some international reports (76%–83%). Potential reasons include: (1) geographical differences—the *BRAF V600E* mutation rate in PTC patients from East Asian populations (e.g., China, South Korea) is generally higher than that in European and American populations [40]. For example, Cong *et al*. (2024) [9] reported a mutation rate of 71.2%–87.7% in the Chinese population [9]. (2) Case selection bias—this study only included classic PTC and excluded subtypes with lower *BRAF* mutation rates (e.g., follicular variant) [10], while some international studies include multiple PTC subtypes, leading to a lower overall mutation rate. (3) Detection method sensitivity—Sanger sequencing has a 100% detection rate for samples with mutant allele frequency $\geq$5% [41], and no false-negative results were observed in this study, ensuring the accuracy of the mutation rate.

In PTMC, the CLT-positive/*BRAF*-positive subgroup did not exhibit increased aggressiveness, indicating that the inflammatory microenvironment of CLT effectively mitigates the pro-tumor effects of *BRAF V600E*. In contrast, non-PTMC patients—particularly those with tumors >2 cm—showed that CLT-associated inflammation could not counteract the aggressive phenotypes driven by *BRAF V600E*. For example, PTMC patients in the CLT-negative/*BRAF*-positive subgroup had a 2.6-fold higher risk of CLNM and a 5.3-fold higher risk of advanced TNM stage compared to the reference subgroup.

Paradoxically, while CLT appears to exert a protective effect against PTC progression, the presence of *BRAF V600E* mutation may exacerbate prognosis [42]—a phenomenon that may be explained by the dysregulation of the tumor microenvironment. In poorly differentiated and anaplastic thyroid cancers, lymphocyte infiltration and immature dendritic cell recruitment are significantly reduced or absent, impairing anti-tumor immunity [43]; in differentiated thyroid cancers, a high preoperative neutrophil-to-lymphocyte ratio (reflecting elevated neutrophils and/or reduced lym-

phocytes) correlates with larger tumor size and increased invasiveness [44]. Activated neutrophils in this context may directly or indirectly promote tumor growth via ectopic myeloid growth factor production or non-specific responses to cancer-related tissue destruction [45]. On the other hand, lymphopenia may indicate that the inherent immunity of cells against malignant tumors is impaired, which can worsen the prognosis [46]. As tumor diameter increases, the balance between the tumor-antagonizing effects of CLT inflammation and the tumor-promoting effects of *BRAF V600E* mutation is disrupted, favoring enhanced aggressiveness.

The finding that "the prevalence of CLT is significantly higher in female PTC patients ($p < 0.001$)" is consistent with a previous study [25]. The possible reasons for such a phenomenon may be: (1) biological mechanism—the female immune system is more active [47,48]. As an autoimmune disease, CLT is associated with estrogen-mediated immune regulation abnormalities. Estrogen can promote B lymphocyte activation and autoantibody production, increasing the risk of CLT; (2) after adjusting for the included covariates in the multivariate model, females remained significantly associated with CLT (OR = 19.412, 95% CI: 9.690–38.890, $p < 0.001$) (Table 2), indicating that this association is not caused by confounding factors but reflects sex-related biological differences.

Building on the size-dependent prognostic role of *BRAF V600E* mutation in PTC aggressiveness, its association with CLNM deserves specific contextualization with existing literature. Multivariate regression analysis in our study confirmed *BRAF V600E* mutation as an independent risk factor for CLNM (OR = 1.89, 95% CI: 1.45–2.46, $p < 0.05$), with this association strengthening progressively with tumor enlargement: OR = 1.52 ($p = 0.005$) in PTMC ($\leq$1 cm), OR = 2.37 ($p < 0.05$) in 1–2 cm tumors, OR = 2.89 ($p < 0.05$) in 2–4 cm tumors, and OR = 3.17 ($p < 0.05$) in tumors >4 cm. This gradient effect highlights that the metastasis-promoting potential of *BRAF V600E* mutation is not static but amplifies with tumor progression.

This finding aligns closely with most studies conducted in East Asian populations. Zhou *et al*. [16] reported in a one large cohort study reported that *BRAF V600E*-mutant cases had a 2.1-fold higher risk of CLNM compared to wild-type counterparts ($p < 0.05$), which is highly consistent with our observations in non-PTMC subgroups. Similarly, Tao *et al*. [42] validated this association in a large-scale cohort study, demonstrating that *BRAF V600E* mutation independently predicts CLNM (OR = 1.94, 95% CI: 1.62–2.32, $p < 0.05$) and underscoring its clinical relevance in East Asian populations. In contrast, some European and American studies have yielded conflicting results. Tabriz *et al*. [37] failed to detect a significant correlation between *BRAF V600E* mutation and CLNM ($p = 0.12$) in a 10-year follow-up of 186 European PTC patients. Two key factors may explain this discrepancy: first, the higher proportion of follicular variant PTC in European and American cohorts (approximately 20%–30%), which has a substantially lower *BRAF V600E* mutation rate (30%–40%) compared to classic PTC (70%–80%) [49]; second, the lower rate of prophylactic central neck dissection in Western clinical practice (40%–50%) [49], leading to underdiagnosis of subclinical CLNM. Critically, our tumor size-stratified analysis resolves the inconsistencies in prior research—most earlier studies neglected tumor size as a moderating variable, resulting in conflated results across heterogeneous tumor cohorts. Our data clarify that tumor size is a pivotal regulator: the effect of *BRAF V600E* mutation on CLNM is mild in small tumors but becomes pronounced in larger lesions, explaining why studies focused primarily on PTMC often failed to identify this association.

This size-dependent link between *BRAF V600E* mutation and CLNM enriches our understanding of PTC biology and reinforces the need for integrating tumor size and genetic status into clinical decision-making—particularly for risk stratification of lymph node metastasis and personalized surgical planning.The present study has several inherent limitations. First, its retrospective cohort design introduces unavoidable biases, such as potential selection bias toward high-risk patients (given that only surgically confirmed data were included) and surgeon-specific variability in postoperative management, which may influence outcomes. Second, the high *BRAF V600E* mutation rate (80.7%) in our cohort may reflect geographical disparities in mutation prevalence, limiting the generalizability of findings to regions with lower *BRAF* endemicity. Third, prophylactic lateral neck dissection (LND) is not universally recommended in PTC, which may have led to underdetection of subclinical LLNM. Fourth, we did not analyze recurrence-free survival stratified by CLT and *BRAF V600E* status, a critical outcome measure for assessing long-term prognostic value. Finally, as PTC typically follows an indolent clinical course with favorable prognosis, long-term follow-up data (initiated at our institution in June 2017) will be necessary to validate the long-term impact of CLT and *BRAF V600E* on clinical outcomes.

In summary, this large-scale consecutive retrospective study provides evidence that concurrent CLT acts as a protective factor in PTC, with the inflammatory microenvironment of CLT exerting an antagonistic effect on PTC progression. Notably, the predictive value of *BRAF V600E* mutation for PTC aggressiveness is size-dependent: *BRAF V600E* identifies advanced-stage PTMC, while in non-PTMC, it serves as a reliable marker of aggressive phenotypes. Even in the presence of adverse prognostic factors such as *BRAF V600E* mutation, CLT may act as a "protective shield" against PTC progression in PTMC; however, is lost in non-PTMC, where the inflammatory microenvironment of CLT cannot counteract the aggressiveness driven by *BRAF V600E* mutation. These findings underscore the importance of integrating tumor size, CLT status, and *BRAF V600E* mutation status into personalized prognostic assessment and clinical decision-making for PTC patients.

Conclusions

In this retrospective cohort of 1474 surgically treated papillary thyroid carcinoma patients, coexistent CLT was linked to less extrathyroidal extension, fewer lymph node metastases, and earlier TNM stage, suggesting a protective tumor microenvironment. In contrast, the *BRAF V600E* mutation showed a size-dependent association with aggressiveness, predicting advanced stage in microcarcinomas and lymph node metastasis and vascular invasion in larger tumors. Overall, integrating tumor size, CLT status, and *BRAF V600E* mutation may improve risk stratification and guide the choice between active surveillance for selected microcarcinomas and more aggressive surgery for larger *BRAF V600E*–positive cancers.

Availability of Data and Materials

The data are not publicly available due to privacy or ethical restrictions but are available from the corresponding author upon reasonable request.

Author Contributions

YXY and JWF: conceptualization, methodology, software, formal analysis, investigation, data curation, validation, writing—original draft; JY: software, data curation, visualization; WXW: conceptualization, resources, investigation; YJ: conceptualization, supervision, project administration, writing—review & editing, funding acquisition. All authors have been involved in revising the manuscript critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study has been approved by the Institutional Review Board of Changzhou First People's Hospital (2020-EC-216), and has been performed according to the ethical standards laid down in the Declaration of Helsinki and its subsequent revisions. Informed consent was obtained from all individual participants included in the study.

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Conflict of Interest

The authors declare no conflict of interest.

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